МАКЕДОНСКА АКАДЕМИЈА НА НАУКИТЕ И УМЕТНОСТИТЕ

ПРЕДИЗВИЦИ ВО ХИРУРГИЈАТА НА УРОГЕНИТАЛНИОТ СИСТЕМ, ТРАНСПЛАНТАЦИЈАТА И МЕДИЦИНАТА

Зборник на научни трудови посветен на акад. Живко М. Попов по повод 70 години од неговото раѓање

MACEDONIAN ACADEMY OF SCIENCES AND ARTS

CHALLENGES IN THE SURGERY OF THE UROGENITAL SYSTEM, TRANSPLANTATION AND MEDICINE

PROCEEDINGS OF SCIENTIFIC WORKS DEDICATED
TO ACADEMICIAN ZIVKO M. POPOV ON THE OCCASION
OF THE 70TH ANNIVERSARY OF HIS BIRTH

SKOPJE 2022

МАКЕДОНСКА АКАДЕМИЈА НА НАУКИТЕ И УМЕТНОСТИТЕ

ПРЕДИЗВИЦИ ВО ХИРУРГИЈАТА НА УРОГЕНИТАЛНИОТ СИСТЕМ, ТРАНСПЛАНТАЦИЈАТА И МЕДИЦИНАТА

ЗБОРНИК НА НАУЧНИ ТРУДОВИ ПОСВЕТЕН
НА АКАД. ЖИВКО М. ПОПОВ
ПО ПОВОД 70 ГОДИНИ ОД НЕГОВОТО РАЃАЊЕ

СКОПЈЕ 2022

Уредник
акад. Љупчо Коцарев

doi: 10.20903/zpopov-2022

CIP – Каталогизација во публикација
Национална и универзитетска библиотека „Св. Климент Охридски“, Скопје

616.61(081)

ПРЕДИЗВИЦИ во хирургијата на урогениталниот систем, трансплантацијата и медицината : зборник на научни трудови посветен на акад. Живко М. Попов по повод 70 години од неговото раѓање / [уредник Љупчо Коцарев]. - Скопје : Македонска академија на науките и уметностите : Macedonian Academy of Sciences and Arts, 2022. - 609 стр.: илустр. ; 17 см

На наспр. насл. стр.: Challenges in the surgery of the urogenital system, transplantation and medicine : proceedings of scientific works dedicated to academician Zivko M. Popov on the occasion of the 70th anniversary of his birth. - Текст напоредно на мак. и англ. јазик. - Фусноти кон текстот. - Слово за животот на академик Живко Попов / Љупчо Коцарев стр. 13-15. - Белешка за авторот стр. 19-30. - Библиографија стр: 31-55. - Резимеа кон одделни трудови

ISBN 978-608-203-350-1

а) Урологија -- Зборници

COBISS.MK-ID 57400325

академик Живко Михаил Попов

Членство во МАНУ: Редовен член од 2009, потпретседател од 2020 година
***Институции*:** Редовен професор по хирургија на Медицинскиот факултет, УКИМ; Клиничка болница Аџибадем Систина, Скопје, Северна Македонија
***Области на работа*:** Урологија/трансплантација на бубрези/урогенитален канцер/ имуногенетски истражувања *e-mail: zivkopopov2000@yahoo.com*
Web of Science Researcher ID: https://publons.com/researcher/AAG-1481-2021/
ORCID ID: https://orcid.org/0000-0002-7178-0927
Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=7004730664

СОДРЖИНА

doi: 10.20903/zpopov-2022-0001 UDK: 929Попов, Ж.

Љупчо КОЦАРЕВ[1]

СЛОВО ЗА ЖИВОТОТ НА АКАДЕМИК ЖИВКО ПОПОВ

Македонската академија на науките и уметностите, ја одбележува седумдесетгодишнината од раѓањето на академик Живко Попов. Академик Попов е човек со повеќе димензии: интелектуалец, лекар, хирург, научник, вљубеник во медицината и во Македонија, визионер, ерудит. Постојат редица поими што се пишуваат на повеќе начини и затоа опишуваат различни концепти/појави. Тоа се поими чии први букви се пишуваат со големи, понекогаш задебелени букви и со мали, понекогаш задебелени букви. Ќе наведам само неколку примера: Слово и слово, **Б**итие и битие, **И**сторија, **и**сторија и историја, **Ж**ивот и живот.

Во првиот пример, синтагмата „слово за Словото“, на пример, се однесува на говор и или слова за Словото, при што „самиот Господ Исус Христос е наречен Слово, преку Кое и Бог говорел“. „Во почетокот беше Словото / И Словото беше во Бога /И Бог беше Слово“ („Свето писмо, Библија“, Скопје, 1990). Оттука, во книгата проповеди „Слова за Словото“, архиепископот охридски и македонски г. г. Стефан ќе напише: „Па, слова ВО КОИ СЀ Е ЗА Словото и околу Словото слова во кои Словото е и повод и причина за Словото, слова во кои сѐ започнува и сѐ завршува со Словото, не можеше да бидат насловени поинаку од ова – ‘Слова за Словото’“.

Поимите **Б**итие битие одиграа клучна улога во 20 век. Затоа треба да му благодариме на Хајдегер. Потпирајќи се на редица мислители (Аристотел, Декарт, Ниче, Кјеркегор, Хусерл), Хајдегер ја истражува фундаменталната перцепција на поимот битие. Според него, постојат два термина: битие и Битие. Во книгата „Битие и Време“ (на германски јазик: *Sein und Zeit*, на англиски јазик: *Being and Time*), тој зборува за

[1] Претседател на Македонска академија на науките и уметностите

онтолошката разлика помеѓу битието и **Б**итието. Хајдегер истакнува дека прашањата за **Б**итието се квалитативно различни од другите ентитети што постојат и нѐ опкружуваат (на пример, карпите, океаните, дрвјата, животните). Тој измисли посебен збор за ова **Б**итие што прашува, бара и се грижи. Тоа е зборот „тука-битие“ или битисување“ (на германски, Dasein), и се однесува на суштества кои можат да го постават прашањето за природата на **Б**итието, што ги прави различни од што било друго во светот. Според него, битисување е начин на живот што го делат членовите на една заедница, слично на јазикот којшто претставува заедничка форма на комуникација.

Разликата меѓу **И**сторија, со големо **И**, и **и**сторија, со мало **и,** ја воведува Мишел Фуко (во 1990 година), како трето поимање на поимот Историја (со големо И), различно од оние на Хегел. Според Ќулавкова, „кога се зборува за историјата, се разликуваат две основни концепции: 1) **и**сторијата сфатена како процес и проток на историски настани, личности и дела сместени во некој простор и во рамки на некој временски тек, па во тој случај зборуваме за историско минато, за реалната историска стварност; 2) **И**сторијата сфатена како повест, како ‘сторија’ и приказна, како историска свест и рефлексија, како јазик и, конечно, како систем на знаења или историска наука“.

Во овој кус текст ќе се обидам да проговорам за разликата меѓу концептите **Ж**ивот и **ж**ивот: (1)**ж**ивот сфатен како процес и проток на настани на битие што се раѓа и умира, на човек, сместен во некој простор и во рамки на некој временски тек, па во тој случај зборуваме за конкретен животен пат, за реална стварност; (2) **Ж**ивот сфатен како состојба што ги разликува животните и растенијата од неорганската материја, вклучувајќи го и капацитетот за раст, размножување, функционална активност и за постојана промена пред смртта, сфатен како постоење на индивидуално човечко суштество или животно, и конечно, како систем на знаења или наука за животот. Ваквото толкување, во принцип, доведува до прашањето за суштината на човековиот живот, а оттаму, до прашањето за положбата на човекот и неговата судбина. Поимот живот игра клучна улога во, на пример, филозофските погледи на Бергсон, Дилтај, Шелери Плеснер. Хајдегер, од друга страна, признава дека е тешко да се пронајде вистинскиот пристап кон проблемот на животот, бидејќи она што е најтешко да се искуси, тоа е самиот живот во неговата специфичност. Според Хајде-

гер, единствениот начин на човековото остварување на неговото присуство во светот е да биде „Битие-во-светот“ (In-derWelt-sein).

Причината за овој кус преглед на различни концепти поврзани со поимите слово, историја, битие и живот, е, од друг агол, да напишам слово за животот на академик Попов по повод седумдесетгодишнината од неговото раѓањето. Не случајно името на славеникот е Живко: во изминатите 70 години, академик Попов егзистираше на начин на „тука-битие“, оставајќи длабоки траги во МАНУ, во современата македонска држава и светската наука. Му посакувам уште многу години да живее, да твори, да ја испишува историјата на македонската држава и да спасува животи.

Во Скопје, 30 октомври 2021 година

ПОГЛАВЈЕ I

АКАДЕМИК ЖИВКО М. ПОПОВ – ЖИВОТ И ДЕЛО

doi: 10.20903/zpopov-2022-0040 UDK: 929Попов, Ж.

Биографски податоци

Академик Живко Михаил Попов е роден пред седумдесет години во Кавадарци, НРМ (СФРЈ). Одраснал во Битола, каде завршил основно училиште (*Гоце Делчев*) и гимназија (*Ј. Б. Тито*), а Медицински факултет во Скопје, во 1976 година. На Клиниките за хируршки болести (Клиника за урологија „Доц. д-р Иван Влашки“) при Медицинскиот факултет, Универзитет „Св. Кирил и Методиј“ (УКИМ) во Скопје, се вработил во 1977 година. Едногодишната воена обврска ја извршил во 1977/78 год. во 47. класа на Санитетската офицерска школа (СОШ) на ЈНА, во Белград и во Осиек (СФРЈ).

Универзитетското образование го почнува како специјалист хирург-уролог во 1983 год., кога ја завршил четиригодишната специјализација по урологија на Медицинскиот факултет во Скопје, а хирург-трансплантолог е од 1986 год., кога завршил и специјализација по трансплантациона хирургија во Универзитетско-клиничкиот центар „Хенри Мондор“, XII париски универзитет, во Франција (кај проф. Клемен-Клод Абу/Clement-Claude Abbou; проф. Жан Овер/Jean Auvert*)*. На 27.11.1990 год., на Медицинскиот факултет при Универзитетот „Св. Кирил и Методиј“ во Скопје ја одбранил својата прва докторска дисертација од областа на медицинските науки со наслов: *Проценка на реактивноста на локорегионалната туморска лимфаденопатија и улогата на лимфаденектомијата во дијагностиката на урогениталниот канцер* (ментор: проф. д-р Благој Бадиев).

Акад. Живко М. Попов своето образование го продолжил во Париз, при што во 1994 год. се здобил со универзитетска диплома за продлабочени студии во хируршките науки (*Diplome d'Etudes Approfondies en sciences chirurgicales, D.E.A.)* на V париски универзитет „Рене Декарт“ (*Rene Descartes*), со писмен испит и јавна одбрана на мемоарот со наслов: *Evaluation de la proliferation et de la surexpression de p53 dans le cancer de la vessie: Valeur pronostique* (директор на студиите: проф. Дидиер Хусен (Didier Houssin).

На 10 ноември 1998 год., ја одбранил својата втора докторска дисертација во Франција од областа на хируршките науки (опција канцерологија), повторно на престижниот V париски универзитет „Рене Декарт“ (*Rene Descartes*), *Faculte de Medecine/UFR Cochin Port Royal,* тезата со наслов: *Marqueurs moleculaires du cancer vesical: valeur pronostique* (ментор: проф. Доминик К. Шопин/Dominique K. Chopin). Јавната одбрана на трудот била оценета со највисока оценка од страна на комисијата со која претседавал претседателот на Француската уролошка асоцијација, проф. Франсоа Рихард/Francois Richard.

Во 2011 год., во рамките на Европската унија на медицински специјалисти (EUMS) се здобил со Европска диплома за трансплантациона хирургија (European Honorary Diploma for Transplantation Surgery), станал член на Европскиот борд по хирургија во Берлин (Felow of European Board of Surgery) и стекнал звање FEBS.

Клиничкиот тренинг и наобразбата ги стекнал во повеќе универзитетски институции во Р Македонија, СФРЈ и во странство. Во согласност со стручните активности предвидени со програмата и планот за специјализација, покрај Хируршките клиники при Медицинскиот факултет во Скопје, во 1982 год., престојувал три месеци на Институтот за урологија и нефрологија во Белград и Уролошката клиника при Клиничкиот центар во Љубљана. Пролетта 1985 год., заминал на неколкумесечна специјализација во Клиниката „Мајо“ на Медицинскиот факултет „Мајо“ во Рочестер (Mayo Clinic, Mayo *Medical School, Rochester*), во Минесота, САД (проф. В. Л. Фурлоу/W. L. Furlow; проф. Хорст Зинке/Horst Zincke), каде што се запознал со употребата на протези во урологијата, импотенцијата, пенисната хирургија, лимфните дисекции кај урогениталниот канцер, радикалните простатектомии, трансабдоминалниот пристап на урогениталните органи и други големи хируршки процедури во онколошката урологија.

Во текот на 1985/1986 год., бил на едногодишна специјализација по трансплантациона хирургија на XII париски универзитет во Клиничкиот центар „Хенри Мондор“, кај реномираните професори Жан Овер/Jean Auvert, Клод Абу/Claude Abbou и Доминик Шопен/Dominique Chopin. Во 1986 год., исто така, престојувал четири месеци кај познатиот проф. Морис Каме/Maurice Camey во Медицинско-хируршкиот центар Фош, *Centre Medico – Chirurgical Foch*, во Париз, заради

едукација во полето на онколошката урологија, посебно за радикалните цистопростатектомии кај канцерот на мочниот меур и неговата супституција, област во која проф. Каме/Camey има сопствени процедури.

Од 1992 до 1994 год., повторно престојувал на XII париски универзитет во Клиничкиот центар и во Центарот за хируршки истражувања „Хенри Мондор", кај професорите Клод Абу (*Claude Abbou*) и Доминик Шопен (*Dominique Chopin*). Во овој период, тој извршил двегодишна суплементарна едукација во полето на бубрежната и мултиорганската трансплантација, онколошката урологија, како и научноистражувачка работа во полето на имунологијата на урогениталниот канцер.

Неговата суплементарна едукација е богата со повеќе кратки стручни престои со цел да се перфекционираат постојните или да се воведат нови хируршки процедури во решавањето на уролошката патологија, од кои се издвојуваат: Универзитетот „Корнел" во Њујорк/*Cornell University*, New York (проф. Нил Бандер/Neal Bander, 1999 г.); *Универзитетскиот клинички центар А. К. Х.*, во Виена, (проф. М. Марбергер/M. Marberger, 2000 и 2002 г.); *Универзитетската Елизабетска болница во Линц*, Австрија, (проф. Јанечек/Janetchek, 2003 г.); Универзитет во Тел Авив, Израел, Клинички центар „Шиба" (*Sheeba*), (проф. Амрам Ајалон/Amram Ayalon, 2003 г.); на Универзитетот во Берн, Швајцарија, (проф. Урс Студер/Urs Studer, 2003 и 2006 г.); Медицински центар „Рабин", болница „Бејлинсон", Оддел за трансплантација на органи, Израел/*Rabin Medical Centre, Beilinson Hospital, Department for Organ Transplantation, Petah Tikva, Izrael* (проф. Ејтан Мор/Eytan Mor, 2013 г.) и други.

Академските именувања и наставната дејност почнале во 1983 год., кога е избран за асистент по предметот хирургија на Медицинскиот факултет во Скопје; асистент по уролошка хирургија со титула странец во 1985/1986 год., во Универзитетските болници „Хенри Мондор" (*Hopital Henri Mondor*) (проф. Жан Овер/Jean Auvert) и „Фош" (*Hopital Foch*) (проф. Морис Каме/Maurice Camey), во Париз, Франција. Во 1991 год. е избран за доцент, во 1996 год. за вонреден професор, а од 2002 год. е редовен професор со два реизбора по предметите Хируршка пропедевтика и Општа хирургија со воена хирургија на Медицинскиот факултет во Скопје. Тој одржува постдипломска настава за специјализанти по урологија, општа хирургија и други допирни

хируршки специјалности, каде што се третираат проблеми од хирургијата на урогениталниот систем. Бил ментор на повеќе успешно одбранети докторски дисертации и магистерски трудови, и сè уште е активно вклучен со три докторанди во третиот циклус студии при Медицинскиот факултет во Скопје.

Повеќегодишните стручни и научноистражувачки престои на клиничките болници во Париз му овозможиле да се здобие и со престижни академски титули на Парискиот универзитет: во 1993/94 г., универзитетски клинички шеф по уролошка хирургија *(Chef de Clinique associe aux Universites)* при Универзитетскиот болнички центар „Хенри Мондор“ (*Centre Hospitalier Universitaire (CHU) Henri Mondor, Faculte de Medicine Creteil, Universite Paris XII Val De Marne*)*;* во учебните 1997/98; 1998/99; 1999/2000; 2001 и 2003 г., стекнал титула универзитетски поканет професор по уролошка хирургија при Клиничкиот центар „Хенри Мондор“ на XII париски универзитет, во Франција *(Professeur Invite des Universites CHU Henri Mondor, Faculte de Medicine Creteil, Universite Paris XII Val De Marne, France*)*,* во рамките на Францускиот национален контингент на професори од прва класа, со полно работно време, по 4 месеци годишно. За време на овие престои на Парискиот универзитет, активно е вклучен во изведувањето настава и менторирањето на студиите за стекнување универзитетска диплома за продлабочени знаења во хируршките науки (*D.E.A.).*

Болничките именувања ги почнал како шеф на отсекот за ендоскопија при Клиниката за урологија, Медицински факултет Скопје (1984 – 1991). Од 1992 до 1995 год., бил шеф на Одделението по општа урологија, а од 1995 до 1999 год., бил назначен за шеф на Одделот по уролошка онкологија. За директор на Клиниката за урологија бил избран во три мандата (1995 – 1997; 1999 – 2002; 2006 – 2012). Во 1996 год., бил назначен за модератор на Хируршките клиники при Клиничкиот центар Скопје, а нивни координативен директор бил во периодот од 1999 до 2002 година. Акад. Попов е основоположник и раководител на Центарот за трансплантација на бубрези при Универзитетската клиника за урологија, Медицински факултет при УКИМ, Скопје (1996 – 2012). Основач и раководител е на Одделот за уролошка хирургија при Специјалната болница за хируршки болести „Филип Втори“ – Скопје (2012 – 2014; 2016 – 2020), а работи и дава свој придонес во развојот на

урологијата во Општата болница „Ремедика“ – Скопје (2014 – 2016), „Неуромедика хоспитал“ (2020 – 2021) и Клиничката болница „Аџибадем – Систина“ (2021–).

Стручни и научни достигнувања

Акад. Живко М. Попов е првиот самостоен македонски трансплантациски хирург, комплетно едуциран и подготвен да ја спроведе оваа комплицирана хируршка методологија во пресадувањето и во решавањето на сите хируршки компликации. Со вкупно направени, под негово раководство, околу 270 пресадувања на бубрези (кадаверични и од жив дарител), главно, во периодот од формирањето на Хируршкиот центар за трансплантација, во 1996 год., па сè до 2012 година. Акад. Попов, со својот трансплантациски хируршки тим, нашата држава ја вброи во оние каде што оваа дисциплина постои и се извршува на европско ниво. Почнувајќи од крајот на 80-тите години на 20-тиот век, кога Клиничкиот центар во Скопје во тогашната СФРЈ претставува референтен центар за една од првите организирани кадаверични трансплантации на бубрези и кога ширум бившите југословенски простори беа првпат пратени десетина презервирани кадаверични бубрези, па сè до денес, оваа медицинска дисциплина перманентно се развива следејќи ги современите процедури во полето на трансплантологијата. Така, во 2002 и 2003 год., под раководство на акад. Попов се изведени и првите четири трансплантации на Балканот, при кои е употребена лапароскопска техника при земањето на бубрегот од живиот дарител, во соработка со проф. Д. Шопен/D. Chopin, од XII париски универзитет, и проф. А. Ајалон/A. Ayalon од Универзитетот во Тел Авив (*Popov et al. Prog.Urol. 2005*). Резултатите од оваа високо диференцирана хируршка дејност, која и денес непречено се изведува и се развива во Центарот за трансплантација на бубрези при Универзитетската клиника за урологија, од страна на учениците и хируршкиот тим на акад. Попов, може да се мерат со секоја европска клиника.

Акад. Живко Попов, едуциран на повеќе странски универзитети, а пред сè на оние во Париз, се формира во професионалец и научник на високо ниво, со широк репертоар на знаења и активности во полето на урогениталната хирургија. Тој е лидер во воведувањето на повеќе тешки хируршки процедури во онколошката урологија, како што се радикалните лимфни дисекции во пределот на ретроперито-

неумот кај тестикуларните карциноми, по што своевремено тој е познат на просторите на СФРЈ. Тој е, исто така, познат и по многубројните операции од областа на пелвичната хирургија на егзереза со илиообтураторни лимфни дисекции и уринарна реконструкција. Тој го воведува трансабдоминалниот хируршки пристап при операциите на туморите на ретроперитонеумот, бубрезите и надбубрежните жлезди, и е еден од лидерите во воведувањето на радикалната простатектомија кај канцерот на простата, како и во реализацијата на неовезиката по радикална цистектомија кај мажот и жената, при малигните тумори на мочниот меур. Прв во Република Македонија ја изведува трансвагиналната суспензија на вратот на мочниот меур по методата на *Raz (UCLA)* кај стрес-инконтиненцијата кај жената. Лидер е во пенисната хирургија на овие простори, воведувајќи ја *Nesbit*-процедурата и *patch*-техниките по Девин/*Devine* кај вродените аномалии на пенисот и *Peyronie*-евата болест, како и радикалните пенектомии со перинеостомија кај канцерот на пенисот. Модерните ендоуролошки методи (перкутана нефролитолапаксија, уретерореноскопијата и др.) за лекување на уринарната калкулоза, при крајот на 80-тите години на минатиот век, станаа една од неговите главни стручни преокупации. Во првата деценија на 21 век, акад. Попов е лидер во воведувањето на лапароскопијата во урологијата, а посебно неговото внимание е насочено кон лапароскопската хирургија на надбубрежните жлезди која веќе станува рутина на нашите простори (Popov et al. *Urology,* 2008*;* Popov et al. *Prilozi,* 2015). Своето богато клиничко, хируршко и научноистражувачко искуство, несебично го пренесува на другите колеги на хируршките клиники, а особено на специјализантите и докторандите.

Резултатите од својата работа, акад. Попов перманентно ги презентира на меѓународни и национални конгреси и ги публикува во реномирани стручни и научни списанија. Како поканет предавач, *chairman*, член на *faculty* и учесник со свои излагања, партиципира на повеќе меѓународни конгреси од кои посебно се издвојуваат оние на *AUA (American Urological Association*): Сан Франциско (1994), Лас Вегас (1995), Орландо (1996 и 2003), Сан Диего (1998), потоа Светскиот конгрес по урологија (*Societe International d Urologie*) во Монтреал (*SIU*, 1999), Сантјаго (*SIU,* 2008) и Атина (*SIU*, 2019), како и на

повеќе конгреси на *EAU* (*European Association of Urology*): Берлин (1994), Женева (2001), Париз (1996, 2012), Бирминген (2002), Конгресот на *ESUOE (European Society for Urological Oncology and Endocrinology)*: Марсеј (1991), Берн (1994), Конгресот на *ESOU* (*European Society of Oncological Urology*): Виена (2004), Академија за хирургија/*Academie de Chirurgie*, Париз, Франција (1995), Научен состанок во Српската академија на науки и уметности (САНУ, 2007), Конгресот на Светското здружение за трансплантација во Сиднеј (TTS, 2008) и други.

Членување во научни асоцијации

- Интернационално здружение по урологија (Societe Internationale d'Urologie – SIU) (1995–). Национален делегат (1998 – 2012).
- Европска уролошка асоцијација – EAU (1998–).
- Европско здружение по уролошка онкологија (член и претставник за Источна Европа во Бордот на Здружението) (2002 – 2006).
- Член на Американската уролошка асоцијација (AUA) (2006–).
- Здружение за трансплантација на франкофонските земји (Societe Fancophone de Transplantation, SFT) (2006–).
- Европско здружение за трансплантација на органи – ESOT (2010–).
- Македонска уролошка асоцијација (претседател: 1999 – 2002, 2010 – 2015).
- Македонско здружение за трансплантација на органи и ткива (2005–).
- Член на Македонското лекарско друштво (МЛД), еден мандат член на Управниот одбор.
- Претседател и организатор на Првиот македонски уролошки конгрес, во соработка со Европската уролошка асоцијација – EAU (2002), во Охрид.
- Претседател и организатор на 7. конгрес на уролозите на Југоисточна Европа (SEEM/EAU, 2011), во Скопје.
- Копретседател и организатор на 5. евроазиски зимски форум, одржан во МАНУ, во Скопје (2013).

Членување во државни и меѓународни тела и комисии

- Член на Федералната комисија за трансплантација при Сојузното министерство за здравство, Белград, Југославија, 1991 – 1992 (министер Драгиша Гачиќ).
- Национален координатор и шеф на „Македонија трансплант“, (координативно тело за трансплантација на органи и ткива), при Министерството за здравство на Р Македонија, (2002 – 2011).
- Член на Советодавното тело на министерот за здравство (2004 – 2006 и 2015 – 2017).
- Член на Одборот за акредитација и евалуација на високото образование во Република Македонија при Министерство за образование и наука (МОН) како претставник на МАНУ, мандат: 2011 – 2015.
- Офицер за врски и член на Одборот на гувернери на Северна Македонија во Меѓународниот центар за генетско инженерство и биотехнологија во Трст, Италија (*Liaison officer and Member of Board of Governors of North Macedonia in International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy*) (2018–).
- Член на Националниот совет за евроинтеграции (НСЕИ) при Собранието на РСМ (2021–).

Национални и меѓународни награди

- 2000 г.: Награда „11 Октомври“ како највисоко општествено признание за повеќегодишни особено значајни достигнувања за Република Македонија во областа на науката и образованието, хирургијата и трансплантацијата на бубрези.
- 2002 г.: Орден за придонес во француската култура и наука „Академска палма“ *(Chevalier dans I Ordre des Palmes Academiques),* со Декрет на Премиерот на Република Франција.
- 2007 г.: Награда „13 Ноември“ на Град Скопје, за особен придонес во развојот и меѓународната афирмација на уролошката хирургија и трансплантацијата на бубрези во градот Скопје и Република Македонија.

- 2007 г. (и 1997 г.): благодарници од Медицинскиот факултет при УКИМ, Скопје, во знак на признание за придонесот во развојот и работата на Факултетот (јубилеи: 1947 – 1997 и 1947 – 2007).
- 2008 г.: Плакета од Универзитетот „Св. Кирил и Методиј“ во знак на признание за придонесот и афирмацијата на Универзитетот.
- 2010 г.: Повелба „Д-р Трифун Пановски“ на Македонското лекарско друштво (МЛД), за исклучителни резултати во унапредувањето на медицинската наука, практиката и развојот на здравствената заштита.
- 2010 г.: Државна награда „23 Октомври“ како највисоко признание за повеќегодишни остварувања за Република Македонија во областа на науката и образованието.

Грантови и проекти

- ***Имунолошка состојба на малигните заболувања*** **(Макропроект на Медицинскиот факултет – Скопје**, раководител проф. П. Колевски. Потпроект за имунолошка состојба на урогениталниот канцер, учесници: Клиника за урологија, К. Ц. Скопје/Републички Завод за трансфузиологија – Скопје, главен истражувач Ж. Попов), оригинална статија:

Ж. Попов. (1993). *Процена на локорегионалната и системска клеточна имунокомпетенција кај урогениталниот канцер*. Извештај на научен проект број 40213089: Имунолошка состојба на малигните заболувања – Универзитет „Св. Кирил и Методиј“, Медицински факултет Скопје, стр. 28–51.

- *Испитување на можностите за неинвазивна дијагностика на карциномот на мочниот меур*, проект на Министерството за образование и наука (учесници ОЕ Хируршки клиники – Клиника за урологија, ЈЗО Клинички центар, Медицински факултет, Скопје; Катедра за молекуларна биологија, ПМФ, Скопје, главен истражувач: **Ж. Попов**), оригинални статии:

S. Panov, D. Roganovic-Zafirova, G. Stavric, G. Yashar, **Z. Popov.** (2004). *High frequency of the HRAS oncogene codon 12 mutation on Macedonian patients with urinary bladder cancer*. Genetics and Molecular Biology, 27, 1, 9–14.

Ж. Попов. (2004). „Извештај за научноистражувачкиот проект број 4023120/0“ (грант од Министерството за образование и наука на РМ).

- Член на Истражувачката група за урогенитални тумори (***GETU- Groupe d'Etudes de Tumeurs Urologiques)*** при 12. Париски универзитет (*CHU Henri Mondor*), директор: проф. Доминик К. Шопен (Dominique K. Chopin) (1992 – 2005), од каде што произлегуваат голем број оригинални публикации во научни списанија со висок фактор на влијание. Во рамките на групата, иницира, зема учество и координира повеќе проекти за клинички студии од областа на уролошката и трансплантационата хирургија и фундаментални научни истражувања од областа на урогениталните тумори и нивната имунологија, со посебен акцент на молекуларните маркери на канцерот на мочниот меур, односно *уротелијалната онкогенеза*, од кои се издвојуваат следните студии:

А – ***Студија за прогностичката вредност на прекумернатa експресија на p53 и на пролиферацијата, оригинални статии:***

Z. Popov, A. Hoznek, M. Colombel, S. Bastuji, C. C. Abbou, D. K. Chopin. (1996). *Evaluation de la surexpression de la protein p53 dans le cancer de la vessie: valeur pronostique,* Chirurgie*,* 121, 6, 461–466,

Z. Popov, A. Hoznek, M. Colombel, S. Bastuji-Garin, MA. Lefrere-Belda, J. Belot, C. C. Abbou, C. Mazerolles, D. K. Chopin. (1997). *The prognostic value of p53 nuclear overexpression and MIB-1 as a proliferative marker in transitional cell carcinoma of the bladder.* Cancer*,* 80 (8): 1472–1481.

Б – Студија за адхезивните молекули (Кадерин- Е), оригинални статии:

S. Gil-Diez de Medina, **Z. Popov**, D. K. Chopin, J. Southgate, G. C. Tucker, A. Delouvee, JP. Thiery, F. Radvanyi. (1999). *Relationship between E-cadherin and fibroblast growth factor receptor 2b expression in bladder carcinomas.* Oncogene, 18 (41): 5722–6.

Z. Popov, S. Gil-Diez de Medina, MA. Lefrere-Belda, A. Hoznek, S. Bastuji-Garin, C. C. Abbou, JP. Thiery, F. Radvanyi, D. K. Chopin. (2000). *Low E-cadherin expression in bladder cancer at the transcrip-*

tional and protein level provides prognostic information. Br J Cancer, 83 (2): 209–2014.

***В – Студија за улогата на EGF или епидермалните фактори на раст кај уротелијалните тумори, оригинални статии**:*

V. Ravery, M. Colombel, **Z. Popov**, S. Bastuji, J-J. Patard, J. Bellot, C. C. Abbou, Y. Fradet. (1995). *Prognostic value of epidermal growth factor-receptor, T138 and T43 expression in bladder cancer.* British Journal of Cancer, 71, 196–200.

Z. Popov, S. Gil-Diex de Medina, V. Ravery, A. Hoznek, S. Bastuji-Garin, M-A. Lefrere-Belda, C. C. Abbou, D. K. Chopin. (2004). *Prognostic value of EGF receptor and tumor cell proliferation in bladder cancer: therapeutic implications.* Urologic Oncology: Seminars and Original Investigations 22, 93–101.

***Г – Проект за истражување на уротелијалните тумори кај претходно дефинирани пациенти со мутации FGFR3, p53 и употреба на BCG, оригинални статии**:*

F. Saint, M-A. Lefrere-Belda, R. Quintela, A. Hoznek, J. J. Patard, J. Bellot, **Z. Popov**, E. S. Zafrani, C. C. Abbou, D. K. Chopin, S. Gil-Diex de Medina. (2004). *Pretreatment p53 Nuclear Overexpression as a Prognostic Marker in Superfitial Bladder Cancer treated WITH Bacillus Calmette-Guerin (BCG).* European Urology 45, 475-482.

- 2007 – 2009: грант од **Шестата рамковна програма** за истражување, технолошки развој и демонстрација на Европската комисија *(Research grant from the 6th Framework Programme on Research, Technological Development and Demonstration of the European Commission-Contract Number:* 037739), со работен наслов: „Интеграција на ДНК, РНК и протеински маркери во дијагнозата и прогнозата на карциномот на мочниот меур“. Во рамките на проектот набавена е опрема за основање банка на ткиво од урогенитален канцер при МАНУ (акад. Попов е главен истражувач за Република Македонија и национален претставник во конзорциумот на проектот).

- 2017 – 2020: **проектна соработка МАНУ – БАН** (Бугарска академија на науките), раководител од македонска страна на проектот со наслов: *Интегрирана имуногенетска анализа на молекуларни маркери на карциномот на мочниот меур релевантни во проценката на рекурентноста и прогресијата на болеста.*

- 2020 – 2022: раководител и главен истражувач на меѓународен научен проект CRP/MKD19-02: *Diagnostic, prognostic and predictive biomarkers for bladder cancer management.* International Centre for Engineering and Biotechnology (**ICGEB Research Grants 2019**), Trieste, Italy.

Публицистичка дејност и научен придонес (сциентометрија)

Три мемоара, од кои два за докторски дисертации и еден за продлабочени знаења во хируршките науки, повеќе извештаи за научноистражувачки проекти. Едитор на бројот 5 од 2000-тата год. на реномираното француско научно списание *Annales d'Urologie,* посветен на бубрежната трансплантација во Република Македонија. Акад. Живко М. Попов како автор и коавтор објавил над 200 научно-стручни трудови, во домашни и меѓународни научни списанија, од кои во *Web of Science* се внесени 85 труда, кои се цитирани 611 пати, а *Hirsch*-овиот индекс изнесува 14. Во научната база *SCOPUS* се вклучени 69 труда кои се цитирани 750 пати, со *Hirsch*-ов индекс 15. Во научната база *Google Scholar* внесени се 182 труда кои се цитирани 1140 пати, а *Hirsch*-овиот индекс изнесува 18 (10.11.2021 година).

За редовен член на Македонската академија на науките и уметностите е избран на 27 мај 2009 година, а е нејзин потпретседател од 2020 година.

doi: 10.20903/zpopov-2022-0041 UDK: 016:929Попов, Ж.

БИБЛИОГРАФИЈА (1978 – 2021) НА АКАД. ЖИВКО М. ПОПОВ

1. A. Stavridis, I. Vlaski, Lj. Vasilev, V. Georgiev, **Z. Popov**, M. Penev, *Primena garamicina u lečenju uroloških bolesnika.* II Simp. za antibiotici i antib. terapija, Belgrad, XI. 1978.
2. Љ. Василев, И. Влашки, Б. Бадиев, А. Ставридис, В. Георгиев, **Ж. Попов**, М. Пенев, *Carcinota на простата - дијагностика и терапија.* X конгрес на лекарите на СРМ, Струга, 1978.
3. А. Ставридис, И. Влашки, Б. Бадиев, Љ. Василев, **Ж. Попов**, М. Пенев, В. Георгиев, *Одразот на простатектомијата врз рехабилитацијата на организмот.* Македонски медицински преглед, год. XXXIII, бр. 1–2, 1979.
4. А. Ставридис, Б. Бадиев, В. Георгиев, М. Пенев, **Ж. Попов**, *Echinococcus renis.* Македонски медицински преглед, год. XXXIV, 3–4, 1980.
5. I. Vlaski, A. Stavridis, M. Penev, **Z. Popov**, V. Georgiev, *Kasni postoperativni rezultati antirefluksnih uretero-cistoneostomija,* IV Internacionalni simpozijum urologa, Niš, maj, 1979. Acta Chirur. Jug., tom 1, 1981.
6. И. Влашки, Б. Бадиев, Љ. Василев, А. Ставридис, М. Пенев, В. Георгиев, **Ж. Попов**, *Оперативно лекување на коралиформната калкулоза на бубрегот.* VI конгрес на уролозите на Југославија, Охрид, 1980. Acta Chirur. Jug., Suppl. 2, 1981.
7. **Ж. Попов**, Н. Костиќ, Б. Доцевски, М. Пенев, *Хируршки пристап на изолираните мезентеријални цисти*. Македонски медицински преглед, год. XXXVI, 1982, 3–4.
8. **Ж. Попов**, А. Ставридис, М. Пенев, А. Настоски, *Примена на цефалоспорински препарати во лекувањето на инфекцијата на уринарниот систем*. VI конгрес на уролозите на Југославија, Охрид, 1980. Acta Chirur. Jug. (Supplement 2), 1981.
9. Љ. Василев, **Ж. Попов**, *Орхиектомија и цитостатска терапија во лекувањето на напреднатите малигноми на простатата*. VI конгрес на уролозите на Југославија, Охрид, 1980. Acta Chirur. Jug. (Supplement 2), 1981.
10. Lj. Vasilev, **Z. Popov**, *Operativno lečenje intrauretralnog condyloma acuminatum kod muškaraca.* VII Kongres urologa Jugoslavije, Beograd, Urološki arhiv, br. 20, 1982.

11. **Z. Popov**, I. Vlaski, R. Nakovski, S. Popovski, *Orchiepididymitis acuta.* Македонско-црногорски медицински денови, Отешево, јуни 1983.
12. Lj. Vasilev, A. Stavridis, **Z. Popov**, *Spontana intraperitonealna ruptura mokračne bešike kod enormne cistolitijaze.* VIII Kongres urologa Jugoslavije, Split, 1984.
13. **Z. Popov**, Lj. Vasilev, *Hirurški tretman Peyronie-eve bolesti pomoču autografta fasciae musculi obliqui externi abdominis*. VIII Kongres urologa Jugoslavije, Split, 1984.
14. Lj. Vasilev, **Z. Popov**, *Formiranje rektalne bešike kod postiradijacionih torpidnih vezikosigmo-vaginalnih fistula*. VIII Kongres urologa Jugoslavije, Split, 1984.
15. D. Chopin, C.C. Abbou, **Z. Popov**, Ph. Lang, C. Buisson, Th. Nebout, J. Auvert, *Use of high dose furosemide for cadaveric renal allograft.* 18eme cours international de transplantation et d'immunologie clinique, Lyon, 12–14, Mars, 1986. Excerpta Medica, Transplantation and Clinical Immunology, Vol. XVIII, 335, 1986.
16. D. Chopin, **Z. Popov**, C.C. Abbou, Ph. Lang, C. Buisson, J. Auvert, *Management of multiple artery grafts during human renal transplantation.* 18^{éme} cours international de transplantation et d'immunologie clinique, Lyon, 12–14, May, 1986. Excerpta Medica, Transplantation and Clinical Immunology, Vol. XVIII, 398, 1986.
17. D. Chopin, **Z. Popov**, C.C. Abbou, Ph. Lang, C. Buisson, J. Auvert, *Use of vena cava for right renal vein prolongation during human renal transplantation*, 18eme cours international de transplantation et d'immunologie clinique, Lyon 12–14, May, 1986. Excerpta Medica, Transplantation and Clinical Immunology, Vol. XVIII, 399, 1986.
18. D. K. Chopin, C.C. Abbou, H.B. Lottmann, **Z. Popov**, Ph. R. Lang, C.L. Buisson, D. Belghiti, M. Colombel and J. Auvert, *Conservative treatment of renal allograft rupture with polyglactin 910 mesh and gelatin resorcin formaldehyde glue.* Journal of Urology, 142, 1–3, 1989.
19. **Z. Popov**, B. Badiev, A. Stavridis, V. Georgiev, *Hirurški pristup u tretmanu urotelijalnih tumora gornjeg urinarnog trakta.* Zbornik radova, VI Internacionalni simpozijum urologa Jugoslavije, Niš, 15–16. X 1987.
20. B. Badiev, **Z. Popov**, M.Penev, *Karcinomi prelaznog epitela bešike kod bolesnika sa urotelijalnih tumora gornjeg urinarnog trakta*. Zbornik radova, VI Internacionalni simpozijum urologa Jugoslavije, Niš, 15–16. X 1987.
21. **Z. Popov**, B. Badiev, V. Georgiev, *Hirurška tehnika "en bloc" kadaverične binefrektomije kod bubrežne transplantacije (video film).* X Kongres urologa Jugoslavije, Bled, 1988.

22. Б. Бадиев, В. Георгиев, **Ж. Попов**, *Местото на перкутаната нефролитотомија (ПНЛ) во ерата на екстракорпоралната литотрипсија (ESWL).* Зборник на трудови, IV конгрес на нефролозите на Југославија, Скопје, 27–30. IX, 1989.

23. Б. Бадиев, В. Георгиев, **Ж. Попов**, *Ендоскопски пристап во дијагноза и третман на уротелијалните тумори кај балканската нефропатија.* Зборник на трудови, IV конгрес на нефролозите на Југославија, Скопје, 27–30. IX, 1989.

24. D.K. Chopin, **Z. Popov**, C.C. Abbou, and J. Auvert, *Use of vena cava to obtain additional lenght for the right renal vein during transplantation of cadaveric kidneys.* Journal of Urology, 141, 1–2, 1989.

25. N. Ivanovski, K. Cakalaroski, A. Oncevski, G. Masin, M. Polenakovic, **Z. Popov**, *Organ donation with living-related donors with cancer*. Прв заеднички состанок на ЕДТА и ЕСОТ, Минхен, 11–14.XII, 1990.

26. **Ж. Попов**, П. Колевски, Б. Бадиев, Ј. Бадиева, *Evaluation of locoregional and sistemic cellular immunity with urogenital cancer*. IX конгрес на Европската асоцијација по урологија (ЕАУ), 1316, Juni 1990, Amsterdam.

27. D.K. Chopin, **Z. Popov**, C.C. Abbou, *Utilisation de la veine cave en transplantation renale. Le prolongement de la veine renale droite*. Annales d'Urologie, 22, No. 5, 1988, 325–327.

28. **Ж. Попов**, Б. Бадиев, *Техника на кадаверична бубрежна експлантација*. IV недела на новини и актуелности во медицината, Скопје, 2-4 јуни 1988, Македонски медицински преглед, год. XLII, Suppl. 8, 1988.

29. К. Чакалароски, Н. Ивановски, Г. Масин, **Ж. Попов**, Б. Бадиев, П. Колевски, *Предтрансплантациски биланс на болни со хронична бубрежна инсуфициенција - кандидат за ренална трансплантација.* IV недела на новини и актуелности во медицината Скопје, 2-4 јуни, 1988, Македонски медицински преглед, год. XLII, Suppl. 8, 1988: 61–66.

30. Б. Бадиев, **Ж. Попов**, П. Колевски, М. Благоевска, Ј. Бадиева, *Имунологија на малигни болести - Макропроект на Медицинскиот факултет во Скопје* „Проценка на локорегионалната и системска имунокомпетенција кај урогениталниот канцер“ – Подпроект.

31. М. Мицуновик, В. Георгиев, **Ж. Попов**, М. Пенев, *Соломоновата операција кај хидроцела.* Македонски медицински преглед, 1–2, 1984: 35–36.

32. K. Cakalaroski, N. Ivanovski, G. Masin, **Z. Popov**, I. Vlaski, *Complications in patients with renal transplantation.* Periodicum Biologorum, Vol. 90, No. 4, 1988: 545–546.

33. N. Ivanovski, K. Cakalaroski, G. Masin**, Z. Popov**, P. Kolevski, M. Polenakovic, *Spontaneous Intestinal Perforation in Renal Transplant Patients,* Transplantation Proceedings, 24; 5 (October), 1992: p. 1761.
34. N. Ivanovski, K. Cakalaroski, G. Masin, M. Polenakovic, **Z. Popov**, P. Kolevski, *Medical Risk and Benefit in Elderly Living Renal Donors-Our Experience.* Transplantaion Proceedings, Vol. 23, No. 5 (October), 1991: p. 2626.
35. K. Cakalaroski, N. Ivanovski, G. Masin, M. Polenakovic, **Z. Popov**, P. Kolevski, *Nonoliguric Reversible Acute Renal Failure in an Obese Living Donor.* Transplantation Proceedings, Vol. 23, No. 5 (October), 1991: p. 2618.
36. **Z. Popov**, P. Kolevski, B. Badiev, I. Daskalov, J. Badieva, *Local and Systemic Cellular Immune Response in Urogenital Tumours.* European Society for Urological Oncology and Endocrinology. 8-th Congress, Marseille, April, 18–20, 1991.
37. **Z. Popov**, Lj. Lekovski, B. Badiev, K. Cakalaroski, N. Ivanovski, P. Kolevski, *Renal Allograft Rupture-Treatment and Long Term Prognosis*. First International Congress on Transplantation in Developing Countries, Singapore, 29th April to 3rd May, 1992.
38. K. Cakalaroski, N. Ivanovski, **Z. Popov**, G. Masin, V. Ristevska, M. Polenakovic, P. Kolevski, *Cyclosporin Provoked Status Epilepticus – Case Report*. First International Congress on Transplantation in Developing Countries, Singapore, 29th April to 3rd May, 1992.
39. N. Ivanovski, K. Cakalaroski, G. Masin, **Z. Popov**, P. Kolevski, *Spontaneous Intestinal Perforation in Renal Transplant Patient.* First International Congress on Transplantation in Developing Countries, Singapore, 29th April to 3rd May, 1992. Transplantation Proceedings, Vol. 24, No. 5 (October), 1992: p. 1761.
40. **Z. Popov**, V. Ravery, M. Colombel, S. Gil-Diez, C.C. Abbou, J. Bellot, D.K. Chopin, *Marqueurs biologiques des tumeurs urotheliales de la vessie.* Groupe d'Etude des Tumeurs Urologiques, CRCHM, Henri Mondor, 94010 Creteil. 87e Congres Francais d'Urologie, Paris, 17–19 Novembre 1993, Progres en Urologie, 3, Suppl. 5, 1993: 91.
41. J.J. Patard, **Z. Popov**, C.C. Abbou, D.K. Chopin, *Evaluation de la reponse immunitaire locale apres instillations endovesicales de BCG dans les tumeurs superficielles de vessie.* Service d'Urologie, Hopital Henri Mondor, Groupe d'Etude des Tumeurs Urologiques, Centre de Recherches Chirurgicales, 94010 Creteil. 87e Congres Francais d'Urologie, Paris, 17–19 Novembre, 1993, Progres en Urologie, 3, Suppl. 5: 92.
42. S. Gil-Diez, M. Colombel, **Z. Popov**, V. Ravery, J.J. Patard, C.C. Abbou, D.K. Chopin, *Hypertrophie benigne de la prostate: Proliferation des cellules basales.* Service d'Urologie, Hopital Henry Mondor, 94010 Creteil, France. 87e

Congres Francais d'Urologie, Paris, 17–19 Novembre, 1993 Progres en Urologie, 3, Suppl. 5: 74.

43. **Z. Popov**, V. Ravery, M. Colombel, C. Mazerolles, J.J. Patard, C.C. Abbou, J. Bellot, D.K. Chopin, *Evaluation of proliferation and p53 overexpression in bladder cancer (oral presentation).* Hopital Henri Mondor, Creteil, Paris; Hopital Purpan, Toulouse, France. 10th Congress of the European Society for Urological Oncology and Endocrinology (ESUOE), March 10–13, 1994, Berne Urological Research, 21, No. 6, 1993: 39.
44. J.J. Patard, **Z. Popov**, B. Muscatelli-Groux, S. Gil-Diez, Y. Fradet, C.C. Abbou, D.K. Chopin, *Local immunological response following intravesical Bacillus Galmette Guerin (BCG) prophylaxis for bladder cancer (oral presentation).* Hopital Henri Mondor, Creteil, Paris, France; Universite Quebec, Canada. 10th Congress of the European Society for Urological Oncology and Endocrinology (ESUOE), March 10–13, 1994, Berne. Urological Research, 21, No. 6, 1993: 107.
45. M. Colombel, **Z. Popov**, S. Gil-Diez, C.C. Abbou, D.K. Chopin, *Analysis of proliferation, cell death and related genes in normal prostate and benign prostatic hyperplasia (BPH) – (oral presentation).* Service d'Urologie, Hopital Henri Mondor, Creteil, Paris, France. 10th Congress of the European Society for Urological Oncology and Endocrinology (ESUOE), March 10–13, 1994, Berne. Urological Research, 21, No. 6, 1993: 033.
46. D.K. Chopin, A. Hoznek, V. Ravery, P. Antiphon**, Z. Popov**, C.C. Abbou, *Resultats du traitement des tumeurs vesicales infiltrant Ie muscle.* (Creteil/Paris). Ann Urol, 1993, 27, No. 1, 42–44.
47. V. Ravery**, Z. Popov**, J. Bellot, C.C. Abbou, D.K. Chopin, *Apport des anticorps monoclonaux dans I'evaluation de I'agressivite des tumeurs de vessie*. (Creteil/Paris) Ann Urol, 27, No. 1, 1993: 45–50.
48. **Z. Popov**, M. Colombel, C. Mazerolles, V. Ravery, J.J. Patard, C.C. Abbou, J. Bellot, D.K. Chopin. (Creteil and Toulouse, France), *Evaluation of proliferation and p53 overexpression in bladder cancer.* American Urological Association (AUA) Eighty-ninth annual meeting, May 14–19, 1994, San Francisco The Journal of Urology, 51, Suppl. 5, 1994: 864.
49. J.J. Patard, **Z. Popov**, B. Muscatelli-Groux, S. Gil-Diez, Y. Fradet, C.C. Abbou, D.K. Chopin, (Creteil, France and Universite Laval, Quebec). *Local immunological response following intravesical Bacillus Calmette Guerin prophylaxis for bladder cancer.* American Urological Association (AUA) Eighty-ninth annual meeting, May 14–19, 1994, San Francisco The Journal of Urology, 51, Suppl. 5, 1994: 978.

50. E. Fontaine, **Z. Popov**, D. Beurton, C.C. Abbou, D.K. Chopin, *Etude de la reponse immunitaire dans I'hipertrophie benigne de la prostate (HBP).* Hopital Henri Mondor, Service d'Urologie, 94010 Creteil, France. 88eme Congres Francais d'Urologie, Paris, 16–18 Novembre, 1994.

51. **Z. Popov**, M. Colombel, A. Hoznek, C.C. Abbou, J. Bellot, D.K. Chopin, *Surexpression de la proteine p53 dans le cancer de la vessie: valeur prognostique.* Hopital Henri Mondor, Service d'Urologie, 94010 Creteil, France. 88eme Congres Francais d'Urologie, Paris, 16–18 Novembre, 1994.

52. D.K. Chopin, **Z. Popov**, V. Ravery, J. Bellot, A. Hoznek, J.J. Patard, C.C. Abou, M. Colombel, *Prognostic factors in superfitial bladder cancer*. Groupe d'Etude des Tumeurs Urologiques, CRCHM, Service d'Urologie, Hopital Henri Mondor, 94010 Creteil/Paris, France. World J Urol., 11, 1993: 148–152.

53. C. Mazerolles, P. Rishmann, D.K. Chopin, **Z. Popov**, B. Malavaud, J. Selves, I. Neulat-Duga, J. Bellot, G. Delsol, *Usefulness of MIB1 monoclonal antibody in assessing the proliferative index in human bladder carcinoma: comparison with Ki-67 antibody.* Histopathology, 25, 1994: 563–568.

54. M. Colombel, S. Gil Diez, E. Fontaine, **Z. Popov**, C.C. Abbou, D.K. Chopin, *Etude comparatimentale de la proliferation et de la mort cellulaire dans I'hypertrophie benigne de le prostate (HBP).* Progres en Urologie, Vol. 4, (Supplement. 1), 1994: 38.

55. F. Radvanyi, S. Gil Diez de Medina, A. Delouvee, **Z. Popov**, C.C. Abbou, J.P. Thiery, D. Chopin, *Loss of KGF receptor mRNA precedes loss of E-Cadherin in transitional bladder tumors*. Proceedings of the Amer. Ass. for Cancer Research, Vol. 36, 1995: 167.

56. **Z. Popov**, A. Hoznek, M. Colombel, J. Bellot, S. Bastuji, C.C. Abbou, D. Chopin, *P53 overexpression and proliferation rates are independent prognostic markers in bladder cancer*. Proceedings of the Amer. Ass. for Cancer Research, Vol. 36, 1995: 630.

57. O.S. Hegazi, A. Khalifa, S. Helal, S. Teleb, **Z. Popov**, J.C. Kouyoumdjian, D.K. Chopin, *P53 nuclear overexpression and proliferation in bilharzial and non bilharzial urinary bladder carcinoma*. Urological Research, Vol. 23, 4, 1995: 53.

58. S. Gil Diez, F. Radvanyi, **Z. Popov**, A. Hoznek, S, Bastuji, J. Bellot, C.C. Abbou, D. Chopin, *Decreased E-Cadherin expression is an independent prognostic indicator in bladder cancer*. Urological Research, Vol. 23, 4, 1995: 144.

59. L. Solomon, S. Gil Diez, M. Colombel, **Z. Popov**, C.C. Abbou, J. Bellot, F. Ramaekers, T. Van der Kwast, D. Chopin, *Biological descriptions of localized carcinoma of the prostate (CAP) organ confined disease*. Urological Research, Vol. 23, 4, 1995: O120.

60. V. Ravery, M. Colombel, **Z. Popov**, S. Bastuji, J-J. Patard, J. Bellot, C.C. Abbou, Y. Fradet, *Prognostic value of epidermal growth factor-receptor, T138 and T43 expression in bladder cancer*. British Journal of Cancer, 71, 1995: 196–200.

61. **Z. Popov**, A. Hoznek, M. Colombel, J. Bellot, C.C. Abbou, D. Chopin, *P53 and proliferation are independent prognostic markers for bladder cancer.* The Journal of Urology, Vol. 153, 4, April 1995: A363.

62. **Z. Popov**, S. Gil Diez, A. Hoznek, F. Radvanyi, J. Bellot, C.C. Abbou, D. Chopin, *Decreased E-Cadherin expression by immunohistochemistry is strongly correlated with stage and grade in bladder cancer*. The Journal of Urology, Vol. 153, 4, April 1995: A524.

63. **Z. Popov**, A. Hoznek, V. Ravery, S. Bastuji, M. Lefrere, J. Bellot, C.C. Abbou, D. Chopin, *Overexpression of epidermal growth factor (EGFR) in bladder cancer is associated with tumor progression but is not an independent prognostic indicator.* The Journal of Urology, Vol. 155, 5, May 1996: A348.

64. D. Chopin, **Z. Popov**, A. Hoznek, A. Lafrere, J. Bellot, C.C. Abbou, *P53 and angiogenesis in invasive bladder cancer,* The Journal of Urology, Vol. 155, 5, May 1996: P. 1518.

65. **Ж. Попов**, А. Станков, Д. Петровски, D. Chopin, *Карактеристики на експресијата на E-Cadherin-от и нејзините соодноси со стадиумот и градусот на канцерот на мочниот меур.* Македонски медицински преглед, 50, Suppl. 23, 1996: 110 (A323).

66. П. Колевски, М. Благоевска, К. Димитровски, А. Христова-Дамчева, Н. Ивановски, **Ж. Попов**, К. Чакаларoски, *Имунолошки подготовки за ренална трансплантација кај болни со хронична бубрежна инсуфициенција*. Македонски медицински преглед, 50, Suppl. 23, 1996: 110 (A325).

67. **Z. Popov**, A. Hoznek, M. Colombel, S. Bastuji, C.C. Abbou, D.K. Chopin, *Evaluations de la surexpression de la proteine p53 dans le cancer da fa vessie: valeur pronostique*. Chirurgie, Vol. 121, Sep., 1996: 461–469.

68. J-J. Patard, B. Muscatelli-Groux, F. Saint, **Z. Popov**, P. Maille, C.C. Abbou, D. Chopin, *Evaluation of local immune response after intravesical bacille Calmette-Guerin treatment for superficial bladder cancer.* British Journal of Urology, 78, 1996: 709–714.

69. N. Ivanovski, K. Cakalaroski, L. Stojkovski, **Z. Popov**, Z. Antova, S. Trajkova, *Kaposi's sarcoma in renal alograft patients*. Македонски медицински преглед, Suppl. 30, 1997: 133–139.

70. N. Ivanovski, **Z. Popov**, P. Kolevski, K. Cakalarski, L. Stojkovski, *Organ shortage in Balkans – Accept everyone.* Transplantation Proceedings, 29, 1997: 3397–3398.

71. N. Ivanovski, K. Cakalaroski, Z. Antova, L. Stojkovski, **Z. Popov**, *Skin malignancies in renal transplant patients*. Nephrol Dyalisis Transplant, 1997: A106.

72. V. Izadifar, J-J. Patard, A. Hoznek, **Z. Popov**, C.C. Abbou, D. Chopin, *P53 nuclear overexpression is associated to bladder tumor recurrence and progression after BCG treatment.* Urological Research, Vol. 25, 1, 1997: O34.

73. **Popov Z**, Hoznek A, Colombel M, Bastuji-Garin S, Lefrere-Belda MA, Bellot J, Abbou CC, Mazerolles C, Chopin DK, *The prognostic value of p53 nuclear overexpression and MIB-1 as a proliferative marker in transitional cell carcinoma of the bladder.* Cancer, 80(8), Oct. 15, 1997: 1472–1481.

74. S. Gil-Diez de Medina, **Z. Popov**, D.K. Chopin, J. Southgate, GC. Tucker, A. Delouvee, JP. Thiery, F. Radvanyi, *Relationship between E-cadherin and fibroblast growth factor receptor 2b expression in bladder carcinomas*. Oncogene, 18, 41, Oct. 7, 1999: 5722–5726.

75. **Z. Popov**, N. Ivanovski, P. Kolevski, *Transplantation rénale en Macédoine*. Annales d'Urologie, Vol. 34, No. 5 (Octobre), 2000: 294–301.

76. N. Ivanovski, **Z. Popov**, P. Kolevski, K. Cakalaroski, G. Spasovski, O. Stankov, O. Stoiceva-Taneva, J. Paneva-Masin, *Transplantation renale utilisant des donneurs vivants de plus 65 ans.* Annales d'Urologie, Vol. 34, No. 5, Octobre 2000: 302–305.

77. P. Kolevski. N. Ivanovski, A. Hristova-Damceva, M. Penev, K. Cakalaroski, Lj. Lekovski, **Z. Popov**, *Épidémiologie du complexe majeur d'histocompatibilité "human leycocite antigenes" dans la population macédonienne*. Annales d'Urologie, Vol. 34, No. 5, Octobre 2000: 306–311.

78. C.C. Abbou, R. Rabii, A. Hoznek, P. Antiphon, **Z. Popov**, D. Chopin, *Prélèvement rénal chez le donneur vivant par voie laparascopique rétropéritonéale ou lomboscopie.* Annales d'Urologie, Vol. 34, No. 5, Octobre 2000: 312–318.

79. P. Kolevski, **Z. Popov**, A. Hristova-Damceva, D. Petrovski, K. Cakalaroski, N. Ivanovski, *Donneurs vivant : aspects imunologiques*. Annales d'Urologie, Vol. 34, No. 5, Octobre 2000: 319–322.

80. **Z. Popov**, N. Ivanovski, Lj. Lekovski, A. Stankov, S. Dohcev, D. Petrovski, K. Cakalaroski, J. Janculev, P. Kolevski, C.C. Abbou, D. Chopin, *Complications chirurgicales après la transplanatation rénale.* Annales d'Urologie, Vol. 34, No. 5, Octobre 2000: 323–329.

81. N. Ivanovski, **Z. Popov**, P. Kolevski, K. Cakalaroski, Z. Antova, G. Spasovski, M. Vickova-Laskovska, G. Zografski, *Cancer apres transplanataion rénale et immunosuppression.* Annales d'Urologie, Vol. 34, No. 5, Octobre 2000: 336–339.

82. K. Cakalaroski, N. Ivanovski, **Z. Popov**, S. Dohcev, P. Kolevski, B. Weil, Ph. Lang, *Les anomalies immunoglobulinémiques mono- et oligoclonales chez les*

transplantés rénaux. Annales d'Urologie, Vol. 34, No. 5, Octobre 2000: 340–344.

83. A. Oncevski, P. Dejanov, V. Gerasimovska, K. Cakalaroski, N. Ivanovski, **Z. Popov**, *Accès vasculaires et insuffisance rénale chronique*. Annales d'Urologie, Vol. 34, No. 5, Octobre 2000: 345–351.
84. **Z. Popov**, S. Gil-Diez de Medina, MA. Lefrere-Belda, A. Hoznek, S. Bastuji-Garin, C.C. Abbou, JP. Thiery, F. Radvanyi, D.K. Chopin, *Low E-cadherin expression in bladder cancer at the transcriptional and protein level provides prognostic information.* Br J Cancer, 83, 2, 2000: 209–14.
85. Lj. Lekovski, A Stavridis, V. Georgiev, **Z. Popov**, M. Penev, O. Stankov, S. Dohcev, D. Petrovski, S. Stavridis, *Exstracorporeal Shock Wave Lithotripsy with Dornier MPL-9000-Ten Year Experience*. Македонски медицински преглед, 54, Suppl. 41, 2000: 39.
86. M. Penev, **Z. Popov**, A. Stavridis, V. Georgiev, Lj. Lekovski, O. Stankov, S. Dohcev, D. Petrovski, *Lithotripsy for Ureteral Lithiasis with the Dornier System with Radiological Location*. Македонски медицински преглед, 54, Suppl. 41, 2000: 42.
87. O. Stankov, **Z. Popov**, A. Stavridis, V. Georgiev, M. Penev, Lj. Lekovski, S. Dohcev, D. Petrovski, S. Stavridis, *Glaufos (Ofloxacin) – Our Experience in the Treatment of UTI-s.* Македонски медицински преглед, 54, Suppl. 41, 2000: 81.
88. D. Petrovski, M. Penev, **Z. Popov**, A. Stavridis, V. Georgiev, Lj. Lekovski, O. Stankov, S. Dohcev, *Yolk Sac Tumor of the testis – Case report.* Македонски медицински преглед, 54, Suppl. 41, 2000: 94.
89. Lj. Lekovski, A. Stavridis, **Z. Popov**, V. Georgiev, M. Penev, O. Stankov, S. Dohcev, D. Petrovski, S. Stavridis, *Nephrectomy - Indications and Complications in 404 Patients in* 8 *Year Period*. Македонски медицински преглед, 54, Suppl. 41, 2000: 99.
90. M. Penev, D. Petrovski, **Z. Popov**, A. Stavridis, V. Georgiev, Lj. Lekovski, O. Stankov, S. Dohcev, S. Stavridis, *Selection of BPH Treatment in Accordance to Urodynamics.* Македонски медицински преглед, 54, Suppl. 41, 2000: 110.
91. **Z. Popov**, N. Ivanovski, P. Kolevski, Lj. Lekovski, O. Stankov, S. Dohcev, D. Petrovski, *Kidney Transplantation in Republic of Macedonia.* Македонски медицински преглед, 54, Suppl. 41, 2000: 42.
92. S. Panov, D. Roganovic-Zafirova, **Z. Popov**, M. Penev, D. Petrovski, G. Jasar, *Detection of H-RAS oncogene codon 12 mutation in paraffin embedded samples of the urinary bladder cancer.* Македонски медицински преглед, 54, Suppl. 41, 2000: 110.

93. С. Банев, П. Цветковски, Л. Спасевска, С. Костадинова, **Ж. Попов**, В. Георгиев, М. Пенев, Љ. Лековски, О. Станков, С. Дохчев, *Дистрибуција на ткивните компоненти кај хиперпластични простатични жлезди.* Зборник на II-от конгрес на хирурзите на Македонија со меѓународно учество, сеп. 2000: 98.

94. Љ. Лековски, М. Пенев, А. Ставридис, **Ж. Попов**, В. Георгиев, О. Станков, Д. Петровски, С. Ставридис, С. Банев, *Континентна уринарна деривација – Studer procedure кај радикална цистектомија поради карцином на мочниот меур.* Зборник на II-от конгрес на хирурзите на Македонија со меѓународно учество, сеп. 2000: 145.

95. G. Spasovski, N. Ivanovski, K. Cakalaroski, **Z. Popov**, J.P. Masin, J. Kamnar, J. Gavrilovski, G. Vidoevski, *Avascular hip necrosis after renal tansplantataion-the importance of an early diagnosis and the role* of a *core decompression.* Nephrol Dyalisis Transplant, 15, 9, 2000: A282

96. N. Ivanovski, **Z. Popov**, P. Kolevski, K. Cakalaroski, L. Stojkovski, G. Spasovski, J. Paneva-Masin, *Use of advanced age donors in living renal transplanataion – ls it justified?* Transplantation Proceedings, 33, 2001: 1227–1228.

97. N. Ivanovski, **Z. Popov**, P. Kolevski, K. Cakalaroski, L. Stojkovski, G. Spasovski, K. Zafirovska, *Living related renal transplantation - the use of advanced age donors.* Clinical Nephrology, 55, 2001: 309–312.

98. G. Spasovski, N. Ivanovski, K. Cakalaroski, A. Sikole, J.P. Masin, **Z. Popov**, G. Zafirovski, J. Gavrilovski, *Risk factors of osteonecrosis development in renal transplant recipients.* Journal of American Society of Nephrology, 12, 2001: A4952.

99. N. Ivanovski, **Z. Popov**, P. Kolevski, K. Cakalaroski, G. Spasovski, A. Sikole, J. Paneva-Masin, *Interleukin-2R antagonists in the prevention of acute rejection in living donor transplantation.* Transplant Proc, 33, 2001: 3203–3204.

100. D. Kuzmanovska, E. Sahpazova, M. Kocova, G. Damjanovski, **Z. Popov**, *Phaeochromocytoma associated with reversible renal artery stenosis.* Nephrol Dial Transplant, 16, 2001: 2092–2094.

101. П. Колевски, А. Димчева-Христова, Н. Ивановски, **Ж. Попов**, *Хистокомпатибилност во антигените од (ХЛА систем) главниот систем на хистокомпатибилност помеѓу примател и дарител при трансплантација на бубрег.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 39.

102. V. Janevski, **Z. Popov**, N. Jankulovski, *Spontaneous intestinal perforation in renal transplant recipients.* Прв национален конгрес по урологија во сора-

ботка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 40.

103. G. Spasovski, N. Ivanovski, **Z. Popov**, K. Cakalaroski, A. Sikole, J. Masin, Lj. Lekovski, G. Zafirovski, J. Gavrilovski, *Hip osteonecrosis-most prominent bone disease in renal transplant recipients.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 41.

104. J. Masin, N. Ivanovski, G. Spasovski, K. Cakalaroski, A. Sikole, P. Kolevski, **Z. Popov**, *Interleukin – 2R antagonists (Dacluzumab or simulect) on the prevention of acute rejection, in living renal transplantation.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија, Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 42.

105. Љ. Лековски, А. Ставридис, В. Георгиев, М. Пенев, О. Станков, С. Дохчев, Д. Петровски, С. Саиди, С. Ставридис, **Ж. Попов**, *Екстракорпорална бранова литотрипсија и класична пиелолитотомија, споредбена студија.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија, Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 44.

106. D. Petrovski, A. Stavridis, V. Georgiev, M. Penev, Lj. Lekovski, O. Stankov, S. Dohcev, S. Saidi, S. Stavridis, **Z. Popov**, *Evaluation of the urodynamic parameters at patients with BPH treated with 5alfa inhibitor and alfa blockator*. Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија, Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 49.

107. S. Panov, D. Roganovic-Zafirova, E. Jovcheva, D. Gligorov, M. Markoska, D. Petrovski, **Z. Popov**, *Non-invasive molecular genetic diagnostics of urinary bladder cancer.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 55.

108. С. Банев, П. Цветкоски, В. Филиповски, А. Ставридис, В. Георгиев, М. Пенев, Љ. Лековски, С. Дохчев, О. Станков, Д. Петровски, С. Ставридис, **Ж. Попов**, *Морфометрија на јадрото кај бенигни и малигни простатични лезии.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 56.

109. **Ж Попов**, О. Станков, М. Кузмановски, С. Банев, *Енормен тумор на тестис.* Прв национален конгрес по урологија во соработка со Европ-

ската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 86.

110. Г. Зафировски, **Ж. Попов**, Г. Спасовски, М. Самарџиски, Т. Пиперковски, С. Антевски, *Остеонекроза на феморалната глава кај пациенти* со *бубрежна трансплантација.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 41.

111. В. Јаневска, С. Банев, С. Костадинова, Л. Спасовска, М. Пенев, **Ж. Попов**, *Корелација меѓу хистолошкиот градус и клиничкиот стадиум на примарните малигни тумори на бубрезите.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 56.

112. **Z. Popov**, O. Stankov, M. Kuzmanoski, N. Jankulovski, E. Basevski, *Mucinous adenocarcinoma arising in urachal cyst.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 87.

113. С. Дохчев, А. Ставридис, В. Георгиев, М. Пенев, Љ. Лековски, О. Станков, Д. Петровски, С. Саиди, С. Ставридис, **Ж. Попов**, *Ортотопска континентна дериватија на урина според методот на Studer.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 57.

114. B. Krstevska, C. Dimitrovski, G. Pemovska, V. Percan, S. Misevska, T. Milenkovic, S. Subevska, **Z. Popov**, *Evaluation of diagnostic methods in decision-making for surgery of adrenal incidentalomas.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13-15 јуни 2002: 59.

115. M. Penev, A. Stavridis, V. Georgiev, Lj. Lekovski, O. Stankov, S. Dohcev D. Petrovski, S. Saidi, S. Stavridis, M. Kuzmanoski, **Z. Popov**, *Lithotripsy or ureteral lithiasis with the dornier system with radiological location.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. 55, Suppl. 51, Охрид, 13–15 јуни 2002: 69.

116. О. Станков, М. Кузманоски, **Ж. Попов**, *Артефициелна ерекција предизвикана од страно тело.* Прв национален конгрес по урологија во соработка со Европската асоцијација по урологија. Македонски медицински преглед, 55, Suppl. 51, Охрид, 13–15 јуни 2002: 77.

117. S. Ristovski, **Z. Popov**, B. Polenakovik, S. Dzikova, M. Sosolceva, B. Trajcevska, Z. Dacevski, *Positive predictive value (PPV) of prostate specific antigen*

*(PSA), digital rectal examination (DRE) and transrectal ultrasonography (TRUS) in diag*nosis, staging and prognosis of prostate cancer in patients with law urinary *tract symptoms (LUTS): 7-year study*. Bju International-Supplement 90 (2002/9).

118. S. Banev, P. Cvetkovski, R. Jovanovic, V. Filipovski, **Z. Popov**, *A case report of* a *teratocarcinoma* of *testis – is it possible?* Abstracts of the XXIVth International Congress of the International Academy of Pathology, 5–10 Oct, 2002, Amsterdam. Histopathology, Vol. 41, Suppl. 1, 2002: 04628.
119. B.L. Krstevska, G. Pempovska, S.J. Misevska, M. Bitovska, **Z. Popov**, B. Bogoeva, *Evaluation of diagnostic methods in decision making for surgery of adrenal gland.* 6th European congress of endocrinology, Lyon, France, 26–30 April, 2003: P0050e.
120. S. Panov, D. Roganovic-Zafirova, E. Jovcheva, D. Gligorov, M. Markoska, **Z. Popov**, *Urinary samples in molecular genetic detection* of *bladder cancer.* Balkan Journal of Clinical Laboratory. IX, 02, I, 2002: OP-XIII.
121. N. Ivanovski, **Z. Popov**, P. Kolevski, K. Cakalaroski, G. Spasovski, J. Masin, K. Zafirovska, *Excellent results of Living Unrelated Renal Transplantation (LURT)–A single Center experience in the Balkans.* Proceedings of the 6th Bantao congress. BANTAO Journal, 1, 2, 2003: 147–148.
122. J. Masin-Spasovska, G. Spasovski, L. Grceva, S. Dzikova, **Z. Popov**, G. Petrusevska, Lj. Lekovski, N. Ivanovski, *Protocol Biopsies of Stable renal Allografts: Our Firs Experience.* BANTAO Journal, 2, 1, 2004: 36–38.
123. S. Panov, D. Roganovic-Zafirova, G. Stavric, G. Yashar, **Z. Popov**, *High frequency of the HRAS oncogene codon 12 mutation on Macedonian patients with urinary bladder cancer*. Genetics and Molecular Biology, 27, 1, 2004: 9–14.
124. N. Ivanovski, **Z. Popov**, P. Kolevski, K. Cakalaroski, J. Masin, K. Zafirovska, *Living emotionally related renal transplantation (LERT) single center experience in the Balkans.* Ann. Transplant 9, 2004: 48–49.
125. **Z. Popov**, S. Gil-Diez-De-Medina, V. Ravery, A. Hoznek, S. Bastuji-Garin, MA. Lefrere-Belda, C.C. Abbou, D.K. Chopin, *Prognostic value of EGF receptor and tumor cell proliferation in bladder cancer: therapeutic implications.* Urol Oncol. 22, 2004: 93–101.
126. F. Saint, MA. Lefrere-Belda, R. Quintela, A. Hoznek, JJ. Patard, J. Bellot, **Z. Popov**, E.S. Zafrani, C.C. Abbou, D.K. Chopin, S.Gil-Diez de Medina, *Pretreatment p53 nuclear overexpression as a prognostic marker in superficial bladder cancer treated with Bacillus Calmette-Guerin (BCG).* Eur Urol. 45, 2004: 475–482.

127. Б. Богоева, С. Банев, **Ж. Попов,** Т. Јосифовски, Н. Јанкуловски, О. Станков, М. Кузманоски, Ј. Јанчулев, Б. Крстевска, Ч. Димитровски, *Лапароскопска адреналектомија: кога и како.* Конгрес на ендокринологија, диабетес и метаболни нарушувања со меѓународно учество, Охрид, 29.IX-02.X 2004. Книга на апстракти (ПС 4/3 Вариа), 141, 2004: 156.

128. B. Bogoeva, S. Banev, V. Filipovski, B. Krstevaska, **Z. Popov**, *Pathomorphological examination of Adrenal Diseases*. Конгрес на ендокринологија, диабетес и метаболни нарушувања со меѓународно учество, Охрид, 29. IX-02.X 2004. Книга на апстракти (ПС 4/3 Вариа), 141, 2004: 160.

129. П. Колевски, А. Димчева-Христова, Н. Ивановски, **Ж. Попов**, *HLA сообноси кај 142 ренални трансплантации.* Конгрес на ендокринологија, диабетес и метаболни нарушувања со меѓународно учество, Охрид, 29.IX-02.X 2004. Книга на апстракти (ПС 4/3 Вариа), Македонски медицински преглед, 58, Suppl. 63, 2004: 16–17.

130. J. Masin-Spasovska, G. Spasovski, S. Dzikova, G. Petrusevska, B. Dimova, Lj. Lekovski, **Z. Popov**, N. Ivanovski, M. Polenakovic, *Comparison* of *histological changes in protocol biopsies at 1 and 6 months after living donor kidney transplantation: do we have to treat borderline and subclinical changes.* Transplant International, Vol. 18, Suppl. 1, 2005: OR-120.

131. G. Spasovski, J. Masin-Spasovska, S. Dzikova, G. Petrusevska, B. Dimova, Lj. Lekovski, **Z. Popov**, N. Ivanovski, *Histological findings in protocol biopsies at 1 and 6 months after living donor transplantation and allograft function at 1 year.* Transplant International, Vol. 18, Suppl. 1, 2005: PO-453.

132. **Z. Popov**, N. Ivanovski, P. Kolevski, G. Spasovski, J. Masin-Spasovska, Lj. Lekovski, *Laparoscopic living donor nephrectomy in the Republic of Macedonia : First four cases*. Transplant International, Vol. 18, Suppl. 1, 2005: PO–561.

133. N. Ivanovski, **Z. Popov**, K. Cakalaroski, J. Masin, G. Spasovski, K. Zafirovska, *Living-unrelated (paid) renal transplantation – ten years later.* Transplant Proc, 37, 2005: 563–564.

134. J. Masin-Spasovska, G. Spasovski, S. Dzikova, G. Petrusevska, B. Dimova, Lj. Lekovski, **Z. Popov**, N. Ivanovski, M. Polenakovic, *The evolution of untreated borderline and subclinical rejections at first month kidney allograft biopsy in comparison with histological changes at 6 months protocol biopsies*. Прилози. Одд. за биол. и мед. науки (МАНУ) = Contributions. Sec. of Biol. and Med. Sci.(MASA), XXVI, 1, 2005: [25]–33.

135. J. Masin-Spasovska, G. Spasovski, M. Polenakovic, S, Dzikova, G. Petrusevska, B. Dimova, Lj. Lekovski, **Z. Popov**, N. Ivanovski, *Chronic allograft nephropathy (CAN) in early renal protocol biopsies: does treatment* of *borderline and subclinical acute rejections prevent development and progression of*

CAN? Прилози. Одд. за биол. и мед. науки (МАНУ) = Contributions. Sec. of Biol. and Med. Sci.(MASA), XXVI, 2, 2005: [91]–103.

136. J. Masin-Spasovska, G. Spasovski, S. Dzikova, L. Grcevska, G. Petrusevska, Lj. Lekovski, **Z. Popov**, N. Ivanovski, *Protocol biopsies in kidney transplant recipients: histologic findings as prognostic markers for graft function and outcome.* Transplant Proc., 37, 2005: 705–708.

137. **Z. Popov**, N. Ivanovski, K. Cakalaroski, P. Kolevski, D. Chopin, *Coelioscopic renal prelevement on living donor. The first four cases in Republic of Macedonia,* Prog Urol., 15*,* 2005: 1227–1228.

138. G. Spasovski, N. Ivanovski, J. Masin-Spasovska, S. Dzikova, L. Grcevska, G. Petrusevska, Lj. Lekovski, **Z. Popov**, M. Polenakovic, *Recurrent glomerulonephritis in living kidney transplantation.* Прилози. Одд. за биол. и мед. науки (МАНУ) = Contributions. Sec. of Biol. and Med. Sci.(MASA), XXVI, 2, 2005: [79]–90.

139. S. Stavridis, V. Georgiev, **Z. Popov**, M. Penev, Lj. Lekovski, O. Stankov, S. Dohcev, D. Petrovski, S. Saidi, M. Kuzmanovski, S. Banev, A. Stavridis, *Radical retropubic prostatectomy for prostate carcinoma; first results* of *the Clinic of Urology in Skopje.* Acta Chirur. Jug., 52, 2005: 37–40.

140. **Z. Popov**, N. Ivanovski, P. Kolevski, O. Stankov, L. Stamatov, *Prelevement renal chez les donneurs vivants par voie laparoscopique; les quatre premiers cas en Republique de Macedoine.* 5e Congres Annuel de la Societe Francophone de Transplantation, Tours, 7–10 Dec., 2005.

141. N. Ivanovski, **Z. Popov**, L. Stamatov, K. Cakalaroski, *Transplantation renale a partir des doneurs vivants repondant a des criteres elargis; I'experience de Skopje.* 5e Congres Annuel de la Societe Francophone de Transplantation, Tours, 7–10 Dec., 2005.

142. K. Cakalaroski, N. Ivanovski, L. Stamatov, Z. Antova, **Z. Popov**, *Rhabdomyolise et insuffisance renale aigue chez un greffe renal du* a *la simvastatin.* 5e Congres annuel de la Societe Francophone de Transplantation, Tours, 7–10 Dec., 2005.

143. J. Masin-Spasovska, G. Spasovski, S. Dzikova, G. Petrusevska, B. Dimova, **Z. Popov**, N. Ivanovski, M. Polenakovic, *Protocol biopsies in kidney transplant recipients at 1 and 6 months in correlation to allograft function.* J Am Soc Nephrol., Philadelphia, Vol. 16, Nov 8–13, 2005: Sa–PO622.

144. J. Masin-Spasovska, G. Spasovski, S. Dzikova, G. Petrusevska, B. Dimova, **Z. Popov**, N. Ivanovski, M. Polenakovic, *Protocol biopsy in kidney transplant recipients 6 months after transplantation compared with 1 month biopsy and correlation* to *allograft function at 1 year.* J Am Soc Nephrol., Philadelphia, Vol. 16, Nov 8–13, 2005: PUB 206.

145. J. Masin-Spasovska, N. Ivanovski, S. Dzikova, G. Petrusevska, B. Dimova, Lj. Lekovski, **Z. Popov**, G. Spasovski, *Early renal protocol biopsies: benefitial effects of treatment of borderline changes and subclinical rejections on the histological changes for chronic allograft nephropathy.* BANTAO Journal, 4, 1, 2006: 88–92.

146. N. Ivanovski, **Z. Popov**, J. Masin-Spasovska, A.H. Dimcevska, P. Kolevski, *First two ABO-incompatible living renal transplantations using, splenectomy, rituximab, plasmapheresis and IVIG as a preconditioning regimen: a single center experience in the Balkans.* Xenotransplantation 13, 2006: 123–125.

147. N. Jankulovski, **Z. Popov**, S. Bojadzieva, P. Validire, J. Gogusev, *Expression of c-KIT oncoprotein in gastrointestinal stromal tumors in adults and children: guideline for diagnosis and treatment.* Прилози. Одд. за биол. и мед. науки (МАНУ) = Contributions. Sec. of Biol. and Med. Sci.(MASA), XXVII; 2, 2006: [59]–70.

148. Л. Петковска, Н. Ивановски, Ч. Димитровски, **Ж. Попов**, З. Переска, Ф. Личоска-Јосифовиќ, *Нарушувања во гликозниот метаболизам кај пациенти со трансплантиран бубрег.* Македонски медицински преглед, 60, 5–6, 2006: 181–257.

149. J. Masin-Spasovska, **Z. Popov**, G. Spasovski, S. Dzikova, G. Petrusevska, K. Cakalaroski, N. Ivanovski, *Faut-il traiter le rejet infra-clinique decouvert suivant le potocol biopsies systematiques du greffon*. Congres Annuel de la Societe Francophone de Transplantation, Tunis, 06–09 Dec., 2006: 256 (PA 69).

150. **Z. Popov**, K. Cakalaroski, P. Kolevski, O. Stankov, O. Ivanovski, D. Petrovski, N. Ivanovski, *Les neoplasmes malignes – de novo apres la transpantation renale – experience d'un centre de greffe unique.* Congres Annuel de la Societe Francophone de Transplantation, Tunis, 06–09 Dec., 2006: 257 (PA 70).

151. K. Cakalaroski, **Z. Popov**, P. Korneti, J. Masin-Spasovska, P. Kolevski, N. Ivanovski, *Les poeines urinaires comme un marqueur precoce d une lesion du greffon renal a partir de donneurs vivants plus ages*. Congres annuel de la Societe Francophone de Transplantation, Tunis, 06–09 Dec., 2006: 301 (PA 114).

152. O. Ivanovski, O. Stankov, M. Kuzmanoski, S. Saidi, S. Banev, V. Filipovski, Lj. Lekovski, **Z. Popov**, *Penile strangulation: two case reports and review of the literature.* J Sex Med., 4, 6, Nov 2007: 1775–1780. Epub 2007 Sep., 21. Review.

153. J. Masin-Spasovska, G. Spasovski, S. Dzikova, G. Petrusevska, Lj. Lekovski, N. Ivanovski, **Z. Popov**, *Do we have to treat subclinical rejections in early protocol renal allograft biopsies?* Transplant Proc, 39, 8, Oct. 2007: 2550–2553.

154. **Z. Popov**, O. Ivanovski, P. Kolevski, O. Stankov, D. Petrovski, K. Cakalaroski, N. Ivanovski, *De novo malignancies after renal transplantation – a single-center experience in the Balkans*. Transplant Proc, 39, 8, Oct. 2007: 2589–2591.

155. **Z. Popov**, A. Stavridis, Lj. Lekovski, M. Penev, S. Dohcev, O. Stankov, D. Petrovski, S. Saidi, M. Kuzmanoski, S. Stavridis, A. Mickovski, S. Banev, G. Zografski, J. Janculev, O. Ivanovski, V. Georgiev, *Urinary diversion: 30 years experience of a single centre in Republic of Macedonia*. Acta Chirur. Jug., 54, 4, 2007: 49–55.

156. G. Spasovski, J. Masin-Spasovska, S. Dzikova, G. Petrusevska, Lj. Lekovski, **Z. Popov**, N. Ivanovski, *Factors associated with allograft function at 1-year – The value of protocol biopsies in kidney transplant recipients.* 44th ERA-EDTA Congress, Barcelona, SPAIN, JUN 22–24, 2007, ERA-EDTA, NDT, Vol. 22, Suppl. 6, 2007, Sap. 463.

157. J. Masin-Spasovska, G. Spasovski, S. Dzikova, G. Petrusevska, Lj. Lekovski, **Z. Popov**, N. Ivanovski, *Treatment of borderline and subclinical rejections in protocol kidney allograft biopsies might influence the progression of chronic allograft nephropathy.* 44th ERA-EDTA Congress, Barcelona, SPAIN, JUN 22–24, 2007, ERA-EDTA, NDT, Vol. 22, Suppl. 6, 2007: Sap. 421.

158. N. Ivanovski, **Z. Popov**, P. Kolevski, *Use of expanded criteria living donors: Efforts to increase the renal transplant donor pool in the Balkans*. Transplant international, Vol. 20, Suppl. 2, Sep. 2007: 221.

159. **Z. Popov**, S. Dohcev, J. Masin, G. Spasovski, O. Ivanovski, Lj. Lekovski, N. Ivanovski, *Pakistan – The new "hope" on the horizon.* Transplant international, Vol. 20, Suppl. 2, Sep. 2007: 228.

160. O. Ivanovski, O. Stankov, M. Kuzmanoski, S. Banev, Lj. Lekovski, **Z. Popov**, *Penile strangulation: presentation of two unusual cases*. Urology, Vol. 70, Issue 3, Sep. 2007: 215–216.

161. O. Ivanovski, I.G. Nikolov, **Z. Popov**, *Mouse model of chronic uraemia: Surgical aspects*. Urology, Vol. 70, Issue 3, Sep. 2007: 315.

162. **Z. Popov**, O. Ivanovski, P. Kolevski, O. Stankov, D. Petrovski, K. Cakalaroski, N. Ivanovski, *De novo malignancies after renal transplantation: a single center experience*. Urology, Vol. 70, Issue 3, Sep. 2007: 120.

163. **Z. Popov**, J. Janculev, O. Stankov, O. Ivanovski, A. Mickovski, J. Masin, P. Kolevski, N. Ivanovski, *ABO-incompatible living renal transplantation: The first two cases on the Balkan Peninsula.* European Urology Meetings, Vol. 70, Issue 3, Sep. 2007: 94.

164. **Z. Popov**, J. Janculev, O. Stankov, O. Ivanovski, J. Masin, P. Kolevski, N. Ivanovski, *Laparoscopic living donor nephrectomy – the first experience in the Balkans*. European Urology Meetings, Vol. 70, Issue 3, Sep. 2007: 94.

165. S. Saidi, S. Dohcev, D. Petrovski, M. Kuzmanoski, S. Stavridis, O. Stankov, M. Penev, Lj. Lekovski, V. Georgiev, A. Stavridis, S. Banev, **Z. Popov**, *The complications after bladder substitution with "Studer-technique" for muscle invasive bladder cancer.* European Urology Meetings, Vol. 2, Issue 5, Oct. 2007.

166. J. Masin-Spasovska, G. Spasovski, S. Dzikova, G. Petrusevska, Lj. Lekovski, N. Ivanovski, **Z. Popov**, *Do we have to treat subclinical rejections in early protocol renal allograft biopsies?* Transplant Proc, 39, 8, Oct. 2007: 2550–2553.

167. **Z. Popov**, N. Jankulovski, T. Josifovski, O. Stankov, M. Kuzmanoski, S. Saidi, S. Stavridis, A. Mickovski, O. Ivanovski, C. Dimitrovski, *Transperitoneal Laparoscopic Adrenalectomy: First Experience from the Balkan Peninsula*. Urology, Vol. 72, Issue 5, Suppl. 1, Santiago de Chile, Nov. 2008: S100–S101. SIU World Uro-Oncology Update.

168. S. Saidi, O. Ivanovski, D. Petrovski, M. Kuzmanoski, S. Stavridis, S. Banev, **Z. Popov**, *Lower urinary tract reconstruction following a cystectomy: experience and results in 20 patients using the "Studer" orthotopic ileal bladder substitution.* Bratisl. Lek Listy, 109, 8, 2008: 353–357.

169. **Z. Popov**, S. Dohcev, J. Masin, I. Rambabova-Busletic, V. Pusevski, O. Stankov, N. Ivanovski, *The organ shortage on the Balkans – Pakistan the promising land*. XXII International Congress of the Transplantation Society, 10–14 August, 2008, Sydney, Australia, Transplantation, No. 896, 2008: 312.

170. N. Ivanovski, J. Masin, S. Dohcev, I. Rambabova-Busletic, V. Pusevski, Z. Popov, **Z. Popov**, *Preemptive expanded criteria in living donor renal transplantation – first choice of treatment of ESRD on the Balkans*. XXII International Congress of the Transplantation Society, 10–14 August, 2008, Sydney, Australia, Transplantation, No. 896, 2008.

171. N. Jankulovski, O. Stankov, SA. Banev, D. Petrovski, A. Mickovski, A. Mitevski, V. Filipovski, **Z. Popov**, *Case report: Isolated malignant peripheral nerve sheath tumor of the kidney*. Прилози. Одд. за биол. и мед. науки (МАНУ) = Contributions. Sec. of Biol. and Med. Sci.(MASA), XXIX, 2, 2009: [361]–369.

172. O. Stankov, O. Ivanovski, **Z. Popov**, *Artificial Penile Bodies–From Kama Sutra to Modern Times*. J Sex Med., 6, Jun. 2009: 1543–1548. https://www.jsm.jsexmed.org/article/S1743-6095(15)32543-1/fulltext

173. S. Saidi, V. Georgiev, S. Stavridis, D. Petrovski, S. Dohcev, Lj. Lekovski, **Z. Popov**, S. Banev, G. Spasovski, *Does prostate specific antigen density corre-*

lates with aggressiveness of the prostate cancer? Hippokratia : quarterly medical journal, 13, 4, 2009: 232–236.

174. P. J. Goebell, S. G. Groshen, B. J. Schmitz-Dräger, [ISBC: P. J. Goebell, ... **Z. Popov**, ... et al.], *p53 immunohistochemistry in bladder cancer – a new approach to an old question / International Study-Initiative on Bladder Cancer (ISBC)*. Urologic Oncology, 28; 4, 2010: 377–388.

175. N. Ivanovski, J. Masin, I. Rambabova-Busljetic, V. Pusevski, Saso Dohcev, Ognen Ivanovski, **Z. Popov**, *The outcome of commercial kidney transplant tourism in Pakistan*. Clinical Transplantation, 25, 1, 2011: 171–173.

176. Masin-Spasovska J., Spasovski G., Dzikova S., Dejanova B., Maleska-Ivanovska V., Petrusevska G., Lekovski L., **Popov Z.**, Ivanovski N, *Ischemia-Reperfusion Injury and Acute Rejection - Effects on Nitric Oxide Levels and Allograft Function and Histology at 1 and 6 Months after Kidney Transplantation*. BANTAO Journal, 7, 1, June 2009: [31]–37.

177. J. Masin-Spasovska, **Z. Popov**, N. Ivanovski, G. Spasovski, *Unusual Clinical Presentation of Colorectal Cancer in a Kidney Transplant Recipient*. BANTAO Journal, 9, 1, June 2011: [46]-48.

178. Saidi S., Georgiev V., Stavridis S., Penev M., Stankov O., Dohcev S., Banev S., Danilovski D., Ivanovski O., **Popov Z.**, *Evaluation of the value of p53 protein expression in the extra-capsular extension of prostate cancer*. Прилози. Одд. за биол. и мед. науки (МАНУ) = Contributions. Sec. of Biol. and Med. Sci.(MASA), XXXII, 2, 2011: [213]-220. http://e20.manu.edu.mk/prilozi/15s.pdf

179. **Popov Z.** = **Попов Ж.**, *Les anomalies vascularies dans la transplantation renale: aspects chirurgicaux = Васкуларни аномалии при бубрежна транс-плантација: хируршки аспекти*. Colloque : Journee de Transplantation Franco-Macedonienne = Француско-македонски ден на трансплантацијата, (Societe Francophone de Transplantation, Македонското Здружение за трансплантација на ткива и органи, МЛД - Здружение на нефролозите), МАНУ, Скопје, Музеј на македонската борба за државност и самостој-ност, 13 мај 2012. (предавач).

180. **Попов Ж**., *Трансплантација на бубрези во Република Македонија - хируршки аспекти*. Стручен состанок по повод 35 години трансплантација на бубрег во Р. Македонија, (МЛД - Здружение на нефролозите и Македонското Здружение за трансплантација на ткива и органи), Скопје, 19.12.2012 (предавач).

181. Saidi S, Stavridis S, Dohcev S, Stankov O, Komina S, Petrusevska G, **Popov Z**, *Incidental Prostate Cancer in Patients Undergoing Radical Cystoprostatectomy in Treatment for Bladder Cancer, Five Years Results*. 5th Uro-

Oncology Winter Congress, 30.01-03.02.2013, Skopje, R.M., The New Journal of Urology, Abstracts, Spec. edition, 2013.

182. Stavridis S., Georgiev V., Penev M., Stankov O., Dohcev S., Saidi S., Ivanovski O., Janculev J., Mojsova M., Srceva M., **Popov Z.**, *Renal Cell Carcinoma With Renal Vein and Inferior Vena Caval Involvement: Surgical Techniques and Outcomes*. 5th Uro-Oncology Winter Congress, 30.01-03.02.2013, Skopje, Macedonia, The New Journal of Urology, Abstracts, Spec. edition, 2013.
183. **Popov Z.**, Kuzmanoski M., Hristov N., Mitrev Z., *Simultaneous Surgical Treatment of The Abdominal Aneurism and Kidney's Tumor*. 5th Uro-Oncology Winter Congress, 30.01-03.02.2013, Skopje, Macedonia, The New Journal of Urology, Abstracts, Spec. edition, 2013.
184. Kuzmanoski M., Stankov O., Jankulovski N., **Popov Z.**, *Mucinous Adenocarcinoma Arising in Urachal Cyst - Case Report*. 5th Uro-Oncology Winter Congress, 30.01-03.02.2013, Skopje, Macedonia, The New Journal of Urology, Abstracts, Spec. edition, 2013.
185. Zafiroski G., **Popov Z.**, Mladenovski S., Kuzmanoski M., Hristov N., Atanasov Z., Mitev K., Mitrev Z., *Urogenital Cancer and Bone Metastasis*. 5th Uro-Oncology Winter Congress, 30.01-03.02.2013, Skopje, Macedonia, The New Journal of Urology, Abstracts, Spec. edition, 2013: 146.
186. Mirchevski J., Mitrev Z., **Popov Z.**, B. Stavreski, T. Angjuseva, E. Idoski, M. Klincheva, *Total Duodenopancreatectomy With Right Radical Nephrectomy and Retroperitoneal Lymph Dissection During a Malignant Tumor of The Pancreas With Surrounding Infiltration*. 5th Uro-Oncology Winter Congress, 30.01-03.02.2013, Skopje, Macedonia, The New Journal of Urology, Abstracts, Spec. edition, 2013.
187. Saidi S., **Popov Z.**, Stavridis S., Janevska V., Panov S., *Digital quantitative immunofluorescent detection of p53 protein in urinary bladder cancer tissue samples*. Прилози. Одд. за биол. и мед. науки (МАНУ) = Contributions. Sec. of Biol. and Med. Sci. (MASA), XXXIV, 1, 2013: 167–175.
188. S. Saidi, S. Stavridis, Dohcev S., Stankov O., Komina S., Petrusevska G., **Popov Z.,** *Incidental prostate cancer in patients undergoing radical cystoprostatectomy in treatment for bladder cancer: our five-year results*. Medicus, 18; 2, 2013: 14–18.
189. Rambabova-Busljetic I., **Popov Z.**, Masin-Spasovska J., Sikole A., Selim Gj., Ivanovski N., *Could living unrelated renal transplantation ameliorate the actual shortage of organs in the Balkan region?* Hippokratia : quarterly medical journal, 17; 3, 2013: 243–245.

190. Saidi S., **Popov Z.**, Stavridis S., Janevska V., Panov S., *Quantitative immunofluorescent detection of p53 protein in urinary bladder cancer samples*. EAU 9th South Eastern European Meeting (SEEM), Thessaloniki, Greece, 1-3 November 2013.

191. Saidi S., **Popov Z.**, Penev M., Stankov O., Stavridis S., Dohcev S., Shabani B., Panov S., *Increased expression of UHRF1 gene in urinary bladder cancer*. EAU 9th South Eastern European Meeting (SEEM), Thessaloniki, Greece, 1-3 November 2013.

192. **Попов Ж.**, *Бубрежна трансплантација во Република Македонија*. Пристапни предавања, прилози и библиографија на новите членови на МАНУ, Скопје, МАНУ, XVII;, 2013: 277–304.

193. **Z. Popov**, P. Kolevski, N. Ivanovski, *Use of expanded criteria in living kidney donors – the crucial factor for improvement of the kidney transplant programme in Republic of Macedonia*. Прилози. Одд. за биол. и мед. науки (МАНУ) = Contributions. Sec. of Biol. and Med. Sci. (MASA), XXXV; 2, 2014: [5]–7, http://manu.edu.mk/prilozi/Contributions_35_2/1–Z_POPOV.pdf

194. S. Pavleska-Kuzmanovska, **Z. Popov**, O. Ivanovski, V. Ristovska, J. Masin-Spasovska, I. Rambabova-Busljetic, N. Ivanovski, *Cyclosporine nephrotoxicity and early posttransplant hyperkaliemia in living – donor renal recipients: Report of 4 cases*. Experimental and Clinical Transplantation, Vol. 12, issue 5, 2014: [479]-483. http://www.ectrx.org/detail/archive/2014/12/5/0/479/0

195. Cakalaroski K., Pavleska-Kuzmanovska S., Danilovska I., Severova V., Trajceska L., **Popov Z.**, Ivanovski N., *Hyperkaliémie associee a la cyclosporine chez les greffes renaux: rapport de deux cas*. Archives of the Balkan Medical Union, 49, Suppl.1, 2014: V-04.

196. K. Cakalaroski, G. Severova, I. Danilovska, L. Trajceska, P. Kolevski, **Z. Popov**, N. Ivanovski, *Réduction de la créatininémie au deuxième jour après la transplantation: un marqueur utile dans la surveillance prolongée du greffon renal*. Archives of the Balkan Medical Union, 49, Suppl.1, 2014: V-05.

197. N. Ivanovski, G. Severova-Andreevska, J. Masin-Spasovska, L. Trajceska, I. Danilovska, K. Cakalaroski, **Z. Popov**, *La transplantation renale ABO incompatible – Lecons de l'experience Balkanique*. Archives of the Balkan Medical Union, 49, 4, Dec. 2014: 397–400, http://umbalk.org/reviews/2014/umb%204'2014%20rezumate.pdf

198. **Z. Popov**, N. Jankulovski, O. Stankov, S. Stavridis, S. Saidi, M. Kuzmanoski, I. Chipurovski, S. Banev, B. Krstevska, O. Ivanovski, C. Dimitrovski, *Laparoscopic adrenalectomy: first single-center experience in the Balkans*. При-

лози. Одд. за мед. науки (МАНУ) = Contributions.Sec. of Med. Sci. (MASA), XXXVI, 3, 2015: [27]–33, http://manu.edu.mk/prilozi/36_3/3.%20Z.%20POPOV.pdf

199. V. Pushevski, P. Dejanov, V. Gerasimovska, G. Petrushevska, A. Oncevski, A. Sikole, **Z. Popov**, N. Ivanovski, *Severe endothelial damage in chronic kidney disease patients prior to haemodialysis vascular access surgery*. Прилози. Одд. за мед. науки (МАНУ) = Contributions. Sec. of Med. Sci. (MASA), XXXVI, 3, 2015: [43]–49, http://manu.edu.mk/prilozi/36_3/5.%20V.%20PUSHEVSKI.pdf

200. M. Polenakovic, **Z. Popov**, *Uro-Oncology Winter Congress : Skopje, Republic of Macedonia (January 30 - February 03, 2013)*. Прилози. Одд. за мед. науки (МАНУ) = Contributions. Sec. of Med. Sci. (MASA), XXXVI, 3, 2015: [157]–160, http://manu.edu.mk/prilozi/36_3/20.%20M.%20POLENAKOVIC,%20Z.%20POPOV.pdf

201. Saidi S., **Popov Z.**, Stavridis S., Panov S., *Alterations of microsatellite loci GSN and D18S51 in urinary bladder cancer*. Hippokratia : quarterly medical journal, 19, 3, 2015: 200-204.

202. **Ж. Попов**, Н. Ивановски, П. Колевски, *Вистината за развојот на трансплантацијата на бубрези во Македонија : писмо*. Дневник, год. XIX, бр. 5711, 09.03.2015: 12.

203. Саиди С., Георгиев В., **Попов Ж.**, Пенев М., Станков О., Дохчев С., Ставридис С., Панов С., *Имунотерапија кај карцином на мочен меур*. Македонски медицински преглед: Зборник на апстракти : симпозиум „Новини во урологијата 2016“, Скопје, 16-17 април 2016 год., Скопје, МЛД, МЗУ, 90, suppl. 92, 2016: 27.

204. Saidi S., Georgiev V., **Popov Z.**, Penev M., Stankov O., Dohcev S., Stavridis S., Ivanovski O., Saidi M., Janevska V., Petrusevska G., Spasovska L., Komina S., Panov S., *Management of primary bladder tumors at the University clinic of urology in Skopje in 2010 during one-year follow up*. Македонски медицински преглед: Зборник на апстракти : симпозиум „Новини во урологијата 2016“, 16-17 април 2016 год., Скопје : МЛД, Македонско здружение по урологија, 90, suppl. 92, 2016: 36–37.

205. **Z. Popov**, *Les liens avec les universités et les centres hospitaliers parisiens et l'influence de l'école françaises sur la médicine et de la chirurgie macédoniennes*. La coopération franco-macédonienne et inter-académique dans l'esprit de nouveaux défis de la Francophonie : travaux du colloque : 15 Mars 2016, Skopje = Македонско-француската меѓуакадемиска соработка во духот

на новите предизвици на франкофонијата : зборник на трудови : 15 март 2016, Скопје, [réd. L.Starova], Skopje, AMSA = Скопје, МАНУ, 2017: [55]–88.

206. N. Ivanovski, K. Cakalaroski, G. Severova, I. Nikolov, O. Ivanovski, **Z. Popov**, *L'histoire triste des acheteurs de rein sur les Balkans*. Communications validées par le secrétariat : 17ème Congrès annuel de la Société Francophone de Transplantation [SFT], La Rochelle - du 12 au 15 décembre 2017 [El. resource], Paris, 2017: [118].
207. K. Cakalaroski, **Z. Popov**, O. Stankov, O. Ivanovski, D. Petrovski, G. Severova, N. Ivanovski, *Les neoplasmes malignes - de novo apres la transplantation renale - experience d'un centre de greffe unique*. Abstract Book : the 21st Session of the Balkan Medical Days, 06-08 October 2017, Sofia, Bulgaria, org.com. L.Traykov, V.Petkova-Dimitrova, [Sofia : Balkan Medical Union-Bulgarian National Section], 2017: 7–8.
208. **Z. Popov**, O. Stankov, S. Stavridis, S. Saidi, S. Banev, O. Ivanovski, C. Dimitrovski, *Laparoscopic Adrenalectomy: First Single Centre Experience On The Balkans*. Abstract Book : the 21st Session of the Balkan Medical Days, 06-08 October 2017, Sofia, Bulgaria, org. com. L.Traykov, V.Petkova-Dimitrova, [Sofia, Balkan Medical Union-Bulgarian National Section], 2017: 35.
209. **Z. Popov**, *Managing vascular anomalies in kidney transplantation*. Conference 2nd Congress of Macedonian Association of Urology, Skopje, Macedonia, 7–9 April 2017 = 2. Конгрес на Македонското здружение по урологија, Скопје, Македонија, 7–9 април 2017.
210. **Z. Popov**, *Macedonia: [First Kidney Transplantation]*. The History of the First Kidney Transplantation : a European Overview / edit. by R. Langer, Budapest, Semmelweis Press, Multimedia Studio, 2019: 160-164, https://www.semmelweiskiado.hu/downloads/1/41/semmelweis_kiado_file_1563261715.pdf
211. G. Severova-Andreevska, I. Danilovska, A. Sikole, **Z. Popov**, N. Ivanovski, *Hypertension after Kidney Transplantation: Clinical Significance and Therapeutical Aspects*. Open access Macedonian journal of medical sciences (OAMJMS) [El. resource], Vol. 7, no. 7, 2019: 1241–1245. https://www.id-press.eu/mjms/article/view/oamjms.2019.264/3233
212. **Z. Popov**, O. Ivanovski, I. Nikolov, G. Spasovski, S. Saidi, S. Stavridis, N. Ivanovski, *Gestion de multiples arteres renales et d'anatomie veineuse peu frequant pendant la transplantation du rain: d'un simple probleme technique a la procedure de securite de greffon*. Posters [El. resource] : 19éme Reunion

Annuelle Societe Francophone de Transplantation, Bordeaux, France : du 3 au 6 Decembre 2019.
http://www.sft-congres.fr/programme-scientifique

213. G. Severova-Andreevska, I. Nikolov, L. Trajceska, I. Rambabova-Busletik, **Z. Popov**, N. Ivanovski, *Low Tacrolimus Clearance Saves the Long Term Graft Function After Kidney Transplantation – 24 Months Single Center Observational Study*. [Abstracts] [El. resource] : Congress of the European Society for Organ Transplantation (ESOT 2019), Copenhagen, Denmark, 15–18 September 2019: BOS211.
https://www5.shocklogic.com/scripts/jmevent/programme.php?Client_Id=%27ESOT%27&Project_Id=%27ESOT19%27&System_Id=1

214. K. Popovska Jankovic, G. Bozinovski, **Z. Popov**, A. J. Dimovski, *FGFR3 and/or CDKN2A/2B somatic mutations are present with high frequency in patients with early stage urothelial cancer*. [Abstracts & Posters] [El. resource] : European Human Genetics Conference (ESHG 2019), Gothenburg, Sweden, June 15–18, 2019: P12.152A.
https://www.abstractsonline.com/pp8/#!/7874/presentation/3100

215. **Z. Popov**, O. Stankov, S. Stavridis, S. Saidi, O. Ivanovski, G. Spasovski, K. Cakalaroski, N. Ivanovski, *Management of Multiple Renal Arteries and Unusual Venous Anatomy During Kidney Transplant: From a Simple Technical Problem to a Graft-Saving Procedure*. Experimental and Clinical Transplantation, Vol. 18, issue 7, 2020: [763]–770.
http://www.ectrx.org/detail/archive/2020/18/7/0/763/0

216. A. Trifunovski, A. Dimovski, S. Dohcev, S. Stavridis, O. Stankov, S. Saidi, M. Gjorgjievska, **Z. Popov**, *Detection of TMPRSS2-ERG Fusion Transcript in Biopsy Specimen of Prostate Cancer Patients: a Single Centre Experience*. Прилози. Одд. за мед. науки (МАНУ) = Contributions. Sec. of Med. Sci. (MASA), XLI, 1, 2020: [5]–14. http://manu.edu.mk/prilozi/41_1/1.pdf

217. O.S. Antonova, S. Rangelov, B. Mladenov, Z. Hammoudeh, D. Nesheva, O. Ivanovski, S. Saidi, G. Petrusevska, C. Slavov, A. Dimovski, Z. Yordanova, D. Plasevska-Karanfilska, **Z. Popov**, A. Galabov, D. Toncheva*, Genotyping of urinary bladder carcinomas for up-regulated EGFR, PIK3CA, FGFR3 and CDKN2A to determine the potential for targeted therapy,* European Journal of Human Genetics, 28 (Suppl 1), 2020: 934-934.

218. A. Trifunovski, S. Dohcev, O. Stankov, S. Stavridis, **Z. Popov**, T*he predictive (prognostic) value of TMPRSS2-ERG in prostate cancer specimen*. Medicus, 25, 3, 2020: 354–360.
http://www.medalb.com/medicus-xxv-3-2020/

219. **Z. Popov**, Lj. Ivkovski, Z. Atanasov, O. Ivanovski, G. Jovic, N. Ivanovski, *Primary Renal Squamous Cell Carcinoma in Native Polycystic Kidney and Ureter 16 Years After Living Donor Kidney Transplant*. Experimental and Clinical Transplantation, Vol. 19, issue 9, 2021: 981-985. http://www.ectrx.org/detail/archive/2021/19/9/0/981/0

220. G. Spasovski, I. Rambabova-Bushljetik, L. Trajceska, S. Dohcev, O. Stankov, S. Stavridis, S. Saidi, **Z. Popov**, *Urinary proteomics in Kidney Transplantation*. Прилози. Одд. за мед. науки (МАНУ) = Contributions. Sec. of Med. Sci. (MASA), XLII, 3, 2021: [7]–16,

http://manu.edu.mk/prilozi/42_3/1.pdf

221. M. Ambardjieva, S. Saidi, R. Jovanovic, J. Janculev, V. Stankov, A. Trifunovski, **Z. Popov**, *Solitary Fibrous Tumor of Adrenal Gland and Review of the Literature*. Прилози. Одд. за мед. науки (МАНУ) = Contributions. Sec. of Med. Sci. (MASA), XLII, 3, 2021: [63]–69, http://manu.edu.mk/prilozi/42_3/7.pdf

222. D. Simonovska, L. Stojkovski, **Z. Popov**, D. Petrovski, Z. Trajkovski, A. Sanevska-Tanevska, *Acute renal failure in intraoperative lesion of both ureters following hysteroadnexectomy-case report*. Journal of Morphological Sciences (JMS), Vol. 4, No. 3, 2021: 76–80, http://www.jms.mk/jms/article/view/272

223. **Z. Popov**, L. Ivkovski, Z.Atanasov, G. Jovic, K. Cakalaroski, N. Ivanovski, *Carcinome epidermoide renal primitive du rein polykystique natif et de l uretere, 16 ans après une greffe de rein de donneur vivant*, 27e Reunion annuelle, Societe Francophone de Transplantation, 7–10 decembre 2021, Geneve.

ПОГЛАВЈЕ II

ТЕМИ ОД УРОЛОШКАТА ОНКОЛОГИЈА

doi: 10.20903/zpopov-2022-0002 UDK: 616.62-006.6-089.84:616.62-008.22

Jovan B. HADŽI-ĐOKIĆ[1]
Vladimir S. VASIĆ[2]

KONTINENTNE URINARNE DERIVACIJE, POSLE RADIKALNE CISTEKTOMIJE ZBOG KARCINOMA MOKRAĆNE BEŠIKE

Abstract

Pošto su utvrđeni principi uspešnog ugrađivanja urinarnog rezervoara, sve je manja zainteresovanost za metode odvođenja urina do stome na koži. Posle kompletne rekonstrukcije urinarnog trakta, pouch se anastomozom dovede do membranozne uretre, a urin se može eliminisati preko mokraćnog kanala. Pošto se pokušalo sa različitim tipovima cistoplastika, primenom sigmoidnog kolona i ileuma, prihvaćena je primena segmenta ileuma u obliku slova U (Camey tip I) iz tri glavna razloga: 1.postoji bolja vaskularizacija koja omogućava preoperativno zračenje, bez rizika od postoperativnog popuštanja anastomoza; 2.manje septičan sadržaj nego u kolonu, što znači manji rizik od infekcije; 3.oblik U segmenta ileuma koji leži na karličnom dnu važan je faktor postoperativne kontinencije i olakšava funkciju abdominalne prese za vreme mokrenja.

Camey tip II, se tehnički razlikuje od metode tipa Camey I po tome što se uzima anza ileuma dužine 70 cm koja se detubularizuje i od nje se formira ileumska bešika. Operacija detubularizovane ileumske bešike tipa Camey II poboljašava kvalitet života operisanih bolesnika u odnosu na istu operaciju tipa.

Vesica ileale Padovana, podrazumeva 40-60 cm ileuma na 20 cm od ileocekumske valvule. Ileumski segment se otvori po antimezenteričnoj ivici i onda se formira ovalni rezervoar posebnom rekonfiguracijom crevnog segmenta. Ureteri se usađuju u rezervoar po tehnici Camey-Le-Duc.

[1] Srpska Akademija Nauka i Umetnosti, Beograd, Srbija
[2] KO Urologije, KBC "Zvezdara", Beograd, Srbija

Ortotopska ileumska bešika sa ekstramuralnom implantacijom uretera, podrazumeva implantiranje uretera u ileumski rezervoar koji je kreiran u obliku slova W. Uradi se detubularizacija ileumskog segmenta po antimezenteričnoj ivici i onda se od lateralnih ivica creva, koje je postavljeno u pomenutu konfiguraciju, šivenjem formiraju dva ležišta za smeštaj oba uretera.

Sigma rektum pouch obuhvata korišćenje 8 cm rektuma i 12 cm sigmoidnog kolona, koji se detubularizuju i od njih se formira urinarni rezervoar.

1. URINARNI REZERVOAR SPROVEDEN DO URETRE – ENTEROCISTOPLASTIKA PO CAMEY-u

Pošto su utvrđeni principi uspešnog ugrađivanja urinarnog rezervoara, sve je manja zainteresovanost za metode odvođenja urina do stome na koži. Posle kompletne rekonstrukcije urinarnog trakta, pouch se anastomozom dovede do membranozne uretre, a urin se može eliminisati preko mokraćnog kanala. Ova značajna inovacija je evidentno poželjan metod, naročito kod mlađih pacijenata. Urinarni pouch koji se anastomozom vezuje za membranoznu uretru može biti Skinner-ova modifikacija Kock-ovog pouch-a, ileocekumski Mainz pouch kako ga je opisao Thiiroff (1987) ili ileumski rezervoar niskog pritiska koji je koristio Studer (1989). Eferentni deo pouch-a se preskače, a najdistalniji i pokretljiviji deo pouch-a, razdvojen od mezenterijuma, anastomozom se vezuje za membranoznu uretru. Ako se pouch anastomozom poveže direktno sa membranoznom uretrom, pražnjenje je zadovoljavajuće uz vrlo malu količinu preostalog urina, a i kontinencija tokom dana. Noćna kontinencija je zadovoljavajuća ukoliko pacijent mokri 1-3 puta tokom noći. Potrebna je reimplantacija uretera u izoperistaltični deo ileuma antirefluksnom tehnikom (Le Due i Camey, 1987). Urodinamske studije pokazuju da talasi pritiska ne prelaze 35 cm H_20, kada se ileumska bešika napuni do vrha, što je potvrdilo da je intralumenski pritisak značajno niži nego pritisak u tubularnom ileumskom segmentu sa indentičnom tenzijom u zidu.

ENTEROPLASTIKA PO METODI CAMEY (TIP I)

Urinarna derivacija nakon radikalne cistektomije se uvek smatrala mutilantnom operacijom. U cilju da se pacijentima pruži radikalan tretman,

a u isto vreme omogući život što bliži normalnom, započelo se sa zamenom bešike segmentom creva od 1958. godine (Camey, 1988). Pošto se pokušalo sa različitim tipovima cistoplastika, primenom sigmoidnog kolona i ileuma, prihvaćena je primena segmenta ileuma u obliku slova U iz tri glavna razloga: 1.postoji bolja vaskularizacija koja omogućava preoperativno zračenje, bez rizika od postoperativnog popuštanja anastomoza; 2.manje septičan sadržaj nego u kolonu, što znači manji rizik od infekcije; 3.oblik U segmenta ileuma koji leži na karličnom dnu važan je faktor postoperativne kontinencije i olakšava funkciju abdominalne prese za vreme mokrenja.

INDIKACIJE

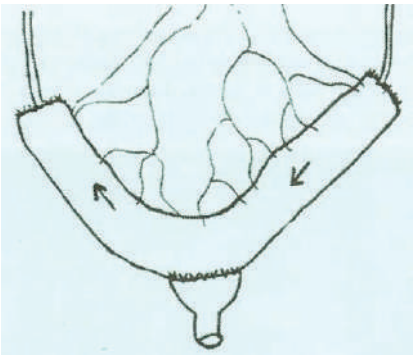
Enteroplastika po Camey-u je zahvat koji je ograničen na pacijente kod kojih se očekuje duži postoperativni život i dobri funkcionalni rezultati. To znači da su pacijenti ispod 70 godina starosti, koji nisu jako gojazni, ne boluju od dijabetesa, srčane ili plućne insuficijencije ili neurološke bolesti pogodni kandidati za ovu intervenciju. Pacijenti moraju biti veoma motivisani, dobro informisani, kooperativni i da shvataju funkciju nove bešike. Takođe je važno da tumor bude infiltrativni, na distanci od vrata mokraćne bešike, a uretra mora biti bez tumora ili suženja ma koje vrste. Gornji urinarni putevi moraju biti normalni. Preoperativno se obavezno mora uraditi uretralna biopsija.

GLAVNI ELEMENT HIRURŠKE TEHNIKE

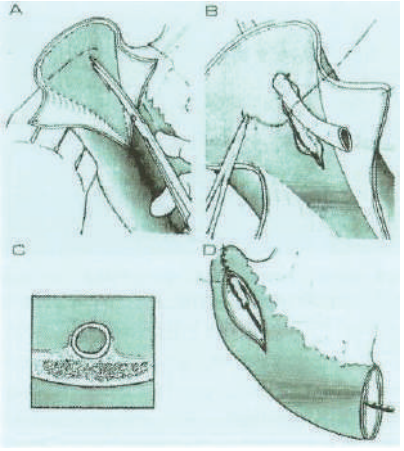
Cistoprostatektomija se izvodi na uobičajen način. Pošto se pristupi semenim kesicama i prostati, disekcija se radi posebno pažljivo u cilju da se očuva zadovoljavajuća kontinencija kao i moguća prezervacija seksualne funkcije.

Disekcija apeksa prostate izvodi se zajedno sa kapsulom, iza Santorinijevog pleksusa i čuvajući sfinkter. Membranozna uretra se otvara transvezikalno, neposredno ispod vrha prostate. Plasiraju se dva lateralna šava buduće ileouretralne anastomoze. Zatim se seče zadnji zid uretre, uz stavljanje zadnjih šavova. Odabere se segment ileuma dužine 35-40 cm, tako da se može napraviti anastomoza sa uretrom bez ikakve tenzije. Napravi se ileoileumska anastomoza. Na sredini antimezenterične strane odabranog segmenta ileuma, napravi se otvor koji odgovara otvoru uretre.

Pažljivo se uradi anastomoza uretre i crevnog segmenta. Ureteri se implantiraju blizu gornjih polova ileumskog segmenta. Na zadnjem zidu napravi se longitudinalna incizija mukoze creva dužine 3,5 cm. Ureter se provuče kroz zid ileumskog segmenta do gornje ivice incizije (mora lagano, opušteno da prolazi). Vrh uretera se koso zaseče i fiksira za donji kraj mukoznog kanala. Ivice mukoze se zatim ušiju lateralno od uretera. Ureterski kateteri se izvedu na uretru, zajedno sa Ch 21 pravim, višestruko perforiranim kateterom, koji mora da se plasira u desnu polovinu segmenta, u cilju evakuacije mukusa lavažom, koji se peristaltikom potiskuje na tu stranu.



Camey-eva enterocistoplastika, tip I.
Anastomoza ileumske anze i uretre, kateter stavljen u desni kraj anze.



Camey-eva enterocistoplastika.
Implantacija uretera u ileum po tehnici Camey-Le Duc.

Ureterski kateteri se obično uklanjaju 12-tog postoperativnog dana, nakon retrogradne cistografije. U slučaju da kontrast izlazi na mestima šava, kateter treba držati još nedelju dana, a zatim ga ukloniti nakon još jedne cistografije. Uretralni kateter se uklanja dva dana posle vađenja ureterskih sondi.

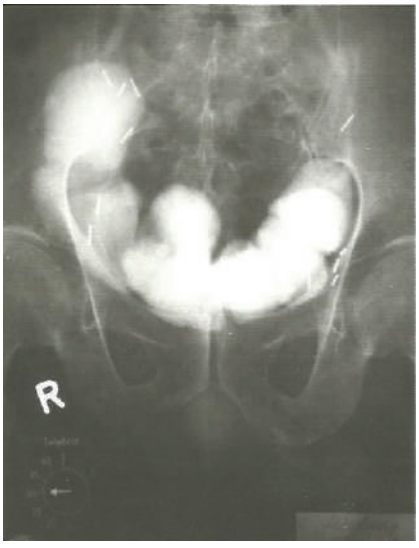
FUNKCIONALNI REZULTATI

Kapacitet ileumske neobešike obično ne prelazi 250 ml. Operisani moraju da mokre 7-8 puta tokom 24 h. Zbog toga im se preporučuje da prazne svoju bešiku svakih 2.5 sata, podrazumevajući i noćno vreme. Postoperativne eventracije su uzrok loših evakuacija mokraćne bešike. Količina izbačene mokraće je dobra kada iznosi 20-22 ml/s. Kontinencija kod ovih bolesnika osigurana je glatkom muskulaturom zadnje uretre i spoljnjeg sfinktera sa njegovim permanentnim tonusom. Dobro urađenom totalnom cistoprostatektomijom ne povređuju se ovi elementi i njihova inervacija koja potiče od unutrašnjeg stidnog nerva i od grana pelvičnog visceralnog pleksusa koji ga prate. Stalni tonus ovih muskularnih struktura dovoljan je da drži ravnotežu pritiska ileumske bešike, utoliko pre što je anastomozom u obliku slova U omogućeno peristaltičkom talasu da prođe s leva na desno, pre ileouretralne anastomoze, preko orificijuma malog kalibra, mekog i zatvorenog pomoću muskularne manžetne poprečno-prugastog sfinktera. Ni jedna kap mokraće ne može da pođe silom, ako ispunjenje neobešike ne dostigne određeni prag.

KOMPLIKACIJE

Komplikacije mogu biti perioperativne i postoperativne. Za razliku od radikalne prostatektomije, kod supstitucione ileocistoplastike, povoljan tok operacije ne zavisi samo od rekonstrukcije vrata mokraćne bešike i prihvatanja neovezike. Ovde je potrebna anatomska i funkcionalna dužina membranozne uretre i intaktni spoljni sfinkter. Tokom disekcije može se javiti masivno krvarenje iz Santorinijevog pleksusa koje je teško kontrolisati, pa su potrebni šavovi koji duboko penetriraju u zid membranozne uretre. U takvim okolnostima treba odbaciti pokušaj ileocistoplastike, pošto ovi šavovi mogu da ugroze funkciju spoljnog sfinktera i time onemoguće redovan tok operacije. Isto se dešava kada je, tokom disekcije, membranozna uretra zasečena suviše nisko. Naglašava se potreba za savršenom

hemostazom u karlici kod cistoprostatektomije, pre izvršene supstitucije bešike. Kada se vrši ureteroileumska implantacija i kada se fiksiraju krajevi plastike, mora se paziti da se ne napravi ureteroileumski ugao koji može uzrokovati uretersku opstrukciju. Ako je mezenterijum suviše kratak mora se odustati od citoplastike, pošto svaka tenzija po liniji šava kod ureteroileumske anastomoze povećava rizik od stvaranja fistule. Postoperativne komplikacije su: gastrointestinalne komplikacije, urološke komplikacije, problemi sa ileumskom bešikom, problemi sa gornjim urinarnim traktom i komplikacije u vezi sa kontinencijom. Urološke komplikacije podrazumevaju ureteroileumske i uretroileumske fistule. Fistule se leče produženom kateterizacijom. Kod 7% pacijenata mogu se pojaviti hernije abdominalnog zida koje se tretiraju uglavnom hirurški. Po pravilu, nova ileumska bešika zadržava funkciju rezervoara, a i evakuaciju urina tokom vremena. Hadži-Đokić i sar. (1993) na 41 ileocistoplastiku (stanje bolesnika praćeno 8 godina) opisuju obostrani VUR kod 1 bolesnika, dilataciju mokraćne bešike kod 2 bolesnika, uretroileumsku fistulu kod 2 bolesnika i noćnu inkontinenciju kod 55% operisanih. Dnevno i noćno mokrenje se nije pogoršavalo tokom vremena (Camey, 1988; Hadži-Đokić, 1993), tako da pacijenti zadržavaju isti tempo mokrenja od operacije.



Descendentna cistografija posle operacije tipa Camey II pokazuje odlično formiranu mokraćnu bešiku.

ENTEROCISTOPLASTIKA TIPA CAMEY II

Tehnički, ova operacija se razlikuje od metode tipa Camey I po tome što se uzima anza ileuma dužine 70 cm koja se detubularizuje i od nje se formira ileumska bešika. Operacija detubularizovane ileumske bešike tipa Camey II poboljašava kvalitet života operisanih bolesnika u odnosu na istu operaciju tipa. Kod detubularizovane mokraćne bešike dnevna kontinencija je bila 100%, a noćna 70%. Ovi pacijenti mokre na 3-4 sata, a pravo stanje se stabilizuje tek 3 meseca nakon operacije. Podaci iz literature zkazuju da je srednji kapacitet mokraćne bešike je 460 ml, a odsustvo rezidualnog urina je ustanovljeno kod 90% operisanih. Autor smatra da enterocistoplastika sa detubularizovanim segmentom ileuma niskog pritiska obezbeđuje izrazito poboljšanje noćne kontinencije. Socijalna reintegracija ovih pacijenata zavisi od restitucije fizioloških funkcija, među kojima kontinencija zauzima prvo mesto. Ileocistoplastika tipa Camey II obezbeđuje i dnevnu i noćnu kontinenciju u više od 90% slučajeva.

ZAKLJUČAK

Principi konstrukcije ileumskog pouch-a niskog pritiska ili ileocekumskog rezervoara su sada vrlo dobro utvrđeni. Ne sme se, međutim, pribegavati tome ako to znači da operacija maligno obolelog pelvisa neće biti kompletna, a ne preporučuju se ni kod pacijenata sa odmaklim karcinomom u donjem urinarnom traktu. Ako se tokom vremena pokaže da ove zamene za bešiku povoljno deluju na funkciju bubrega, možda će se više cistektomija raditi bez preoperativne iradijacije, iako ona nije potpuno kontraindikovana ovom rekonstruktivnom operativnom zahvatu, ako postoji zdravo tkivo koje bi se obložilo oko delova zahvaćenih anastomozom. Kod starijih pacijenata sa malignim oboljenjem u maloj karlici, jednostavnija metoda ileumskog konduita će ipak imati svoje mesto u urološkoj hirurgiji.

LITERATURA

1. Camey M.: The future of total bladder substitution. In: Hohenfelnner R., Wammack R. (Eds.): Continet urinary diversion. Churchill Livingstone, 57-61, 1992.

2. Camey M., Le Duc A.: Lenterocystoplastie apres cystoprostatectomie totale pour cancer de la vessie. Annaled de Urologie, 13:114-123, 1979.
3. Camey M., Botto H., Richard F.: Compications of the Camey procedure. Urologie clinics of Nort America. Vol 15, N°2, 249-255, 1988.
4. Hadži-Đokić J,, Mićić S., Tulić C., Vuksanović A., Bojanić N.: Komplikacije Camey-ove operacije. XII Kongres udruženja urologa Jugoslavije, Vrnjačka Banja, Zbornik radova, abst. 107,1993.
5. Hadži-Đokić J., Vuksanović A., Džamic Z., Tulić C: The Camey enterocisto-plasty after radical cystoprostatectomy: complications; Acta Urologica Italica May-June 1999; Vol. XIII-No.3, 163-166.
6. Hadži-Đokić J., Tomić M., Mićić S., Tulić C.: Naši rezultati sa zamenom mokraćne bešike segmentom ileuma posle cistektomije zbog karcinoma mokraćne bešike. Zbornik radova IX Kongresa urologa Jugoslavije - Beograd, 1986., 399-403.
7. Hadži-Đokić J.: Zamena mokraćne bešike segmentom ileuma po Camey-u posle radikalne cistoprostatektomije - indikacije i naši rezultati. X Kongres urologa Jugoslavije, Bled, 1988. Zbornik rezimea., p 16.
8. Leandry R, Rossignol G., Ramon J., Gauthier J. R.: Complications de la vessie ileal de replacement: experience de 405 patients. Progrès en Urologie. Vol 1, Suppl. N°4, abstr. 58, 1991.
9. Le Duc A., Camey M., Teilac R: An original antireflux ureteroileal implan-tation technique: long term follow up. Journal of Urology, 137: 1156-1158, 1987.
10. Merricks J. W.: A continent substitute bladder and urethra. In: Kong L. R., Stone A. R., Webster G. D. (Eds.): Bladder reconstruction and continent urinary diversion. Year Book Medical Publishers, Chicago, 179-203, 1987.
11. Reddy P. K., Longe P. H., Fraley E. E.: Total bladder replacement using detubularized sigmoid colon: tehnique and results. Journal of Urology, 145: 51-55, 1991.
12. Studer U. E., Furger R: Die psyhosoziale Reintegration des urostomiepatienten. Schweiz Med. Wochenschr., 111: 1834-1836, 1981.
13. Studer U. E., Casanova G. A., Motaz A. E., Zingg E. J.: Results with a bladder substitute from cross folded segments (Goodwins cup patch technique). Progress in Urology, 1: 13- 27, 1991.
14. Studer U. E., Ackerman D., Casanova G. A., Zingg E. J.: Three years eperience with an ileal low pressure bladder substitute. British Journal of Urology, 63: 43-52, 1989.

2. DRUGE HIRURŠKE TEHNIKE STVARANJA ORTOTOPSKOG URINARNOG REZERVOARA

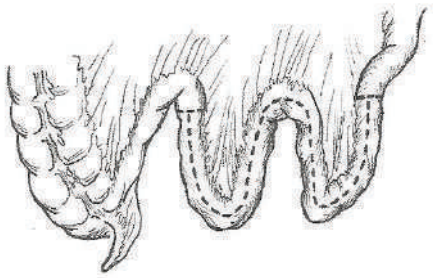
VESICA ILEALE PADOVANA

Indikacija za ovu operaciju je invazivni karcinom ograničen na mokraćnu bešiku kod muškaraca. Prema mišljenju autora operacije, preporučuju se preoperativne biopsije uretre kao i staging limfadenektomija. „Nerve-sparing" cistoprostatektomija se preporučuje kod određenih bolesnika. Uzima se 40-60 cm ileuma na 20 cm od ileocekumske valvule. Ileumski segment se otvori po antimezenteričnoj ivici i onda se formira ovalni rezervoar posebnom rekonfigu racijom crevnog segmenta. Ureteri se usađuju u rezervoar po tehnici Camey-Le-Duc. U postoperativnom toku, ureterske sonde se vade 8. postoperativnog dana, retrogradna cistografija rezervoara se radi posle tri nedelje. Uretralni kateter se uklanja kada ekstravazacija na cistografiji ne postoji. Pacijentu se savetuje, da uči da mokri pritiskajući trbušne mišiće uz relaksaciju karličnog dna. Intravenska urografija i videourodinamski testovi se sprovode na 3, 6 i 12 meseci a uretroskopija na godinu dana. Prednost ove metode je to što ostavlja intaktan ileocekumski region. Izolovani segment ileuma koji se koristi je kratak, što redukuje pojavu metaboličkih komplikacija. Ovako konstruisana ileumska bešika omogućava dobru kontinenciju tokom dana, sa rizikom od inkontinencije tokom noći. Takođe, veliki je kapacitet ileumske bešike (oko 450- 600 cm^3), a dobri su i udaljeni rezultati. Ureteroileumska anastomoza nosi izvesne tehničke rizike. Najvažnije je sprečiti nastajanje stenoze. Slične ovoj tehnici su i operacije po Studer-u i Hautmann-u, koje su dosta popularne kod urologa širom sveta koji se bave ovom problematikom. Ove tehnike se dosta često primenjuju, i u literaturi su opisani dobri onkološki i funkcionalni rezultati, prilikom kontrole i praćenja ovih bolesnika.

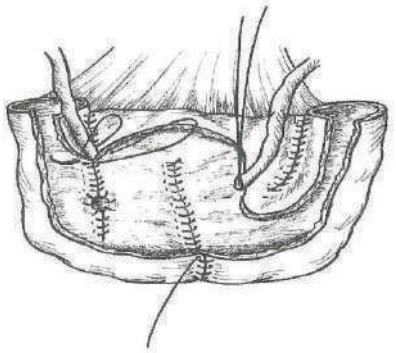
ORTOTOPSKA ILEUMSKA BEŠIKA SA EKSTRAMURALNOM URETERSKOM IMPLANTACIJOM

Ova tehnika se koristi kod implantacije normalnih ili diktiranih uretera u ileumski rezervoar koji je kreiran u obliku slova W. Izoluje se segmet ileuma u dužini oko 40 cm i pripremi se za W-konfiguraciju. Uradi se detubularizacija ileumskog segmenta po antimezenteričnoj ivici i onda se od

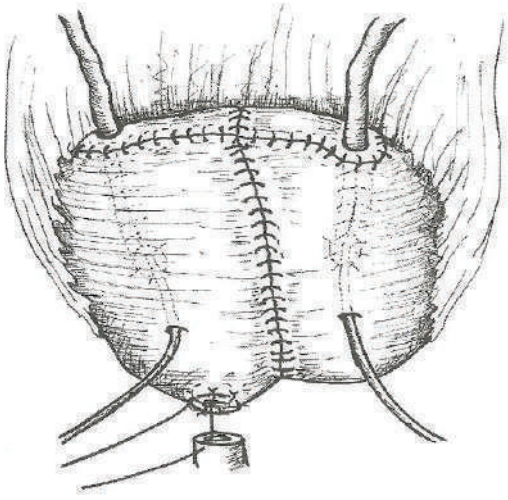
lateralnih ivica creva, koje je postavljeno u pomenutu konfiguraciju, šivenjem formiraju dva ležišta za smeštaj oba uretera. Kada su ureteri postavljeni i fiksirani za ležišta, onda se oni produžnim šavom mukoze prepokrivaju. Stavljaju se ureterske sonde koje se provlače kroz zid pauča napolje, i nakon toga rezervoar se potpuno zatvara. Na vrhu rezervoara obrazuje se otvor čija se mukoza evertira, da bi se prevenirala stenoza. Potom se preko uretralnog katetera uradi anastomoza između rezervoara i uretre. Ova tehnika se primenjuje da bi se izbegla pojava ureterointestinalne stenoze ili refluksa.



Ileumska bešika sa ekstramuralnom implantacijom uretera, 40 cm segmenta distalnog ileuma se izoluje i aranžira u W-konfiguraciju.



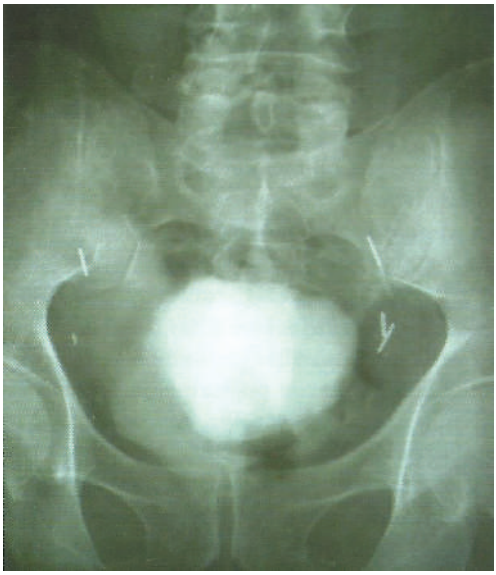
Ureter je spatuliran i anastomoziran za intestinalnu mukozu, a onda je tunel zatvoren preko produžnim šavom. Dužina tunela je oko 2 cm.



Rezervoar je formiran, ureterske sonde su provučene
kroz zid prednjeg zida neobešike.

FOCH-POUCH (Z PLASTIKA)

Tehnikom detubularizacije postignut je normalan kapacitet bešike, prezervacija sfinktera i rana perinealno-abdominalna reedukacija, koja počinje odmah posle operacije omogućujući kontinenciju. Implantacija uretera u bešiku po tehnici Camey-Le-Duc prevenira vezikoureterski refluks. Kod dobro izvedene operacije, gde je korišćena bilo koja tehnika ortotopske zamene mokraćne bešike, treba da se postignu sledeći rezultati: dobra kontinencija, zadovoljavajući kapacitet bešike sa malim intravezikalnim pritiskom i kompetentan antirefluksni mehanizam (Benson, 1996). Apsolutne kontraindikacije za implantaciju ovakve bešike su: urinarna stres-inkontinencija, oštećen rabdosfinkter, veoma oštećena bubrežna i funkcija jetre, i onkološki procesi na uretri i ozbiljne bolesti creva.



Foch pouch descendentna cistografija pokazuje dobro razvijenu mokraćnu bešiku.

Kod bolesnika kod kojih je urađena „nerve-sparing“ cistektomija i koji su mlađi od 65 godina je značajno bolja i dnevna i noćna kontinencija. Prema podacima World Health Organisation (WHO) frekvencija distribucije primene urinarnih derivacija na više od 7.000 bolesnika je sledeća: ortotopska neobešika u 47% slučajeva; ileumski konduit u 33%; analna diverzija u 10%; kontinentna kutana diverzija u 8%; inkontinentna kutana diverzija u 2% (Hautmann, Abol-Enein, 2007).

LITERATURA

1. Abol-Enein H, Ghoneim MA. A novel ureteroileal reimplantation tehnicque: the serous lined extramural tunnel. A preliminary report. J Urol 151 (1994) 1193.
2. Benson MC, Olsson CA. Continent urinary diversion. Urol Clin North Am 1999; 26: 125— 147.
3. Botto H. What is the best bladder replacement. Ann Urol (Paris) 2005. Nov; 39 suppl 5: S120-5.

4. Hautmann RE, de Petriconi R, et all. The ileal neobladder: Complications and functional results in 363 patients after 11 years of follow up. J Urol 1999; 161: 422-7.
5. Hautmann RE, Volkmer GG, Schumacher MC, Gschwend JE, Studer UE. Long-term results of standard procedures in urology: the ileal neobladder. World J Urol 2006; 24: 305-14
6. Hautmann RE. 15 years experience with the ileal neobladder. What have we learned? Urologe A. 2001 sep; 40(5): 360-7.
7. Hautmann RE, Miller K, Steiner U, Wenderoth V. The ileal neobladder: 6 years of experience with more than 200 patients. J Urol 1993 Jul; 150(1): 40-5.
8. Hautmann RE. Urinary diversion: ileal conduit to neobladder. J Urol 2003; 169: 834-42.
9. Rowland RG, Mithcell ME, Bihrle R, Kahnoski RJ, Piser JE. Indiana continent urinary reservoir. Journal of urology (1987), 137: 1136-1139.

3. SIGMA REKTUM POUCH – URINARNA DERIVACIJA

Sigma rektum pouch (Mainz-poch II) su u urološku praksu uveli Margit Fish i Rudolph Hohenfellner 1991. godine. Glavni elementi i značaj ove hirurške metode su detubularizacija sigmoidnog kolona i rektuma i isto-strana anastomoza creva bez prekidanja kontinuiteta, što omogućava nizak pritisak u crevnom rezervoaru i eliminiše visoki pritisak koji bi izazivale kontrakcije creva. Ova metoda takođe, omogućava bolju zaštitu gornjeg urinarnog trakta i kontinenciju. Takođe, operativna tehnika je prosta i laka. Glavni napredak ove procedure je detubularizacija creva i mali pritisak u crevnom rezervoaru. Pošto sa cistektomijom dolazi do anatomskog i funk-cionalnog gubitka mokraćne bešike, ova metoda omogućava mokrenje na anus ukoliko je analni sfinkter prethodno bio kompetentan. Ona takođe može biti korišćena da bi se izvršila konverzija ureterosigmoidostomije ili kolon konduita. Indikacije za ovu vrstu hirurške intervencije su i ireparabilne veziko-vaginalne fistule i intersticijalni cistitis.

OPERATIVNA TEHNIKA

Originalna hirurška tehnika koju su objavili Fish i Hohenfellner 1991. godine podrazumeva, korišćenje 8 cm rektuma i 12 cm sigmoidnog kolona, koji se detubularizuju i od njih se formira urinarni rezervoar. Ureteri se

usađuju u rezervoar submukozno, po metodi Goodwin-Hohenfellner. Hadži-Đokić i saradnici su objavili i modifikaciju ove metode, pri čemu je glavna razlika od originalne tehnike implantacija uretera po metodi Camey-Leduc. Ureteralne sonde koje su plasirane u uretere izvlače se na anus i fiksiraju za kožu perianalnog predela. Poznati urolozi iz Egipta (Mansoura) M. Ghoneim i H. Abol-Enein su publikovali metodu pod nazivom „Double folded sigma rectum pouch", koja se može uspešno primenjivati i kod dilatiranih uretera.

PREGLED LITERATURE

Fish i Hohenfellner su 1993. godine objavili seriju od 70 uspešno operisanih pacijenata. J. Hadži-Đokić i D. Bašić su 2006. godine objavili seriju od 210 operisanih pacijenata pri čemu su naročito obratili pažnju na kvlaitet života operisanih bolesnika. Turski autor Fengiz Girgin sa saradnicima je uporedio tri metode urinarnih derivacija (Cock pouch, Cock neobladder i sigma rectum pouch). Kod 58,6% pacijenata je urađen sigma rektum pouch. Oni su konstatovali da je ova metoda bez komplikacija kod pacijenata koji žele da budu suvi i da je kontinencija bila zastupljena kod 100% pacijenata. Nemački urolog Bastian (2004. godina) je objavio svoje rezultate kod 41 pacijenta, gde je prmenjivao tri različite tehnike usađivanja uretera. Rezultati su pokazali da nema razlike u pojavi komplikacija kod ove tri vrste implantacije uretera. Takođe, su konstatovali da je ovo sigurna i zadovoljavajuća derivacija kod tri grupe pacijenata. Autori iz Srbije, I. Ignjatović i D. Bašić su publikovali svoje dvanaestogodišnje iskustvo na 67 pacijenata. Oni su zaključili da je ova hirurška tehnika prosta i sigurna procedura u odnosu na komplikacije, kontinenciju i kvalitet života. Gruzijski autori (Zhvania, 2012), su objavili svoje rezultate na 320 operisanih bolesnika. Oni su zaključili da je ova forma urinarne derivacije metod izbora kod pacijenata kod kojih uretra ne može biti korišćena. Autori iz Egipta (Hammouda, 2006. godine) su objavili svoja iskustva na 95 operisanih pacijenata, gde je primenjen double folded recto-sigmoid pouch zbog dilatiranih uretera. Rezltati su bili zadovoljavajući.

KOMPLIKACIJE

Kod sigma rektum pouch hirurške tehnike mogu se javiti rane komplikacije u prva tri meseca od hirurške intervencije. To su unilateralna hidronefroza, bilateralana hidronefroza, akutni pijelonefritis, akutna bubrežna

insuficijencija i rektalna hemoragija. Ove komplikacije se javljaju u oko 8,5% slučajeva (J. Hadži-Đokić, 2006). Kasne komplikacije se javljaju tri meseca posle hirurške intervencije. U njih spadaju ventralna hernija, unilaterlna i bilateralna stenoza uretera. One se javljaju u oko 9% slučajeva (J. Hadži-Đokić, 2006). U lečenju stenoza kod ureterointestinalne anastomoze je primenjivana balon kateter dilatacija ili metalni štreker stent sa uspehom od 60-80%. Ukoliko se sa ovim procedurama nije uspelo, onda je primenjena perkutana nefrostomija. Ovi pacijenti su u 98% slučajeva bili kontinentni i danju i noću. Ukoliko se uporedi kvalitet života kod sigma rektum pouch operacije i ilealnog konduita, zaključeno je da su pacijenti gde je urađen sigma rektum pouch mnogo zadovoljniji svojim stanjem (J. Hadži-Đokić 1999, Tulić 1999).

ZAKLJUČAK

Sigma retkum pouch nema mnogo postoperativnih ranih i kasnih komplikacija. To je laka operativna tehnika sa brzim postopertivnim oporavkom. Kvalitet života pacijenata je zadovoljavajući. To je zadovoljavajuća urinarna derivacija posle radikalne cistektomije u slučajevima gde ortotopska urinarna derivacija ne može da se primeni.

LITERATURA

1. Dzamić Z, Hadzi-Djokić J, Tulić C, Vuksanović A, Lalić N, Aćimović M, Janicić A, Durutović O. Quality of life in patients with a modified Mainz-pouch II urinary diversion. Acta Chir Iugosl. 1999; 46(1 Suppl): 11-3
2. Morgan MA, Polan ML, Melecot HH, Debru B, Sleemi A, Husain A. Experience with a low-pressure colonic pouch (Mainz II) urinary diversion for irreparable vesicovaginal fistula and bladder extrophy in East Africa. Int Urogynecol J Pelvic Floor Dysfunct. 2009 Oct; 20(10): 1163-8
3. Maksimovic HM, Markovic BB, Pejcic T, Hadzi Djokic J, Markovic Z, Yachia D. Interventional radiology methods of treating ureteral strictures developed on anastomoses after the radical cystectomy in Mainz pouch II and ileal conduit derivation. Acta Chir Iugosl. 2007; 54(4): 83-7
4. Dzamic Z, Hadzi Djokic J, Acimovic M, Jovanovic M, Filimonovic J, Tulic C, Ladjevic N, Milkovic B, Pejcic T, Radosavljevic R. Modified Mainz pouch II urinary diversion and quality of life. Acta Chir Iugosl. 2007; 54(4): 57-62

5. Fisch M, Hohenfellner R. Sigma-rectum pouch (Mainz pouch II). BJU Int. 2007 Apr; 99(4): 945-60
6. Hadzi-Djokic JB, Basic DT. A modified sigma-rectum pouch (Mainz pouch II) technique: analysis of outcomes and complications on 220 patients. BJU Int. 2006 Mar; 97(3): 587-91
7. Bao J, Yue Z, Wu G, Shi W, Wang W. Technique and results in total laparoscopic radical cystectomy with sigmoidorectal pouch (Mainzpouch II) - an initial experience. Exp Ther Med. 2017 May; 13(5): 1749-1752
8. Sfoungaristos S, Mykoniatis I, Poulios E, Paikos D, Hatzichristou D. Percutaneous Nephrolithotomy in a Patient with Mainz Pouch II Urinary Diversion: A Case Report. Sfoungaristos S, Mykoniatis I, Poulios E, Paikos D, Hatzichristou D. Prague Med Rep. 2016; 117(4): 198-203
9. Castillo OA, Miranda-Utrera N. Laparoscopic cystectomy and intracorporeal continent urinary diversion (Mainz II) in treatment for interstitial cystitis. Actas Urol Esp. 2014 Apr; 38(3): 200-4
10. Zhvania G, Mshvildadze Sh, Managadze G, Khvadagiani G. Results of radical cystectomy with Mainz pouch II diversion (single institution experience). Georgian Med News. 2012 Oct; (211): 7-13

Jovan B. HADŽI-ĐOKIĆ
Vladimir S. VASIĆ

CONTINENTAL URINARY DERIVATIONS, AFTER RADICAL CYSTECTOMY DUE TO BLADDER CANCER

Abstract

Since the establishment of the principles of successful implantation of the urinary reservoir, the interest in methods of eliminating urine by the stoma on the skin has significantly decreased. After a complete reconstruction of the urinary tract, the pouch is brought to the membranous urethra by anastomosis, and urine can be eliminated through the urethra. Having tried different types of cystoplasty, using the sigmoid colon and ileum, the use of the U-shaped segment of the ileum (Camey type I) was accepted for three main reasons: 1. there is a better vascularization that allows preoperative radiation, without the risk of postoperative anastomosis; 2. less septic content than in the colon, which means a lesser risk of

infection; 3. the shape of the segment of the ileum that lies on the pelvic floor is an important factor of postoperative continence and facilitates the function of the abdominal press during urination.

The Camey type II differs technically from the Camey I method in terms of taking a 70 cm long anza of the ileum, which is detubularized and forms an ileal bladder. The Camey II type surgery, involving detubularized ileum segment, improves the quality of life of operated patients compared to the same surgery type I.

Vesica ileale Padovana involves 40-60 cm of ileum 20 cm above the ileocecum valve. The ileum segment is opened along the antimesenteric edge and then an oval reservoir is formed by a special reconfiguration of the intestinal segment. The ureters are implanted in the reservoir using the Camey-Le-Duc technique.

An orthotopic ileal bladder with extramural implantation of the ureters involves implantation of the ureter into the ileal reservoir created in the shape of the letter W. Detubularization of the ileal segment along the antimesenteric edge is performed and then the lateral edges of the intestine, set in the said configuration, are sutured in the form of two beds, where both ureters will be implanted.

A sigma rectum pouch involves the use of 8 cm of rectum and 12 cm of sigmoid colon, which are detubularized in order to form a urinary reservoir.

doi:10.20903/zpopov-2022-0003 UDK: 616.62-006.6-089.84:616.62-008.22

Сашо ДОХЧЕВ[1], Александар ТРИФУНОВСКИ[1], Оливер СТАНКОВ[1], Сотир СТАВРИДИС[1], Димитар ТРАЈКОВСКИ[1], Јосиф ЈАНЧУЛЕВ[1], Гоце НИНЕСКИ[1], Живко ПОПОВ[1,2]

ОРТОТОПСКА ДЕРИВАЦИЈА НА УРИНА ПО СТУДЕР (STUDER) – постоперативни аспекти –

Извадок

Карцином на мочниот меур претставува втора најчеста уролошка малигност во светот со инциденца кај мажите, во 2012 година, од 5,3 на 100 000 лица и стапка на смртност од 1,9 на 100 000. Ефикасна метода во третманот на канцер на мочниот меур со висок степен на малигност и избегнување на креирање стома, претставува радикалната цистектомија со ортотопска деривација на урина по методата на Студер (Studer). Како резултат на продолженото време на контакт на уринатa со цревната слузница, при оваа техника може да се јават одредени метаболни проблеми и електролитни дисбаланси. Исто така, поради влијанието што го има интервенцијата врз терминалниот илеум, може да се појават состојби на малапсорпција и дехидратација. Методата на Студер (Studer) претставува форма на континентна деривација, но не е ослободена од проблеми поврзани со континентноста. Стапката на континентност, во голема мера, варира меѓу различни студии, во зависност од дефинирање на тоа, што се подразбира под уринарна инконтиненција. Процедурата, исто така, бара сеопфатна комуникација и советување меѓу пациентот и хирургот, особено во постоперативното одржување на неовезиката, а проблемите поврзани со актот на мокрење се регистрирани и квантифицирани преку уродинамички иследувања. Пациентите со ортотопска супституција имаат, главно, подобар

[1] University Clinic of urology, Clinical Campus Mother Teresa, Skopje, N. Macedonia.

[2] Macedonian Academy of Sciences and Arts, Skopje; Medical Faculty, University "SS Cyrill and Methodius", Skopje, North Macedonia.

квалитет на живот (QOL) и подобра сексуална функција, во споредба со оние пациенти со други типови на уринарна диверзија.

Клучни зборови: карцином на мочен меур, ортотопска деривација на урина, неовезика.

Карциномот на мочниот меур е деветти најчест карцином во светот. По канцерот на простатата, тој претставува втора најчеста уролошка малигност во светот, со инциденца кај мажите од 5,3 на 100 000 лица и со стапка на смртност од 1,9 на 100 000. 5-годишната преваленца е 25,4 на 100 000 [1]. Карциномот на мочниот меур е дефинитивно дијагностициран и верификуван преку хистолошки преглед на примероци од ткиво со што се одредува и степенот на диференцијација на клетките (градус на тумор) и неговото ширење (стадиумот на тумор – stage). Стадиумот на карциномот е одреден со употреба на системот за одредување на степенот на локално ширење на туморот (T), присуство на карциномот во регионалните лимфни јазли (N) или присуство на далечни метастази (M). Златен стандард во третманот за канцер на мочен меур со висок степен на малигност, односно на мускулна инвазија, претставува радикалната цистектомија со продолжена лимфаденектомија и уринарна диверзија, која опфаќа илеален кондуит, континентен резервоар за урина и ортотопска деривација со креирање на неовезика (нов мочен меур).

Видот на извршената диверзија на уринатa зависи од склоноста на пациентот и на хирургот, асоцираниот коморбидитет и од очекуваниот квалитет на живот (QoL) постоперативно. Откако Барденхеер од Келн ја извршил првата цистектомија за тумор на мочниот меур, во 1887 година, хируршкиот предизвик не е само да се отстрани заболениот орган туку колку што може приближно да се супституира неговата функција. Повеќе од 100 години подоцна, радикалната ексцизија на мочниот меур со ортототпска неовезика останува најефикасната метода со која се избегнуваат потребата од формирање на стома и ризикот од стомачни компликации со придружни физички и психосоцијални ефекти. Над 60 % од пациентите третирани со илеален кондуит се чувствувале помалку комплетни и целосни, биле загрижени за истекување на уринатa од стомакот и чувствувале срам

поради стомата [2]. Оперативната техника опишана од Студер (Studer) опфаќа издвојување на дистален илеален сегмент, негово отворање по должина на антимезентеричната страна и формирање резервоар [3, 4]. Уретероинтестиналната анастомоза се изведува на проксималниот дел на резервоарот, а дисталниот дел се анастомизира со мембранозната уретра. Кандидати се пациентите со мускулатурата на мочниот меур во T2a, T2b стадиум, и без метастази; пациенти со мултифокален и рекурентен тумор рефрактерен на повторен TURT; CIS-рефрактерен на имунотерапија и отсуство на тумор на вратот на мочниот меур [5, 6].

Искуствата во Македонија од ваков тип интервенција, првпат се презентирани на Националниот конгрес за уролози на Македонија, во соработка со ЕАУ, во 2002 година, од група автори од Универзитетската клиника за урологија – Скопје. Контраиндикации за ортотопската реконструкција на неовезиката се оштетување на бубрезите и на црниот дроб, цревни заболувања и карцином на апексот на простатата, односно на вратот на мочниот меур.

ВАЖНИ ПОСТОПЕРАТИВНИ АСПЕКТИ

Електролитен дисбаланс – ѕидот на новиот мочен меур е обложен со цревна слузница која е попропустлива за уринарните електролити отколку уротелиумот. Како резултат на тоа, метаболните проблеми може почесто да се појават поради продолжено време на контакт на уринатa со цревната слузница отколку со инконтинентната уринарна диверзија. Уринатa содржи повисоки концентрации на калиум, водород и хлориди. Овие јони се апсорбираат во замена со натриум и бикарбонат од крвотокот, што резултира во хиперхлоремична, хиперкалемична метаболна ацидоза и со синдром на загуба на сол [4]. Овие состојби клинички се манифестираат со летаргија, замор и дехидрираност. Важно е навремено да се открие и да се третира метаболната ацидоза бидејќи може да доведе до ресорпција на калциумот од коските и до хипофосфатемија, што резултира со остеопороза и остеомалација. Метаболната ацидоза може да предизвика хиперкалциурија, со што го зголемува ризикот за развој на бубрежна калкулоза, а освен тоа уринатa е поконцентрирана од интрацелуларниот домен, и ова ги поттикнува поместувањата на течностите од ѕидовите на неовезиката

кон уринатa и води до дехидратација. Кај еден пациент од вкупно 44, колку што беа опфатени во примерокот на анализата во студијата на Универзитетската клиника за урологија – Скопје, по оперативниот зафат се забележале појави на анорексија, чувство на гадливост, губиток на тежина и констатирана е перзистирачка метаболна ацидоза. Третирано е со NaHCO3, соодветен хигиенско-диететски режим и со контролирано внесување на храна и течности.

Другите материи од уринатa, како што се креатинин, уреа и амонијак, излачувани во уринатa, исто така, се апсорбираат од мукозата на цревата. Затоа, бубрежната инсуфициенција претставува контраиндикација за постоењето на новиот мочен меур (неовезика) бидејќи тоа би било дополнително оптоварување на оштетените бубрези за да се излачат реапсорбираните отпадни продукти од уринатa. Тоа се однесува и за функцијата на црниот дроб бидејќи зголеменото оптоварување на оштетениот црн дроб за метаболизирање на реапсорбираните продукти од уринатa, како што е амонијакот, може да го зголеми ризикот од инсуфициенција на црниот дроб [7].

Хигиенско-диететски режим – пациентите кои се подложни на креирање на неовезиката имаат поголем ризик за појава на илеус, во просек, на петтиот ден постоперативно, во споредба со оние пациенти кај кои се формира илеален кондуит (14,8 % наспроти 5,5 %, P = 0,018) [8]. Во анализата извршена на нашата клиника на 44 пациенти, регистрирани се 5 случаи на постоперативен илеус кои беа разрешени со конзервативен третман. Затоа, императив претставува присуството на доволно гликогенски резерви во црниот дроб со помош на предоперативни додатоци на јаглехидрати и протеини. Потребно е да се надоместуваат соодветни диетални влакна постоперативно, за да се намали можноста за опстипација, како и да се следи внесувањето на витамин Б 12 и на фолна киселина. Ова е важно поради потенцијалното влијание на оперативната интервенција над терминалниот илеум, кој претставува единствен дел од цревата што може да ги апсорбира овие хранливи материи, а кој станува значително покус постоперативно. Намалување на должината на терминалниот илеум, исто така, ја засега реапсорпцијата на жолчните киселини, што резултира во малапсорпција на маснотии, а тоа води кон дијареја и дехидратација. Ваквите проблеми се поизразени ако операцијата вклучува и ресекција на

илеоцекалната валвула бидејќи микроорганизмите од колонот (на пр. бактериоиди) може да навлезат во илеумот и да ги раздвојат жолчните киселини од нивните конјугати, а добиените слободни жолчни киселини претставуваат емулгатори на маснотии [4, 9]. Пациентите со нов мочен меур (неовезика, неоциста), исто така, треба да бидат обезбедени со адекватна хидратација бидејќи потенцијално може да изгубат значителна количина вода во урината.

Континентност – неовезиките (неоцистите) претставуваат форма на континентна деривација на урината, што не значи дека се целосно ослободени од проблемите поврзани со континенцијата. Стапките на континенција варираат зависно од хируршката техника и времетраењето по операцијата. Вкупните стапки на континенција кај неовезиките се движат од 22,3 % до 63,2 %, одредено на 12 месеци постоперативно, и 17,7 – 74,5 %, над 40 месеци постоперативно [10, 11]. Стапките на континенција во текот на денот варираат од 21,4 % до 99,0 % на 3 до 48 месеци постоперативно [10]. Модификацијата на Abol-Enein и Ghoneim, односно W-уринарниот резервоар, која е најчесто користената техника, има стапка на континентност во текот на денот од 93,3 % [2]. Севин (Sevin) и сор. демонстрираат постепено зголе-мување на стапката на континентност во текот на денот, во временски период: на 6, 12, 24, 36 и ≥ 48 месеци постоперативно, при што стапките на дневната континенција се 63 %, 70 %, 76 %, 88 % и 92 % [12]. Дневната континентност во анализата на 20 испитаника од нашата клиника укажува на присутност кај 14 пациенти (70 %), а ноќна конти-нентност кај 45 %, односно 9 пациенти по 6 месеци од интервенцијата [5]. Понатаму, не е утврдена значителна разлика во однос на застапеноста на инконтиненцијата во текот на денот помеѓу пациентите со моди-фициран нов мочен меур со S-оформен резервоар и здрава контролна популација без уринарна диверзија (10 % наспроти 9,3 %, P = 0,1) [12].

Пациентите со неовезика се помалку континенти навечер отколку во текот на денот. Во текот на ноќта, 18,9% – 79%, 74 %, 23 %, 77,6 % и 44 % од пациентите биле континентни на 12, 38, 44, 48 и 54 месеци постоперативно [10, 12]. Модификацијата на Abol-Enein и Goneim на уринарниот резервоар има висока стапка на континентност во текот на ноќта, од 80 % при средно следење од 38 месеци [2]. Повторно, Севин (Sevin) и сор. демонстрираат постепено зголемување

на стапката на ноќна континенција, со текот на времето, одредувано на 6, 12, 24, 36 и ≥48 месеци постоперативно, при што стапките на ноќна континенција се 55 %, 62 %, 73 %, 85 % и 90 % [12]. При споредување на здрави испитаници на слична возраст, пол и коморбидитети, пациентите со модифициран нов мочен меур со S-оформен резервоар имаат значително повисока стапка на ноќна инконтиненција (28 % наспроти 3,7 %, P = 0,003) [13].

Недостатокот на стандардизација во одредувањето на континентноста, потоа различните хируршки техники при реконструкција на новиот мочен меур и варијациите во однос на водењето на евиденцијата кај пациентите откриваат широк спектар на стапки на континентност помеѓу различни студии.

Одржување на неовезика – законот Лаплас диктира дека зголемувањето на радиусот на резервоарот ќе ја зголеми затегнатоста на ѕидот на мочниот меур, со што ќе се предизвика поголемо оштетување на ткивото. Кога се зголемува притисокот во сферичниот резервоар, затегнатоста на ѕидот се зголемува како резултат на влијанието на истиот фактор, доколку дебелината на ѕидот и радиусот на резервоарот останат исти [3]. Меѓутоа, во неовезиката, секое понатамошно ширење над капацитетот, ќе доведе до раст на големината, а со тоа и на радиусот на резервоарот, што ќе води кон истегнување и истенчување на ѕидот на меурот. Треба да се избегнува преголема дистензија, особено во раниот постоперативен период, за да се избегне оштетување на ткивото што заздравува, а тоа би се постигнало со соодветна обука за одржување на неовезиката од страна на пациентот [3].

Во непосредната постоперативна фаза, супрапубичниот катетер останува во неовезиката постоперативно, со што се овозможува соодветно хируршко закрепнување, пред да се почне со обуката. Се прави редовно 6-8 часовно плакнење на мочниот меур преку катетерот со 100 ml физиолошки раствор од 0,9 % NaCl, за да се намали ризикот од затнување на катетерот. Цистограмот се изведува во текот на 2-3 недели, постоперативно, за да се потврди дека новиот резервоар е непропустлив [4]. Отстранувањето на уринарниот катетер и испитувањето на неовезиката се вршат со цел да се следи волуменот на уринатa во мочниот меур (void volume) и количината на урина што се задржала по микцијата (post void volume), и доколку е потребно, се врши обука

за интермитентна самокатеризација на неовезиката. Во нашите случаи, исто така, неовезиката се плакне со 20 или 40 мл физиолошки раствор на секои 4 часа и партиклите од мукус се аспирираат. По вадењето на катетерот се даваат упатства за актот на мокрење, односно тоа да се изведува во седечка положба, со релаксација на пелвичната мускулатура и со помош на Валсалва-маневар. Сите пациенти можат да мократ, прво во седечка положба, потоа стоејќи, а капацитетот на мочниот меур постепено расте од 250 мл до 400 мл по 6-тиот месец.

Техниките за мокрење се важни поради фактот што отсуствува контрактилноста која природно е присутна во природниот мочен меур. Се даваат упатства на пациентите да ја празнат неовезиката во седечка положба, свесно да ги релаксираат мускулите на сфинктерот и леваторната мускулатура на карлицата, и притоа да применат Валсалва-маневар за да го испразнат новиот мочен меур. Доколку е потребно, пациентите, исто така, можат да го зголемат својот интраабдоминален притисок со наведнување напред или да извршат нежен рачен притисок над долниот дел на стомакот и супрапубичната регија [4].

Втор важен момент е постепеното зголемување на капацитетот на новиот мочен меур со зголемување на капацитетот од 150 до 200 ml до 400 до 500 ml подолгорочно. За време на раните фази, честото празнење на неовезиката се изведува 2-3 часа на ден и на 3-часовен временски интервал ноќно време, за да се избегне инконтиненција со прелевање на уринатa (ischiuria paradoxa) [14, 15]. Интервалите на празнење може потоа да се зголемат постепено во текот на следните неколку недели, за да се постигне целта да се празни мочниот меур на секои 5-6 часа во текот на денот и само еднаш во текот на ноќта. Исто така е важно да се напомене дека големата количина на постмикциска урина ќе бара потреба таа почесто да се празни бидејќи новиот мочен меур побргу се полни. Капацитетот на новиот мочен меур постепено расте, а со тоа расте и интервалот на мокрење од 4 на 6 часа. Исто така, се следи и нивото на електролитите, креатининот, бикарбонатите во крвта, како и на плазминиот витамин Б 12.

Пациентите кои се соочуваат со прашање за несоодветното празнење дури и по извршувањето на правилните маневри, можеби ќе треба да размислат за интермитентна самокатетеризација. Пациентите со поголем индекс на телесна маса (body mass index) се изложени на

повисок ризик од појавата на несоодветно празнење на новиот мочен меур (или 1,5; 95 % CI: 1,06 – 2,15, P = 0,023) [10].

Уродинамички промени – етиологијата на инконтиненцијата кај неовезиките, исто така, е испитана со помош на уродинамички студии. Мултиваријантната анализа има откриено дека големиот волумен на урината што се детектира по актот на микција, фреквенцијата и максималната амплитуда на неинхибираните контракции на мазната мускулатура на неовезиките, се најзначајните параметри асоцирани со ноќната инконтиненција. Фармакотерапијата има улога во подобрувањето на континентноста, при што едно краткорочно испитување даде ветувачки резултати при третман со оксибутинин (5 мг 3 пати на ден) и верапамил (240 мг еднаш на ден) соодветно со 70 % и 55 % клиничко подобрување [16].

Со уродинамички анализи (урофлоуметрија) и со податоци од 20 континентни испитаника на нашата клиника е утврдено дека капацитетот на мочниот меур во просек изнесува 420 мл (250 – 660 мл). Max Flow Rate 11 мл/сек (5 – 20 мл/сек), Average Flow rate 7 мл/сек (4 – 12 мл/сек), прво чувство за мокрење е на 300 мл (220 – 350 мл), а Peak pressure (cm water) е 26 (13 – 45) [5].

Покрај инконтиненцијата, кај некои пациенти се јавува слабо празнење на неовезиката или „хиперконтиненција“, односно ретенција. Одредени компликации на неовезиките, како што се електролитните дисбаланси, ацидозата, инфекциите на уринарниот тракт и формирањето калкули во резервоарот, се поизразени кај тие пациенти. Финалниот облик на новиот мочен меур и должината на сегментот на цревата може да влијаат на волуменот на постмикционата резидуална урина. Резидуалната постмикциона урина во анализата на уролошката клиника во Скопје, во просек изнесува 80 мл (30 – 220), а кај двајца пациенти е регистрирана ретенција на урина и тие беа повторно катетеризирани [5].

Сексуална функција – ефектите врз еректилната функција и сексуалната желба се важни размислувања кои треба да се земат предвид при цистектомија. Токму затоа се развиени техники кои водат кон зачувување на невроваскуларните петелки кои ги инервираат гени-

талиите со што се помага во зачувување на предоперативната сексуална функција. Билатералното зачувување на невроваскуларните петелки при формирање на илеална неовезика се покажало дека постигнува 2-годишна стапка на потенција до 60 %, во споредба со стапка од 30 %, постигната со едностранн техники на презервација на петелката [17]. Постојат контрадикторни наоди. Дури и со процедури за презервација на нервите, мажите со уринарни диверзии и неовезики искусуваат значително пониски стапки на ерекција (35,5 наспроти 83,3 %, P = 0,003) и намален квалитет на ерекција (22,2 наспроти 83,3 %, P = 0,002) од испитаниците кои се без диверзии [13]. Неодамнешната серија случаи, исто така, ги поддржа овие наоди, односно Асгари (Asgari) и сор. откриваат дека мажите доживуваат значително влошување и на еректилната функција и на сексуалната желба постоперативно, без оглед на видот на извршената диверзија на урината [18]. 5 пациенти од група на 20 испитаника во студија на нашата клиника за урологија кои се согласија да дадат информации, се пожалија на проблеми со ерекцијата, а тие се конзервативно третирани.

Помеѓу 23,7 – 35,5 % од пациентите задржуваат адекватна еректилна функција по конструкцијата на неовезика [11]. Некои студии покажуваат дека конструкцијата на неовезика води кон подобра заштита на сексуалната функција, во споредба со илеалниот кондуит. Поточно, 35,0 % од мажите во групата на илеална неовезика биле во можност да постигнат и да одржат ерекција доволна за пенетрација во текот на сексуалниот однос, во споредба со 9,8 % од мажите во групата на креирани илеални кондуити (P = 0,006). Покрај тоа, повеќемина мажи во групата на континентна илеална неовезика ја оценуваат својата сексуална желба како висока или многу висока, во споредба со мажите во групата на инконтинентни илеални кондуити (45,0 % наспроти 24,4 %, P = 0,01), исто така, Асгари (Asgari) и сор. откриваат дека повеќемина мажи со континентна илеална неовезика имаат пријавено непроменета сексуална желба во споредба со пациентите кои имаат инконтинентен илеален кондуит (17,5 % наспроти 7,2 %, P = 0,01) [18].

Квалитет на живот – подразбира широк поим; вообичаено е утврдуван со голем број параметри кои помагаат да се утврди влијанието кое промената го има врз одреден аспект од животот. Споре-

дувањето во QoL е придружено со недостатокот на стандардизација, во однос на употребата на разни видови прашалници, и релевантноста на податоците во секој од нив. Некои мерења се насочени кон утврдување на QoL воопшто, додека други се поспецифични кон начините на кои уринарната диверзија (пренасочување) може да влијае на животот. QoL кај пациентите кои имаат континентна неовезика најчесто се споредува со животот што го живеат оние пациенти со илеален кондуит, веројатно затоа што ова се двете најчести процедури кои се изведуваат за диверзија на урината.

Фуџисава (Fujisawa) и сор. го споредуваат QoL во однос на здравствената состојба кај 56 пациенти и не наоѓаат статистички значајна разлика во однос на параметрите за бодување помеѓу пациентите кои имаат ортотопска неовезика и оние со илеален кондуит. Сепак, пациентите со неовезика или илеален кондуит имаат значително пониско ниво на физичко функционирање и нарушено емоционално и социјално функционирање, во споредба со општата популација [19].

Меѓутоа, постојат спротивставени наоди во други студии. Во проспективната студија на Синг (Sing) и сор., пациентите кои биле подложени на ортотопска реконструкција на мочен меур, забележале значително подобро физичко функционирање ($P \leq 0,001$), емоционален статус ($P \leq 0,01$), социјално функционирање ($P = 0,01$) и целосен здравствен статус/QoL ($P \leq 0,002$) во текот на првите 18 месеци, постоперативно, во споредба со пациентите кај кои е формиран илеален кондуит [18].

Асгари (Asgari) и сор. наоѓаат сигнификантно поголема стапка на задоволство во секојдневниот живот кај оние пациенти со континентна ортотопска неовезика во споредба со оние со илеален кондуит (76,2 % наспроти 52,8 %, $P = 0,002$) [18]. Контрадикторни наоди се присутни во студијата на Тулин (Tulin) и сор., кои откриваат дека 37 % од оние со ортотопска неовезика пријавиле дека тоа негативно влијае на нивниот начин на спиење, во споредба со 22 % кај оние пациенти со уретеростомија. Нарушувањата на начинот и фреквенцијата на празнење во текот на денот и ноќта беа статистички значително повисоки кај оние пациенти со ортотопичен нов мочен меур, наспроти пациентите со илеален кондуит, консеквентно, 29 % наспроти 19 % [14], а единствена можна причина за пациентите со неовезика да немаат подо-

бар QOL од оние пациенти со илеален кондуит е дека зголемена инциденца на нарушувањата на спиењето негативно влијае на нивото на енергијата и QoL во текот на денот [14].

ЗАКЛУЧОК

Ортотопската реконструкција на неовезиката има голем број единствени предности и недостатоци во споредба со другите форми на уринарната диверзија. Оваа процедура бара сеопфатна комуникација и советување меѓу пациентот и хирургот, за да се земат предвид сите аспекти за постапката. Важните прашања вклучуваат спречување на метаболичките компликации, надминување на проблемот со уринарната инконтиненција, особено за време на раната постоперативна фаза и согласност на пациентот со режимот на обука за маневрирање со новиот мочен меур. Покрај тоа, потенцијалните пациенти треба внимателно да бидат избрани, имајќи ги предвид контраиндикациите за оваа операција, со цел намалување на ризикот од постоперативните компликации. Стапките на континенција варираат во голема мера меѓу различни студии, во зависност од времето по операцијата, видот на реконструкцијата и дефинирање на тоа што се подразбира под уринарна инконтиненција во студиите. Стапките на инконтиненција на урина се повисоки во текот на ноќта отколку во текот на денот, а тоа го намалува квалитетот на спиењето. Пациентите со ортотопска супституција имаат главно подобар квалитет на животот (QOL) и сексуална функција, во споредба со оние пациенти со илеалните кондуити.

КОРИСТЕНА ЛИТЕРАТУРА И ИЗВОРИ

1. GLOBOCAN. (2012). *Estimated Cancer Incidence, Mortality and Pre-valence Worldwide in 2012*. International Agency for Research on Cancer. 2012. [Last cited on 2014 Jan 23]. Достапно на: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx
2. Gakis, G., Stenzl, A. (2010). Ileal neobladder and its variants. *Eur Urol Suppl.* 9: 745–53. [Google Scholar].

3. Basford, J. R. (2002). The Law of Laplace and its relevance to contemporary medicine and rehabilitation. *Arch Phys Med Rehabil.* 83: 1165–70. [PubMed] [Google Scholar].
4. Herdiman, O., Ong, K., Johnson, L., Lawrentschuk, N. (2013). Orthotopic bladder substitution (Neobladder): Part II: Postoperative complications, management, and long-term follow-up. *J Wound Ostomy Continence Nurs*. 40: 171–80. [PubMed] [Google Scholar].
5. Saidi, S., Ivanovski, O., Petrovski, D., Kuzmanovski, M., Stavridis, S., Banev, S. et al. (2007). *Lower urinary tract reconstruction following a cystectomy : Expirience and result in 20 patients using "Studer" orthotopic ileal blader substitution.* Bratisl Lek Listy; 109(8): 353–357.
6. Popov, Z., Stavridis, A., Lekovski, L., Penev, M., Dohcev, S., Stankov, O. et al. (2007). Urinary diversion: 30 years experience of a single centre in Republic of Macedonia. ActaChirJugosl; 54(4): 49–56.
7. Ong, K., Herdiman, O., Johnson, L., Lawrentschuk, N. (2013). Orthotopic bladder substitution (neobladder): Part I: Indications, patient selection, preoperative education, and counseling. *J Wound Ostomy Continence Nurs.*; 40: 73–82. [PubMed] [Google Scholar].
8. Erber, B., Schrader, M., Miller, K., Schostak, M., Baumunk, D., Lingnau, A. et al. (2012). Morbidity and QoL in BC patients following cystectomy and urinary diversion: A single-institution comparison of ileal conduit versus orthotopic neobladder. *ISRN Urol 2012*: 342–796. [PMC free article] [PubMed] [Google Scholar].
9. Thorstenson, A., Jacobsson, H., Onelöv, E., Holst, J. J., Hellström, P. M., Kinn, A. C. (2007). Gastrointestinal function and metabolic control after construction of an orthotopic ileal neobladder in BC. *Scand J Urol Nephrol.*; 41: 14–9. [PubMed] [Google Scholar].
10. Meyer, J. P., Drake, B., Boorer, J., Gillatt, D., Persad, R., Fawcett, D. (2004). A three-centre experience of orthotopic neobladder reconstruction after radical cystectomy: Initial results. *BJU Int.*; 94: 1317–21. [PubMed] [Google Scholar].
11. Studer, U. E., Burkhard, F. C., Schumacher, M., Kessler, T. M., Thoeny, H., Fleischmann, A. et al. (2006). 20-years experience with an ileal orthotopic low pressure bladder substitute – Lessons to be learned. *J Urol.*; 176: 161–6. [PubMed] [Google Scholar].
12. Sevin, G., Soyupek, S., Armagan, A., Hoscan, M. B., Oksay, T. (2004). Ileal orthotopic Neobladder (modified Hautmann) via a shorter detubularized ileal segment: experience and results. *BJU Int.*; 94: 355–9. [PubMed] [Google Scholar].
13. Protogerou, V., Moschou, M., Antoniou, N., Varkarakis, J., Bamias, A., Deliveliotis, C. (2004). Modified S-pouch neobladder vs ileal conduit and a

matched control population: A quality-of-life survey. *BJU Int.*; 94: 350–4. [PubMed] [Google Scholar].
14. Thulin, H., Kreicbergs, U., Wijkström, H., Steineck, G., Henningsohn, L. (2010). Sleep disturbances decrease self-assessed QoL in individuals who have undergone cystectomy. *J Urol.*; 184: 198–202. [PubMed] [Google Scholar].
15. Steers, W. D. (2000). Voiding dysfunction in the orthotopic neobladder. *World J Urol.*; 18: 330–7. [PubMed] [Google Scholar].
16. El-Bahnasawy, M. S., Shaaban, H., Gomha, M. A., Nabeeh, A. (2008). Clinical and urodynamic efficacy of oxybutynin and verapamil in the treatment of nocturnal enuresis after formation of orthotopic ileal neobladders. A prospective, randomized, crossover study. *Scand J Urol Nephrol.*; 42: 344–51. [PubMed] [Google Scholar].
17. Kessler, T. M., Burkhard, F. C., Perimenis, P., Danuser, H., Thalmann, G. N., Hochreiter, W. W. et al. (2004). Attempted nerve sparing surgery and age have a significant effect on urinary continence and erectile function after radical cystoprostatectomy and ileal orthotopic bladder substitution. *J Urol.*; 172: 1323–7. [PubMed] [Google Scholar].
18. Asgari, M. A., Safarinejad, M. R., Shakhssalim, N., Soleimani, M., Shahabi, A., Amini, E. Sexual function after nonnerve-sparing radical cystoprostatectomy: A comparison between ileal conduit urinary diversion and orthotopic ileal neobladder substitution. *Int Braz J Urol.* 2013; 39: 474–83. [PubMed] [Google Scholar].
19. Autorino, R., Quarto, G., Di Lorenzo, G., De Sio, M., Perdonà, S., Giannarini, G. et al. (2009). Health related QoL after radical cystectomy: Comparison of ileal conduit to continent orthotopic neobladder. *Eur J Surg Oncol.*; 35: 858–64. [PubMed] [Google Scholar].

Sasho DOHCHEV, Aleksandar TRIFUNOVSKI, Oliver STANKOV,
Sotir STAVRIDIS, Dimitar TRAJKOVSKI, Josif JANCULEV,
Goce NINESKI, Zivko POPOV

ORTHOTOPIC DERIVATION OF URINE BY STUDER
- postoperative aspects -

Abstract

Bladder cancer is the second most common urological malignancy in the world with a male incidence in 2012 of 5.3 / 100,000 and a mortality

rate of 1.9 / 100,000. An effective method in the treatment of high-grade bladder cancer of malignancy and avoidance of stoma formation is radical cystectomy with orthotopic urine derivation by the Studer method. Due to the prolonged time of urine contact with the intestinal mucosa in this technique, certain metabolic problems and electrolyte imbalances may occur. In addition to the impact that the intervention has on the terminal ileum, conditions of malabsorption and dehydration can occur. The Studer method is a form of continent derivation, but it is not excluded from problems related to the continent itself. The rate of continence varies widely between different studies depending on what is considered to be urinary incontinence. The procedure also requires comprehensive communication and counseling between the patient and the surgeon, especially in the postoperative maintenance of neobladder, and problems related to the act of urination are registered and quantified through urodynamic examinations. Patients with orthotopic replacement have a predominantly better quality of life (QOL) and sexual function, compared with those with other types of urinary diversion.

Keywords: bladder cancer, orthotopic urine derivation, neobladder.

doi: 10.20903/zpopov-2022-0004 UDK: 616.62-006.6-07/-08

Sava MIĆIĆ[1,2] Vladan ANDREJEVIĆ[2] and Ivan VUKOVIĆ[2]

NEOPLASM IN A BLADDER DIVERTICULUM

Abstract

Bladder diverticulum is an outpouching of the urothelial mucosa lining from the muscular layer of the bladder. Primary neoplasms arising in vesical diverticula are rare, they occur in around 1% of all bladder carcinoma. Most malignant tumors in vesical diverticula are of transitional type (about 78%), followed by squamous cell carcinoma (17%), a combination of transitional and squamous cell types (2%), and adenocarcinoma (2%). Painless hematuria is the cardinal symptom for diverticular tumors. Modern diagnostic tools like ultrasound, computed tomography (CT) and nuclear magnetic resonance (NMR) enable a more accurate and precise diagnosis of the neoplasm in a bladder diverticulum. From 1961 to 2020, a series of 36 patients with bladder diverticulum cancer was presented and the data show that incidence of tumors today is similar to several decades ago. Most of the patients had p GII and T2 stage disease, therefore the most common surgical treatment was either diverticulectomy or partial cystectomy or radical cystectomy. Recurrent tumor, in our group, was evident in 8 patients with transitional cell carcinoma. Six patients with recurrent tumors were alive for >36 months, while 2 died of the disease within 21 months. No recurrence was noted in 10 patients, and they lived more than 48 months after the operation. We can conclude that therapeutic results are better today than in previous decades due to better diagnostic methods and a more radical surgical approach.

Key words: bladder diverticulum, tumor in bladder diverticulum

[1] Uromedica Polyclinic, Belgrade

[2] Clinic of Urology, Clinical Center of Serbia, University of Belgrade

Introduction

Bladder diverticulum is a herniation of the mucosa lacking a muscle layer. This results in a loss of contractility and urine stasis in the diverticulum. Vesical diverticula are congenital or acquired. Acquired diverticula are, by far, more common and are secondary to lower urinary tract obstruction, such as benign prostatic hyperplasia, vesical neck contracture, urethral stricture, or neurogenic bladder. Most bladder diverticula are small and asymptomatic. This lesion may be complicated by inflammation, calculus, infection and malignancy (Kong et al, 2013). Chronic irritation of urine stasis inside the diverticulum results in chronic infection and inflammation and then facilitates the development of neoplasm. In men with bladder diverticulum, mucosal inflammation, ulceration, dysplasia, squamous metaplasia, and leukoplakia were found in 84% of men who underwent diverticulectomy (Kelalis and McLean, 1967).

Painless hematuria is the cardinal symptom for diverticular tumors, as in ordinary bladder tumors. According to Melekos et al (1987), hematuria was present in 87.5% of patients with neoplasms occurring in the diverticula and in 100% of patients with tumors elsewhere in the bladder.

Primary neoplasms arising in vesical diverticula are rare, around 1% of all bladder carcinoma (Idrees et al, 2013). In 0.8% to 13% of patients with vesical diverticulum, neoplasms develop within the diverticulum (Fox et al, 1962; Prakash et al, 2010). As in other bladder tumors, diverticular neoplasms are most prevalent in men above the age of 40. Most malignant tumors in vesical diverticula are of transitional type (about 78%), followed by squamous cell carcinoma (17%), a combination of transitional and squamous cell types (2%), and adenocarcinoma (2%) (Shirai et al, 1984). Prakash et al (2010) reported that the prevalence of bladder diverticulum is more common in men as compared with women (31.6% vs 9%) and also, they found the presence of bladder diverticulum in 23.4% in cadavers.

Due to its rarity, neoplasm in the diverticulum remains infrequently encountered in general practice. Bladder tumors within a diverticulum are difficult to diagnose. Bladder diverticulum neoplasms are characterized by early transmural invasion and a tendency for higher histopathological grades, which allow for prompt diagnosis and crucial treatment. An outpourching in the bladder urothelium through the muscularis propria can complicate the delivery of local therapies, and lack of muscle invasive disease in

a bladder diverticulum can make decisions on extirpation more difficult. Based on ultrasound examination, cystoscopic evaluation, bimanual examination and computerized tomography (CT) or nuclear magnetic resonance (NMR) findings, tumors were classified as superficial (Ta, Tis), superficially invasive confined to diverticulum (T1) or extra diverticular (T3+). Patients with superficial or superficially invasive disease were treated either conservatively with repeat transurethral resection, or with partial or radical cystectomy. Current practice suggests that intradiverticular neoplasm is often stage and treated aggressively as it is thought that it is more likely to disseminate (Walker et al, 2014; Idrees et al, 2013). Unifocal bladder neoplasm limited to the diverticulum and with good bladder function are considered for partial cystectomy, or diverticulectomy. Of course, the finding of carcinoma in situ excludes the partial cystectomy

Materials and methods

We analyzed 96 men with vesical diverticula in the period from 1961 to 1980 (Micic and Ilic, 1983). Of these patients 13 had primary tumors in the diverticulum. Patients with concomitant tumors elsewhere in the bladder were excluded. From 1981 until 2001, we examined 11 patients with primary carcinoma of a bladder diverticulum out of the 101 of patients with vesical diverticulum and 12 out of 88 patients with bladder diverticula were diagnosed with primary carcinoma of the diverticulum from 2001 to 2020. The whole group of men with carcinoma of bladder diverticulum consisted of 36 patients in period of 1961-2020

Results

In our group of patients with diverticular tumor, 25 had symptoms such as hematuria in (69%), urinary tract infection with pyuria in 28 (77%), urinary obstruction in 26 (72%), and fever in 5 (13%). Patients underwent excretory urography from 1961-1998 (Fig 1) and after 1998 they underwent an ultrasound (Fig 2), CT, or NMR with contrast. Cystoscopy was also used in diagnostic evaluation. Tumors were classified as superficial (Ta, Tis), superficially invasive confined to diverticulum (T1) or extra diverticular (T3+). Histopathologic examination revealed transitional cell carcinoma in 28 (77%) patients (4 patients with pG1, 10 patients – pG2 and 14 patients -

pG3), squamous cell carcinoma in 5, and adenocarcinoma in 3. Four patients underwent a transurethral resection of the tumor. Diverticulectomy was done in 10 and partial or total cystectomy was completed in 22 patients.

Recurrent tumor, in our group, was evident in 8 patients with transitional cell carcinoma and was of a higher stage than the primary tumor in 6. Six patients with recurrent tumor were alive for >36 months, while the 2 died of the disease within 21 months. The patients with squamous cell carcinoma and adenocarcinoma died of disseminated disease within 16 and 22 months postoperatively. No recurrence was noted in 10 patients and they remained alive more than 48 months after the operation.

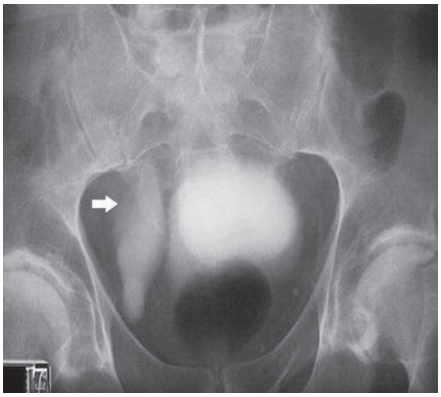


Figure 1. IVF with cystogram showing bladder diverticulum with neoplasm

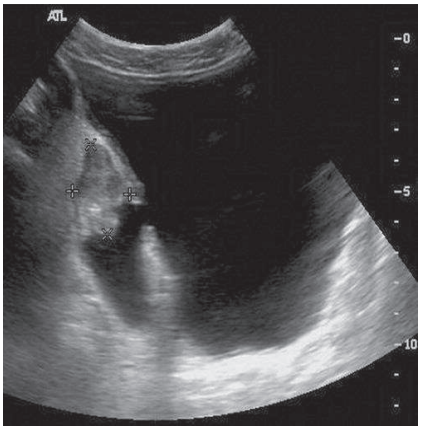


Figure 2. Ultrasound picture of neoplasm in bladder diverticulum

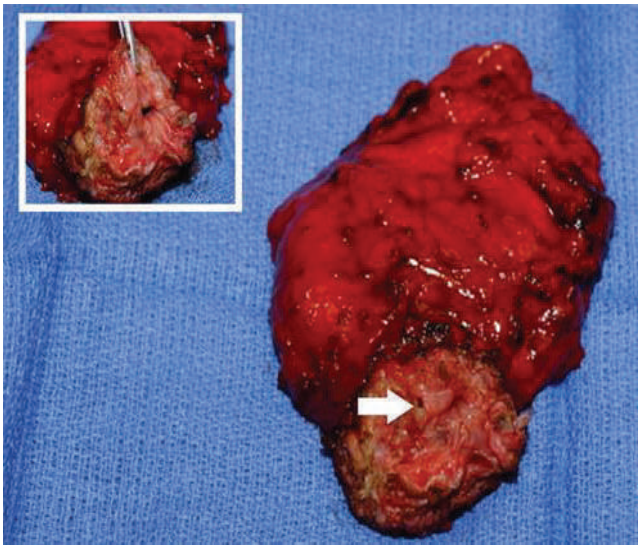


Figure 3. Extracted bladder with neoplasm in diverticulum

Discussion

Intradiverticular bladder carcinomas are malignant epithelial neoplasms arising within a diverticulum of the urinary bladder. Neoplasm arising in urinary bladder diverticulum are uncommon, but not rare and their incidence ranges between 0.8 and 10% (Melekos et al 1987)

These tumors usually occur in aged patients with bladder outlet obstruction, rarely on congenital diverticula. Inflammation, metaplasia, and dysplasia are commonly seen in the vesical diverticula. The urinary bladder is the most frequent site for urinary tract cancers. More than 95% of primary bladder cancers are of epithelial origin, most commonly transitional cell tumors. Squamous cell carcinoma and adenocarcinoma account for a minority of epithelial tumors and mesenchymal neoplasms are rare.

Golijanin et al (2003) published 39 cases, the series on diverticular neoplasms, and found that the most important factor in outcome is the clinical stage, regardless of the histological grade. In their study of 39 patients, 33% presented with superficial disease, 33% with superficially invasive tumors and 33% with invasive (extra diverticular) disease. They treated patients with superficial or superficially invasive disease either conservatively with repeat transurethral resection, or with partial or radical

cystectomy. Patients with extra diverticular extension were treated with partial or radical cystectomy when amenable to surgical extirpation. The 5-year disease specific survival rate for their group was 72 ± 5.4%. Significant differences in the 5-year disease specific survival rate were observed among patients presenting with superficial tumors (83 ± 9%), superficially invasive tumors (67 ± 7%) and extra diverticular disease (45 ± 14%).

Garzotto et al (1996) suggest multimodal therapy, combining diverticulectomy with chemotherapy or preoperative radiotherapy. They showed a disease-specific survival rate of 89%, and they suggested a significant benefit from systemic chemotherapy and RT when combined with surgery for these neoplasms.

In their review of 2,642 patients with radical cystectomy, Hu et al (2014) found 1,991 patients (75 %) who met the inclusion criteria (patients who underwent radical cystectomy for curative intent for primary urothelial cancer). The median follow-up for the urothelial cancer in bladder diverticula group (10.3 years) was comparable to that of patients without urothelial cancer in the bladder diverticula (12.9 years, $p = 0.91$). A total of 77 patients (4%) had urothelial cancer in the bladder diverticula. Of these, 44 (57 %) had the highest pathologic stage of tumors within the bladder diverticula. The remainder ($n = 33$) were found in association with separate, more pathologically advanced tumors.

Considering the risk of the upstaging, therapy decisions should not be based solely on diagnostic cystoscopy and transurethral resection, but should also include radiologic imaging, as was suggested by the "Young Academic Urologist" working group on Urothelial Carcinoma of European Association of Urology (Voskuilen et al, 2018). They found that the upstaging of diverticular tumors was frequent (55%), indicating an inaccuracy in clinical staging in urothelial carcinoma of a bladder diverticulum. Also, the group underline that partial cystectomy may represent a feasible alternative of radical cystectomy in carefully selected urothelial carcinoma of a bladder diverticulum, with equivalent oncological results, but the urologist should be aware of the potential underestimation of tumor extent of carcinoma in a bladder diverticulum.

In review of 36 patients with carcinoma arising from a bladder diverticulum, we demonstrated during the first period of analysis (1961-1980) that treatment, which consisted of transurethral resection and radical

cystectomy, showed a poor survival rate. The last period, the following 30 years, showed better diagnostic possibilities, computed tomography, and nuclear magnetic resonance. In addition, earlier radical surgical procedures enabled better results and survival, especially after 1990, and with the introduction of aggressive chemotherapy the survival rate improved even more. This diagnostic and therapy improvement led to much a better survival rate of patients with urothelial cancer of a bladder diverticulum over the last 20 years.

In conclusion, transurethral resection of neoplasm in a bladder diverticulum seems to be adequate for men with superficial non-invasive urothelial cancer. There is a smaller number of cases, even with modern diagnostic capabilities, due to patients reporting these problems later on to their doctor. We can say that diverticulectomy or partial cystectomy, and radical cystectomy are a safer therapy for patients and provide a better survival rate.

RERERENCES

1. Kong MK, Zhao X, Kheterpal E, Lee P, Taneja S, Lepor H, Melamed J, Deng FM. Histopathologic and clinical features of vesical diverticula. Urology 2013; 82: 141-47.
2. Kelalis PP, McLean P. The treatment of diverticulum of bladder. J Urol 1967; 98: 349-352.
3. Idrees MT, Alexander RE, Kum JB, Cheng I. The spectrum of histopathologic findings in vesical diverticulum: implications for pathogenesis and staging. Hum Pathol.2013; 44: 1223-32.
4. Fox M, Power RF, Bruce AW: Diverticulum of the bladder: Presentation and evaluation of treatment of 115 cases. Br J Urol 1962; 34: 286.
5. Prakash Rajini T, Bhardwaj AK, Jayanthi V, Rao K, Sing G. Urinary bladder diverticulum and its association with malignancy: an anatomical study on cadavers. Rom J Morphol Embryol 2010; 51: 543-545.
6. Shirai T, Arai M, Sakata T. Primary carcinomas in urinary bladder diverticula Acta Pathol.Jap 1984; 34: 417-424.
7. Walker NF, Gan C, Olsburg J, Khan MS. Diagnosis and management of intra-diverticular bladder tumors. Nat Rev Urol 2014, 11(7): 383-90.
8. Micic S and Ilic V. Incidence of neoplasm in vesical diverticula. 1983, J of Urology, vol 129: 734-735.

9. Melekos MD, Asbach HW, Barbalias GA. Vesical diverticula: etiology, diagnosis, tumorigenesis, and treatment. Analysis of 74 cases. Urology. 1987; 30: 453-457.
10. Golijanin D, Yossepowitch O, Bek SD, Sogani P, Dalbagni G. Carcinoma in a bladder diverticulum: presentation and treatment outcome. J Urol 2003; 170(5): 1761-1764.
11. Garzotto MG, Tewari A and Wajsman Z. Multimodal therapy for neoplasms arising from a vesical diverticulum. J Surg Oncology 1996, vol62 (1): 56-48.
12. Hu B, Satkunasivam R, Schuckman A, Miranda G, Cai J, Daneshmand S. Urotelial cancer in bladder diverticula: outcomes after radical cystectomy World J Urology 2014. DOI 10.1007/s00345-014-1472-5
13. Voskuilen CS, Seiler R, Rink M, Poyet C, Noon A, Roghmann F, Necchi A, Aziz A, Lavolle A et al . Urothelial carcinoma in bladder diverticula: multicentric analysis of characteristics and clinical outcomes. European Urology Focus, 2020; 6: 1226-1232.

doi: 10.20903/zpopov-2022-0005 UDK: 616.71-033.2-08:616.6-006.6

Ѓорѓе ЗАФИРОСКИ[1], Живко ПОПОВ[2]

НОВИНИ ВО ЛЕКУВАЊЕТО НА КОСКЕНИТЕ МЕТАСТАЗИ КАЈ УРОЛОШКИТЕ МАЛИГНИТЕТИ

Апстракт

Коските се најчеста локализација при метастазирање на уролошките малигнитети. Коскените метастази кај уролошките неоплазми често се придружени со силно изразен морбидитет, болка и нарушување на функцијата на зафатениот дел. Навремената дијагноза и правилното лекување со новите протоколи може да го намалат морбидитетот, да го подобрат квалитетот на животот и да го продолжат преживувањето. Цел на трудот е да се анализираат пациентите со уролошки малигнитети и со скелетни метастази и да се прикажат резултатите од новините во лекувањето на коскените метастази кај уролошките малигнитети. Кај коскените метастази од хормонорезистентен карцином на простата или од уротелиален карцином на везика уринарија, третманот со золендронична киселина или деносумаб може да ги намали компликациите од скелетните проблеми. Кај пациентите со скелетни метастази од карцином на везика уринарија, таквиот третман може да го продолжи и преживувањето. Кај коскените метастази од карцином на бубрег и кај герм-клеточните тумори, лекувањето со бисфосфонати или со деносумаб не дава толку добри резултати. Кај герм-клеточните тумори, коскените метастази може да се лекуваат со хемотерапија со цисплатин. Солитарните коскени метастази од уролошките малигнитети може да се лекуваат со хируршка ресекција на метастазата и ендопротетско заместување. Овие скелетни метастази може да се лекуваат и со радиотерапија или стереотактичка радио-

[1] Болница „Неуромедика“ - Скопје; Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, РС Македонија

[2] Македонска академија на науките и уметностите - Скопје; Медицински факултет, Универзитет „Св.Кирил и Методиј“, Скопје, РС Македонија

терапија, особено кај скелетни метастази од карциоком на бубрег кои, често, при хируршките ресекции се придружени со масивни крвавења. Во секој случај, коскените метастази кај уролошките малигнитети треба да се лекуваат со золендронична киселина или со деносумаб, а потоа да се одлучи дали ќе се лекуваат со радиотерапија или со хируршка ресекција. Со примената на новите методи на лекување кај голем процент од пациентите со коскени метастази од уролошки малигнитети се постигнува подобар квалитет на живот и подолго преживување.

Клучни зборови: коскени метастази, уролошки малигнитети, хируршка ресекција, радиотерапија, золендронична киселина, деносумаб.

Вовед

Уролошките малигнитети на простатата, бубрезите и везика уринарија почесто се придружени со коскени метастази. Околу една третина од пациентите со карцином на бубрег и везика уринарија имаат коскени метастази (1).

Голем дел од пациентите со хормонално резистентен карцином на простата развиваат скелетни метастази (1, 2). Инциденцата на проблеми со скелетот кај уролошките малигнитети може да надмине и 50 % од случаите. Во последниве дваесетина години постојат неколку нови опции за лекување на пациентите со метастази на коските кај уролошките малигнитети (1, 2, 5, 8, 9, 11). Во трудов се изнесени пристапи во лекувањето на одделни форми на уролошките малигнитети и метастази во коските. При болни коскени метастази се применува палијативна радиотерапија, дадена во различни шеми на фракционирање. Во такви случаи може да се контролира болката во околу 30 % од случаите (5, 6, 7). Кај примената на стереотактичната радиотерапија, искуствата се ограничени. Кај метастазите на коска од карцином на простата, болката може да се контролира ефективно, како и појавата на патолошка фрактура. Нема сигурни докази дека во такви случаи радиотерапијата или стереотактичната радиотерапија на коскените метастази може да го продолжи преживувањето (1, 6, 7).

Кај карцином на бубрег, коските се честа метастатска локализација со честота од 24 % до 51 %. Скенот на коските со Тц 99 м често дава псевдонегативна појава на сликите. Повеќето коскени метастази

се агресивни, остеолитични. Коскените метастази кај карцином на бубрег, според Зекри (Zekri) и соработниците, во 71 % од случаите се остеолитични, во 18 % од случаите се остеобласни и кај 11 % од случаите се мешани (1). Агресивните остеолитични метатази често доведуваат до тешки морбидитети како резултат од компликациите настанати во врска со скелетот. Хиперкалцемијата и патолошките фрактури создаваат сериозни проблеми кај пациентите.

Уротелијалните карциноми се претставени со најчестиот карцином на везика уринарија, следен со карцином на бубрегот, со карличен карцином и уретеријален карцином. 70 % од карциномите на везика уринарија се суперфицијални и може да се менацираат со трансуретрална резекција, 60-70 % рецидивираат, а 20-30 % прогресираат во повисок степен на малигност. Радикалната цистектомија е стандардна и ефикасна опција за лекување на операбилните карциноми на везика уринарија. При поставувањето на дијагнозата на карцином на везика уринарија, кај 20 % од случаите има висцерални метастази. Хемотерапијата е честа терапевтска опција при присуството на метастази. Коските се најчесто застапени метастази кај карцином на везика уринарија. Лоша прогноза имаат пациентите со карцином на везика уринарија кои се резистентни на конвенционалната хемотерапија. Потребни се нови терапевтски опции кај напреднати случаи на карцином на везика уринарија.

Карциномот на простата дава голем процент на метастази во коските. Дури во иницијалната фаза, околу 25 % од пациентите имаат скелетни метастази. Коскените метастази се откриваат со скен на коските со Тц 99 м. Околу 90 % од хормонално резистентните карциноми на простата развиваат коскени метастази. Присуството или отсуството на коскени метастази е еден од поважните фактори кој влијае на прогнозата и на видот на селекцијата на терапевтскиот пристап. Уште во 2004 г., трајалите покажале дека доцетакселот го подобрува преживувањето кај хормонорезистентните карциноми на простатата. Исто така, золендроничната киселина ги редуцира скелетните компликации. Комбинацијата на доцетаксел, зомета и преднизолон е главен третман кај хормонорезистентните карциноми на простатата со коскени метастази. Лекувањето на карциномот на простата со андрогени депривациски лекови доведува до коскена загуба. Ваквата терапија ја зголемува коскената ресорпција во случаите по простатектомијата или по радиотерапијата, ја зголемува телесната тежина, доведува до анемија, исхемија на срце, до остеопороза, коскени фрактури. Овие ком-

пликации доведуваат и до намалување на преживувањето кај карцином на простата. Познавајќи ги овие факти, потребно е да се превенираат скелетните компликации и другите проблеми. Евидентно е дека пациентите со карцином на простата и со скелетни метастази имаат значително подолго преживување во споредба со другите карциноми. Поради тоа, во менаџментот на коскените метастази, важна улога има нивното лекување, како и лекувањето на многубројните скелетни проблеми. Во последниве неколку години, воведен е и нов протокол на лекување со моноклонални антитела – деносумаб. Деносумабот дава подобри резултати во компарација со золендроничната киселина.

Милер (Miller) и соработниците покажале потенцијална клиничка корист од деносумаб со доцетаксел, при што доаѓа до намалување на коскената ресорпција и до забележливо зголемување на шансите за преживување (11).

Герм сел (Germ cell) се тумори со интермедијарна прогноза – семиноми, и со лоша прогноза, кои не се семиноми. Кај оваа група карциноми, коскените метастази се ретки и се појавуваат во околу 0,5 % од случаите, и нема специјални препораки за нивниот менаџмент.

Карциномите на супрареналната жлезда се ретки и ретко даваат коскени метастази.

Карциномот на пенисот ретко дава коскени метастази и нивното лекување е слично како и кај сите коскени метастази.

Материјали и методи

Во периодот од 2000 до 2019 година, анализирани се 75 пациенти со хистолошки верифициран уролошки канцер . Од 75 пациенти, кај 34 (45,3 %) се детектирани коскени метастази. Следењето на болните се одвиваше во период од 6 месеци до 10 и повеќе години (просечно, 41 месец). Уролошките карциноми се поделени во стадиуми: прв стадиум, локализиран во зафатениот орган; втор стадиум, регионален, каде што се зафатени и крвните садови и блиските лимфни жлезди; и трет стадиум, со оддалечени метастази во коските или во другите висцерални органи. Евалуирани се повеќе варијабли како што се: видот на карциномот, стадиумот на болеста, т. е. присуството или

отсуството на скелетни метастази, дали се солитарни или мултипни и дали се присутни и висцерални метастази, и преживувањето, користејќи го Каплан-Мејеровиот (Kaplan-Meier) метод. За детекција на коскените метастази, користени се следните имеџинг-техники: нативна радиографија, компјутерска томографија (КТ), сцинтиграфија со Тц 99м, а по потреба и магнетна резонанса (МР) и позитрон-емисиона томографија (ПЕТ-КТ), како и лабораториски испитувања: крвна слика, калциум, фосфор, алкална фосфатаза, ПСА и специфични тумормаркери. Одлуката за видот на лекувањето се одредуваше индивидуално, во зависност од видот на малигномот, стадиумот на болеста, локализацијата на коскената метастаза, возраста на пациентот и можностите за најсоодветен третман.

РЕЗУЛТАТИ

Анализирани се 75 пациенти со уролошки малигнитети, од кои 34 беа со коскени метастази (45,3 %) и пристапите во нивното лекување, како и резултатите од преживувањето. Најчести знаци кај пациентите со коскени метастази беа болка, оток и нарушена функција на зафатената локализација. За поставување дијагноза, применети се стандардните методи.

Табела 1 Видови уролошки малигни тумори – (75)

[Вид карцином]	[број]	[проценти]	[коскени метастази]	број [проценти]
Карцином на простата	43	58,40 %	21	48,8 %
Карцином на бубрег	12	16,43 %	5	33,3 %
Карцином на везика ур.	8	10,95 %	4	37,5 %
Карцином на тестиси	6	8,21 %	1	16,6 %
Карцином на супрарен. жл.	4	2,73 %	2	50 %
Карцином на пенис	2	2,73 %	1	50 %
Вкупно	**75**	**100 %**	**34**	**45,3 %**

Табела 2 Сензитивност и специфичност на различни имејџинг-техники користени за детекција на коскени метастази

Вид техника	КТ	МР	ПЕТ-КТ	Сцинтиграфија со Тц 99м
Сензитивност %	73	91	90	86
Специфичност	95	95	97	81

Од вкупно 75 пациенти со уролошки малигнитети, 34 (45,3 %) имаат коскени метастази, а од нив 22 (64,7 %) пациенти имаат мултипни коскени метастази и други висцерални метастази, додека 12 (35,3 %) имаат солитарни коскени метастази. Пациентите со солитарни коскени метастази, во компарација со оние со мултипни коскени метастази и со висцерални метастази, имаа подолг период на преживување ($p< 0.001$).

Табела 3 Лекување на пациентите со коскени метастази кај уролошки малигнитети

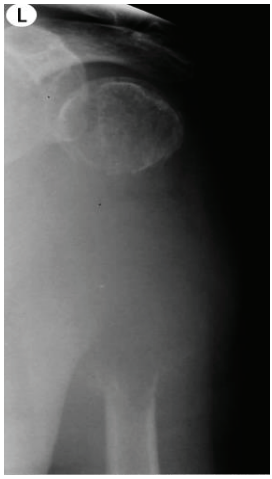
Вид лекување	број на пациенти	проценти
Хормонотерапија	21	61,7 %
Золендронична киселина	27	79,4 %
Деносумаб	9	26,5 %
Оперативно лекување	8	23,5 %
Радиотерапија	11	32,3 %

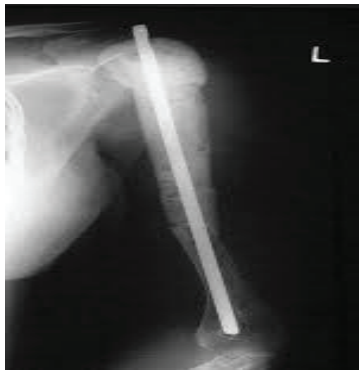
- Еден пациент може да биде лекуван со повеќе видови терапија.

Табела 4 Преживување на пациенти со уролошки малигнитети и коскени метастази

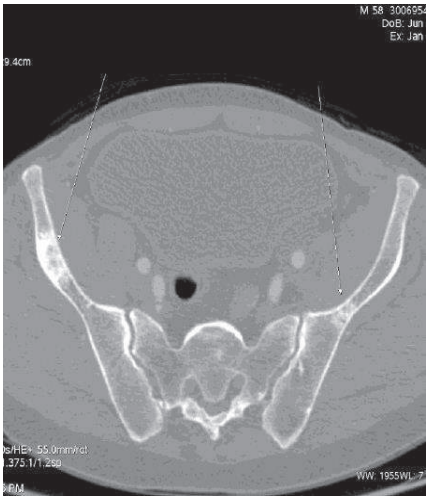
Вид малигнитет	[1 г. преживување]	[5 г. преживување]	[10 г. преживување]
Простата (21)	15 (71,4 %)	5 (23,8 %)	3 (14,3 %)
Бубрег (5)	4 (80 %)	2 (40 %)	2 (40 %)
В. урин (4)	3 (75 %)	1 (25 %)	0
Тестис (1)	1 (100 %)	1 (100 %)	0
Надб. жл (2)	2 (100 %)	1 (50 %)	1 (50 %)
Пенис (1)	1 (100 %)	0	0
Вкупно 34	26 (76,5 %)	10 (29,4 %)	6 (17,6 %)

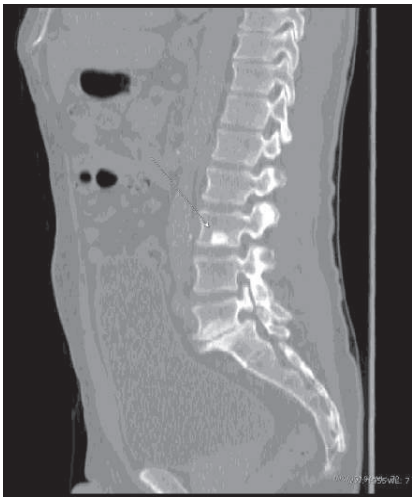
Нашата серија брои 75 пациенти со уролошки малигнитети, од кои 34 имаат коскени метастази. Кај 22 пациенти регистрирани се мултипни коскени метастази и висцерални метастази, а кај 12-мина, солитарни коскени метастази види слика 1а и 1б и слика 2а и 2б).





Слика 1а: Солитарна метастаза на лев хумерус од карцином на бубрег.
Слика 1б: Метастаза на лев хумерус, третирана со ресекција и реконструкција.





Слика 2а: Мултипни метастази на карлица од карцином на простата.
Слика 2б: Мултипни метастази на 'рбетен столб од карцином на простата.

Нашата серија на уролошки малигнитети и коскени метастази е мала и не би можело да се донесат релевантни податоци за веродостојноста на должината на преживување. Потребна е поголема серија на болни и обработка на многу варијабли за да се добие подобра слика за преживување кај пациентите со уролошки малигнитети и коскени метастази. Сепак, охрабрува тоа што има значаен процент на петгодишно и десетгодишно преживување кај овие пациенти. Имајќи ја предвид и понапреднатата возраст на пациентите, резултатите се уште попредизвикувачки. Затоа новините во лекувањето на пациентите со уролошки малигнитети и коскени метастази треба да се применуваат и да се изнаоѓаат нови пристапи за подолго преживување на пациентите.

Дискусија

Коскените метастази се јавуваат во разни фази на болеста кај пациентите со уролошки малигнитети и претставуваат честа појава. Новините во лекувањето на коскените метастази го подобруваат

квалитетот на животот и го продолжуваат преживувањето кај одредени форми на уролошки малигнитети. Пред повеќе од 100 години Стивен Пеџет (Stephen Paget) опишал „семе и почва“, при што метастатските канцер-клетки се опишани како семе, а тие ја преферираат „почвата“ што ја претставува коскениот матрикс. Коската е одлична средина за метастатските клетки со многу видови фактори за раст. Кога остеокластите ја разградуваат коската, се ослободуваат фактори за раст, што создаваат плодна почва за растеж на канцер-клетките. Метастатските клетки директно не ги активираат остеокластите. Тие ги активираат стромалните клетки преку паратиреоидните хормонски рецептори во остеокласната диференцијација и активација (1, 2, 3). Тие ја зголемуваат продукцијата на РАНКЛ, кои имаат централна улога во остеокласната диференцијација и активација. РАНКЛ е протеин на клеточната мембрана, го афицира имунолошкиот систем, контролира коскена регенерација и ремоделација и претставува регулаторен ген на апоптозата (1, 2). Во нашата серија на коскени метастази кај уролошките малигнитети се забележуваат случаи со долгорочно преживување, по 10-15 години од откривањето на скелетните метастази со примена на бисфосфонати, моноклонални антитела, радиотерапија или оперативно лекување, слично како и во други објавени студии. Во одреден број случаи, прво се откриени скелетните метастази, а потоа и примарниот уролошки малигнитет. Золендроничната киселина има инхибиторна улога за коскената ресорпција, што доведува до статистичка редукција на скелетните компликации (1, 2, 10, 11). Познато е дека карциномот на бубрег има најосетливи коскени метастази при инхибиција на коскената ресорпција. Деносумабот ја инхибира матурацијата на остеокластите, нивното врзување за РАНКЛ, но има и директен антиканцероген ефект (1, 2, 3, 4, 8, 10, 11). Денес, различни водичи не даваат специјални препораки за терапија на солитарните коскени метастази кај карцином на простата. Хируршката ресекција на коскената метастаза и ендопротетското заместување или фиксирањето со остеосинтетски материјал кај пациенти со карцином на бубрег или на простата се препорачуваат во индивидуални, селектирани случаи и го подобруваат квалитетот на животот (9). За процена на вредноста на хируршките интервенции потребни се поголема серија на случаи и подолго време на опсервација. Слични резултати се реферираат и кај коскените метастази од карцином на везика уринарија при третман со радиотерапија,

золендронична киселина, со деносумаб или оперативна ресекција, или со комбинација на овие методи (4, 5, 6, 7). Треба да се има предвид дека при примената на золендроничната киселина или деносумабот, потребни се следење на калциумот поради опасност од хиперкалцемија и превенција од остеонекроза на вилицата, со примена на Д-витамин и на препарати на калциум (8). Сè уште не е познато дали радиотерапијата го продолжува животот кај пациенти со коскени метастази од карцином на простата (5). Наспроти тоа, золендроничната киселина или деносумабот покажува дека ги контролира болките во коските и ги редуцира скелетните компликации од нив (2, 3, 4). Што се однесува на коскените метастази кај герм-цел туморите и кај туморите на надбубрежната жлезда, кои се сигнификантно поретки, би требало тие индивидуално да се третираат, како и другите уролошки малигнитети.

При одлука за хируршка интервенција кај коскените метастази од уролошките малигнитети, потребни се индивидуална процена и преземање нови превентивни процедури како што се: емболизацијата и балон-техниката за да се намали губењето крв за време на хируршката интервенција, што во одредени случаи може да биде сериозно.

Новините во лекувањето на скелетните метастази кај пациентите со уролошки малигнитети даваат можност за нивно подобро менаџирање со сите расположливи средства.

ЗАКЛУЧОК

Коскените метастази кај уролошките малигнитети се чести. Кај пациентите со уролошки малигнитети и коскени метастази се применува лекување со золендронична киселина или деносумаб, кој(а) може да ја контролира болката и да го превенира понатамошно ширење на метастазите, како и редуцирањето на скелетните компликации. Во отсуство на одговор на оваа терапија, се применува и радиотерапија со хемотерапија, или стереотактична радиотерапија, која би имала слични ефекти. Кај солитарните коскени метастази, по добра подготовка и анализа може да се примени и хируршка ресекција со ендопротетска замена или друг вид остеосинтетска фиксација. Герм-цел туморите поретко даваат коскени метастази. Кај нив се применува хемотерапија со цисплатин, и доколку е потребно, се постапува по истите протоколи

како и кај коскените метастази од другите видови уролошки малигнитети. Примената на новите методи во лекувањето на коскените метастази кај пациентите со уролошки малигнитети ги контролира болките и го подобрува квалитетот на животот, а не ретко доведува и до повеќегодишно преживување. За дефинитивни заклучоци, потребни се поголема серија пациенти и подолг временски период на нивно следење.

РЕФЕРЕНЦИ

1. Froehner, M., Holscher, T., Hakenberg, O.W. et al. (2014). *Treatment of Bone Metastases in Urologic Malignancies*. Urol Int; 93: 249–256.
2. Poon, M., Zeng, I., Lam, H. et al. (2013). *Incidence of Skeletal-Related Events Over Time From Solid Tumour Bone Metastases Reported in Randomized Trials Using Bone-Modifying Agents*. Clin Oncol (R Coll Radiol); 25: 435–444.
3. Sayrol, P. J., Armstrong, A. J., Fizzazi, K., Freendland, S. et al. (2013). *New and emerging therapies for bone metastases in genitourinary cancers*. Eur Urol, 63: 309–320.
4. Smith, M. R., Saad, F., Coleman, R. et al. (2012). *Denosumab and Bone-Metastasis Free Survival in Man with Castration – Resistant Prostate Cancer: Results of Phase 3, Randomised, Placebo-Controlled Trial*. Lancet; 379: 39–46.
5. Lutz, S., Berk, l., Chang, E. et al. (2011). *American Society for Radiation Oncology (ASTRO): Paliative Radiotherapy for Bone Metastases: an ASTRO evidence-based guideline*. Int Radiat Oncol Biol Phys; 79: 965–976.
6. Chow, E., Van der Lynden, Y. M., Roos, D. et al. (2014). *Single Versus Multiple Fractions of Repeat Radiation for Painful Bone Metastases: a randomized controlled,non inferiority trial*. Lancet Oncol; 15: 164–171.
7. Tree, A. C., Khoo, V. S., Eeles, R. A. et al. (2013). *Stereotactic Body Radiotherapy for Oligometastases*. Lancet Oncol; 14: 28–37.
8. Saad, F., Gleason, D. M., Murray, R. et al. (2002). *Zolendronic Acid Prostate Cancer Study Group: A randomized, placebo-controlled trials of zolendronic acid in patients with hormone-refractory metastatic prostate carcinoma*. J Natl Cancer Inst; 94: 1458–1468.
9. Fuchs, B., Trousdale, R. T., Rock, M. G. (2005). *Solitary Bony Metastasis from Renal Cell Carcinoma: significance of surgical treatment*. Clin Orthop Relat Res; 431: 187–192.

10. McKay, R. R., Lin, X., Perkins, J. J. et al. (2014). *Prognostic significance of Bone Metastases and Bisphosphonate Therapy in Patient with Renal Cell Carcinoma*. Eur Urol; 66(3)502–509.
11. Wirth, M., Tammela, T., Cicalelese, V. et al. (2014). P*revention of bone metastases in patients with high-risk nonmetastatic prostate cancer treated with zolendronic acid: efficacy and safety results of the Zometa European Study (ZEUS)*. Eur Urol, Epub ahead of print.

Gjorgje ZAFIROSKI, Zivko POPOV

NOVELTIES IN THE TREATMENT OF BONE METASTASES IN UROLOGICAL MALIGNITIES

Abstract

The bones are the most common location of metastatic urological malignancy. Bone metastases in urological neoplasms are often accompanied by severe morbidity, pain, and dysfunction of the affected area. Early diagnosis and proper treatment with new protocols can reduce morbidity, improve quality of life and prolong survival. The aim of the paper is to analyze patients with urological malignancies and skeletal metastases and to present the results of innovations in the treatment of bone metastases in urological malignancies. In bone metastases from hormone-resistant prostate cancer or urothelial carcinoma of the urinary bladder, treatment with zoledronic acid or denosumab may reduce complications of skeletal related problems. In patients with skeletal metastases from bladder carcinoma, such treatment may prolong survival. In bone metastases from renal cell carcinoma and germ cell tumors, treatment with bisphosphonates or denosumab does not work as well. In germ cell tumors, bone metastases can be treated with cisplatin chemotherapy. Solitary bone metastases from urological malignancies can be treated with surgical resection of the metastasis and reconstruction of the defect. These skeletal metastases can also be treated with radiotherapy or stereotactic radiotherapy, especially in skeletal metastases from renal cell carcinoma, which are often accompanied by massive bleeding during surgical resections. In any case, bone metastases in urological malignancies should be treated with zoledronic acid or deno-

sumab, and then it should be decided whether they will be treated with radiotherapy or surgical resection. The application of new methods of treatment in a large percentage of patients with bone metastases from urological malignancies achieves a better quality of life and improved survival.

Keywords: bone metastases, Urologic malignancies, surgical resection, radiotherapy, zolendronic acid, denusimab.

doi: 10.20903/zpopov-2022-0006 UDK: 616.65-006.6-037

Slobodan RISTOVSKI[1]

PROSTATE CANCER AFTER SURGERY FOR BENIGN PROSTATIC HYPERPLASIA

Abstract

Objective: To determine the incidence and characteristic of prostate cancer in patients with previous BPH surgery.

Materials and Method: In a retrospective study between 2002 and 2015, we analyzed patients who developed prostate cancer after surgery for BPH. Patients were examined by age, prostate volume, IPSS score, type and duration of the drug therapy, PSA values before and three months after surgery, and the type of BPH surgery. In patients with prostate cancer, we estimated the time between BPH surgery and the occurring of cancer, Gleason score, TNM stage, type of therapy and survival. Follow-up for BPH patients was 3 months and for the prostate cancer (PCa) group five years. Cox regression was used to determine the influence of various variables on the incidence of prostate cancer after BPH surgery.

Results: Incidence of prostate cancer was 1.69% (9 of 532 BPH surgeries) and was diagnosed significantly ($p<0.001$) more in patients who underwent open prostatectomy versus TURP. The mean time between BPH surgery and diagnosis of prostatic cancer was 7.2 years and did not correlate with the investigated parameters. The value of IPSS in the BPH group was significantly higher compared to before PCa surgery ($p=0.012$). In the PCa group, PSA values decreased from 2.30±0.83 to 0.95±0.38ng/ml after three months and in the BPH group from 1.98±0.84 to 0.54±0.33ng/ml. PSA

[1] University Surgical Clinic “St. Naum Ohridski”, Urology Dept., Skopje, RN Macedonia

reduction rate for the PCa group was 58.4±11.6% versus 70.7±0.58% in the BPH group. In the Age-adjusted analysis, the PSA reduction rate was 0.050 (0.001-0.937) HR (CI). In the PCa group, the serum PSA levels were increased by 6.5 times (mean 14.97ng/ml) (p=0.001) compared to the BPH group. Before BPH surgery, the mean prostate volume was 60, 4 ccm, 5.3 ccm greater than in the cancer group. Two PCa patients had bone metastases. Radical prostatectomy was performed in 5 cases and four were treated with LHRH agonists and antiandrogens. One died three years after PCa diagnosis. Conclusions: PSA reduction rate was a borderline significant predictor of prostate cancer after BPH surgery.

Keywords: benign prostate hyperplasia, prostate cancer, TURP, open prostatectomy, PSA reduction rate.

INTRODUCTION

There is controversy over whether benign prostatic hyperplasia or BPH surgery increases the risk of prostate cancer. Prostate cancer and benign prostatic hyperplasia are the two most common urological diseases in older men. Prostate cancer is the second most commonly diagnosed cancer in men, accounting for 15% of all cancers diagnosed (1). Otherwise, BPH is present in 70% of men over 70 years, and of all diagnosed prostate cancers, 80% of them had at the same time and BPH. Clinical BPH is present over 50% in men over 80 years old. The incidence of prostate cancer increases about 15 years later than with BPH. In both diseases, the incidence and mortality increase with age (1-3). There exists a worldwide difference in incidence between developed countries in Europe and America and Asia and the African continent (4,5). Both conditions are associated with epidemiological, hormonal, anatomical factors as well as the impact of inflammation, metabolic syndrome, and genetic alterations (6,7,8,9).

As anatomical connections, most cancers originate in the peripheral zone while BPH usually develops in the transition zone of the prostate (8,9). Well known 5ARI therapy for BPH reduced the relative risk of prostate cancer by detecting prostate cancer by 25% over 7 years and reducing prostate size as well as reducing the incidence of low-risk cancer (10-13). The ratio of estrogens to androgens increases by 40% in older men and this

may affect the natural course of BPH and CaP (14). Asians often have a diet containing phytoestrogens, which impact the lower prevalence of BPH and CaP compared to a Western diet (15).

Inflammation is associated with a 7.7-fold higher incidence of BPH. A fast-growing prostate may be a risk factor in developing prostate cancer. Several studies suggest an association between BPH and prostate cancer with certain genetic aberrations (16).

Large epidemiological and randomized controlled trials indicate a higher incidence of prostate cancer in patients who previously had BPH surgery. No causal link has been established between BPH surgery and the incidence of prostate cancer (17, 18).

MATERIALS AND METHODS

In a retrospective study in the period of 2002 to 2015, we analyzed the number and characteristics of prostate cancers that occurred in the group of 532 patients who had previous BPH surgery. Patients who underwent surgery for BPH had follow up meetings for three months. Before surgery, the patient's PSA values were determined, as well as prostate volume, IPSS score, and type and duration of previous drug treatment. At this point, the PSA value was controlled. Patients with an elevated PSA at above 4 ng/ml had a prostate biopsy, and in case of any positive findings, they were excluded from the study as well as all patients who presented T1a-b stage on histopathological finding.

Patients who developed prostate cancer were analyzed for PSA values, prostate volume, IPSS score, type of BPH surgery, period between BPH surgery and prostate cancer diagnosis, TNM stage, Gleason score, as well as the type of therapy and survival. Patients with PCa had follow-up meetings for a period of 5 years. A statistical analysis of all examined parameters was performed in patients with prostate cancer as well as determining any correlation with the parameters in the period of BPH surgery.

Statistical Analysis: Comparisons between the normally distributed variables were made with an independent Student t-test. If a non-normally distributed variable was involved in the comparison then non-parametric methods were used. For comparisons of non-numeric variables, the Chi-

squared test was used. To determine the relationship between numeric variables, Pearson's correlation was used. Cox regression was used to determine the influence of various variables on the incidence of prostatic cancer after BPH surgery. Hazard Ratios are given with 95% confidence intervals. We used SPSS statistical software (version 22.0 IBM, Armonk, NY, USA), and a two-tailed $p < 0.05$ was considered significant. Data are shown as mean ± standard deviation unless specified otherwise.

RESULTS

Between 2002 and 2015, 532 patients underwent BPH surgery. Transurethral resection of the prostate (TURP) was performed in 476 patients (89.5%) and open prostatectomy (OP) in 56 (10.5%) cases. In a period of 3.1 to 12.4 years after BPH surgery, nine patients (1.69%) were diagnosed with prostate cancer (PCa), six in the group with TURP (1.26%), and three (5.35%) in the group with open prostatectomy (p=0.001). Table 1.

Twenty-two patients with clinical BPH were excluded from the study because of a finding of incidental, T1a, T1b prostate carcinoma.

Table 1
Types of Benign Prostatic Hyperplasia Surgery done 2002-2015

	BPH surgery (%)	TURP(%)	OP(%)
BPH(n,%)	532 (100)	476 (89,5)	56 (10,5)
PCa(n,%)	9 (1.69)	6 (1.26)	3 (5.36) p=0.001

BPH- benign prostatic hyperplasia, TURP- transurethral resection of the prostate, OP- open prostatectomy,

In twenty-nine patients who had questionable digito-rectal examination results and PSA values, prostate biopsies were done. In three of them, prostatic carcinoma was diagnosed and they were excluded from the study.

The time from BPH surgery to the diagnosis of prostate cancer ranged from 37 to 149 months, with an average of 92.8 months (7.7 years).

The first two patients with prostate cancer were detected in 2011, followed by two in 2013, two in 2014, and three in 2015.

The average age in the PCa group was 74.4±1.68 (66-82) years, which is 7.2 years higher than the age in the BPH group. The age at the time of BPH operation correlates with the age at the time of prostatic cancer operation (R=0.770; p=0.015). The IPSS score before the BPH surgery was 29.4±0.74 (25-33) points. The IPSS score after cancer diagnosis was 22.9±2.1 (12-33) points, which is 6.5 points lower than in the BPH group (p=0.012). The mean prostate volume before BPH surgery was 60.4 ccm (26-92 ccm) and in the PCa group, 55.1 ccm (27-97), which greater in volume by 5.3 ccm than in the cancer group. Table 2.

Table 2
Prostate cancer group: clinical parameters

PCa N0	PCa age	PCa: year of diagnosis	Time from BPH surgery to PCa diagnosis months (years)	IPSS-BPH	IPSS before PCa diagnosis	Type of BPH surgery	PVol-BPH (ccm)	PVol-PCa (ccm)
1	74	2013	121 (10.1)	33	29	OP	59	45
2	82	2014	149 (12.4)	29	33	TURP	35	67
3	77	2015	91 (7.6)	30	23	OP	90	97
4	66	2015	87 (7.2)	28	23	TURP	37	32
5	76	2014	59 (4.9)	31	20	TURP	68	27
6	71	2011	80 (6.7)	29	12	OP	92	55
7	76	2015	85 (7.1)	25	22	TURP	26	60
8*	79	2011	126 (10.5)	29	17	TURP	85	35
9	69	2013	37 (3.1)	31	27	TURP	52	78
Mean +/-SD	74.4± 1.68		92.8±11.5 (7.7±0.95)	29.4± 0.74	22.9±2.10		60.4±8.32	55.1±7.71

PCa- prostate cancer, BPH- Benign prostatic hyperplasia, IPSS- The International Prostate Symptom Score, TURP- Transurethral resection of prostate, OP- open prostatectomy, PVol-prostate volume, * - death

The PSA value in the cancer group ranged from 6.9 to 45.9 ng/ml, a mean of 15.0 ng/ml before the prostatic biopsy. The PSA level before BPH surgery was 2.3 ng/ml (1.1 to 3.4), and after three months those values were at 0.3 to 1.6 ng/ml with a mean of 0.94 ng/ml. In the cancer group, the PSA levels were 6.5 times higher (p=0.001) compared to at the time of BPH surgery. PSA before BPH surgery correlated with PSA 3 months after BPH surgery (R=0.816; p=0.007). PSA before BPH surgery was significantly higher than 3 months postoperatively (p=0.001). PSA 3 months after BPH surgery was significantly lower than before prostatic biopsy (p=0.009).

All the patients in the cancer group had enlarged prostate volumes, significantly raised levels of PSA, and elevated IPSS score.

Five out of nine patients with prostatic cancer used alpha-blockers as monotherapy for BPH and 4 patients used combination therapy with alpha-blockers (AB) and 5 alpha-reductase inhibitors (5ARi). The mean duration of medical therapy before BPH surgery was 17.2 (3-35) months. A longer duration of medical therapy was associated with a lower IPSS before BPH surgery (R=0.757; p=0.018). There was no correlation found between alpha-blockers and combination therapy, duration of its usage, and the appearance of prostate cancer.

Regarding the Gleason score, six patients had 3+3 (International Society of Urological Pathology- (ISUP) 1), and three of them had a score of 3+4 (ISUP 2). Three out of four patients with 5ARi therapy had a Gleason score of ISUP1 and one of ISUP-2. Seven patients were in the T2N0M0 stage, and two with metastatic disease in the T2NXM1b, and T3NXM1b stage.

Radical prostatectomy was performed in four patients and the other five were treated with hormonal therapy (LHRH agonists and antiandrogens). The average age in the PCa group who underwent radical prostatectomy was 70.5 years (66-76), which is lower by 7.1 years in comparison to the PCa group treated with hormonal therapy, 77.6 years (74-82). One patient died 3 years after the diagnosis of prostate cancer was established, at the age of 82, after he was treated with LHRH antagonists.

The patients in the prostate cancer group had a significantly higher PSA 3 months after BPH surgery than patients in which cancer was not diagnosed (p= 0.014). PSA reduction rate was significantly lower in patients in which prostatic cancer was diagnosed after BPH surgery than in those in the BPH group (p=0.032). Table 3.

Table 3

Prostate cancer group: clinical parameters PSA, MT, BPH surgery. Gleason score, PCa therapy TM stage

PCa No	PSA before BPH surgery	PSA 3 months after BPH surgery	PSA before prostate biopsy	Type of MT before BPH surgery	Type of BPH surgery	Gleason score)	Treatment of PCa	TNM stage
1	2.1	0.6	9.1	AB+5ARi	OP	3+3	HT	T2NxMx
2	2.7	1.1	19.4	AB	TURP	3+3	HT	T2N0M0
3	1.3	0.8	45.9	AB	OP	3+4	HT	T3NxM1b
4	1.8	0.9	7.8	AB	TURP	3+4	RP	T2N0M0
5	3.1	1.3	13.7	AB+5ARi	TURP	3+4	RP	T2 NoM0
6	2.1	1.0	10.2	AB+5ARi	OP	3+3	RP	NoM0
7	1.1	0.3	7.4	AB	TURP	3+3	HT	T2NxMx
8	3.4	1.6	6.9	AB	TURP	3+3	HT	T2NxM1b
9	3.1	0.9	14.3	AB+5ARi	TURP	3+3	RP	T2NxMo
Mean±SD	2.3±0.27	0.9±0.13	15.0±4.1					

PCa –Prostate cancer, BPH-Benign Prostatic Hyperplasia, IPSS-The International Prostate Symptom Score, PSA-Prostate Specific Antigen AB-alpha blocker, OP-Open Prostatectomy; TURP- Transurethral Resection of Prostate, MT-Medical Therapy, AB- alfa blocker, 5ARi- 5 alfa reductase inhibitor, HT- hormonal therapy, RP- radical prostatectomy

In the Cox regression analysis of the predictors of cancer incidence in patients after BPH surgery, only the PSA reduction rate was a borderline predictor in the unadjusted analysis. In the PCa group, PSA values decreased from 2.30±0.83 to 0.95±0.38 ng/ml after three months and in BPH group from 1.98±0.84 to 0.54±0.33 ng/ml. PSA reduction rate was 58.4±11.6% for the PCa group compared with 70.7±0.58% in the BPH group.

In the Age-adjusted analysis for HR (CI), the PSA reduction rate was 0.050 (0.001-0.937) and this was the sole predictor of prostate cancer incidence after benign prostate surgery (p=0.048). Table 4.

Table 4
Cox regression analysis of predictors of prostatic cancer after benign prostatic surgery

Variable	**Unadjusted Analysis HR (CI)**	**p-value**	**Age Adjusted Analysis HR (CI)**	**p-value**
BPH Surgery Age	0.980 (0.851-1.129)	0.777	0.980 (0.851-1.129)	0.777
BPH-IPSS	0.924 (0.987-1.301)	0.924	0.908 (0.744-1.300)	0.908
Prostate Volume (ccm)	1.011 (0.980-1.044)	0.484	1.013 (0.979-1.048)	0.452
PSA prior to BPH surgery (ng/ml)	1.045 (0.419-2.607)	0.925	1.123 (0.406-3.111)	0.823
PSA 3months postop (ng/ml)	3.842 (0.632-23.35)	0.144	4.797 (0.736-31.25)	*0.101*
PSA reduction rate (%)	0.055(0.002-1.697)	*0.097*	0.050 (0.001-0.937)	0.048
AB+5ARi	4.432 (0.532-36.91)	0.169	4.435 (0.533-36.93)	0.168
AB	0.226 (0.027-1.879)	0.169	0.225 (0.027-1.877)	0.168
BPH surgery:TURP	0.540 (0.121-2.417)	0.420	0.524 (0.116-2.373)	0.401
BPH surgery: OP	1.851 (0.414-8.284)	0.420	1.901 (0.421-8.658)	0.401

BPH-Benign Prostatic Hyperplasia; IPSS- International Prostatic Symptom Score; PSA-Prostatic Specific Antigen; AB-Alpha blocker, 5ARi-5 alpha reductase inhibitors, TURP-Transurethral Resection of Prostate; OP-Open Prostatectomy;

DISCUSSION

The main finding in our study was a low incidence and low mortality of prostate cancer after BPH surgery. We also found that a PSA reduction rate, three months after BPH surgery, could be a predictor of prostate cancer.

Only 1.69% of patients with previous BPH surgery developed prostate cancer. Patients with prostate cancer who were treated with TURP compared to open prostatectomy (OP) had a 4.2-fold lower incidence. A Swedish study by Chokkalingam et al. reported an incidence rate twice as high (2.96%) for prostate cancer after BPH surgery in a group of 1748 patients who were treated with TURP and 824 treated with open prostatectomy. They reported two times more patients with TURP than OP but most of them underwent surgery before the 1980s, when TURP was routinely used (17).

A Danish study by Ørsted et al. observed an incidence rate 3.48% in PCa patients with 77,698 men who received surgical BPH treatment, and Kanno et al., from Japan, presented a similar incidence rate of 3.2% of patients with prostate cancer over 1 to 7 years in a cohort of 407 patients with TURP (18, 19). Carlson et al., with a cohort of 7,901 patients with previous TURP (1982-1997), reports an increased standardized incidence ratio (SIR) for prostate cancer [1.26, CI 95% (1.17–1.35)] but no increased standardized mortality ratio (SMR), [0.59, CI 95% (0.47–0.73)](20). By contrast, Ørsted et al. found that clinical BPH was associated with a two- to three-fold increased risk of PCa incidence and a two- to eight-fold increased risk of PCa mortality. The authors emphasized that this data should not be used to infer causality. The studies of Kanno, Karlsson, and Ørsted presented an incidence rate twice as high of ours. Armenian et al. also observed an increased risk of prostate cancer incidence after BPH surgery (21).

But studies by Greenwald et al. on 800 men with BPH and Simons et al. on sample size on 4,800 men with BPH, did not find an association between BPH and an increase risk of prostate cancer (22, 23).

Ørsted et al. reported the median age at PCa diagnosis was 72 yr. for PCa patients and 75 yr. for BPH patients. This was 2.2 years and eight years higher than in our study, respectively.

We reported a period of 7.7 years from BPH surgery to the diagnosis of prostate cancer. In Wolff, study time of appearance of all cancer cases was up to 7 years (24). Chokkalingam et al. found that patients with TURP developed prostate cancer after 6.5 years and patients with open prostatectomy, one year later. Ørsted et al. presented the median time to diagnosis of PCa after surgery for clinical BPH at 3 years (range: 0-27yr). In Tanaka, 7 of 319 cases of prostatic cancer had previous BPH surgery 22 months to 15 years prior (25).

Hua L, in a Chinese study from 2004, analyzed twelve cases of prostate cancer after BPH surgery which appeared after 10 months to 14 years, with the average at 5-6 years.

We did not find a significant difference in the time of occurrence of prostate cancer in TURP and OP subgroups (7.5 years versus 8.1 years). In a study from Japan by Kanno et al., from 1995 to 2003, 13 (3.2%) of 407 patients, all with TURP, developed prostate cancer over 1 to 7 years. Kato et al. presented case of prostate cancer fourteen months after open prostatectomy in 1996 (26).

All studies showed a period of diagnosis of prostate cancer after BPH surgery from 10 months to 27 years.

Regarding the IPSS score, our PCa patients were severely symptomatic before the diagnosis of prostate cancer. Hua L et al. reported mild to heavy symptoms according to IPSS (21).

We presented reduction values of PSA after three months at 0.94 ng/ml, which was a higher percentage of the reduction compared with the study by Wolff et al., where PSA was reduced from 6.8 ng/ml to 2.2 ng/ml after 48 months. We found that PSA levels were 6.5 times higher compared to the time of BPH surgery. In the Tanaka study all prostate cancer cases presented a significant elevation of the PSA (6.4-399 ng/ml) at the time of cancer diagnosis.

An important finding was a PSA reduction rate of 58.4% 3 months after BPH surgery compared to 70.7% in patients with BPH surgery who did not develop prostate cancer ($p = 0032$). This was similar to Wolff's findings (24).

According to TNM classification and Gleason score, our patients were at a low stage and grade but two had a metastatic disease. Kanno's findings show that 6 of 13 patients were moderately differentiated. The other 6 had poorly differentiated cancer and one had a ductal carcinoma of

the prostate. Hua L described that of twelve cases, 3 were at the T2 stage, 3 at T3, and six had metastasis (21).

In our study, one patient died of prostate cancer with bone metastases. In Hua L's study, 3 of 12 patients died with a metastatic disease.

The limits of our investigation include the small number of patients with prostate cancer and the short follow-up period of patients with BPH surgery. Our study was done in the PSA era, while large epidemiological studies were from the pre-PSA era.

CONCLUSION

BPH surgery did not increase risk of prostate cancer. PSA reduction rate was the sole predictor of prostate cancer incidence after benign prostate surgery.

BIBLIOGRAPHY

1. Ferlay, J., et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 2015. 136: E359. https://pubmed.ncbi.nlm.nih.gov/25220842
2. Colonna M, Danzon A, Delafosse P, et al. Cancer prevalence in France: time trend, situation in 2002 and extrapolation to 2012. Eur J Cancer 2008; 44: 115–22.
3. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. Ann Oncol 2007; 18: 581–92.
4. Gades NM, Jacobson DJ, Girman CJ, Roberts RO, Lieber MM, Jacobsen SJ. Prevalence of conditions potentially associated with lower urinary tract symptoms in men. BJU Int 2005; 95: 549–53.
5. Homma, Y., Kawabe, K., Tsukamoto, T., Yamanaka, H., Okada, K., Okajima, E., … Aso, Y. (1997). Epidemiologic Survey of Lower Urinary Tract Symptoms in Asia and Australia Using the International Prostate Symptom Score. International Journal of Urology, 4(1), 40–46. doi:10.1111/j.1442-2042.1997.tb00138.x
6. Andersson, S.-O., Rashidkhani, B., Karlberg, L., Wolk, A., & Johansson, J.-E. (2004). Prevalence of lower urinary tract symptoms in men aged 45-

79 years: a population-based study of 40 000 Swedish men. BJU International, 94(3), 327–331. doi:10.1111/j.1464-410x.2004.04930.
7. Antonio Alcaraz, P. H. (April 2009). Is There Evidence of a Relationship between Benign Prostatic Hyperplasia and Prostate Cancer? Findings of a Literature Review. European Urology, Volume 55 Issue 4, 864-875.
8. Nickel JC, Roehrborn CG, O'Leary MP, Bostwick DG, Somerville MC, Rittmaster RS. The relationship between prostate inflammation and lower urinary tract symptoms: examination of baseline data from the REDUCE trial. Eur Urol 2008; 54: 1379–84.
9. Ozden C, Ozdal OL, Urgancioglu G, Koyuncu H, Gokkaya S, Memis A. The correlation between metabolic syndrome and prostatic growth in patients with benign prostatic hyperplasia. Eur Urol 2007; 51: 199–206.
10. Bostwick DG, Burke HB, Djakiew D, et al. Human prostate cancer risk factors. Cancer 2004; 101(Suppl 10): 2371–490.
11. McVary KT. BPH: epidemiology and comorbidities. Am J Manag Care 2006; 12: S122–8.
12. Debruyne F, Barkin J, van Erps P, Reis M, Tammela TL, Roehrborn C, on behalf of the ARIA3001, ARIA3002 and ARIB3003 Study Investigators. Efficacy and safety of long term treatment with the dual 5-a-reductase inhibitor Dutasteride in men with symptomatic benign prostatic hyperplasia. Eur Urol 2004; 46: 488–95.
13. McConnell JD, Bruskewitz R, Walsh P, et al. The effect of finasteride on the risk of acute urinary retention and the need for surgical treatment among men with benign prostatic hyperplasia. N Engl J Med 1998; 338: 557–63.
14. Andriole G, Brawley O, Tammela T, Rittmaster R. Baseline characteristics of patients in the REDUCE chemoprevention study. Eur Urol Suppl 2005; 4(3): 184.
15. Sim HG, Cheng CW. Changing demography of prostate cancer in Asia. Eur J Cancer 2005; 41: 834–45.
16. Randazzo, M., et al. A positive family history as a risk factor for prostate cancer in a population-based study with organized prostate-specific antigen screening: results of the Swiss European Randomized Study of Screening for Prostate Cancer (ERSPC, Aarau). BJU Int, 2016. 117: 576. https://pubmed.ncbi.nlm.nih.gov/26332304)
17. Chokkalingam, A. P. (2003). Prostate carcinoma risk subsequent to diagnosis of benign prostatic hyperplasia: a population-based cohort study in Sweden. Cancer 98, 1727-1734 (. Cancer, 98, 1727-1734.
18. Orsted DD, Bojesen SE, Nielsen SF, Nordestgaard BG. Association of clinical benign prostate hyperplasia with prostate cancer incidence and mortality revisited: A nationwide cohort study of 3,009,258 men. EurUrol. 2011; 60: 691

"https://www.ncbi.nlm.nih.gov/pubmed/21705134" "https://scholar.google.com/scholar_lookup?journal=EurUrol&title=Association+of+clinical+benign+prostate+hyperplasia+with+prostate+cancer+incidence+and+mortality+revisited:+A+nationwide+cohort+study+of+3,009,258+men&author=DD+Orsted&author=SE+Bojesen&author=SF+Nielsen&author=BG+Nordestgaard&volume=60&publication_year=2011&pages=691-8&" \t "_blank" Google Scholar]

19. Karlsson CT, W. F. (2011 Nov). Risk of Prostate Cancer after Trans Urethral Resection of BPH: A Cohort and Nested Case-Control Study. . Cancers (Basel), 8; 3(4): 4127-38.
20. Kanno H, U. S. (2006 May;). Prostate cancer development after transurethral resection of the prostate- histopathological studies of radical prostatectomy specimens. Nihon Hinyokika Gakkai Zasshi., 97(4): 649-59.
21. Hua L, Z. J. (2004 Aug;). Prostate cancer after prostatectomy for benign prostatic hyperplasia. Zhonghua Nan Ke Xue,, 10(8): 612-613.
22. Buckley BS, L. M. (2011 Nov;). Risk of prostate cancer associated with benign prostate disease: a primary care case-control study, Br J Gen Pract., 61(592): e684-91.
23. Schenk JM, K. A. (2011 Jun). Association of symptomatic benign prostatic hyperplasia and prostate cancer: results from the prostate cancer prevention trial. Am J Epidemiol., 173(12): 1419-28.
24. "https://pubmed.ncbi.nlm.nih.gov/?term=Wolff+JM&cauthor_id=11326651" J M Wolff , "https://pubmed.ncbi.nlm.nih.gov/?term=Boekels+O&cauthor_id=11326651" O Boekels, "https://pubmed.ncbi.nlm.nih.gov/?term=Borchers+H&cauthor_id=11326651" H Borchers , "https://pubmed.ncbi.nlm.nih.gov/?term=Jakse+G&cauthor_id=11326651" G Jakse , "https://pubmed.ncbi.nlm.nih.gov/?term=Rohde+D&cauthor_id=11326651" D Rohde Altered prostate specific antigen reference range after transurethral resection of the prostate Anticancer Res Nov-Dec 2000; 20(6D): 4977-80.
25. Tanaka Y, A. H. (2001 Jan;). Prostatic cancer developing after transurethral resection of the prostate for benign prostatic hyperplasia]. Hinyokika Kiyo., 47(1): 11-4.]
26. Kato Y, N. S. (1996 Nov;). A case of prostate cancer diagnosed one and half year after retropubic prostatectomy for benign prostatic hypertrophy]. Hinyokika Kiyo., 42(11): 907-9.

doi: 10.20903/zpopov-2022-0007 UDK: 616.45-006-079.4(497.7)"2015/2020"

Brankica KRSTEVSKA[1], Zivko POPOV[2]

DIAGNOSIS AND MANAGEMENT OF PATIENTS WITH ADRENAL INCIDENTALOMAS: OUR EXPERIENCE

Abstract

Introduction: Adrenal incidentalomas (AIs) are adrenal masses which are incidentally discovered with imaging technology. They are non-functional and functional, benign, and malignant lesions.

Aim: We present a 5-year study of diagnostic and follow up of adrenal incidentalomas with special emphasis on their hormonal activity and differentiation from benign to malignant masses.

Material and methods: A group of 26 patients with AIs were investigated at the Internal Medical Centre "Srce". In all patients, clinical examination, CT, and hormonal tests were performed.

Results: Of the 26 patients (10 male and 16 female), aged between 25 and 80, the median tumour size was 33 mm, unilateral AIs in 96.1%, located on the right side in 52%, 96% were benign masses, of which 81% were non-functional adrenal masses. Non-functional asymptomatic autonomous cortisol secretion tumours were 22.2%, 7.7% were pheochromocytoma, 3.8% was Cushing's syndrome, adrenocortical carcinoma in 3.8%, and Schwannoma in 3.8%. Statistically significant positive correlation was found between HTA and secreting adrenal tumours ($r=0.426$, $p<0.05$), and between the post- suppressive level of cortisol and the size of adrenal tumour ($r= 0.560$, $p<0.05$). Age, symptoms and DMT2 have statistically significant impact on the prediction of

[1] Internal Medicine Centre "Srce", Skopje; Medical Faculty, University "SS Cyrill and Methodius", Skopje, North Macedonia

[2] Macedonian Academy of Science and Arts, Skopje; Medical Faculty, University "SS Cyrill and Methodius", Skopje, North Macedonia

tumour masses larger then 4 cm ($p<0.05$). 53.8% of patients underwent surgery and the others are followed.

In this small study of 26 cases with AIs, 11.5% were benign hyper-secreting tumours, and only one patient (3.8%) had a malignant tumour. Appearance of symptoms, age, or tumour masses larger than 4 cm are more likely to be secreting or malignant tumours.

Keywords: adrenal incidentalomas, pheochromocytoma, "autonomous cortisol secretion", tumour size.

Introduction

Adrenal incidentalomas (AIs) are adrenal masses, generally 1 cm or more in diameter, discovered incidentally with radiological examination, performed for other medical indication than adrenal diseases [1]. With modern radiological imaging techniques such as abdominal ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI), this entity appears more frequently in clinical practice and causes more clinical dilemmas [2]. The prevalence of AIs on autopsy series was reported to be about 8%, and 4% on the radiological series with a tendency to increase in the future [3, 4]. The prevalence rises with ageing. AI affects about 0.2% of patients between 20 and 30 years of age, while it affects 7% of patients over 70 years of age. This entity is a more common disease between the ages of 50 and 70 years mainly in patients with diabetes mellitus type 2 (DMT2), hypertension (HTA), and obesity. In an ageing society, this problem will become a more frequent risk in diagnosis and management.

AIs are benign or malignant and non-functional or functional tumours. The differential diagnosis includes a non-functioning adrenal adenoma, functional tumours (Cushing's syndrome, pheochromocytoma, primary aldosteronoma), primary adrenocortical carcinomas, metastases, and other adrenal masses (myelolipoma, cyst, Schwannoma, ganglioneuroma) [5]. They should be managed based on the functional status and the potential for malignancy. Primary adrenocortical carcinomas are rare, but metastases from extra adrenal carcinomas (lung, breast, colon, melanoma, lymphoma) are more common. Some of these lesions may be identified easily, but it is

difficult to distinguish adenoma from carcinoma. In the past two decades, the growing literature of data has allowed for recommendations of diagnosis and for decisions to be developed, such as how to treat AIs, based on clinical, hormonal and radiographic testing [1, 7]. The tumour size and radiological appearances are two key predictors for malignant AIs. Adrenal masses ≥ 4 cm or radiologically suspicious AIs are recommended for removal with total adrenalectomy. Functional tumours (Cushing's syndrome, pheochromocytoma, primary aldosteronoma) need urgent therapy. Non-functional adrenal incidentalomas may be asymptomatic hypersecreting adenoma known as autonomous cortisol secretion (subclinical Cushing's syndrome) with a high risk for morbidity and for surgery [7, 8]. Small and non-functional ones predominate in AIs and they should be followed-up.

Material and methods

In this retrospective study, conducted from September 2015 to September 2020, at the Internal Medicine Centre "Srce", Skopje, 26 patients were admitted with adrenal masses incidentally discovered via radiological imaging techniques. Evaluation of all patients with adrenal incidentaloma includes clinical, hormonal, and radiological testing.

Careful history was taken and physical examinations were performed which looked for the symptoms and signs of adrenal hormonal excess (Cushing's syndrome, pheochromocytoma, aldosteronism) and malignant disease in patients with AI.

Demographic characteristics of the patients were: age, sex, diameter and side of the lesion, endocrine function, BMI, type 2 diabetes mellitus, hypertension, histological findings from surgical adrenalectomy, or follow- up.

The following hormonal tests were performed: measurement of basal cortisol and basal adrenocorticotropic hormone (ACTH), in a fasting state at 8:00 AM. Out of 26 patients, 18 with AIs underwent a 1- mg dexamethasone suppressive test. The criteria for cortisol over secretion [1, 5] were: post-dexamethasone serum cortisol level at <50 nmol/l is considered “normal”; post-dexamethasone serum cortisol level between 51 to 138 nmol/l indicates “possible autonomous cortisol secretion” and a level of cortisol above 138

nmol/l suggests “autonomous cortisol secretion”. The basal level of ACTH < 10 pg/ml confirms an “autonomous cortisol secretion”.

A urine collection after 24 hours for metanephrine and vanillyl-mandelic acid (VMA) was used for the evaluation of adrenal medulla.

CT, as the radiological method, was used primarily in detecting adrenal lesions and in distinguishing benign from malignant lesions.

Laparoscopic adrenalectomy was indicated in adrenal lesions > 4 cm with radiological characteristics consistent to malignancy, functional tumours and autonomous cortisol secretion.

The study was done according to the Helsinki Declaration.

Statistical Analysis.

Data are presented as average, median and percentages. The results were analysed with SPSS, version 21. Pearson correlation tests and the general linear model were used. The level of significance was set to $p<0.05$.

Results

Our study included 26 patients (16 female and 10 male), at a mean age of 54.5 ± 15.7 (range, 25-80) years (Table 1). Six patients were younger than 40, and 20 were older than 40 years. The number of patients with adrenal masses according to age was: 2 patients between 20 and 29 years; 4 patients between 30 and 39 years; 5 patients between 40 and 49; 4 patients between 50 and 59; and 11 patients were >60 years or older (Figure 1).

Table 1.

Demographic, clinical, hormonal, and radiological characteristics of patients with adrenal incidentalomas

No of patients	26
mean age (years)	54.5±15.7
female/male	16/10 (1.6)
Tumor size (median, mm)	33
Unilateral	25/26 (96.1%)
right	13/25 (52%)

left	12/25 (48%)
Bilateral	1/26 (3.8%)
cortisol (nmol/l)	449.8±155.5
1mg-DST (without suppression, cortisol>138nmol/l)	4/18 (22,2%)
1mg-DST (suppression between 50−138nmol/l)	5/18 (27.7%)
1mg-DST (suppression <50nmol/l)	9/18 (50%)
ACTH (pg/ml)	23.3±16.3
VMA (median, umol/dU)	31
metanephric (median, umol/dU)	2.15
BMI (kg/m2)	26.59±3,89
BMI>25kg.m2	15/26 (57%)
DMT2	6/26 (23.06%)
HTA	12/26 (46.1%)
with symptoms	20/26 (76.9%)
operat.	14/26 (53.8%)
Tu secreting Cushing	1/26 (3.8%)
Pheochromocytoma	2/26 (7.7%)
malignant (adenocarcinoma)	1/26 (3.8%)
incident of death during 5 years	2/26 (7.7%)

Presented results are averages ±SD and percentages. Abbreviations: 1mg-DST = 1mg dexamethasone suppressive test; ACTH=adrenocorticotropic hormone VMA=Vanillylmandelic acid BMI=body mass index; DMT2=diabetes mellitud type 2; HTA= arterial hypertension.

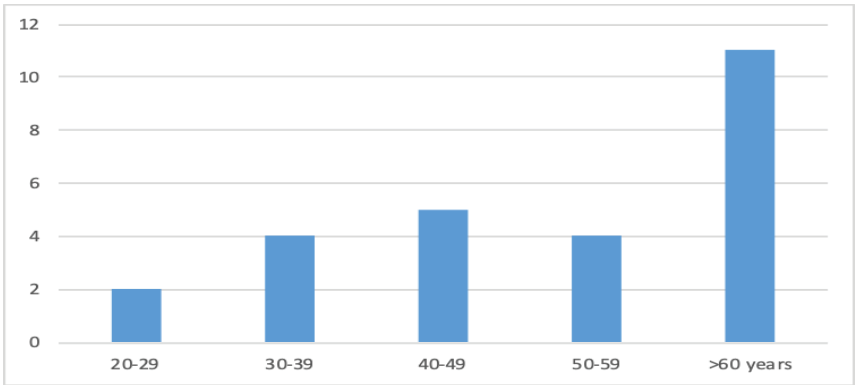


Figure 1 – Number of patients with adrenal masses according to age

Most of the patients had unilateral adrenal incidentaloma (96.1%) and only one patient (3.8%) had bilateral adrenal incidentalomas. Thirteen patients (52%) had adrenal incidentalomas located on the right side, 11 (42.2%) on the left side, and 1 had (3.8%) bilateral incidentalomas. The median size of the tumour was 33 mm (range 1-12cm). Fourteen patients had adrenal masses smaller than 40 mm, and twelve larger than 40 mm (Figure 2).

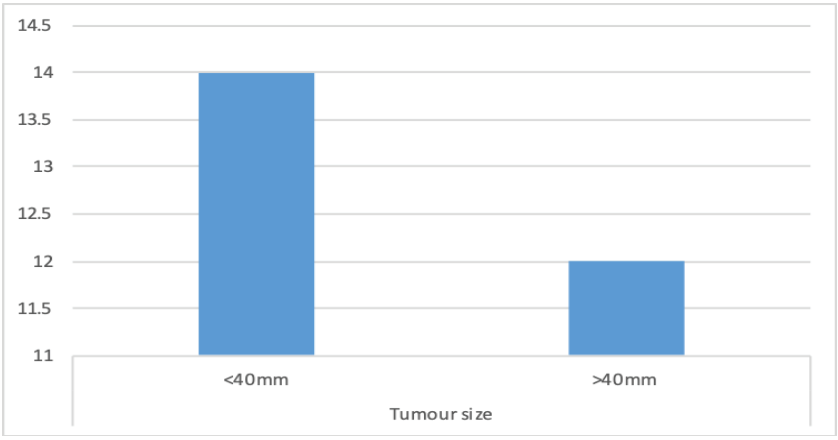


Figure 2 – Number of patients with adrenal masses according tumour size

Non-functional adrenal masses were found in 81%. The frequency of apparent functional tumours (Cushing's syndrome and pheochromocytoma) was 11% (Figure 3). Autonomous cortisol secretion was diagnosed in 22.2%

asymptomatic patients with non-functional adenoma, while in 27.7% they were diagnosed as probable autonomous cortisol secretion (Figure 4).

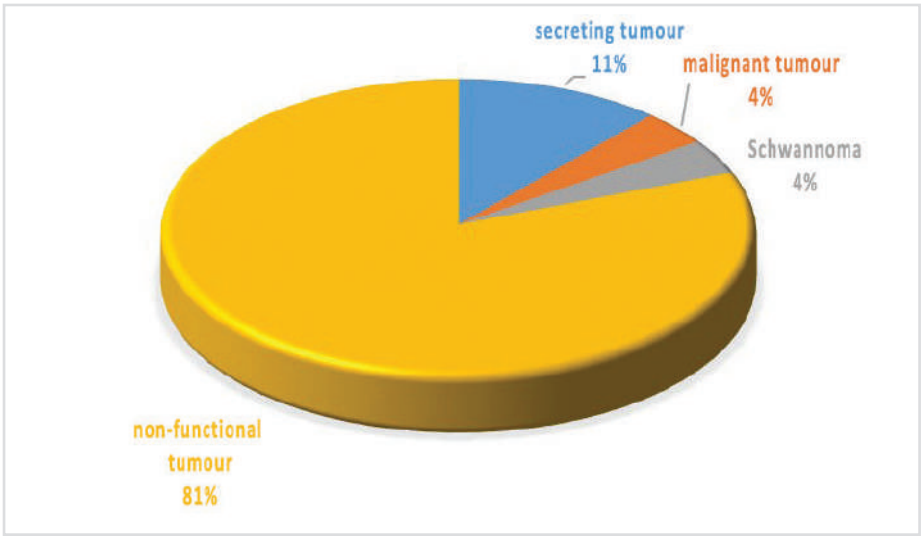


Figure 3 – Type of adrenal tumours

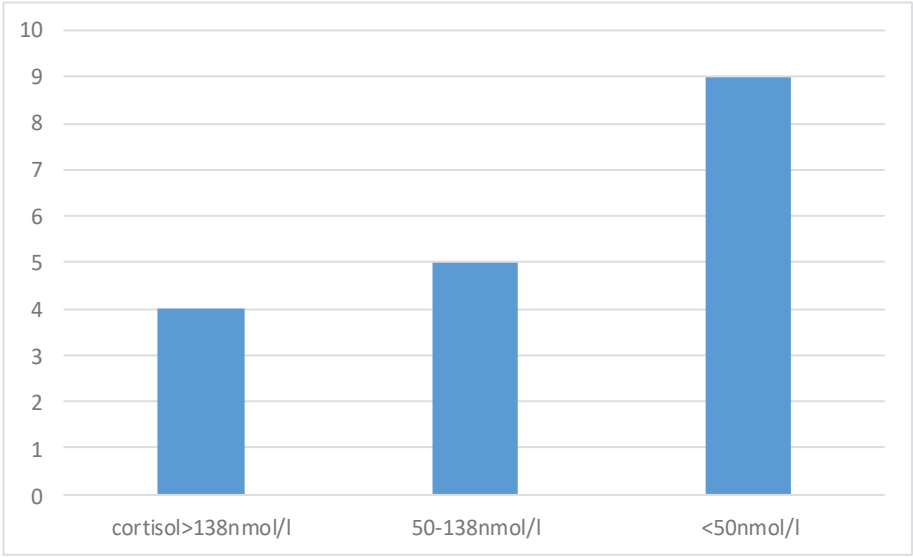


Figure 4 – Number of patients according the results of cortisol after low dose dexamethasone test

One patient (3.8%) had primary adrenocortical carcinoma with the diameter of 8.2 cm, confirmed via radiological characteristics and a histopathologic diagnosis. He died 5 months after surgery from local and distinct metastases.

One young patient (3.8%) had a large tumour 12 cm in diameter with radiological characteristics for adrenal malignant lesion, but histological diagnosis confirmed Schwannoma.

23.06% of the patients with AIs had type 2 diabetes, hypertension in 46.1%, overweigh/obesity in 57%, and other different symptoms in 76% (Table 1).

Statistically significant positive correlation was found between HTA and secreting adrenal tumours ($r=0.426$, $p<0.05$). HTA and age have significant impact on secreting adrenal tumours, as a dependent variable ($p<0.05$) (Table 2).

Table 2

Effect of patients' characteristics on secreting adrenal tumours
Dependent Variable: secreting adrenal tumors

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	2,608[a]	9	,290	3,537	,012
age	**,974**	**4**	**,243**	**2,972**	**,049**
sex	,259	1	,259	3,165	,093
Simpt.	,193	1	,193	2,353	,143
DMT2	,145	1	,145	1,774	,200
HTA	**,465**	**1**	**,465**	**5,675**	**,029**
Error	1,392	17	,082		
Total	4,000	26			

a. R Squared = ,652 (Adjusted R Squared = ,468)
Abbreviations:DMT2= diabetes mellitus type 2; HTA= arterial hypertension.

Age, symptoms, and DMT2 have a statistically significant impact on the appearance or prediction of adrenal masses larger than 4 cm ($p<0.05$) (Table 3).

Table 3

Effects of patients' characteristics on tumour size
Dependent Variable: tumour size

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	60,103[a]	9	6,678	59,836	,000
age	**3,383**	**4**	**,846**	**7,579**	**,001**
Simpt.	**,883**	**1**	**,883**	**7,908**	**,012**
DMT2	**,681**	**1**	**,681**	**6,103**	**,024**
HTA	,158	1	,158	1,414	,251
sex	,010	1	,010	,091	,766
Error	1,897	17	,112		
Total	62,000	26			

a. R Squared = ,969 (Adjusted R Squared = ,953)

Abbreviations: DMT2= diabetes mellitus type 2; HTA= arterial hypertension.

Of 26 patients, more than a half, 53.8%, underwent surgery, and the others are being followed-up with. The incidence of death within 5 years was 7.7%.

Discussion

Adrenal incidentaloma is a common endocrine disease. The prevalence in autopsy series is 8%, and on CT series more than 4% [1,3,4]. In patients younger than 30 years it is rare, with a prevalence of 0.2%. The prevalence increases with age and it is estimated to be more than 7% in patients of 70 years or older [5].

Regarding demographic characteristics, more AIs were found in patients older than 40 years and the peak of prevalence was above 60 years old. At 60 years old, the patients underwent imaging examinations for others reasons than adrenal diseases.

Furthermore, AIs are found predominantly more in females than in males. This population probably undergoes more physical exams for gastro-intestinal, kidney, and gynaecological diseases.

According to demographic characteristics, the data from a Korean Study, the COAR (Co-work of Adrenal Research), an [9] Italian study, [10] and ours show that the mean age of diagnosis of AIs is 55 years versus 58 and 54.5, respectively. In the Italian study and in ours, masses were commonly found in females, 58.16% versus 61.53%; the median size of tumours (computed tomography measurement) was 3.0 cm (range 0.5 to 25 cm) versus 3.3 cm (range from 1 to 12 cm). We accept a new recommendation [1] for defining AI if it is >1 cm in diameter. In the present study, unilateral lesions were detected in 96.1%, bilateral adrenal masses in 3.8%, and those located on the right side at 52%. Many studies, however, have reported that the left side location is a more frequent finding [11,12].

Evaluation of endocrine status of patients with adrenal incidentaloma is the cornerstone of management [1,5,7,13]. In general, every patient should be screened for glucocorticoid excess, and for adrenal catecholamine and mineralocorticoid excess in selected patients with hypertension and hypokalaemia [14].

Ten to 15% of AIs secreting hormones were in excess. In this study of 26 patients, 81% have non-functional adrenal masses, while 11% were functional; 7.7% were pheochromocytoma and 3.8% was Cushing's syndrome. Patients with symptoms and signs of hypercortisolism should be screened and diagnosed as soon as possible. We found only one patient with Cushing's syndrome who was operated by laparoscopic adrenalectomy. An untreated patient is at high risk for morbidity and mortality [15,16].

Among non-functional AIs asymptomatic patients could be found without the obvious stigmata of Cushing's syndrome, named subclinical Cushing's syndrome or autonomous cortisol secretion [1,7,16]. These patients have hypertension, type 2 diabetes mellitus, obesity, and osteoporosis, as a result of continuous endogenous exposure to cortisol secretion. In our study, autonomous cortisol secretion tumours were at 22.2%, using a 1-mg overnight dexamethasone suppressive test [1], which was performed in 18 out of 26 patients. Four patients with proven autonomous cortisol secretion were at the median age of 45.5 years and the median tumour size was 4.9 cm. We found a statistically significant positive correlation between the level of

cortisol after 1 mg DST and the adrenal tumour size ($r=0.560$, $p<0.05$). Also, there is a statistically significant positive correlation between HTA and secreting adrenal tumours ($r=0.426$, $p<0.05$). These results are supported by other studies that found the cortisol level of post 1- mg DST was an independent cardiovascular risk factor [18,19,20]. This data justifies our decision for surgery. Generally, age, degree of cortisol excess, general health, co-morbidities, and patients' preference should be considered when deciding for surgery [1,7]. Diabetes, hypertension and obesity were improved after surgery; similar data was reported in other studies [5].

Pheochromocytoma is a catecholamine secreting tumour that is found in 5% of adrenal incidentaloma [7,17]. In this study, 7.7% were pheochromocytoma. One patient has a silent pheochromocytoma without hypertension, elevated urinary metanephrine, and a tumour size of 7.7 cm. This was our criteria for surgery. The other young patient (33 years old) has hypertension, with normal urinary metanephrine, and a tumour size of 3.2 cm. Both cases show a different imaging phenotype on CT. Careful examination should be completed. If the results of 24-hour urinary catecholamine metabolites are normal, the measurement of fractionated plasma free metanephrines may be useful. Therefore every patient with AI should be screened for pheochromocytoma, even if the patient is normotensive or asymptomatic before surgery, because silent pheochromocytoma could be lethal [21].

Primary aldosteronism is 1% of adrenal incidentaloma [1]. We did not find any patients with aldosteronoma, but no renin/aldosterone ratio screening was performed since hypokalaemia was not reported in any of the patients.

Primary adrenocortical carcinoma cancer is rare, with an incidence of 1 to 2 cases per 1 million persons [21,22]. In this study, one patient (3.8%) had primary adrenocortical carcinoma with a diameter of 8.2 cm, confirmed on radiological characteristics and histological diagnosis. He died 5 months after surgery from local and distinct metastases. In the cohort of 2005 patients with adrenal incidentalomas, adrenocortical carcinoma was found in 4.7% [7]. The size and imaging characteristics are two major predictors for malignancy [23,24].

Bilateral adrenal masses are found in 15% of the patients with adrenal incidentalomas. In this study, 3.8% were bilateral incidentalomas. Differential diagnosis includes bilateral metastases, bilateral incidentaloma, congenital adrenal hyperplasia or infiltrative lesions. Patient follow-up is recommended, as it is in unilateral adrenal incidentaloma. If one of the adrenal masses grows more than 20% from the initial radiological measurement, surgery is indicated [7,25].

CT is used at the initial diagnosis in patients with adrenal masses. It is a useful tool in distinguishing benign from malignant lesions, but not the functionality of the tumour. In all AIs, a non-contrast (unenhanced) CT is recommended as the first line of investigation with the determination of HU. An adenoma with <10 HU is consistent in lipid-rich benign adenoma. A contrast-enhanced washout CT may be used as the next test for characterizing the adrenal incidentaloma. CT characteristics and tumour size were used in all patients in our study, as part of the strategy for surgery or follow-up.

According to European and other recommendations, surgical treatment is necessary in selected patients [1, 14, 25]. Laparoscopic adrenalectomy is the first line of treatment for patients with AI [26, 27]. In our study, 53.8% of patients underwent surgery, by an expert surgeon. The histopathological findings were: 3 functional adenoma, 3 autonomous cortical secretion, 1 adrenocortical carcinoma, 3 cysts, 1 Schwannoma, and 3 adrenocortical adenomas.

About 46.2% of patients are sent for follow-up, without evidence of growth of the tumour or hyperfunctioning.

Conclusion

In this small study of 26 cases with adrenal incidentalomas, 11.5% were benign hypersecreting tumours and there was only one patient (3.8%) with a malignant tumour. Appearance of symptoms, age, or tumour masses larger than 4 cm are more likely to be secreting or malignant tumours. A lifelong follow-up for patients is needed in order to establish undiscovered cases of malignant or hypersecreting tumours among non-operated patients.

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Fassnacht M, Arlt W, Bancos I, et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol. 2016;175 (2):G1–G34.
2 Marty M, Gaye D, Perez P, et al. Diagnostic accuracy of computed tomography to identify adenomas and benign tumors amongst adrenal incidentalomas in an endocrinological population [published online ahead of print 21 February 2018]. Eur J Endocrinol. doi: 10.1530/EJE-17-1056.
3. Barzon I, Sonino N, Fallo F, et al. Prevalence and natural history of adrenal incidentalomas. European Journal of Endocrinology 2003, 149: 277-285.
4. Kobayashi S, Iwase H, Matsuo K, et al. Primary adrenocortical tumors in autopsy records--a survey of "Cumulative Reports in Japan" from 1973 to 1984. Jpn J Surg. 1991 Sep; 21(5):494-8.
5. Sherlock M, Scarsbook A, Abazz A, et al. Adrenal Incidentaloma. Endocrine Reviews , Volume 41, Issue 6, December 2020, Pages 775- 820.
6. Kloos RT, Gross MD, Francis IR, et al. Incidentally discovered adrenal masses. Endocr Rev. 1995; 16(4): 460–484
7. Young WF Jr. Clinical practice. The incidentally discovered adrenal mass. N Engl J Med. 2007; 356 (6): 601–610.
8. Nieman LK. Approach to the patient with an adrenal incidentaloma. J Clin Endocrinol Metab. 2010; 95(9): 4106–4113.
9. Ahn SH, Kim JH, Baek SH, et al. Characteristics of Adrenal Incidentalomas in a Large, Prospective Computed Tomography-Based Multicenter Study: The COAR Study in Korea. Yonsei Med J. 2018 Jun; 59(4): 501-510.
10. Mantero F, Terzolo M, Arnaldi G, et al. Study Group on Adrenal Tumors of the Italian Society of Endocrinology . A survey on adrenal incidentaloma in Italy. J Clin Endocrinol Metab. 2000; 85 (2): 637–644.
11. Hao M, Lopez D, Luque-Fernandez MA, et al. The lateralizing asymmetry of adrenal adenomas. Endocr Soc. 2018; 2(4): 374-385.
12. Reginelli A, Grezia DG, Izzo A, et al. Imaginig of adrenal incidentaloma: our experience. International Journal of Surgery 12 (2014)S 126 S131.
13. Vassilatou E, Vryonidou A, Michalopoulou S, et al. Hormonal activity of adrenal incidentalomas: results from a long-term follow-up study. Clin Endocrinol (Oxf). 2009; 70 (5): 674-679.

14. Kapoor A. Morris T, Rebello R. Guidelines for the management of incidentally discovered adrenal mass. Can Urol Assoc J 2011; 5(4): 241-7. DOI:10.5489/cuaj.11135

15.Giordano R, Marinazzo E, Berardelli R, et al. Long-term morphological, hormonal, and clinical follow-up in a single unit on 118 patients with adrenal incidentalomas. Eur J Endocrinol. 2010; 162 (4): 779-785

16. Debono M, Bradburn M, Bull M, et al. Cortisol as a marker for increased mortality in patients with incidental adrenocortical adenomas. J Clin Endocrinol Metab. 2014; 99 (12): 4462-4470.

17. Kokko JP, Brown TC, Berman MM. Adrenal adenoma and hypertension. Lancet. 1967; 1(7488): 468-70

18. Morelli V, Palmieri S, Lania A, et al. Cardiovascular events in patients with mild autonomous cortisol secretion: analysis with artificial neural networks. Eur J Endocrinol. 2017/; 177(1):73-83. doi: 10.1530/EJE-17-0047. Epub 2017 May 3.

19. Park J, De Luca A, Dutton H, et all: Cardiovascular outcomes in autonomous cortisol secretion and non-functioning adrenal adenoma: a systemic review. J Endocr Soc. 2019; 3(5): 996-1008.

20. Di Dalmazi G, Vicennati V, Garelli S, et al. Cardiovascular events and mortality in patients with adrenal incidentalomas that are either non-secreting or associated with intermediate phenotype or subclinical Cushing's syndrome: a 15-year retrospective study. Lancet Diabetes Endocrinol. 2014; 2(5): 396–405.

21. Cho YY, Suh S, Joung JY, et al. Clinical characteristics and follow-up of Korean patients with adrenal incidentalomas. Korean J Intern Med. 2013; 28 (5): 557-564.

22. Lenh B-V, Duc N-M, Thong P-M. Non-functioning adrenocortical carcinoma. Radiol Case Rep. 2020 Jun: 16(6) 1329-1334.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8049877/#

23. Kiselijak-Vasiliades K, Bancos I, Hamrahian A, et al. American Association of Clinical Endocrinology Disease State Clinical Review on the Evaluation and Management of Adrenocortical Carcinoma in an Adult: a Practical Approach. Endocr. Prac 2020; 26(11): 1366-1383.

24. Yener S, Ertilav S, Secil M, et al. Prospective evaluation of tumor size and hormonal status in adrenal incidentalomas. J Endocrinol Invest. 2010; 33 (1): 32-36

25. Terzolo M, Stigliano A, Chiodini I, et al. Italian Association of Clinical Endocrinologists AME position statement on adrenal incidentaloma. Eur J Endocrinol. 2011; 164 (6): 851-870.
26. Ballo A, Corallino D, Ortenzi M, et al. Cancer risk in adrenalectomy: are adrenal lesions equal or more than 4 cm a contraindication for laparoscopy? Surgical Endoscopy. Published online: 01 March 2021. https://doi.org/ 10.1007/s00464-021-08380-7
27. Popov Z, Jankulovski N, Stankov O, et all. Laparoscopic adrenalectomy: first single center experience in the Balkan. Prilozi. December 2015; 36 (3): 27-33.

Бранкица КРСТЕВСКА, Живко ПОПОВ

ДИЈАГНОЗА И МЕНАЏМЕНТ НА ПАЦИЕНТИ СО АДРЕНАЛНИ ИНЦИДЕНТАЛОМИ: НАШИ ИСКУСТВА

Резиме

Вовед. Инциденталомите на надбубрежните жлезди (АИ) се случајно откриени надбубрежни маси со имиџиг техниките. Тие се нефункционални или функционални, бенигни или малигни лезии.

Цел. Презентирање на 5-годишно искуство со дијагностицирање и следење на надбубрежните инциденталоми, со посебен акцент на нивната хормонална активност и диференцијација на бенигни од малигни маси.

Материјал и методи. Група од 26 пациенти беа вклучени во оваа ретроспективна студија спроведена во Центарот по интерна медицина „Срце“. Кај сите пациенти е извршен клинички преглед, КТ и хормонални тестови.

Резултати. Од 26 пациенти (10 мажи и 16 жени), на просечна возраст од 54,5 (опсег, од 25 до 80) години, со просечна големина на туморот од 33 mm, едностранн беа 96,1 %, и тоа на десната страна во 52 % од случаите. 96 % беа бенигни маси, од кои 81 % беа несекретирачки тумори. Нефункционална асимптоматска автономна секреција на кортизол имаше кај 22,2 %, 7,7% беа феохромоцитоми, 3,8 %

Кушингов синдром, адренокортикален карцином во 3,8 % и Шванома во 3,8 %. Пронајдена беше статистички значајна позитивна корелација помеѓу ХТА и секреторните надбубрежни тумори ($r = 0,426$, $p < 0,05$) и помеѓу пост-супресивното ниво на кортизол и големината на надбубрежниот тумор ($r = 0,560$, $p < 0,05$). Возраста, симптомите и DMT2 имаат статистички значително влијание на појавата на туморските маси поголеми од 4 см ($p < 0,05$). 53,8 % од пациентите беа оперирани, а другите се следеа.

Заклучок. Во оваа мала студија со 26 случаи со надбубрежни инцидентоломи, 11,5 % биле бенигни хиперсекретирачки тумори, а само еден пациент (3,8 %) имаше малиген тумор. Присуството на симптоми, возраста или туморски маси поголеми од 4 см имаат поголема веројатност да бидат сектеритачки или малигни тумори.

Клучни зборови: надбубрежни инциденталоми, феохромоцитом, „автономна секреција на кортизол“, големина на тумор.

doi: 10.20903/zpopov-2022-0008 UDK: 616.45-006-079.4(497.7)"2015/2020"

Viktor STANKOV[1], Sasho DOHCHEV[1], Sotir STAVRIDIS[1], Skender SAIDI[1], Ognen IVANOVSKI[1], Bashkim SHABANI[1], Dimitar TRAJKOVSKI[1], Aleksandar TRIFUNOVSKI[1], Slobodan GURMESHEVSKI[1], Asim MUSA[1], Oliver STANKOV[1], Nikola JANKULOVSKI[2], Zivko POPOV[1,3]

LAPAROSCOPIC SURGICAL TREATMENT FOR ADRENAL TUMORS, A RETROSPECTIVE ANALYSIS

Abstract

Objective. Laparoscopic adrenalectomy has rapidly replaced open adrenalectomy as the procedure of choice for benign adrenal tumors. The aim of this study was to evaluate the long-term results of 105 consecutive laparoscopic and open adrenalectomies performed during a period of 14.5 years at the University Clinic of Urology in Skopje. We aimed to present our experience with this procedure. In addition, we compare the clinical outcomes of laparoscopic (LA) vs. the open adrenalectomies (OA) performed at our institution.

Patients and methods: A retrospective analysis of patients operated on for adrenal tumors was conducted. From May 2005 to August 2020, one hundred adrenalectomies were performed on 105 patients since laparoscopic adrenal surgery was introduced in our country. There were 48 men and 57 women, aged 23 to 73 years. All patients were assessed regarding their demographic data, hormonal status, operative time, estimated blood loss,

[1] University Clinic of urology, Clinical Campus Mother Teresa, Skopje, N. Macedonia.

[2] University Clinic of digestive surgery, Cinical Campus Mother Teresa, Skopje, N. Macedonia.

[3] Macedonian Academy of Sciences and Arts, Skopje; Medical Faculty, University "SS Cyrill and Methodius", Skopje, North Macedonia.

complications, size of the tumor, number of patients requiring blood transfusion, hospital stay and conversion to open surgery for LA.

Results: In 93 patients, the laparoscopic procedure was completed successfully. In 12 cases, the laparoscopic procedure was converted to an open one. Operative time for laparoscopic adrenalectomies ranged from 45 to 120 minutes. The average postoperative hospital stays for laparoscopic adrenalectomy ranged from 1 to 2 days (1.5 days), versus 5 to 20 days for patients who underwent the open or converted procedure. LA proved superior to OA, resulting in less estimated blood loss, shorter operating time, shorter time to resumption of oral intake, shorter postoperative hospital stay and less analgesic requirements. During the follow-up of 3 to 96 months, no tumor recurrence and/or metastasis developed.

Conclusions: Laparoscopic adrenalectomy should be the treatment of choice for all benign adrenal tumors. Laparoscopic resection of large adrenal tumors necessitates experience in open and advanced laparoscopic surgery. Our results concur with other retrospective reviews comparing laparoscopic and open adrenalectomy, demonstrating unequivocal advantages in terms of reduced length of hospital stay, blood loss, return of bowel function, functional recovery, and post-operative morbidity

Keywords: Adrenal tumors, Laparoscopic adrenalectomy, Laparoscopy

Introduction

The surgical approach to the adrenal gland varies widely according to the size and the endocrine nature of the tumor, with the anterior transperitoneal, posterior and flank extraperitoneal and thoracoabdominal approaches being preferable in individual cases. The incision of open adrenalectomy is quite large, leading to significant postoperative pain that may necessitate the use of epidural catheters, with consequent increased wound morbidities, cosmetic defect, and longer hospital stay [1, 2]. Morbidity after open adrenalectomy has been reported to be as high as 40%, with the mortality rate in the range of 2% to 4% [1]. However, the small size of the adrenal gland, the benign nature of most adrenal tumors, and the difficulty of gaining access to the organ by open surgery, together with the improvement

of cross-sectional imaging (CT and MRI) to define the lesion preoperatively, make the laparoscopic approach particularly suitable for adrenalectomy [3].

Twenty two years ago, Gagner et al. [4] reported their first experience with the transperitoneal laparoscopic adrenalectomy (LA) in three cases of benign adrenal pathology. In the early 1990s, the indications for LA included small benign lesions [5], with larger lesions or phaeochromocytomas being approached very cautiously, with malignancy being considered an absolute contraindication. The indications included various pathological conditions, e.g. aldosterone-producing adenoma, Cushing's disease, nonfunctioning adenoma and other rare pathologies (adrenal cyst and myelolipoma). The groups in favor of laparoscopic surgery were gaining experience rapidly and many early contraindications (e.g. obesity, phaeochromocytoma, large adrenal lesions, previous abdominal surgery and malignancy) proved not to be absolute, but rather related to the team's surgical skills [6].

This type of operation is presently considered to be the ‘standard of care” for most adrenal diseases requiring surgery because of its functional efficacy and all the typical advantages inherent in minimally invasive surgery. More than 20 years after the initial description of this surgical procedure we present one of the first LA experiences in the Balkan region, with a particular focus on the indications, results, and complications. We compare these data with those from open surgeries performed in our Institution. In addition, we present a case of giant splenic artery aneurism mimicking adrenal tumor, treated successfully by the laparoscopic approach.

Patients and methods

During the study period, one hundred and five consecutive patients, aged from 23 to 73 (an average age of 55 years) underwent unilateral LA adrenalectomy in our institution, including 65 right and 40 left sided. Abdominal CT and ultrasound were the radiologic tools used for diagnosing the adrenal masses, unless otherwise indicated. There were 31 OA performed mostly in the period before LA was introduced. The LA procedures

were performed by a few surgeons at the University Clinic of Urology. All patients were assessed regarding their demographic data, complete endocrinological status, operative time, estimated blood loss, complications, size of the tumor, number of patients requiring blood transfusion, hospital stay, and conversion to open surgery for LA. The postoperative course was carefully reported, especially concerning the time of initial eating and ambulation, as well as any postoperative complications and the length of the hospital stay.

The transperitoneal approach for laparoscopic adrenalectomy was used in all cases of LA. The surgical technique was that described by Hamilton [7]. We routinely employ a 60° flank position, with the bed flexed, in order to elevate the surgical area and to widen the space between the costal arch and the iliac crest for adequate port placement. Figure 1 shows the exact placement of the trocars. We prefer this approach since the anatomic landmarks for a safe procedure are well visualized. Our primary target was early control of the main adrenal vein. This had two benefits: it avoided attacks of hypertension resulting from manipulation of the adrenal gland, and it facilitated complete mobilization of the gland, as the adrenal vein is the main structure anchoring the gland in place [2]. The exposure of the inferior vena cava (IVC) on the right side and the left renal vein on the left side was used for early control of the main adrenal vein. On the right side, after the liver has been lifted, the posterior peritoneum was incised to expose the inferior vena cava and the adrenal gland (Figure 2). Because of the small size of the adrenal vessels, vascular endoclips were sufficient for their control (Figure 3). The dissection progresses superomedially, laterally, posteriorly, and, finally, posteriorly. After the dissection of the adrenal mass was completed, it was entrapped in a plastic bag (Figure 4), then retrieved via an extended muscle-splitting portside incision. The left sided LA was performed mostly using 3 ports, whereas for the right-sided LA we used 4 ports.

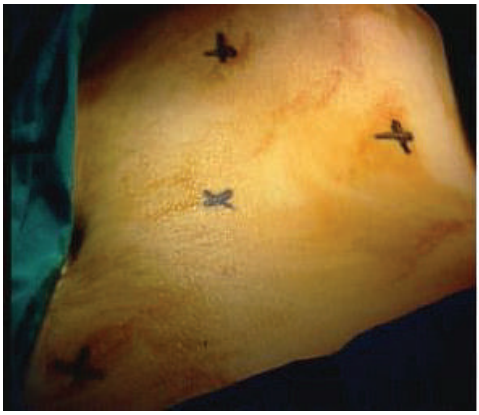


Figure 1 – Flank semilateral position and exact placement of the trocars (left sided adrenalectomy

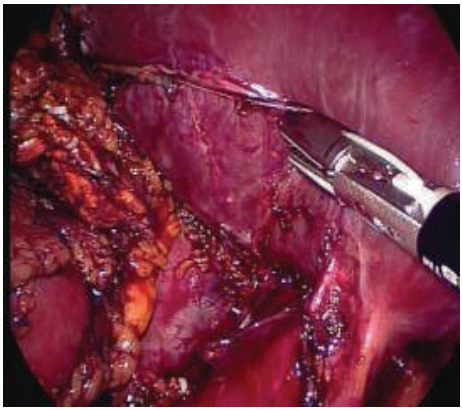


Figure 2 – Incision of the posterior peritone To expose the inferior vena cava and the adrenal gland

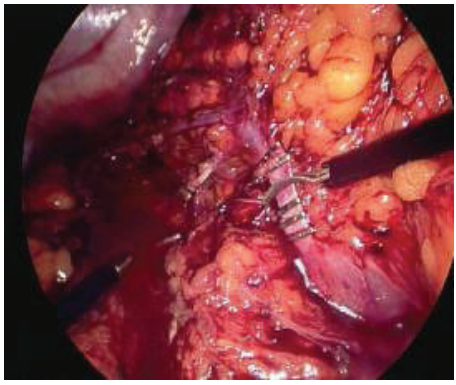


Figure 3 – Division of the adrenal vein after dissection and clipping

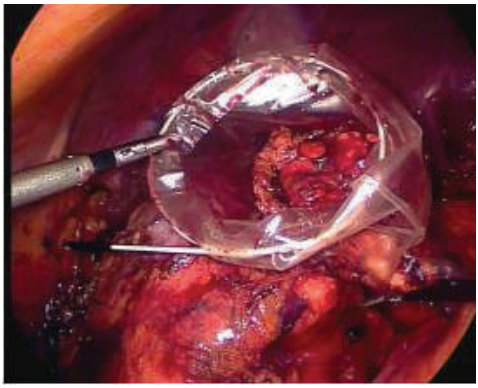


Figure 4 – Adrenal mass entraped in a plastic bag

The laparoscopic procedure was successsfully completed in all cases except in 12, which was converted to open surgery primarly for patient safety as well as better surgical efficacy.

Operative time for laparoscopic adrenalectomies ranged from 45 to 120 minutes (median 80 minutes). The average postoperative hospital stay for laparoscopic adrenalectomy ranged from 1 to 2 days (mean 1.5 days), versus 5 to 20 days in patients who underwent an open or converted

procedure. The definitive histopathological diagnosis for LA included: Conn's syndrome (17 patients), Cushing's syndrome (33 patients), pheochromocytoma (39 patients), incidentaloma (10 patients) and other tumors (6 patients). (Table 1). Another 31 cases were performed via transabdominal subcostal approach. The definitive histopathological diagnosis for OA were: Conn's syndrome (4 patients), Cushing's syndrome (10 patients), pheochromocytoma (4 patients), incidentaloma (7 patients), malignancy between one metastatic RCC (3 patients), suprarenal cyst (2 patients) and oncocytoma (1 patient) (Table 1).

Table 1

Postoperative histopathological diagnosis

Histopathology	Laparoscopic adrenalectomy	Open adrenalectomy
Conn's syndrome	17	4
Cushing syndrome	33	10
Pheochromocytoma	39	4
Incidentaloma	10	7
Malignancy (one metastatic	x	3
Suprarenal cyst	x	2
Oncocytoma	x	1

Table 2

Demographic and operative data

	Laparoscopic	Open adrenalectom	p
Number	105	31	
Female/male	57/48	17/14	
Size of the tumor (cm) range	4.6 ± 0.4	6.1 ± 0.7	$p < 0.5$
Mean surgical time (hours)	1.5 ± 0.9	3.7 ± 0.3	p = NS
Blood loss avg (ml)	120 (50–350)	371 (150–450)	$p < 0.01$
Time to resumption of oral intake (days)	1.9	4.4	$p < 0.001$
Postoperative hospital stay (days)	1,5 ± 0.5	8.7 ± 1.5	p = 0.01

Discussion

In the present study we present our initial experience with LA, with a particular focus on the indications, results, and complications, comparing these data with those from the open surgery performed at the University Clinic of Urology. At present, LA should be considered the 'platinum standard' for the treatment of the vast majority of adrenal diseases, including malignnancy that is confined to the organ [8]. In addition, LA can be performed safely and efficiently in patients with significant comorbidities and in patients with large adrenal tumors [9]. In the early series, the operative times were prolonged, but it quickly became clear that the operation was a safe and feasible procedure. Several large single-surgeon and single-institution studies demonstrated excellent results beyond the initial learning curve. For example, Suzuki and associates in 1999 reported 24 complications among 75 consecutive patients (32%). Five patients (6.6%) were converted to open surgery. The operative time was 227 minutes on the left and 210 minutes on the right with minimal blood loss [10]. Lezoche and colleagues have just recently reported their experience with 214 consecutive cases of laparoscopic transperitoneal adrenalectomy with a mean operating time of 80 minutes and a mean hospital stay of 2.5 days [6]. There were no deaths, 2% of the patient had complications, and 6% underwent conversion to open surgery. In our series, the conversion rate (11.42%) is in accordance with the literature. The hospitalization period in the first half of our series was 2.2 ± 0.3 days. Many of our patients leave the hospital within 2 days after successful LA. Likewise, the average surgical time for LA procedures was longer in the first half of series than in the later operations ($p < 0.03$).

Our results concur with other retrospective reviews comparing laparoscopic and open adrenalectomy, demonstrating unequivocal advantages in terms of reduced length of hospital stay, blood loss, return of bowel function functional recovery and post-operative morbidity. In contrast, we did not achieve any cost savings in the laparoscopic approach; in fact, the charges were higher in this group. However, the operative and hospitalization costs represent only a fraction of the overall costs attributable to the medical care of these patients. Cost savings result from a quicker return to normal activities, shorter disability, and rapid return to society as compared to open

procedures. These savings may be more difficult to quantitate, however. From a technical standpoint, large adrenal masses are difficult to dissect laparoscopically. Several authors limit the laparoscopic adrenalectomy to lesions less than 6 cm in size, whereas others perform laparoscopic adrenalectomy on tumors up to 13 cm in diameter, with no morbidity. Extensive experience in advanced laparoscopic techniques and in open adrenal surgery are mandatory to manipulate and excise large tumors with a laparoscopic approach.

At present, laparoscopic adrenalectomy for invasive malignant tumors is contraindicated. En bloc extensive resections as nephrectomy, hepatectomy, and splenectomy are not well suited to the laparoscopic technique. Several cases of laparoscopic adrenalectomy for malignant tumors, followed by local recurrence and disseminated abdominal carcinomatosis within a few months, have been published. Although most institutions perform open procedures for adrenal cancer, the number of laparoscopic adrenalectomies carried out for malignant tumors is a notable phenomenon over the past few years.

In conclusion, LA is not easier, quicker or cheaper; nor does it avoid the need for general anesthesia. It has taken considerable time and effort in the operating room to develop a safe and effective operation procedure. Early ligation of the central adrenal vein to facilitate pharmacologic control in pheochromocytocma has been emphasized in reports and textbooks. One of our patients with an 6 cm pheochromocytoma developed hypertension during laparoscopic adrenalectomy, and was successfully treated with hydralazine. An extensive search of the literature showed that hemodynamic instability during surgery has been successfully treated. Other investigators sugest that the laparoscopic approach to pheochromocytoma decreases the intraoperative release of catecholamines compared to the open technique, thereby minimizing the risk of a hypertensive crisis. Laparoscopic adrenalectomy is currently the gold standard for all benign adrenal diseases. Our series confirms that laparoscopic adrenalectomy is a safe and effective procedure associated with low morbidity, short hospital stay, and recovery. However, the procedure necessitates experience in open adrenal surgery, advanced laparoscopic techniques, and familiarization with new technologies. Careful

preoperative planning and support from the endocrinology team minimize the occurrence of intraoperative or postoperative complications.

REFERENCES

1. McLeod MK. Complications following adrenal surgery. J Natl Med Assoc. 1991; 83: 161–164.
2. El-Kappany HA, Shoma AM, El-Tabey NA, et al. Laparoscopic adrenalectomy: a single-center experience of 43 cases. J Endourol. 2005; 19: 1170–1173.
3. Lezoche E, Guerrieri M, Feliciotti F, et al. Anterior, lateral, and posterior retroperitoneal approaches in endoscopic adrenalectomy. Surg Endosc. 2002; 16: 96–99.
4. Popov Z, Jankulovski N, Stankov O, Stavridis S, Saidi S, Kuzmanoski M, Chipurovski I, Banev S, Krstevska B, Ivanovski O, Dimitrovski C. Laparoscopic Adrenalectomy: First Single-Center Experience in the Balkans. Pril (Makedon Akad Nauk Umet Odd Med Nauki). 2015; 36(3): 27-33. doi: 10.1515/prilozi-2015-0075. PMID: 27442393.
5. Gagner M, Lacroix A, Bolte E. Laparoscopic adre- nalectomy in Cushing's syndrome and pheochromo- cytoma. N Engl J Med. 1992; 327: 1033.
6. Gagner M, Lacroix A, Prinz RA, et al. Early experi- ence with laparoscopic approach for adrenalectomy. Surgery. 1993; 114: 1120–1124; discussion 1124–1125.
7. Lezoche E, Guerrieri M, Crosta F, et al. Perioperative results of 214 laparoscopic adrenalectomies by an- terior transperitoneal approach. Surg Endosc. 2008; 22: 522–526.
8. Hamilton BD. Transperitoneal laparoscopic adrena- lectomy. Urol Clin North Am. 2001; 28: 61–70.
9. Cobb WS, Kercher KW, Sing RF, et al. Laparoscopic adrenalectomy for malignancy. Am J Surg. 2005; 189: 405–411.
10. Chan JE, Meneghetti AT, Meloche RM, et al. Pro- spective comparison of early and late experience with laparoscopic adrenalectomy. Am J Surg. 2006; 191: 682–686.
11. Suzuki K, Ushiyama T, Ihara H, et al. Complications of laparoscopic adrenalectomy in 75 patients treated by the same surgeon. Eur Urol. 1999; 36: 40–47.

12. Castilho LN, Mitre AI, Arap S. Laparoscopic adrenal surgery in a Brazilian center. J Endourol. 2003; 17: 11–18.
13. Popov Z, Ivanovski N, Cakalaroski K, et al. [Co- elioscopic renal prelevement on living donor. The first four cases in Republic of Macedonia]. Prog Urol. 2005; 15: 1227–1228.
14. Saw EC, Ku W, Ramachandra S. Laparoscopic resec- tion of a splenic artery aneurysm. J Laparoendosc Surg. 1993; 3: 167–171.
15. Watanabe Y, Sato M, Abe Y, et al. Three-dimen- sional arterial computed tomography and laparos- cope-assisted splenectomy as a minimally invasive examination and treatment of splenic aneurysms. J Laparoendosc Adv Surg Tech A. 1997; 7: 183–186.
16. Leung KL, Kwong KH, Tam YH, et al. Laparoscopic resection of splenic artery aneurysm. Surg Endosc. 1998; 12: 53.
17. Meinke AK, Floch NR, Dicorato MP. Laparoscopic options in the treatment of splenic artery aneurysms. Surg Endosc. 2002; 16: 1107.
18. Reardon PR, Otah E, Craig ES, et al. Laparoscopic resection of splenic artery aneurysms. Surg Endosc. 2005; 19: 488–493.
19. Gagner M, Lacroix A, Bolte E, 1992 Laparoscopic adrenalectomy in Cushing's syndrome and pheochromocytoma. N Engl J Med. 327: 1033.
20. Thomson GB, Grant CS, van Heerden J, et al, 1997 Laparoscopic versus open posterior adrenalectomy: a case-control study of 100 patients. Surgery. 6: 132-136.
20. Schell SR, Talamin MA, Udelsman R, 1998 Laparoscopic adrenalectomy. In: Advances in surgery. Mosby-year book Inc. 32: 333-350.
21. Lezoche E, Guerriri M, Paganini AM, et al, 2000 Laparoscopic adrenalectomy by the anterior transperitoneal approach: results of 108 operations in unselected cases. Surg Endosc. 14: 920-925.
22. Ramacciato G, Mercantini P, Pietromaria A, et al, 2005 Ten years of laparoscopic adrenalectomy: Lessons learned from 104 procedures. Am Surg. 71: 321-325.

doi:10.20903/zpopov-2022-0009

UDK: 616.62-006.6-02:613.81
616.62-006.6-02:613.84

Viktor STANKOV[1], Skender SAIDI[1], Saso DOHCEV[1], Sotir STAVRIDIS[1], Josif JANCULEV[1], Aleksandar TRIFUNOVSKI[1], Oliver STANKOV[1], Zivko POPOV[1,2]

ASSOCIATION OF TOBACCO SMOKING AND ALCOHOL CONSUMPTION WITH URINARY BLADDER CANCER AGRESSIVENESS

Abstract

Objectives: Bladder cancer is the most common malignancy in the urinary system and is associated with high incidence, recurrence, and mortality rates. The influence of common smoking and drinking habits on the urinary bladder cancer is still controversial. The main goal of our study was to identify the association of tobacco smoking and alcohol consumption on the tumor characteristics at the time of bladder cancer diagnosis.

Methods: We have evaluated selected data from 70 patients with confirmed urinary bladder cancer regarding their smoking and alcohol consumption. The clinicopathological tumor characteristics include: a number of vesical tumors revealed at first cystoscopy (single or multiple tumors), histopathological grade (G1, G2 and G3) and T-classification stage (binary categories: superficial and muscle-invasive).

Results: The patient's smoking habits were found to be statistically significantly associated with the histological grade of differentiation (p=0.027) and T-classification (p=0.021). Alcohol consumption was associated with the number of primary tumors at the cystoscopy (p=0.042).

[1] University Clinic of Urology, Clinical Center, Vodnjanska str. 17, MK-1000, Skopje, Republic of North Macedonia

[2] Macedonian Academy of Sciences and Arts, Skopje, Republic of North Macedonia

All other comparisons between the clinicopathological data and patient's habits reveal differences that were not significant (p>0.05).

Conclusions: The results suggest that smoking was associated with the histological grade of differentiation and T-classification grade as one of the most important indicators of tumor aggression in the patients with UBC. Alcohol consumption was associated with the number of primary tumors revealed by cystoscopy at the time of first diagnosis.

Keywords: urinary bladder cancer; tobacco smoking; alcohol consumption.

INTRODUCTION

Bladder cancer is the most common malignancy in the urinary system and is associated with high incidence, recurrence, and mortality rates [1]. Urothelial (transitional cell) carcinoma is the most frequent histological type that accounts for nearly 90% of all bladder cancers.

According to GLOBOCAN, urinary bladder cancer (UBC) was the ninth most common malignancy worldwide in 2012, with 430,000 newly diagnosed cases [2]. In the Republic of North Macedonia, the mortality rate for this cancer type is 101 per 100,000 for the year of 2017, as reported by the Institute of Public Health [3].

It has been generally accepted that smoking is the most frequent risk-factor, implicated in approximately half of all bladder cancer cases [4]. According to the National Institutes of Health-AARP Diet and Health Study Cohort, cigarette smoking is a major risk factor for urinary bladder cancer, increasing the risk by 3.89 and 4.65, in men and women, respectively [5]. Other well-established risk factors are occupational exposure to aromatic amines and polycyclic aromatic hydrocarbons and genetic predisposition, while the links with dietary factors and environmental pollution are less evident. Some of the previous studies indicate that the intensity of smoking is significantly associated with more aggressive UBC at the time of diagnosis [6].

However, the association between alcohol consumption and bladder cancer incidence and major tumor characteristics are inconsistent among the studies. The results of an earlier, large meta-analysis of UBC cases indicated

a slightly increased risk of 1.3 for alcohol-drinkers, compared with non-drinkers [7]. Some recent case-control studies also suggested an increased risk for UBC in alcohol ever-drinkers [8]. The exact impact of alcohol on bladder carcinogenesis, including various alcoholic beverages, drinking pattern, and modification by other risk factors remains uncertain despite the relatively large number of published studies.

The aim of this study was to examine the association of tobacco smoking and alcohol consumption with some clinicopathological characteristics of tumor aggressiveness at the time of first diagnosis of UBC.

MATERIALS AND METHODS

Study design and patient population

In this retroprospective, observational study we have evaluated a selected demographic and clinicopathologic characteristics of 70 patients with histopathologically confirmed UBC, treated by transurethral resection of bladder tumor (TURBT) at the University Clinic of Urology in Skopje between October 2009 and March 2011. Clinicopathological parameters that were evaluated included: the number of vesical tumors revealed at the first cystoscopy (single or multiple tumors), histopathological grade (G1, G2 and G3) and T-classification stage (superficial: pTa or pT1, and muscle-invasive: pT2 or pT3) according to WHO classification. For this study, considering the number of patients, we have used only the binary categories: superficial and muscle-invasive. Grading and tumor staging were considered at the time when the tissue sample from the primary tumor was obtained.

Assessment of patient tobacco smoking and alcohol consumption

Patient data considering tobacco and alcohol consumption were obtained by a questionnaire.

For the purpose of this study, we have defined the smoking habits of the patients into the following four categories: non-smokers, light-smokers (≤20 cigarettes/day), moderate-smokers (21-60 cigarettes/day) and heavy-smokers (>60 cigarettes/day).

Alcohol consumption was stratified into categories by using predefined levels: nondrinkers, moderate-drinkers (~1 drink/day) and heavy-drinkers

(>1 drink/day). The definition of a standard drink and the approximate frequency of drinking was according to the previously accepted criteria [10].

Only the patients with complete data regarding both clinicopathological and demographic parameters are recruited in this study. The study was approved by the Ethical Committee of the Urology Clinic at the Medical Faculty in Skopje (No. 03-1165 from December 28, 2009) and signed informed consent was obtained from each patient recruited for the study.

Statistical analysis

Correlations between the patient's habits and selected clinicopathological characteristics of tumor aggressiveness were analyzed by a Fisher's exact probability test (two tailed) using XLStat 2016 installed on Microsoft Excel 2016. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

In this study, we evaluated the data from 70 patients with histopathologically confirmed UBC regarding selected demographic and clinicopathological data (Table 1).

Table 1

Relevant demographic and clinicopathological patient characteristics

Age	**Minimum**	**Maximum**	**Average**	**SD**
	38	79	64.3	9,29
			n	**%**
Gender	Males		61	87.14
	Females		9	12.86
Smoking habit	Non-smokers		23	32.86
	Light-smokers		8	11.43
	Moderate-smokers		26	37.14
	Heavy-smokers		13	18.57
Alcohol consumption	Non-drinker		47	67.14
	Moderate-drinker		22	31.43

	Regular-drinker	1	1.43
Histological grade of differentiation	Grade 1	5	7.14
	Grade 2	42	60.00
	Grade 3	23	32.86
T-classification	Superficial	53	75.71
	Muscle-invasive	17	24.29
Number of primary tumors at cystoscopy	Single	42	60.00
	Multiple	28	40.00

The average age is 64.3 ± 9.29 years (range 38-79). Males make up the majority of the group of patients (87.14%) and females were only 12.86% of the group.

It is obvious from the data shown that less than one-third of the patients are non-smokers (32.86%), while rest consume tobacco to various degrees.

Regarding alcohol consumption, more than two-thirds of the patients are non-drinkers, less than one-third consume moderate amounts of alcoholic beverages and an insignificant percent (1.43%) are regular-drinkers.

The association of tobacco smoking and alcohol drinking habits with the histological grade of tumor differentiation is presented in Table 2.

Table 2

Association of tobacco smoking and alcohol consumption with the histological grade of tumor differentiation

Histological grade of differentiation		**Grade 1**		**Grade 2**		**Grade 3**		***p*** *
		n	**%**	**n**	**%**	**n**	**%**	
Smoking habit	Non-smokers	1	20.00	18	42.86	4	17.39	**0.027**
	Light-smokers	2	40.00	6	14.29	0	0.00	
	Moderate-smokers	2	40.00	13	30.95	11	47.83	
	Heavy-smokers	0	0.00	5	11.90	8	34.78	
Alcohol consumption	Non-drinker	5	100.00	26	61.90	16	69.57	1.000
	Moderate-drinker	0	0.00	15	35.71	7	30.43	
	Regular-drinker	0	0.00	1	2.38	0	0.00	

* Fisher's exact test (two-tailed)

We found statistically significant differences between the patient's smoking habits and histological grade of differentiation ($p<0.05$). Moderate and heavy-smokers are dominantly found in the subgroup of patients with Grade 3 tumors, while non-smokers and light-smokers are more frequently found with Grade 2 and 1 tumors.

However, differences considering alcohol drinking were not significant ($p>0.05$).

Further analysis was performed considering superficial or muscle-invasive T-classification of the primary tumor.

The results of the analysis are presented in the Table 3.

Table 3

Association of tobacco smoking and alcohol consumption with the pathological T-classification of the primary tumor

T-classification of the primary tumor		**Superficial**		**Muscle-invasive**		***p*** *
		n	**%**	**n**	**%**	
Smoking habit	Non-smokers	20	33.33	3	35.71	**0.021**
	Light-smokers	8	8.33	0	9.52	
	Moderate-smokers	19	45.83	7	35.71	
	Heavy-smokers	6	12.50	7	19.05	
Alcohol consumption	Non-drinker	38	71.70	9	52.94	0.266
	Moderate-drinker	14	26.42	8	47.06	
	Regular-drinker	1	1.89	0	0.00	

* Fisher's exact test (two-tailed)

We found that the differences between the subgroups of patients with superficial or muscle-invasive tumors regarding smoking habits are statistically significant ($p<0.05$). By contrast, there are no significant differences regarding alcohol consumption habits.

Analysis of the cystoscopically detected number of primary tumors in association with smoking and alcohol drinking are presented in the Table 4.

Table 4

Association of tobacco smoking and alcohol consumption with the number of the primary tumors revealed by cystoscopy

Number of the primary tumors		Single		Multiple		p *
		n	%	n	%	
Smoking habit	Non-smokers	12	28.57	11	39.29	0.474
	Light-smokers	6	14.29	2	7.14	
	Moderate-smokers	15	35.71	11	39.29	
	Heavy-smokers	9	21.43	4	14.29	
Alcohol consumption	Non-drinker	32	76.19	15	53.57	**0.042**
	Moderate-drinker	9	21.43	13	46.43	
	Regular-drinker	1	2.38	0	0.00	

* Fisher's exact test (two-tailed)

The differences between the distribution of patients with single vs. multiple primary tumors detected by cystoscopy regarding tobacco smoking habits are not statistically significant (p>0.05). Interestingly, those distribution differences are significant considering alcohol consumption (p<0.05).

DISCUSSION

It is already established that tobacco smoke contains different carcinogenic compounds, including 4-aminobiphenyl, 2-naphthylamine, aromatic amines, and other substances which are associated with UBC in patients with smoking habits [10]. In addition, the risk of developing this cancer type increases with the number of cigarettes and the years smoked [11]. There is some evidence that smoking not only promotes carcinogenesis but also influences the tumor's behavior.

Alcohol (ethanol), however, has not been shown to be carcinogenic in animal studies. However, numerous epidemiological studies and meta-analyses suggest that alcohol consumption habits are associated with an increased risk for various types of malignant tumors including liver, larynxgeal, and urothelial cancer [12]. Alcoholic beverages contain different compounds that, along with their metabolic products, are excreted through

the urinary tract. Some compounds, such as acetaldehyde, which could be detected in the urine of alcohol-drinkers have a potential for DNA damage and are thus classified as carcinogenic [13]. Indirectly alcohol may facilitate or promote the effects of other carcinogens that are present in the diet or other environmental exposures.

The current empirical concept of "field change" implies that UBC patients have a significantly higher risk of new cancer development elsewhere in the urinary tract [14]. In support of this thesis, patients with UBC often have *in situ* cancers or dysplasia at different urothelial locations, and these tumors are polyclonal and in various progressive stages. Some carcinogens in tobacco are eliminated by renal excretion and could have prolonged contact with the bladder urothelium.

In our study we have evaluated selected data from 70 patients with confirmed urinary bladder cancer regarding their smoking and alcohol consumption data.

The patients' smoking habits were found to be statistically significantly associated with a histological grade of differentiation (p=0.027) and T-classification (p=0.021) as one of the hallmarks of tumor aggression. This finding is concordant with growing evidence that tobacco smoking is a well-established risk factor in UBC development and tumor aggressiveness, negatively affecting the clinical outcome [6]. Many studies and meta-analyses that have been conducted over the last few decades support these conclusions, although there are also some discordant or inconsistent results in the literature.

In the retrospective study by Pietzak et al. (2015), they analyze a total of 740 tobacco-smoking patients who were diagnosed with bladder cancer from 1987 to 2009. The authors concluded that heavy smokers have a greater statistical risk of having high-grade tumors with invasion of the detrusor muscle upon first diagnosis [15]. A meta-analysis of 26 studies indicates that tobacco smoking is an important risk factor for the development of UBC, and this negatively affects further patient outcomes [16]. Conversely, Ros and collaborators found no association between smoking behavior and the aggressiveness of the UBC tumor among male patients, while the results were not consistent regarding the female patients [17].

Regarding alcohol consumption, in the present study we found evidence that this habit was associated with the number of primary tumors

at the cystoscopy (p=0.042). Although several epidemiologic studies have been conducted to investigate the association between the alcoholic beverage consumption, UBC risk, and tumor aggressiveness, the results are not as clear and consistent as in the case of smoking.

The meta-analysis by Bagnardi et al. (2001) has found that alcohol consumption was not associated with increased risk of UBC [12]. A similar conclusion was inferred from a large meta-analysis of Pelucchi et al. (2012) and de Menezes et al. (2013) [18, 19].

However, an increased risk of UBC was found in heavy-drinkers in a large cohort study in the Netherlands. The results of this study, however, do not suggest that alcohol consumption is an important risk-factor for UBC [20]. Moderate alcohol consumption was found to be associated with overall cancer risk, including bladder cancer, in a recently conducted study from US cohorts [21]. The possible effects of alcoholic beverage consumption on UBC tumor aggressiveness still needs to be examined.

In conclusion, the results of our study suggest that habitual smoking was associated with the histological grade of differentiation and T-classification grade as one of the most important indicators of tumor aggression in patients with UBC. On the other hand, alcohol consumption was associated with the number of primary tumors revealed by cystoscopy at the time of first diagnosis. These results, along with a number of published studies, suggest it should be advised to avoid tobacco smoking and the consumption of alcoholic beverages in the patients with UBC.

REFERENCES

1. Zhang L, Wu B, Zha Z, Qu W, Zhao H, Yuan J. Clinicopathological factors in bladder cancer for cancer-specific survival outcomes following radical cystectomy: a systematic review and meta-analysis. BMC Cancer. 2019; 19(1):716. doi: 10.1186/s12885-019-5924-6.
2. Ferlay, J. et al. GLOBOCAN 2012v1.0, Cancer Incidence and Mortality Worldwide. IARC Cancer Base No. 11. Lyon, France: International Agency for Research on Cancerhttp://globocan.iarc.fr (Date of access: 21.11.2019).
3. Институт за јавно здравје на Република Македонија. Малигни неоплазми во Република Македонија. 2015-2017.
4. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 100E. Lyon, 2012.

5. Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC. Association between smoking and risk of bladder cancer among men and women. JAMA. 2011; 306(7):737-45. doi: 10.1001/jama.2011.1142.
6. Barbosa ALA, Vermeulen SHHM, Aben KK, Grotenhuis AJ, Vrieling A, Kiemeney LA. Smoking intensity and bladder cancer aggressiveness at diagnosis. PLoS One. 2018; 13(3):e0194039. doi: 10.1371/journal.pone.0194039.
7. Zeegers MP, Tan FE, Verhagen AP, Weijenberg MP, van den Brandt PA. Elevated risk of cancer of the urinary tract for alcohol drinkers: a meta-analysis. Cancer Causes Control. 1999; 10(5):445-51. doi: 10.1023/a:1008938520686.
8. Zaitsu M, Nakamura F, Toyokawa S, Tonooka A, Takeuchi T, Homma Y, et al. Risk of Alcohol Consumption in Bladder Cancer: Case-Control Study from a Nationwide Inpatient Database in Japan. Tohoku J Exp Med. 2016; 239(1):9-15. doi: 10.1620/tjem.239.9.
9. Greenfield TK, Kerr WC. Alcohol measurement methodology in epidemiology: recent advances and opportunities. Addiction. 2008; 103(7):1082-99. doi: 10.1111/j.1360-0443.2008.02197.x.
10. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene. 2002; 21(48):7435-51. doi: 10.1038/sj.onc.1205803.
11. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, et al. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol. 2013; 63(2):234-41. doi: 10.1016/j.eururo.2012.07.033.
12. Bagnardi V, Blangiardo M, La Vecchia C, Corrao G. Alcohol consumption and the risk of cancer: a meta-analysis. Alcohol Res Health. 2001; 25(4):263-70.
13. Zaitsu M, Nakamura F, Toyokawa S, Tonooka A, Takeuchi T, Homma Y, Kobayashi Y. Risk of Alcohol Consumption in Bladder Cancer: Case-Control Study from a Nationwide Inpatient Database in Japan. Tohoku J Exp Med. 2016; 239(1):9-15. doi: 10.1620/tjem.239.9.
14. Smith JA Jr. Patients with superficial transitional cell carcinoma of the bladder and their smoking status. Cancer. 1999; 86(11): 2192-4.
15. Pietzak EJ, Mucksavage P, Guzzo TJ, Malkowicz SB. Heavy Cigarette Smoking and Aggressive Bladder Cancer at Initial Presentation. Urology. 2015; 86(5):968-72. doi: 10.1016/j.urology.2015.05.040.

16. Rink M, Crivelli JJ, Shariat SF, Chun FK, Messing EM, Soloway MS. Smoking and Bladder Cancer: A Systematic Review of Risk and Outcomes. Eur Urol Focus. 2015; 1(1):17-27. doi: 10.1016/j.euf.2014.11.001.
17. Ros MM, Bueno-de-Mesquita HB, Kampman E, Buchner FL, Aben KKH, Egevad L et al. Fruit and vegetable consumption and risk of aggressive and non-aggressive urothelial cell carcinomas in the European Prospective Investigation into Cancer and Nutrition. European Journal of Cancer. 2012; 48(17):3267-77. doi.org/10.1016/j.ejca.2012.05.026.
18. Pelucchi C, Galeone C, Tramacere I, Bagnardi V, Negri E, Islami F,et al. Alcohol drinking and bladder cancer risk: a meta-analysis. Ann Oncol. 2012; 23(6):1586-93. doi: 10.1093/annonc/mdr460.
19. de Menezes RF, Bergmann A, Thuler LC. Alcohol consumption and risk of cancer: a systematic literature review. Asian Pac J Cancer Prev. 2013; 14(9):4965-72. doi: 10.7314/apjcp.2013.14.9.4965.
20. Zeegers MP, Volovics A, Dorant E, Goldbohm RA, van den Brandt PA. Alcohol consumption and bladder cancer risk: results from The Netherlands Cohort Study. Am J Epidemiol. 2001 Jan; 153(1):38-41. doi: 10.1093/aje/153.1.38.
21. Cao Y, Willett WC, Rimm EB, Stampfer MJ, Giovannucci EL. Light to moderate intake of alcohol, drinking patterns, and risk of cancer: results from two prospective US cohort studies. BMJ. 2015; 351:h4238. doi: 10.1136/bmj.h4238.

doi: 10.20903/zpopov-2022-0010 UDK: 616.65-006.6-089.84-06:616.62-008.22

Јован ИВЧЕВ[1,2]

КОНЗЕРВАТИВЕН ТРЕТМАН НА УРИНАРНА ИНКОНТИНЕНЦИЈА ПО РАДИКАЛНА ПРОСТАТЕКТОМИЈА – НАШЕ 5-ГОДИШНО ИСКУСТВО

Вовед

Уринарната инконтиненција е една од најчестите постоперативни компликации на радикалната простатектомија која значајно го нарушува квалитетот на животот на пациентите.

Постоперативната уринарна инконтиненција не зависи од големината на простатичната жлезда, од претходни трансуретрални ресекции на простатата, стадиумот на болеста или презервацијата на нервно-васкуларниот сноп, туку, во најголема мера, од: презервацијата на предната компонента на пругавиот сноп на надворешниот сфинктер и дорзалниот венски комплекс (1), предоперативно високо ниво на специфичниот антиген на простата (PSA) над 10нг/мл, возраста на пациентот (над 70 години) и дадена предоперативна ендокрина терапија (2).

Процентот на пациенти со постоперативна уринарна инконтиненција во разни студии покажува големи варијации, од 6 до 69 % (3, 4, 5). Големата разлика се објаснува со употребата на повеќе различни методи за процена на инконтиненцијата, со изборот на соодветни пациенти за анкетирање и изборот на дијагностички критериуми за одредување на степенот на инконтинентност (6). Третманот на пост-

[1] Факултет за медицински науки, Универзитет „Гоце Делчев“, Штип, РС Македонија
[2] Градска општа болница „8 Септември“ – Скопје, РС Македонија

165

простатектомичната инконтиненција (PPI) во првите 18 месеци по операцијата е конзервативен и тој се состои од: физички вежби за јакнење на пелвичното дно, електростимулација на пелвичната мускулатура, екстракорпорална магнетна инервација (ExMI) и медикаментозна терапија со антимускаринski лекови (solifenacin, oxibytynin chloride…), инхибитори на фосфодиестераза-5-ензимот – PDE5 (tadalafil), антидепресиви и анксиолитици (duloxetine) и α-адренергични агонисти. Перзистентната стрес-постоперативна инконтиненција по 18 месеци на конзервативен третман се квалификува како трајна и таа се решава оперативно, со вградување на артефициелен уретрален сфинктер, ретроуретрална трансобтураторна полипропиленска лента (sling) или со трајна катетеризација. Процентот на трајна пост-простатектомична инконтиненција во разни студии варира од 4 до 8,4 %, а процентот на пациенти кај кои се воспоставува „целосна уринарна контрола“ изнесува само околу 32 %, поради што оваа постоперативна компликација претставува сè уште сериозен уролошки проблем кој бара комплексен мултимодален пристап на третман (9).

Цели

– Утврдување на процентот и степенот на уринарна инконтиненција кај пациенти оперирани од карцином на простата во нашата установа.

– Утврдување на зависноста на постоперативната уринарна инконтиненција од предоперативното ниво на простата специфичниот антиген (PSA) и возраста на пациентот.

– Утврдување на ефикасноста од конзервативниот третман со моно или двојна терапија со антимускаринski препарати и инхибитори на фосфодиестераза-5-ензимот – PDE5.

Материјал и методи

Во студијата опфатени се 186 пациенти со дијагностициран карцином на простата, оперирани на уролошкото одделение во ГОБ „8 Септември“, во периодот од 1.1.2016 до 29.3.2021 година. Дијагнозата и стадиумот на болеста се поставени врз основа на патохистолошката анализа на доставените примероци од трансуретрална ултрасонографска биопсија на простатичната жлезда. Кај 177 пациенти направена е ретропу-

бична радикална простатектомија, а кај 9, лапароскопска екстраперитонеална радикална простатектомија. Од нив, постоперативно конзервативно се лекувани 67 пациенти, поради стрес или ургентна инконтиненција.

Дијагнозата на видот и степенот на инконтинентност кај пациентите се поставени врз основа на анамнестичките податоци (*Questionnaire Urinary Incontinence Diagnostic* – QUID-анкета), физикалниот преглед, цистоскопијата и анализата на постоперативните контролни прегледи. Статистичките податоци се обработени и прикажани се во вид на процентуална застапеност, средна вредност и табеларен приказ на компаративните анализирани вредности.

Резултати

Од вкупно 186 пациенти кај кои е направена операција радикална простатектомија, постоперативно поради стрес или ургентна уринарна инконтиненција конзервативно се лекувани 67 (36,02 %) пациенти, кај 8 (4,30 %) пациенти констатирана е трајна инконтинентост, додека кај 111 (59,67 %) е воспоставена нормална уринарна континентност (дијаграм 1).

Дијаграм 1 – Постоперативна стрес и трајна уринарна инконтиненција од вкупно 186 направени радикални простатектомии

Просечното времетраење на постоперативниот конзервативен третман изнесува 6 месеци (од 2 до 12 месеци).

Од вкупно 75 пациенти со УИ, кај 8 (10,66 %) пациенти се констатирани трајна инконтинентност и индициран траен катетер или пелена. Останатите 67 (89,33 %) пациенти се лекувани конзервативно со антимускаринскиот препарат solifenacin (од 5 или 10 mg), и тоа:

– кај 52 (69,33 %) пациента како монотерапија, во траење од 2 до 6 месеци;

– кај 8 (10,66 %), во комбинација со tabl. mirabergon од 50 mg, во траење од 4 до 12 месеци и

– кај 7 (9,33 %), во комбинација со tabl. tadalafil од 5 mg, од првиот до третиот постоперативен месец (табела 1).

Табела 1

Видови конзервативен третман користен кај пациентите со постоперативна уринарна инконтиненција

Вид конзервативен третман на постоперативните уринарни инконтиненции	Број на пациенти	%
solifenacin a 5 (10) mg	52	69,33
solifenacin 10 mg + mirabergon 50 mg	8	10,66
solifenacin 10 mg + tadalafil 5 mg	7	9,33
Трајна уринарна ретенција (уринарен катетер/пелена)	8	10,66

Значајно намалување на симптомите на ургентна и стрес-инконтиненција по 4-месечна монотерапија, терапија со tabl. solifenacin (од 5 или 10 mg), забележена е кај 71,15 %, со tabl. solifenacin (од 5 или 10 mg) во комбинација со tabl. mirabergon од 50 mg кај 50 %, а со tabl. solifenacin 10 mg во комбинација со tabl. tadalafil од 5 mg кај 85,71 % од лекуваните пациенти. Подобрување на состојбата по 6-месечна монотерапија со soli-

fenacin забележена е кај 80,76 %, а со двојна терапија со solifenacin и mirabergon, кај 87,5 % од пациентите (табела 2).

Табела 2

Видови медикаментозен конзервативен третман на умерени и средно тешки форми на постоперативна уринарна инконтиненција

Вид третман	Подобрување по 2 месеца	Подобрување по 4 месеца	Подобрување по 6 месеца	Подобрување по 12 месеца
solifenacin 10 mg	22 (44,30 %)	37 (71,15 %)	42 (80,76 %)	47 (90,38 %)
solifenacin 10 mg + mirabergon 50 mg	2 (25,0 %)	4 (50,0 %)	7 (87,5 %)	7 (87,5 %)
solifenacin 10 mg + tadalafil 5 mg	5 (71,42 %)	6 (85,71 %)	/	/

Дискусија

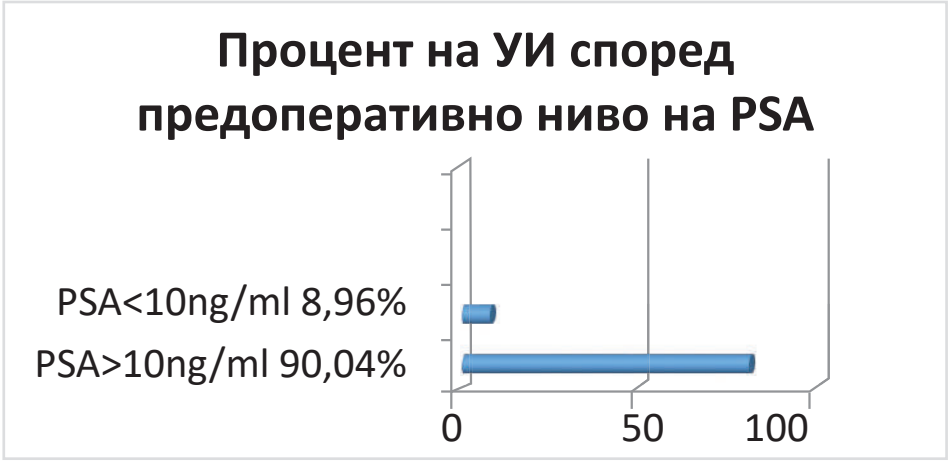
Уринарната инконтиненција претставува најчеста компликација по радикална простатектомија (отворена, лапароскопска или роботска), и таа претставува сериозно нарушување на квалитетот на животот кај пациентите.

Процентот на трајната постоперативна инконтиненција во разни студии варира од 4 % до 8 %, додека процентот на умерен и средно тежок степен на уринарна инконтиненција (понатаму во текстот УИ) во првата постоперативна година варира од 36 % до 41 % (7, 8).

Од вкупно 75 пациенти со УИ, кај 8 (10,66 %) пациенти констатирана е трајна инконтинентност и индициран траен катетер или пелена. Кај останатите 67 (89,33 %) пациенти, антимускаринскиот препарат solifenacin (од 5 или 10 mg) е користен како лек на избор и тоа: кај 52 (69,33 %) пациенти како монотерапија, во траење од 2 до 6 месеца; кај 8 (10,66 %), во комбинација со tabl. mirabergon од 50 mg, во траење од 4 до 12 месеца, а кај 7 (9,33 %), во комбинација со tabl. tadalafil од 5 mg, од првиот до третиот постоперативен месец.

Процентот на трајна уринарна инконтинентност од 4,30 % и постоперативната стрес-инконтиненција од 36,02 %, дијагностицирана кај оперираните пациенти во нашата установа во последните 5 години, укажуваат дека ефикасноста од оперативното лекување е на приближно ниво со другите современи хируршки установи.

Оштетениот уретрален сфинктер и намалената должина на уретрата за време на оперативниот зафат генерално се потврдени во многу студии како главен етиолошки фактор за настанувањето на постоперативната УИ (8). Сè уште високиот процент на постоперативни уринарни инконтиненции и во оваа студија укажува на потребата од постојано подобрување на оперативната техника, со цел да се овозможи поадекватна презервација на двата уретрални сфинктери. Предоперативното ниво на PSA од над 16,7 нг/мл кај 61 (91,04 %) од 67 конзервативно лекувани пациенти по радикална простатектомија, ги потврдува досегашните студии во кои постоперативната уринарна инконтиненција во голем дел се јавува кај пациенти со предоперативно ниво на PSA над 10нг/мл (2) (дијаграм 2).



Дијаграм 2 – Процент на постоперативни уринарни инконтиненции според предоперативното ниво на простата специфичниот антиген (PSA)

Просечната старост од 70,2 години, на конзервативно лекуваните пациенти по радикална простатектомија, во испитуваната група, се потврдува како дополнителен ризик-фактор за појава на постоперативна УИ. Кај ниеден од оперираните пациенти не е дадена неоадјувантна антиандрогена терапија, што претставува основа за понатамошна анализа и евентуална примена како дел од предоперативната подготовка на пациентите со дијагностициран карцином на простатичната жлезда.

Заклучок

– Навремено поставената дијагноза на карцином на простата кај пациентите со PSA под 10 нг/мл претставува еден од основните предуслови за превенција од постоперативна уринарна инконтинентност.

– Конзервативниот третман на постоперативната уринарна инконтиненција со антимускаринскиот препарат solifenacin, како монотерапија или во комбинација со β-3 адренергичен агонист (mirabergon), овозможува висок процент на излекуваност или намалување на симптомите на уринарната ургентност и инконтинентност по 4-6 месеци од почетокот на терапијата.

– Примената на антимускаринскиот препарат tabl. solifenacin од 5 мг паралелно со tabl. tadalafil од 5 мг, непосредно по изведената радикална простатектомија, во период до 3 месеци, претставува значајна превенција од појавата на постоперативна стрес-инконтиненција.

Клучни зборови: карцином на простата, радикална простатектомија, уринарна инконтиненција, антимускаринска терапија.

ЛИТЕРАТУРА

1. Campbell's Urology. (1992). Sixth Edition. Philadeelphia,W. B. Saunders C. 75:2884.
2. Minei, S., Iwamura, M., Uchida, T., Koshiba, K. (1997). Urinary Continence Following Radical Prostatectomy. *Japanese Journal of Clinical Oncology*, Volume 27, Issue 2, April 1997, Pages 71–75.
3. Poon, M., Ruckle, H., Bamshad, B. R., Tsai, C., Webster, R. et al. (2000). R*adical retropubic prostatectomy: bladder neck preservation versus reconstruction*. *J* Urol. 2000;163: 194.
4. Bishoff, J. T., Motley, G., Optenberg, S. A., Stein, C. R., Moon, K. A. et al. (1998). *Incidence of fecal and urinary incontinence following radical perineal and retropubic prostatectomy in a national population*. J Urol. 1998; 160:454.
5. Kretschmer, A., Hubner, W., Sandhu, J. S., Bauer, R. M. (2016). *Evaluation and management of postprostatectomy incontinence: a systematic review of current literature. Eur* Urol Focus;2(3): 245–259.
6. Wei, J. T., Dunn, R. L., Marcovich, R., Montie, J. E., Sanda, M. G. (2000). *Prospective assessment of patient reported urinary continence after radical prostatectomy*. *J* Urol. 2000;164: 744–748.
7. Hoyland, K., Vasdev, N., Abrof, A., Boustead, G. (2014). *Post-Radical Prostatectomy Incontinence: Etiology and Prevention*. Rev Urol; 16(4): 181–188).
8. Borges, R. C., Tobias-Machado, M., Gabriotti, E. N., Winter dos Santos Figueiredo, F. Bezerra CA, Glina S. BMC Urol 2019; 19**:** 32.
9. Stanford, J. L., Feng, Z., Hamilton, A. S., Gilliland, F. D., Stephenson, R. A., Eley, J. W. (2000). Urinary and sexual function after radical prostatectomy for clinically localized prostate cancer: the Prostate Cancer Outcomes Study. JAMA, 283 (3) (2000), pp. 354–360 Jan 19.
10. Broome, B. A. (2003). *The impact of urinary incontinence on self-efficacy and quality of life. Health Qual Life Outcomes.*, 1 (2003), p. 35. Aug 22.
11. MacDiarmid, S. (2003). *Antimuscarinic Therapy for the Treatment of Mixed Incontinence*. Rev Urol. 2003; 5 (Suppl 8): S18–S25.
12. Appell, R. A. (1997). *Clinical efficacy and safety of tolterodine in the treatment of overactive bladder: a pooled analysis*. Urology. 1997; 50: 90–96.
13. Chancellor, M., Freedman, S., Mitcheson, H. D. et al. (2000). *Tolterodine, an effective and well tolerated treatment for urge incontinence and other overactive bladder symptoms*. Clin Drug Invest. 2000; 19: 83–91.

14. Talcott, J. A., Rieker, P., Propert, K. J., Clark, J. A., Wishnow, K. I., Loughlin, K. R., Richie, J. P. et al. (1997). *Patient-reported impotence and incontinence after nerve-sparing radical prostatectomy*. J Natl Cancer Inst. 1997; 89: 1117–23.
15. Braslis, K. G., Santa-Cruz, C., Brickman, A. L., Solloway, M. S. *Quality of life 12 months after radical prostatectomy*. Br J Urol. 1995; 75: 48–53.
16. Sacco, E., Prayer-Galetti, T., Pinto, F., Fracalanza, S., Betto, G. (2006). *Urinary incontinence after radical prostatectomy: incidence by definition, risk factors and temporal trend in a large series with a long-term follow-up*. BJU Int. 2006; 97 (6): 1234–41.
17. Kretschmer, A., Hubner, W., Sandhu, J. S., Bauer, R. M. (2016). *Evaluation and management of postprostatectomy incontinence: a systematic review of current literature*. Eur Urol Focus. 2016; 2(3): 245–59.

Jovan IVCHEV

CONSERVATIVE TREATMENT OF URINARY INCONTINENCE AFTER RADICAL PROSTATECTOMY – OUR 5 YEARS OF EXPERIENCE

Abstract

Introduction: Urinary incontinence is one of the most common postoperative complications of radical prostatectomy, which significantly impairs the quality of life of the patients. Urinary incontinence after radical prostatectomy is most commonly associated with: preservation of the anterior component of the striped bundle of the external sphincter and dorsal venous complex (1), preoperatively high prostate-specific antigen (PSA) levels above 10 ng / ml, age over 70 years and given preoperative endocrine therapy (2). The percentage of patients with postoperative urinary incontinence in various studies shows large variations of 6 to 69% (3,4,5,6).

Objectives:

- Determination of the percentage and degree of urinary incontinence in patients operated for prostate cancer in our institution.

- Determination of the dependence of postoperative urinary incontinence on the preoperative level of PSA and the age of the patient.

- Determination of the effectiveness of treatment with mono or double therapy with antimuscarinic drugs and inhibitors of phosphodiesterase 5 enzyme - PDE5.

Material and methods: The study included 186 patients with radical prostatectomy operated on the urology department at the GCH "September 8", in the period from 01.01.2016 to 29.03.2021. Of these, 75 patients were treated conservatively due to stress or urgent incontinence.

Results: Significant reduction in symptoms after 4 months of monotherapy with tabl. Solifenacin (5 or 10 mg) was observed in 71.15%, with tab. Solifenacin (5 or 10 mg) in combination with tabl. Mirabergon 50mg at 50% with tabl.Solifenacin 10 mg in combination with tabl. Tadalafil 5mgr in 85.71% of treated patients. Improvement of the condition after 6 months of Solifenacin monotherapy was observed in 80.76% and with dual therapy of Solifenacin and Mirabergon in 87.5% of patients.

Discussion: The percentage of permanent urinary incontinence of 4.30% and postoperative stress incontinence of 36.02%, diagnosed in this study in the last 5 years, indicates that the efficiency of the surgical treatment is approximately at the level of other modern surgical institutions. The still high percentage of postoperative urinary incontinences indicates the need for continuous improvement of the surgical technique, in order to enable more adequate preservation of both urethral sphincters. The preoperative PSA level of over 16.7 ng / ml in 61 (91.04%) of 67 conservatively treated patients after radical prostatectomy confirms that postoperative urinary incontinence occurs largely in patients with preoperative PSA levels above 10ng / ml (2).

Conclusion:

- Early diagnosis of prostate cancer in patients with PSA below 10 ng / ml is one of the basic prerequisites for prevention of postoperative urinary incontinence.

- Conservative treatment of postoperative urinary incontinence with the antimuscarinic drug Solifenacin, as monotherapy or in combination

with β-3 adrenergic agonist (Mirabergon), provides a high percentage of cure or reduction of symptoms of urinary urgency and incontinence after 4-6 months.

- The application of tabl.Solifenacin 5mg simultaneously with tabl. Tadalafil 5 mg, immediately after the radical prostatectomy, lasting up to 3 months, is a significant prevention of postoperative stress incontinence.

Keywords: prostate cancer, radical prostatectomy, urinary incontinence, antimuscarinic therapy

ПОГЛАВЈЕ III

ТРАНСПЛАНТАЦИЈА НА БУБРЕЗИ

doi: 10.20903/zpopov-2022-0011 UDK: 616.61-089.843-06(497.2)"2018/2019"

Dimitar MLADENOV[1], Vladislav MLADENOV[1], Plamen DIMITROV[1], Vasil VASILEV[1], Krassimir YANEV[1], Marincho GEORGIEV[1]

EARLY POSTOPERATIVE UROLOGICAL AND SURGICAL COMPLICATIONS AFTER KIDNEY TRANSPLANTATION BY A LIVING AND CADAVERIC DONOR

Abstract

Organ transplantation and kidney transplantation, in particular, has aroused public interest and excited the medical community for centuries. Nowadays, kidney transplantation is an established surgical method for the treatment of end-stage chronic renal failure with good long-term results. Despite the continuous progress and development of transplantology, a number of postoperative surgical and urological complications still occur today, which could compromise the success of this operative method.

Aim: Our aim is to update the knowledge in transplantology and to summarize our data on early postoperative urological and surgical complications after kidney transplantation.

Material and methods: This study is based on a retrospective analysis of the disease history of 35 patients who underwent kidney transplantation at the Clinic of Urology at the University Hospital "Alexandrovska", Sofia for the period from 02.2018 to 12.2019. All possible early surgical and urological complications were followed up, including symp-

[1] Clinic of Urology, Medical University University Hospital "Alexandrovska", 1, "Georgi Sofiiski" Str., 1431 Sofia, Bulgaria

tomatic and asymptomatic manifestations that do not require active invasive treatment.

Results: The team focused on complications such as ureteral stricture, urinary retention, ureteral necrosis, urinoma, DJ stent problems, hematoma, vascular stenosis, thrombosis, lymphocele, urinary tract infection, hernia or inflammation in the area of the surgical wound. Early postoperative complications were found in 46% of the observed transplant patients. Urological complications were found in 26% of them. The incidence of urinary tract infections in transplanted patients was 26%. During the follow-up period there were no cases of urinary retention, stricture of the ureter and urinoma in the group of patients. The incidence of surgical complications in the transplanted patients was 29%. The incidence of postoperative hematomas was 23%. Postoperative lymphocele was diagnosed in one patient, which shows a complication rate of 3% for this diagnosis. The incidence of surgical wound infection in transplanted patients during the follow-up period was 3%. No cases of venous or arterial thrombosis and hernia in the area of the operative wound were observed during the follow-up period.

Conclusions: Our results show that the frequency of the different early postoperative urological and surgical complications correspond to the data found in literature from other transplant centers. The average length of hospital stay in the Clinic of Urology, University Hospital "Alexandrovska" for transplanted patients may be extended due to the specific nature of the health care system in the country. The transplantation activities in our clinic correspond to the world standards.

Keywords: kidney, transplantation, urological, surgical, complications

INTRODUCTION

Brief historical data on kidney transplantation

Organ transplantation as well as kidney transplantation in particular has aroused public interest and excited the medical community for many years. The first scientifically documented transplants date back to the 19th century, when it was mainly in the form of skin transplants. During the first 60 years of the 20th century, the rapid development of antiseptics, anesthe-

siology, and vascular surgery created better conditions for the development of organ transplantation. In 1902, the Austrian surgeon H. Ullman from Vienna performed the first kidney transplantation on a dog, which was assessed as technically successful. He implanted the kidney in the dog's neck area, anastomosing the ureter on the skin so that he could monitor diuresis - the kidney excreted urine for five days. In 1906, the first attempts at kidney transplantation from animals to humans were made by Dr. Mathieu Jaboulay from Lyon, but these operations proved unsuccessful. In 1933, Dr. Yu Yu Voronoy from Kiev performed the first kidney transplantation from a cadaver by. The recipient lived for 4 days and the transplanted kidney never worked. In 1952, the first kidney transplantation from a living donor (mother of a child) was performed by Prof. Hamburger in Paris. Unfortunately, the transplantation had a very short period of success. On December 23, 1954, Dr. Joseph Murray from Boston performed the first successful kidney transplantation. The transplantation was performed between identical twins - isotransplantation. Oscar Creech and Keith Reemtsma transplanted a kidney and a heart from a chimpanzee to a human. The survival of several of the patients for months suggests that in the presence of quality immunosuppressive therapy, organ transplantation between different species (xenotransplantation) is possible. Thomas Starzl tried to transplant a kidney from a baboon to a patient in a very severe condition, but the operation was unsuccessful. In 1967 Eurotransplant organization was founded by van Rood in Leiden. He succeeded to prove that HLA compatibility played a key role in the acceptance of the new organ by recipients and their survival. In 1968, “The Report of the Ad Hoc Committee of Harvard Medical School to Examine the Definition of Brain Death” set new horizons for the development of transplantology, recognizing the state of irreversible coma as a state of death.

The first kidney transplantation in Bulgaria was performed in 1968 in Pirogov Hospital by Prof. Minkov, et al. The recipient was a child, whose solitary kidney was removed after an injury. The patient underwent transplantation of two kidneys placed in the pelvis. On February 1, 1969, Prof. N. Atanasov and Prof. St. Lambrev performed the first organ transplantation

in Alexandrovska Hospital, where a 42-year old woman who was suffering from endemic nephropathy received a kidney transplant. The Clinic of Urology of UMHAT "Alexandrovska", over time, has become a leading center in the country for kidney transplantation.

Kidney transplantation today

Kidney transplantation is now an established surgical method for the treatment of end-stage of chronic renal failure with good long-term results. Organs are transplanted from patients with diagnosed brain death and from living donors. Compared to dialysis (hemodialysis, peritoneal dialysis), kidney transplantation significantly improves the quality of life of the patients, their physical endurance, promotes social integration and reduces the incidence of chroniodialysis-related diseases. From an economic point of view, kidney transplantation leads to a significant reduction of treatment costs for patients with end-stage chronic renal failure. Successful kidney transplantation, including follow-up of the patient during the first year, costs as much as two years of dialysis. The average duration of transplanted kidney function after transplantation is 9 years. There are cases in which good graft function was observed after 20 years or more. Life expectancy in transplanted patients is significantly longer than in dialysis patients.

Postoperative complications of kidney transplantation

Despite the continuous progress and development of transplantology, a number of postoperative surgical and urological complications still occur today. They could compromise the success of this surgical method. Such postoperative complications mean for the patients both development of additional ailments and hospitalizations.

Surgical postoperative complications include vascular problems such as venous and arterial thrombosis of the graft, renal artery stenosis, lymphocele etc. Other complications are related to the surgical wound - infections, dehiscence and hernias. Hemorrhagic complications include diffuse tissue bleeding and bleeding from the vascular anastomoses. The most common complications are related to the operative wound (between 12-36%), followed by hematomas (between 2 and 25%) and the least common are vascular complications (between 1 and 12%).

Urological complications also affect the postoperative period in kidney transplantation. These include urine leakage from the vesico-ureteroneostomy, urinoma, ureteral obstruction, urinary tract infections, vesicoureteral reflux, urolithiasis. The urological complications after a successful kidney transplantation have a frequency rate between 2-37%.

The timely diagnosis and appropriate treatment of surgical and urological complications is of great importance. There are numerous, mostly retrospective studies in the literature on the frequency of surgical and urological complications after kidney transplantation and their relevant risk factors. Most articles focus on single surgical or urological complications. Studies that summarize all possible complications are extremely rare.

AIMS AND TASKS

Our goals and tasks were to update our knowledge in transplantology, to acquaint ourselves with the current scientific literature and data on this issue, and to summarize our data on early postoperative urological and surgical complications after kidney transplantation. We also set for ourselves the goal to evaluate our results and compare them with the data available in contemporary medical literature on this topic.

MATERIAL AND METHODS

This study is based on a retrospective analysis of the disease history of 35 patients who underwent kidney transplantation in the Clinic of Urology, University Hospital "Alexandrovska", Sofia for the period from 02.2018 to 12.2019 (Table 1.). The cohort of patients consists of 28 men and 7 women. The mean age of the patients was 43 years (median 42 years). The mean age of women and men was 41 and 44 years, respectively. One of the men received a second kidney transplant. The study included transplantations from living and cadaveric donors. In our study, the number of kidney

transplants from a living donor was 13 and those from a cadaveric donor was 22. The follow-up period for early postoperative complications was 60 days after surgery.

Table 1

Kidney transplantations, performed from a living and cadaveric donor in the Clinic of urology, University Hospital "Alexandrovska", Sofia for the period from 02.2018 to 12.2019.

Year	2018	2019	Total
Cadaveric donor	13	9	22
Living donor	5	8	13
Total	18	17	35

The following were causes for chronic renal failure in the cohort of patients: chronic glomerulonephritis in 21 patients, nephrosclerosis in 5 patients, hypertensive nephropathy in 4 patients, polycystosis in 3 patients, and chronic pyelonephritis in 2 patients (Table 2.).

Table 2

Causes for chronic renal failure (CRF) in the cohort of patients

Causes for CRF	Number of patients (35)
Chronic glomerulonephritis	21
Nephrosclerosis	5
Hypertensive nephropathy	4
Polycystosis	3
Chronic pyelonephritis	2

It is difficult to draw conclusions about the epidemiology of the disease from the place of residence of the recipients because of increased migration of the population within the country (Table 3.).

Table 3

Distribution of the transplanted patients from living and cadaveric donors by the place of residence.

Residence	Patients	Residence	Patients	Residence	Patients
Aytos	1	Devin	1	Petrich	1
Balchik	1	Dobrich	2	Plovdiv	1
Bezmer	1	Kotel	1	Pokrovnik	1
Belene	1	Kresna	1	Ribnovo	1
Breznitsa	1	Krumovitsa	1	Sofia	4
Burgas	1	Merdanya	1	Srem	1
Varna	3	Oven	1	Stara Zagora	2
Voyvodino	1	Pazardjik	1	Targovishte	1
Godetch	1	Pernik	2	Shumen	1

Methodology of kidney transplantation in the Clinic of Urology, University Hospital "Alexandrovska", Sofia

The kidney is prepared on a specially organized work table before performing the transplantation itself. An in-depth examination and assessment of the whole organ is performed – it is examined for anatomical features and abnormalities of the vessels and the ureter, for the integrity of the renal capsule and for the presence of any other abnormalities. The perirenal adipose tissue is removed until the renal capsule is presented. The renal vein and artery are dissected and if lateral branches are present they are ligated. The ureter is dissected of excess tissue while maintaining its blood supply.

In the Clinic of Urology, University Hospital "Alexandrovska", Sofia kidney transplantation is performed heterotopically, retroperitoneally, contralaterally in the respective fossa iliaca. A slightly inclined pararectal incision is made 2 cm above crista iliaca anterior superior. After incising the adipose tissue, the abdominal muscles are dissected in a dull way. The

peritoneum is pushed medially and thus the retroperitoneal space is reached, where the iliac vessels are dissected.

The external iliac artery and vein are carefully freed from the surrounding lymphatic pathways, which are ligated. This is followed by the formation of the venous anastomosis (end to side) between the donor renal vein and an external iliac vein by a running polypropylene 5-0 or 6-0 suture. Similarly, the arterial anastomosis (end to side) is formed between the donor renal artery and the iliac artery. After removing the clamps from the venous and, subsequently, from the arterial circulation, the kidney is inspected for turgor, perfusion and areas without blood supply.

Upon successful revascularization of the transplanted kidney, the ureteroneocystostomy is performed. The implantation of the donor ureter is performed using the Lich-Grégoire extravesical technique in the area of the bladder dome. The bladder is filled in advance with sodium chloride solution, then opened between two threads – bladder wall holders. This is where the implantation of the ureter is performed, after its shortening, longitudinal incision at the distal end and intubation with a DJ stent. The anastomosis is performed with a 5-0 monofilament resorbable suture and a running suture through all layers of the bladder wall. Bassini-Röhl antireflux plasty is performed to finish the ureteroneocystostomy.

The mean operative time for the kidney transplantation in the Clinic of Urology, University Hospital "Alexandrovska", Sofia is 186 minutes. The average duration of surgery for a cadaveric donor transplantation is 195 minutes, whereas living donor transplantation has an average operative time of 171 minutes.

RESULTS

All possible early surgical and urological complications were followed up, including these with symptomatic and asymptomatic manifestations. The research team monitored the patients for ureteral stricture, urinary retention, urethral necrosis, urinoma, DJ stent related problems, hematoma, vascular narrowing, thrombosis, lymphocele, urinary tract infection, hernia or inflame-mation in the area of the surgical wound.

In the cohort of 35 patients, early complications were manifested in 16 patients (46%) - 8 patients (23%) transplanted from a living donor and 8 patients (23%) transplanted from a cadaveric donor. Both urological and surgical complications were observed in 3 patients during the follow-up period (Table 4.).

Table 4

Frequency of the early urological and surgical complications in the transplanted patients for the period from 02.2018 to 12.2019 in the Clinic of Urology, University Hospital "Alexandrovska", Sofia.

Early complications	Total	Men	Women
Number of patients	35	28	7
Number of patients with urological complications	9 (26%)	7	2
Urinary infections	9 (26%)	7	2
Number of patients with surgical complications	10 (29%)	9	1
Hematoma	8 (23%)	7	1
Lymphocele	1 (3%)	1	0
Surgical wound infection	1 (3%)	1	0

22 kidney transplantations from cadaveric donors were performed during the period from 02.2018 to 12.2019 at the Clinic of Urology, University Hospital "Alexandrovska", Sofia. Out of them, 17 of the recipients were men and 5 were women. Early urological complications were observed in 4 patients (18%), 3 (18%) of whom were men and 1 was a woman (20%). Four transplant patients had a urinary tract infection which required antibiotic treatment. Early surgical complications were observed in 5 patients (23%) transplanted from a cadaveric donor, all of the patients men. We observed 3 (14%) cases of hematoma, 1 (5%) lymphocele and 1 (5%) infection of the surgical wound. All three of the patients diagnosed with hematoma, required revision of the surgical intervention (Table 5.).

Table 5

Frequency of early urological and surgical complications in patients transplanted from a cadaveric donor for the period from 02.2018 to 12.2019 in the Clinic of urology, University Hospital "Alexandrovska", Sofia.

Early complications	Total	Men	Women
Number of patients	22	17	5
Number of patients with urological complications	4 (18%)	3 (18%)	1 (20%)
Urinary infections	4 (18%)	3 (18%)	1 (20%)
Number of patients with surgical complications	5 (23%)	5	0
Hematoma	3 (14%)	3	0
Lymphocele	1 (5%)	1	0
Surgical wound infection	1 (5%)	1	0

13 kidney transplantations from a living donor during the period from 02.2018 to 12.2019 at the Clinic of Urology, University Hospital "Alexandrovska", Sofia. Out of these transplantations, 11 of the recipients were male and 2 were female. Early urological complications were observed in 5 patients (38%), 4 (36%) of whom were men and 1 was (50%) female. Five transplanted patients had a urinary tract infection which required anti-biotic treatment. Early surgical complications were observed in 5 patients (38%) transplanted from a living donor, 4 (36%) of whom were male and 1 (50%) was female. Five (38%) postoperative hematomas were diagnosed and in 4 of the patients surgical revision was necessary (Table 6.).

Table 6

Frequency of the early urological and surgical complications in patients transplanted from a living donor for the period from 02.2018 to 12.2019 in the Clinic of Urology, University Hospital "Alexandrovska", Sofia.

Early complications	Total	Male	Female
Number of patients	13	11	2
Number of patients with urological complications	5 (38%)	4 (36%)	1 (50%)
Urinary infections	5 (38%)	4 (36%)	1 (50%)
Number of patients with surgical complications	5 (38%)	4 (36%)	1 (50%)
Hematoma	5 (38%)	4 (36%)	1 (50%)

The average hospital stay of the transplanted patients in the Clinic of Urology, University Hospital "Alexandrovska" was 16 days, ranging between 9 and 23 days. The average hospital stay of recipients from a cadaveric donor, who developed early complications was 15 days, compared to 14 days for patients without complications. The average hospital stay of recipients from a living donor, who developed early complications was 18 days, compared to 17 days for patients without complications. Our results do not show a significant difference in the mean hospital stay in transplanted patients from living and cadaveric donors, who develop or do not develop early postoperative complications.

DISCUSSION

Kidney transplantation is a routine method of treatment for patients with end-stage of chronic renal failure, that shows good long-term results [2]. A significant decrease in the incidence of urological and surgical postoperative complications has been achieved over the years with the development of transplantology and different minimally invasive techniques [4]. This is of great importance, because the occurrence of such complications after kidney transplantation may lead to increased morbidity and mortality in transplanted patients [20].

UROLOGICAL COMPLICATIONS

The occurrence of urological complications after kidney transplantation is relatively common. These complications are divided into early and late, according to their time of onset. The frequency of these complications is between 3% and 37%, according to the modern scientific literature data [1]. In the Clinic of Urology, University Hospital "Alexandrovska", Sofia 35 patients underwent transplantation in the period from 02.2018 to 12.2019, and 9 of them had some urological complications. This shows that the incidence of urological complications in transplanted patients during this period is 26%, which corresponds to the data in international literature on

the subject. It is necessary to specify the various criteria for assessing these complications, in order to present our results in more depth. It is noteworthy, that different authors present the urological complications differently, which explains the relatively wide range of their frequency. Some authors note only these complications, which require surgery [23]. Others include complications, such as reflux, macroscopic hematuria and urinary tract infections [29]. There are researchers, who classify the appearance of lymphocele after kidney transplantation as a urological complication. Our results include both asymptomatic or conservatively treated complications, as well as those, that required invasive intervention. Lymphoceles in our study were classified as a surgical complication. Our results also take into account the type of donation. A distinction is made between patients transplanted from a living and a cadaveric donor. The incidence of urological complications was 38% in patients transplanted from a living donor and 18% in patients transplanted from a cadaveric donor.

Urinary tract infections

In the Clinic of Urology, University Hospital "Alexandrovska", Sofia, 35 patients underwent transplants for the period from 02.2018 to 12.2019 and 9 of them were diagnosed with postoperative urinary tract infections. This shows, that the incidence of urinary tract infections in patients who underwent a transplant during this period is 26%. In patients, who received a transplant from a living donor, the frequency is higher - 38%. Patients, who received a transplant from a cadaveric donor show a frequency of UTI of 18%. Postoperative hematomas around the graft were also found in 3 patients, diagnosed with urinary tract infection, requiring revision of the kidney transplant.

Urinoma

The formation of an urinoma is an early urological complication. Most often they are in the area of ureteroneocystostomy. The cause of urinomas could be necrosis of the ureter, surgical error during the anastomosis, insufficient length of the ureter, lesion during explanation or dissection of the ureter. The incidence of urinomas after kidney transplantation has been noted between 1% and 12% in the scientific literature. Some authors, such as Gonzalo Rodriguez et al. [16], Hernandez et al. [18/19] and

Burmeister et al. [6] note all cases of postoperative urinomas. Other authors, such as Dinckan et al. [9], Nie et al. [25] and Streeter et al. describe necrosis of the ureter as a separate complication. According to their results, its frequency is between 0.3% and 2.9%.

No cases of urinoma were observed in the transplant patients in our clinic during the follow-up period.

Strictures of the ureter

Ureteral strictures are mainly late urological complications. They could be due to a surgical problem in the area of ureteroneocystostomy, torsion of the transplanted kidney and ureter or fibrosis, as a result of ischemia [17]. The data show a frequency between 1% and 8%. Streeter et al. describe a higher incidence of ureteral strictures in transplanted patients from a living donor, than from a cadaveric donor, but the difference remains statistically insignificant [36]. The treatment of ureteral strictures in transplanted patients may include percutaneous nephrostomy, balloon dilatation of the ureter and stent fixation. In complicated cases, when the stricture is in the area of the anastomosis, it is possible to perform reimplantation of the ureter. A ureteroplasty is performed when the stricture is in the proximal or middle part of the ureter [3].

In the transplanted patients in our clinic no cases of stricture of the ureter were observed in the follow-up period.

Urinary retention

Urinary retention in transplanted patients can occur as both an early and a late urological complication, depending on whether there is supravesical, vesical or subvesical obstruction [10]. As an early complication, it may be a result of edema or kinking of the ureter, clotting and compression by a hematoma or lymphocele in the iliac fossa. As a late complication, urinary retention occurs as a result of ureteral stenosis or obstruction after the removal of the ureteral stent. Urinary retention may also be due to strictures of the urethra or meatus, benign prostatic hyperplasia, urolithiasis

and an acute rejection of the graft [36]. The frequency of urinary retention after kidney transplantation is between 2% and 20%, according to scientific data [35]. The wide frequency range is due to the different designs and criteria of the various studies. The incidence of supravesical retention is between 2.9% and 10.5%, according to the scientific data [24].
No cases of urinary retention were observed in the transplanted patients in our clinic during the follow-up period.

SURGICAL COMPLICATIONS

This group includes vascular complications (venous or arterial thrombosis, arterial stenosis), surgical wound complications (dehiscence, infection, hernia) and hemorrhagic complications [8]. The current scientific literature shows that the frequency of surgical complications varies between 15% and 34% [30, 31]. These values depend on what the different authors consider to be a surgical complication and what the follow-up period is of the patients.

In the Clinic of Urology, University Hospital "Alexandrovska", Sofia, 35 patients underwent transplants for the period from 02.2018 to 12.2019 and 10 of them developed surgical complications. This shows that the incidence of surgical complications in patients who received a transplant during this period is 29%. In patients who received a transplant from a living donor, the frequency is higher - 38%. Patients who received a transplant from a cadaveric donor show a frequency of 18% for these complications.

Hematoma

Hematomas after kidney transplantation are most common in the first postoperative days. The cause of postoperative hematomas is most often bleeding from the small non-ligated hilum vessels of the graft or from the small, retroperitoneally located vessels of the recipient. Particularly at risk are overweight patients and those taking platelet aggregation inhibitors or anticoagulant therapy [27]. Postoperative hematomas lead to delayed graft function and prolonged dialysis treatment with an increased risk of additional hemorrhage. Often, the postoperative bleeding in transplanted patients stops spontaneously. Recent publications on this topic show an incidence of postoperative bleeding and the appearance of hematomas between 3% and 25% [26]. In cadaveric donation, this frequency is slightly

higher between 7% and 25.4% [28]. In living donation, the incidence of postoperative hematomas is between 3.9% and 17.1% [22].

In the Clinic of Urology, University Hospital "Alexandrovska", Sofia, 35 patients underwent transplants for the period from 02.2018 to 12.2019 and 8 of them developed postoperative hematoma. This indicates, that the incidence of hematomas in patients who received a transplant during this period is 23%. In patients who received a transplant from a living donor, the frequency is higher - 38%. Patients who received a transplant from a cadaveric donor show a frequency of 14%. A revision of the surgical intervention was required in 7 of the patients with postoperative hematoma. From a therapeutic point of view, it is appropriate for large hematomas to have broad indications for revision in order to prevent secondary infections or abscesses. Small symptomatic hematomas could be drained percutaneously.

Lymphocele

Lymphoceles are considered an early complication after kidney transplantation. Most often they are a result of insufficient ligation of the perivascular lymphatic vessels in the area of the iliac vessels of the recipient or of the hilar lymph nodes of the graft [14]. The new antiproliferative immunosuppressive drugs, organ rejection, drug therapy with diuretics, anticoagulants and high-dose steroids are also risk factors for lymphocele formation in the iliac fossa. In most cases lymphoceles do not manifest clinically and are resorbed spontaneously without any therapeutic intervenetion. Percutaneous drainage could be performed in cases of uncomplicated symptomatic lymphocele. If this treatment is unsuccessful then laparoscopic or open technique is used for peritoneal fenestration of the lymphocele. The incidence of lymphocele after kidney transplantation is in the range between 5% and 39%, according to scientific data [33]. Incidence is lower and varies between 1% and 18% when we consider only symptomatic cases [5]. The incidence of lymphocele in patients who received a transplant from a cadaveric donor is higher - 33.9%, according to the current scientific data [15] than in patients who received a transplant from a living donor (12.4% - 24 , 3%) [12]. The incidence of symptomatic lymphocele in patients, who

received a transplant from a cadaveric donor is also higher (12.9% - 15.7%), than in patients who received a transplant from a living donor (1.4% - 5%) [30, 31].

In the Clinic of Urology, University Hospital "Alexandrovska", Sofia, 35 patients underwent transplants for the period from 02.2018 to 12.2019 and 1 of them developed lymphocele, which provides an incidence of 3%. No lymphoceles were observed in the postoperative period of patients who received a transplant from a living donor. For the patients who received a transplant from a cadaveric donor, one patient developed a lymphocele. This patient remained asymptomatic during the time of observation and did not require invasive treatment. The incidence of lymphocele in this group of patients was 5%.

Surgical wound infection

Complications of the operative wound is not a small matter regarding complications after kidney transplantation. They often lead to prolonged hospital stay, frequent rehospitalizations and impaired graft function [20]. The reasons for the increased risk of infection are due to the contact of the surgical area with potentially contaminated urine during ureteroneocystostomy and subsequent immunosuppression. Risk factors for this complication are obesity, diabetes, urinoma, lymphocele, revision of the surgical wound and the use of new and stronger immunosuppressive drugs. According to available scientific data, the incidence of surgical wound infection after kidney transplantation is between 1% and 20% [7]. El Hag et al. and Kocak et al. investigated the incidence of surgical wound infections in transplanted patients from a living donor. In their group of patients the frequency of this complication was similarly between 2% and 15% [11, 22]. In the Clinic of Urology, University Hospital "Alexandrovska", Sofia, 35 patients underwent transplants for the period from 02.2018 to 12.2019, and in 1 of them a postoperative infection of the operative wound was detected. The incidence of surgical wound infection in transplanted patients during this period was therefore 3%. No wound infections were observed in the postoperative period in patients, who received a transplant from a living donor. In patients, who received a transplant from a cadaveric donor, one patient developed inflammation of the surgical wound. In this case revision of the surgical wound was not necessary. The inflammatory process was controlled conservatively with regular dressings, surgical wound cleansing

and antibiotic therapy. The incidence of surgical wound infection in this group of patients was 5%.

Thrombosis

Renal artery or vein thrombosis is an early surgical complication after a kidney transplantation. These are rare but are extremely serious complications, as they can lead to loss of the transplanted kidney, due to lack of collateral blood supply [20]. The performance of a timely thrombectomy is essential to preserve the transplanted kidney. In the case of renal vein thrombosis, an urgent revision is necessary in order to avoid rupture of the graft. Often the cause of thrombosis is a technical error with a lesion of the vascular intima, kinking or vascular torsion. Other risk factors are hemodynamic instability of the patient, multiple graft vessels and underlying coagulation disorders. Poor kidney function and arteriosclerotic changes in the recipient's vessels can also lead to venous or arterial thrombosis of the graft. A hematoma or a lymphocele in the area of the iliac fossa can compress the renal vein and also cause venous thrombosis. There is data in the literature that the incidence of venous and arterial thrombosis after kidney transplantation varies between 0.3% and 7% [34].

In the transplanted patients at our clinic there are no cases of venous or arterial thrombosis for the follow-up period.

Hernia

The incidence of hernia in the area of the surgical wound after kidney transplantation is described in the available literature with an incidence between 1% and 18% [13]. Risk factors for the occurrence of hernias are performed revisions of the operative wound, multiple transplantation procedures, obesity, aging, and the use of antiproliferative immunosuppressive drugs.

This postoperative complication was not observed in the group of patients we studied.

In our group of patients, the average hospital stay does not differ significantly for patients with or without postoperative complications. This

may be due to the fact that the average time for postoperative monitoring for all transplanted patients in our clinic depends on some features of the healthcare system in Bulgaria.

SUMMARY AND DISCUSSION

Early postoperative complications were observed in 46% of the patients who underwent transplants. Urological complications were developed in 26% of them. The incidence of urinary tract infections in patients who underwent transplants was 26%. We did not observed any urinary retention, stricture of the ureter, or urinoma during the follow-up period.

The incidence of surgical complications in patients who underwent transplants was 29%. One patient in our group developed postoperative lymphocele, showing a complication rate of 3%. Surgical wound infection was found in 3%. No cases of venous or arterial thrombosis and hernia in the area of the operative wound were observed during the follow-up period.

CONCLUSIONS

1. The frequency of individual early postoperative urological and surgical complications corresponds to the literature data from other transplant centers.

2. The average time of hospital stay for transplanted patients in our clinic could depend on some features of the healthcare system in the country.

3. Our results suggest that the Clinic of Urology, University Hospital "Alexandrovska", Sofia performs transplantation activities that are adequate to world standards in kidney transplantation.

BIBLIOGRAPHY

1. Akoh J A, Opaluwa A S, Weller D: Urological complications of renal transplantation: Reducing the risk. Saudi journal of kidney diseases and transplantation 2009, 20, 1005-1009.
2. Andrews P A: Renal transplantation. BMJ 2002, 324: 530-534.

3. Asadpour A, Molaei M, Yaghoobi S: Management of ureteral complications in renal transplantation: prevention and treatment. Saudi journal of kidney diseases and transplantation 2011, 22: 72-74.
4. Benoit G, Delmas V, Gillot C, Hureau J: Anatomical bases of kidney transplantation in man. Anatomia Clinica 1984, 6: 239-245.
5. Bischof G, Rockenschaub S, Berlakovich G, Langle F, Muhlbacher F, Fugger R, Steininger R: Management of lymphoceles after kidney transplantation. Transplant international 1998, 11: 277-280.
6. Burmeister D, Noster M, Kram W, Kundt G, Seiter H: Urological complications after kidney transplantation. Der Urologe 2006, 45: 25-31.
7. Dean P G, Lund W J, Larson T S, Prieto M, Nyberg S L, Ishitani M B, Kremers W K, Stegall M D: Wound-healing complications after kidney transplantation: a prospective, randomized comparison of sirolimus and tacrolimus. Transplantation 2004, 77: 1555-1561.
8. Dimitroulis D, Bokos J, Zavos G, Nikiteas N, Karidis N P, Katsaronis P, Kostakis A: Vascular complications in renal transplantation: a single-center experience in 1367 renal transplantations and review of the literature. Transplantation proceedings 2009, 41: 1609-1614.
9. Dinckan A, Tekin A, Turkyilmaz S, Kocak H, Gurkan A, Erdogan O, Tuncer M, Demirbas A: Early and late urological complications corrected surgically following renal transplantation. Transplant international 2007, 20: 702-707.
10. Duty B D, Barry J M: Diagnosis and management of ureteral complications following renal transplantation. Asian Journal of Urology 2015, 2: 202-207.
11. El Hag M E, El Imam M, Omran M, Idris M, Elsheikh A, Elsabig M, Miskeen M: Renal Transplantation GEZIRA Hospital for Renal Disease and Surgery (GHRDS). Sudanese Journal of Public Health 2009, 4: 265-274.
12. El-Mekresh M, Osman Y, Ali-El-Dein B, El-Diasty T, Ghoneim M A: Urological complications after living-donor renal transplantation. BJU international 2001, 87: 295-306.
13. Flechner S M, Zhou L, Derweesh I, Mastroianni B, Savas K, Goldfarb D, Modlin C S, Krishnamurthi V, Novick A: The impact of sirolimus, mycophenolate mofetil, cyclosporine, azathioprine, and steroids on wound healing in 513 kidney-transplant recipients. Transplantation 2003, 76: 1729-1734.

14. Glass L L, Cockett A T: Lymphoceles: diagnosis and management in urologic patients. Urology 1998, 51: 135-140.
15. Goel M, Flechner S M, Zhou L, Mastroianni B, Savas K, Derweesh I, Patel P, Modlin C, Goldfarb D, Novick A C: The influence of various maintenance immunosuppressive drugs on lymphocele formation and treatment after kidney transplantation. The Journal of urology 2004, 171: 1788-1792.
16. Gonzalo Rodriguez V, Rivero Martinez M D, Trueba Arguinarena J, Calleja Escudero J, Muller Arteaga C, Fernandez del Busto E: Diagnosis and treatment of urological complications in kidney transplants. Actas Urologicas Espanolas 2006, 30: 619-625.
17. Guardiola Mas A, Sanchez Gascon F, Gimeno L, Llorente Vinas S, Lopez Cubillana P, Nicolas Torralba J A, Banon Perez V J, Valdelvira Nadal P: Urologic complications in renal transplantation. Study of 250 cases. Actas Urologicas Espanolas 2001, 25: 628-636.
18. Hernandez D, Rufino M, Armas S, Gonzalez A, Gutierrez P, Barbero P, Vivancos S, Rodriguez C, de Vera J R, Torres A: Retrospective analysis of surgical complications following cadaveric kidney transplantation in the modern transplant era. Nephrology, Dialysis, Transplantation 2006, 21: 2908-2915.
19. Hernandez D, Rufino M, Bartolomei S, Gonzalez-Rinne A, Lorenzo V, Cobo M, Torres A: Clinical impact of preexisting vascular calcifications on mortality after renal transplantation. Kidney international 2005, 67: 2015-2020.
20. Humar A, Matas A J: Surgical complications after kidney transplantation. Seminars in dialysis 2005, 18: 505-510.
21. Humar A, Ramcharan T, Denny R, Gillingham K J, Payne W D, Matas A J: Are wound complications after a kidney transplant more common with modern immunosuppression? Transplantation 2001, 72: 1920-1923.
22. Kocak T, Nane I, Ander H, Ziylan O, Oktar T, Ozsoy C: Urological and surgical complications in 362 consecutive living related donor kidney transplantations. Urologia internationalis 2004, 72: 252-256.
23. Krol R, Ziaja J, Chudek J, Heitzman M, Pawlicki J, Wiecek A, Cierpka L: Surgical treatment of urological complications after kidney transplantation. Transplantation proceedings 32006, 8: 127-130.
24. Minnee R C, Surachno S, Kox C, ten Berge I J, Aronson D C, Idu M M: Is a selective splinted ureterocystostomy protocol feasible in renal transplantation? An analysis of 475 renal transplantations. Transplant international 2006, 19: 558-562.

25. Nie Z L, Li Q S, Jin F S, Zhang K Q, Zhu F Q, Huo W Q, Ma Q: Urological complications in 1223 kidney transplants. Zhonghua yi xue za zhi 2009, 89: 1269-1271.
26. Parada B, Figueiredo A, Mota A, Furtado A: Surgical complications in 1000 renal transplants. Transplantation proceedings 2003, 35: 1085-1086.
27. Pawlicki J, Cierpka L, Krol R, Ziaja J: Risk factors for early hemorrhagic and thrombotic complications after kidney transplantation. Transplantation proceedings 2011, 43: 3013-3017.
28. Perez Fentes D A, Blanco Parra M, Toucedo Caamano V, Romero Burgos R, Punal Rodriguez J A, Varo Perez E: Surgical complications after kidney transplantation. Research based on 185 cases. Actas Urologicas Espanolas 2005, 29: 578-586.
29. V, Leisinger H J, Pascual M, Jichlinski P: Urological complications in renal transplantation from cadaveric donor grafts: a retrospective analysis of 20 years. Urologia internationalis 2005, 75: 144-149.
30. Risaliti A, Corno V, Donini A, Cautero N, Baccarani U, Pasqualucci A, Terrosu G, Cedolini C, Bresadola F: Laparoscopic treatment of symptomatic lymphoceles after kidney transplantation. Surgical endoscopy 2000, 14: 293-295.
31. Risaliti A, Sainz-Barriga M, Baccarani U, Adani G L, Montanaro D, Gropuzzo M, Tullissi P, Boscutti G, Lorenzin D, Mioni G, Bresadola F: Surgical complications after kidney transplantation. Giornale italiano di nefrologia 2004, 21 Suppl 26: 43-47.
32. Rodriguez G V, Martinez R M, Arguinarena T F, Martin M S, De Castro Olmedo C, Del Busto F E: The use of double J stent for prevention of urological complications in kidney transplants. Actas Urologicas Espanolas 2008, 32: 225-229.
33. Sansalone C V, Aseni P, Minetti E, Di Benedetto F, Rossetti O, Manoochehri F, Vertemati M, Giacomoni A, Civati G, Forti D: Is lymphocele in renal transplantation an avoidable complication? American Journal of Surgery 2000, 179: 182-185.
34. Seow Y Y, Alkari B, Dyer P, Riad H: Cold ischemia time, surgeon, time of day, and surgical complications. Transplantation 2004, 77: 1386-1389.

35. Srivastava A, Sinha T, Madhusoodanan P, Karan S C, Sandhu A S, Sethi G S, Kotwal S V, Bhatyal H S, Sood R, Gupta S K, Verma P P: Urological complications of live related donor renal transplantation: 13 years' experience at a single center. Urologia internationalis 2006, 77: 42-45.
36. Streeter E H, Little D M, Cranston D W, Morris P J: The urological complications of renal transplantation: a series of 1535 patients. BJU international 2002, 90: 627-634.

doi: 10.20903/zpopov-2022-0012 UDK: 616.61-089.843(497)

Ninoslav IVANOVSKI[1], Jelka MASIN[2], Perko KOLEVSKI[3],
Olivera STOJCEVA-TANEVA[4], Zivko POPOV[5]

USE OF ELDERLY LIVING KIDNEY DONORS – 20 YEARS OF EXPERIENCE IN THE BALKANS

Abstract

The Balkan region has changed dramatically over the past 20 years. Despite transplantation efforts, dialysis remains the standard way of treating end stage renal disease. Living renal transplantation is still the predominant transplant activity. Seeking to solve this problem, we decided to accept expanded criteria for living donors including the elderly, marginal, unrelated, and ABO incompatible individuals. We present our 20 years of experience with 230 living donor renal transplantations, using elderly individuals, including 90 individuals older than 65 years (mean age 68 ± 4.5; range = 65 - 88; ED group). The predominantly haploiddentical recipients had a mean age of 45 ± 6 (range = 18 - 56 years). Sequential immunesuppressive protocols were used in all cases including induction with antithymocyte-globulin or interleukin-2 receptor antagonists. We analysed the

[1] Clinical Hospital "Zan Mitrev" Skopje, Medical Faculty, University "SS Cyrill and Methodius, Skopje, North Macedonia (e-mail: nivanovski@yahoo.com)

[2] University Clinic of Nephrology, Medical Faculty, Skopje, North Macedonia

[3] Medical Faculty, University "SS Cyrill and Methodius", Skopje, North Macedonia

[4] Centre for haemodialysis "Diaverum" Skopje, Medical Faculty, Skopje, North Macedonia

[5] Macedonian Academy of Sciences and Arts Skopje; Medical Faculty, University "SS Cyrill and Methodius, Skopje, North Macedonia
(e-mail: zivkopopov2000@yahoo.com)

5-year Kaplan-Meier graft survival rate, rejection episodes, delayed graft function, and renal function for comparison with the outcomes of 110 kidneys from younger donors (mean age = 53.4 years; range 25 - 62; YD group) and haploidentical recipients (mean age 32.2; range = 16 - 42). This was performed within the same period. The 3- and 5-year cumulative graft survival rates in the ED group were 81% and 72% compared with 85% and 81% in the YD group, respectively (P>0.9, NS). The incidences of acute rejection episodes were also comparable for both groups (19% and 17%, respectively). Delayed graft function occurred in 15% of the ED group but only in 8% in YD group. The serum creatinine value at the end of 60 months of follow up was 146.04 μmol/L in the ED group versus 123.38 μmol/L in the YG group (P< .001). There were no major surgical complications in either group. We recommend the use of elderly living donors as a valuable source of kidneys, especially in countries where deceased donor transplantation has not yet been established.

Keywords: Kidney transplant, elderly donors, increased donor pool

INTRODUCTION

In the last 30 years, the Balkan region in South Eastern Europe has changing dramatically. According to the EDTA – ERA registry data, the incidence and the prevalence of CKD patients in the Balkan countries is similar with those in Western Europe. However, transplant activity is still very low when compared with the developed countries, and dialysis remains the usual means of treatment. The shortage of available organs for kidney transplantation has led to several strategies in order to expand the donor pool. Our transplant centre promoted the strategy of acceptance of expanded criteria of living donors, including advanced age donors (over 65 years), marginal, unrelated, and ABO incompatible donors (1, 2).

Increased incidence of end-stage renal disease and an actual shortage of organs has led to the introduction of expanded criteria of organ donors including older donors. Thus, over the past decade older donors, (living or deceased) have become a relevant source of organs. Since 1999, the United Network for Organ Sharing reported an increase in kidney donors above age 65, an increase of 33% living donors and 26% for deceased donors. Initial

single centre reports of patient and graft survival of the recipients of kidneys from living donors 50 - 71 years old after 5 years are encouraging. Patient and graft survivals were comparable to those of recipients of younger living donor kidneys, and GFR appeared stable, though at a lower level (3, 4,).

Our first experience of 28 older living donor recipients was published in the same journal 2001, but, despite the encouraging results, the significance was limited due to the limited number of transplant patients.

MATERIAL AND METHODS

We performed a 5 year cumulative graft survival study in a total of 230 patients who underwent living donor renal transplantation in our Centre over the last 20 years. The living related (91.5%) and unrelated (8.5%) kidney donors were accepted according to our policy of widely acceptance of all potential donors. (1) Among the 230 subjects, 90 received kidneys from donors over 65 years of age (elderly donor group – EDG, mean age 68, range 66 - 86). The usual work-up for elderly donors was applied including the careful elimination of any potential risks associated with the donation process. Diabetic and nonregulated hypertensive elderly donors were excluded. The lower level of donor's GFR accepted for transplantation was 65 ml/min. Predominantly haploidentical children make up the recipient group (EDG) with a mean age of 45 ± 6 (range 35 - 58) years. The usual preservation procedure with Euro-Collins solution was used. All recipients were treated with the Quadruple Sequential Immunosuppressive protocol including an induction therapy (ALG, ATG or Il-2R antagonists) and triple drug maintenance therapy with Micophenolat Mofetil or Azathioprin, Prednisolone and Cyclosporine A. Over the last 10 years (2000 to 2010), we used protocols with CyA minimisation corresponding to the Co trough level between 75 to 100 ng/m, full dose of MMF (2 gr/day) and induction with IL-2R antagonists or ATG for unrelated donors. The results were compared with the recipient's group of 140 patients (mean age 32.2, range 16 - 40 years) with the living donors younger then 65 years (YDG, mean age 53.4,

range 30 - 62). There was no statistical difference between the groups, warm and cold ischemia times, anastomosis time, HLA mismatches, racial distribution and number of unrelated donations.

RESULTS

The 3 and 5 years Kaplan Meier cumulative death censored graft survival rate in the EDG was 81% and 72%, compared with 85% and 81% in the YDG without statistically significant differences (Log rank test: p = 0.6567). Delayed graft function appeared in 15 patients in the EDG (16%) and 7 in the YDG (5%). Serum creatinine after 5 years of follow-up was 146.04 ± 33.9 in the EDG compared with 123.38 ± 31.8. The rate of rejection episodes was low in both groups of patients: 16 (17%) and 17 (16%). The results are presented on Table 1 and Figure 1.

There were no significant surgical complications among the recipients and renal donors.

Table 1

Living donor recipient and transplant characteristics

	Age of the Donors		p
	>65 (68.3 years)	>65 (53.4 years)	< 0;01
N(%)	90 (40%)	140(60%)	<0;01
Age (years)	45.6 (35-58)	32.2 (16-40)	<0,01
Male	55.4%	57.6%	ns
Related	85 (94%)	125 (89%)	ns
Nonrelated	5 (6%)	15 (11%)	ns
Cause of ESRD			
Glomerulonephritis	28%	29%	ns
Nephroangiosclerosis	20%	22%	ns
Lithiasis – Pyelonephritis	14%	16%	ns
Polycystic kidney disease	12%	13%	ns
Diabetes	7%	5%	ns
Others	19%	15%	ns
HLA mismatch	2.2	2.8	ns
WIT	3‘	3’	ns
CIT	3.3 h	3.4 h	ns
Preservation	Euro-Colins	Euro-Collins	

Surgical complications			
Wound infections	8 (9%)	11 (8%)	ns
Lymphocells	6 (6.6%)	10 (7%)	ns
Ruptures	1	1	
Arterial kinking	3 (3.3%)	3 (2%)	ns
Renal artery stenosis	5 (5.5%)	7 (5%)	ns
Renal artery thrombosis	1	1	
DGF	15 (16%)	7 (5%)	0.001
Rejection episodes	16 (17%)	17 (15%)	ns
Serum creatinine	146±33	123±31	0.001

WIT – Warm Ischaemia Time
CIT – Cold Ischaemia Time
DGF – Delayed Graft Function

DISCUSSION

Our findings confirmed the excellent clinical outcomes achieved with transplantation from older living donors, even over 65 years old. The excellent 5 graft survival rate in the EDG (74%) is not statistically different from those obtained with the younger donors (81%). Comparing our previous results regarding use of elderly living donors published in the same journal 10 years ago, it is clearly demonstrated that there are better short time outcomes regarding graft survival. The cumulative death censored graft survival rate of 74% after 5 years in the EDG is significantly better compared with 67% in the previous study. (5) The better graft survival rate is confirmed also in the YDG (74% vs. 81%, respectively). The introduction of new immunosuppressive protocols with ATG or IL-2R antagonists as an induction, a full dose of Micophenolat Mofetil as a part of a regular triple drug immunosuppression, and CNI minimisation protocols clearly contributed to better outcomes of living transplantation using elderly donors. Although the serum creatinine after 5 years of follow up was more among recipients of older living transplantation (146 μmol compared with those with younger donors (123 μmol/l), the results are still very satisfactory. Both values of serum creatinine are clearly superior when compared with

the graft function in deceased donor transplantation after 5 years (3, 6). DGF in our experience is still a clinically relevant problem when using elderly donors, but we strongly believe that the use of modern preservation solutions (HTC, Wisconsin, Celsior..) instead of Euro-Collins may contribute in minimising the problem. Regarding rejection episodes, we experienced a relatively low percentage (16 and 17% for both groups of the recipients), and this is also confirmed in daily clinical practice all over the world. The low percentage of rejection episodes may also contribute to better short and long term survival of renal transplantation.(7)

In summary, we confirm an excellent 5 years graft survival rate with living donors in our study cohort of 90 transplant recipients, even with those patients over 65 years of age. These results underline our policy to accept elderly donors as a valuable source of organs in the Balkans as well to recommend this practice to all regions where a deceased donor program is still underdeveloped. Thus, for further relevant conclusions regarding use of elderly living donors as a valuable source of organs, further additional long term survival studies are needed.

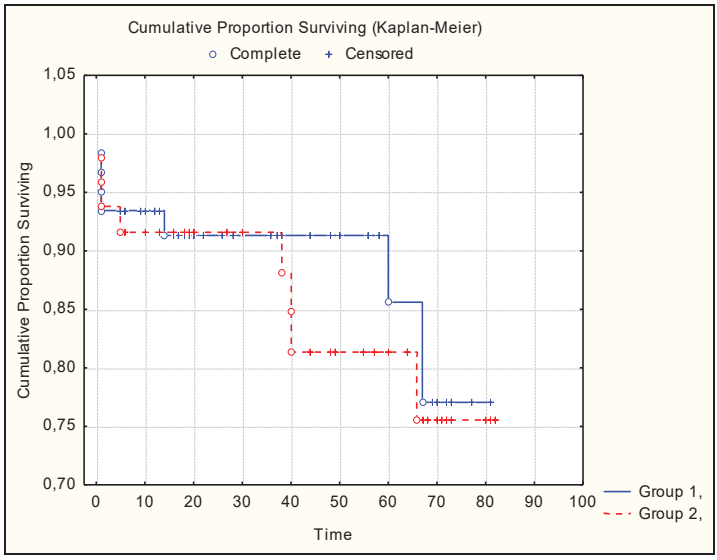


Figure 1 – Cumulative survival of kidney graft recipients regarding age
N=219
Log rank test: p = 0.6757
Group 1 – donor age < 65
Group 2 – donor age > 65

REFERENCES

1. Ivanovski N, Popov Z, Kolevski P. The Donor Organ Shortage on the Balkans: Accept Everyone. Transplant Proc 1997; 29: 3397
2. Iordanous Y, Seymour N, Young A, et al. Recipient Outcomes for Expanded Criteria Living Kidney Donors: The Disconnect Between Current Evidence and Practice. Am J Transplant 2009; 9: 1558
3. Gill SJ, Gill J, Rose C et al. The Older Living Kidney Donor: Part of the solution to the organ shortage. Transplantation 2006; 82: 1662
4. Abulezz RS. Kidney Transplantation From Older Donors: Proceed with Caution. Kidney Int 2010; 78: 637
5. Ivanovski N, Popov Z, Kolevski P et al. Use of advanced age donors in living renal transplantation – is it justified? Transplant Proc 2001; 33: 1227
6. Chavalitdhamrong D, Gill J, Takemoto S et al. Patient and Graft Survival from Deceased Kidney Donors Age 70 Years and Older: An Analysis of the Organ Procurement Transplant Network/United Network of Organ Sharing Database. Transplantation 2008; 85: 1573
7. Serur D, Saal S, Wang J et al. Deceased-donor kidney transplantation: improvement in long term survival. Nephrol Dial Transplant 2011; 26: 317

doi: 10.20903/zpopov-2022-0013 UDK: 616.61-089.843-097

Перко КОЛЕВСКИ[1], Нинослав ИВАНОВСКИ[2], Живко ПОПОВ[3]

ХЛА ВО ТРАНСПЛАНТАЦИЈА НА БУБРЕГ

Апстракт

Сознанијата за ХЛА-системот, за неговата структура и за улогата во имуната одбрана на организмот овозможија да се оствари широк процес на трансплантација на органи и ткива. Неговиот огромен полиморфизам покажа нагласување на индивидуалноста на секоја личност и бара остварување на најголема хистокомпатибилнност помеѓу примателот и донорот на органот. Раководење со процесите на препознавање туѓо од свое, подготвување на туѓите структури за презентирање на имуниот систем и активација на целокупниот одбранбен систем говорат за важната улога на ХЛА-системот во процесите на прифаќање-отфрлање на трансплантатот. Кај серија од 142 трансплантации следени се ХЛА-односите помеѓу паровите прима-тел-дарител и при најголем број од трансплантираните болни (117 – 82, 39 %), меѓу нив и дарителите постоеле 2 инкомпатибилности, односно постоела хаплоидентичност. Поголем број од нив имаат функционални бубрези по првата, односно по третата година, а одреден број и по 10 години. Сле-

[1] Medical Faculty, University “SS Ciryll and Methodius”, Skopje, Republic of North Macedonia (e-mal: p.kolevski@itm.org.mk)

[2] Clinical Hospital “Zan Mitrev” Skopje, Medical Faculty Skopje, Republic of North Macedonia (e-mail: nivanovski@yahoo.com)

[3] Macedonian Academy of Sciences and Arts Skopje; Medical Faculty, University “SS Cyrill and Methodius”, Skopje, Republic of North Macedonia
(e-mail: zivkopopov2000@yahoo.com)

дена е ХЛА-алоимунизацијата настаната од претходни трансфузии или бременост и нејзиното влијание врз прифаќањето на трансплантатот. Најголем број од болните не примале трансфузии (64 – 45,77 %) или, пак, се во групата до 5 трансфузии. ХЛА-антителата почнуваат да се појавуваат кај болните во групата до 5 трансфузии, додека во групата болни до 10, односно над 10 трансфузии, значајно се присутни. Најголем број болни не примале трансфузии (64 – 45,77 %) или, пак, се во туупата до 5 трансфузии. ХЛА-антителата почнуваат да се појавуваат кај болните во групата до 5 трансфузии, додека во групата болни до 10, односно над 10 трансфузии, значајно се присутни. Дискутиран е процесот на имуномодулација кај целните трансфузиолошки протоколи. Нагласена е важноста на вкрстената проба, навременото откривање на појавата на отфрлање и следењето на имуносупресијата преку определување на нивото на Cd3, CD4 и CD8, кои треба да се движат во нормални граници.

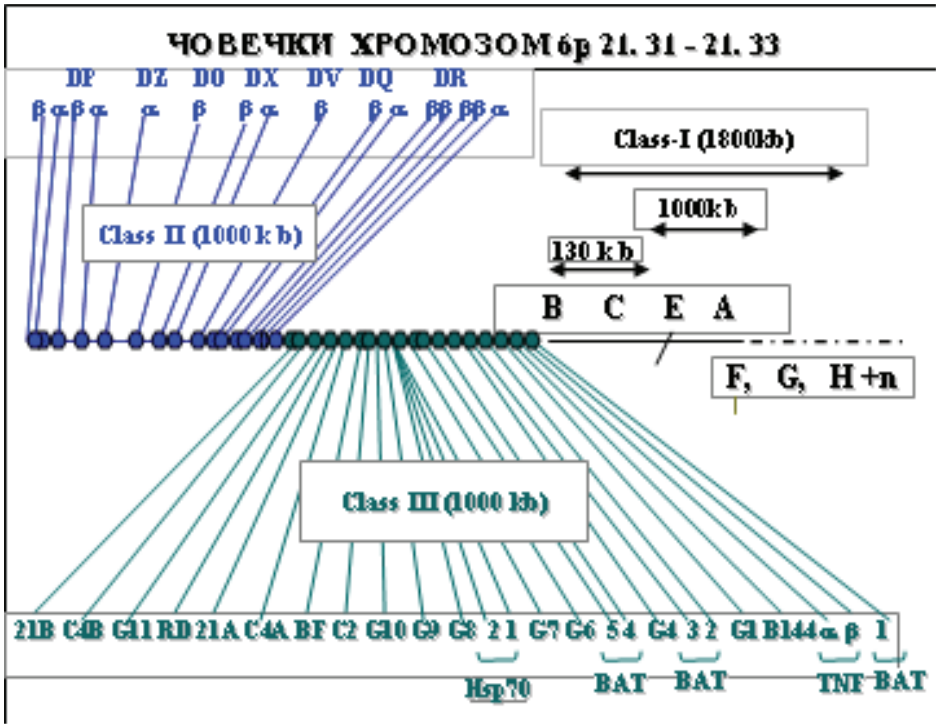
ХЛА-СИСТЕМ

ХЛА-системот (Human Leucocyte Antigen или изворно наречен Human Leucocyte Locus A), односно СМН (Complex Major Histocompatibility), претставува збир на гени кои детерминираат појава на антигени најшироко присутни во организмот со воспоставување и контрола на голем број имунолошки функции со особено значење за животен опстанок.

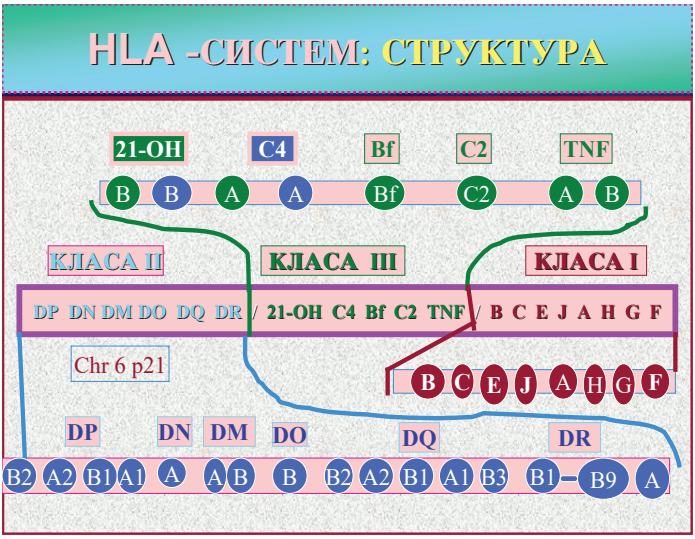
Во основа на имуната одбрана се наоѓа процесот на препознавање на туѓите структури од своите и невоспоставување имун-одговор против себе, кој се стекнува уште интраутерино и е генетски детерминиран. За да се разбере оваа карактеристика, неопходно е да се запознаат структурата и функциите на СМН-системот.

Структура на ХЛА-антигени

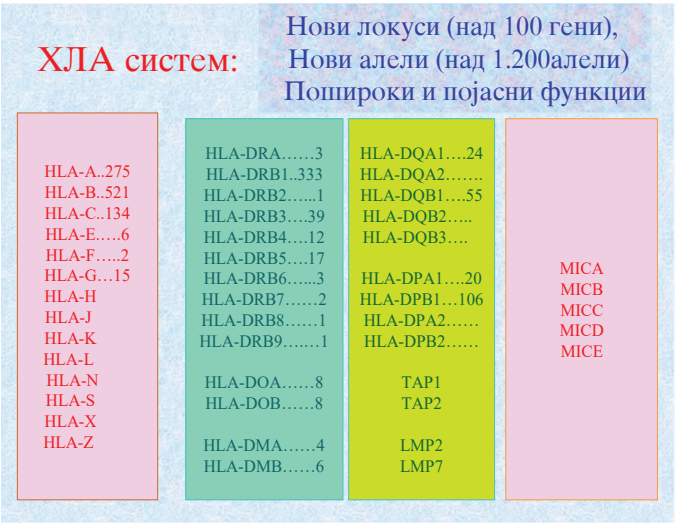
Молекулите на ХЛА-системот се определени од генетски комлекс присутен на кусиот крак на 6-тиот хромозом (6р 21.31 – 21.33).



Тој содржи гени подредени во три класи, и тоа:
I класа, со локусите B, C, E, J, A, F, G, H;
III класа, со локусите 21-OHB, C4B,G11...21OHA, C4A Bf, C2, TNF, BAT и II класа, со локусите DP, DN, DO, DX, DV, DQ, DR.



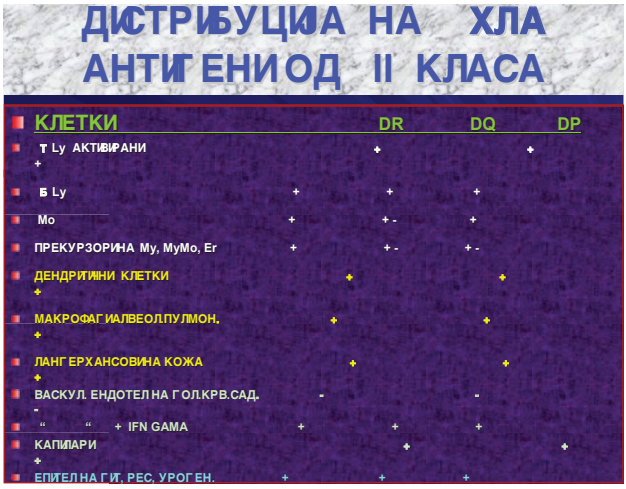
Бројот на гените не е краен, се претпоставува дека ги има повеќе од 1.000, кои детерминираат појава на огромен број алели, па така, овој систем има најголем полиморфизам.

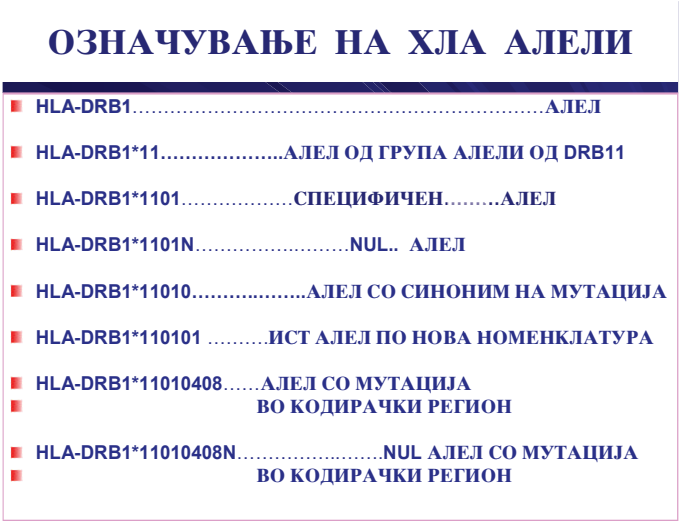


ХЛА-молекулите од I класа се наоѓаат на површината на сите клетки со јадро, додека молекулите од гените од Г-локусот од I класа

се наоѓаат само во трофобластот, од целата II класа се наоѓаат, пред сè, на Б-лимфоцити, потоа на Т-активирани лимфоцити, на макрофагите, во ендотелот на капиларите и во епителот на гастроинтестиналниот, респираторниот и урогениталниот систем.

3.





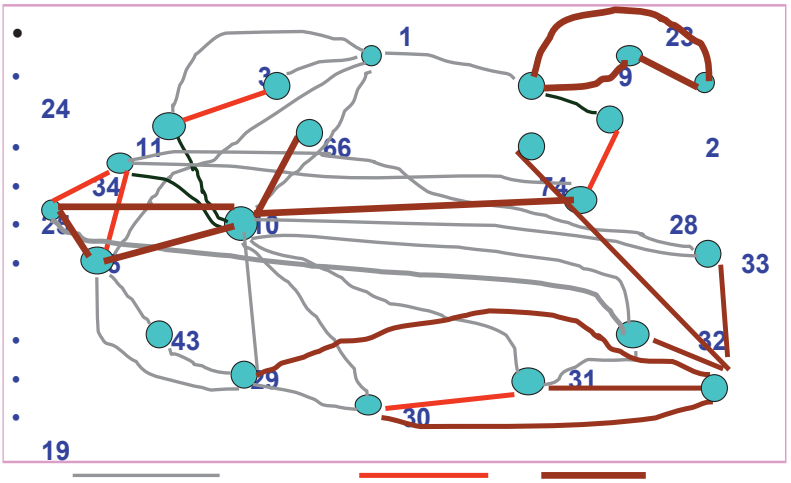
Тромбоцитите од II класа немаат антигени, а од I класа имаат. На нивната експресија може да влијаат цитокините, други имунолошки структури и некои вируси. ХЛА-молекулите се експресирани над кле-

точната мембрана. Тие се подвижни (мобилни), може да бидат ендоцитозирани, рециклирани и ослободени во околината. Сите локуси експресираат молекули над клеточната мембрана, освен HLA-E, HLA-F и HLA-H. Молекулите од прва класа се синтетизираат со T/2 од 8 до 10 часа, а втора класа T/2 за 36 часа. Антигените од првата класа се состојат од еден гликопептиден ланец со 44.000 далтони MT- тежок синџир кој во екстрацелуларниот дел има три домени (алфа 1 состав до 90 аминокиселини, алфа 2 од 90-182 а.а. и алфа 3 0183 д0273 а.а.) и еден лесен синџир кој е прилепен за него – бета 2 микроглобулин – 11500д и до 99а.а. Во трансмембранскиот дел има 26 а.а., во интрацитоплазматскиот дел граден е од 30 д035 а.а. II класа има два гликопептидни синџири – тежок, со 32000 MT –34000 д (во надворешниот дел има алфа 1 домен, со 85-88а.а., алфа 2 домен, со 95а.а.), и лесен, со 27-29000MT (бета 1 со 95а.а., бета 2 со 95а.а.). Исто така, содржи трансмембрански дел и интрацитоплазматски дел.

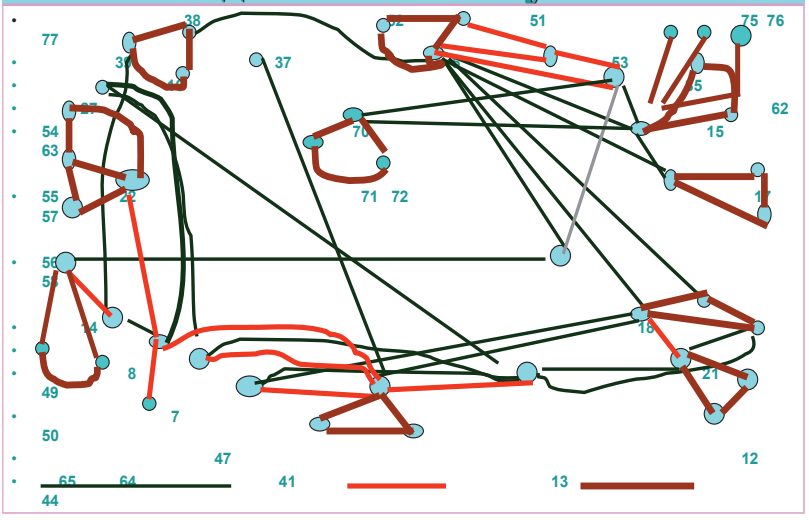
Посебни карактеристики на ХЛА-антигени во прифаќање-отфрлање на трансплантатот

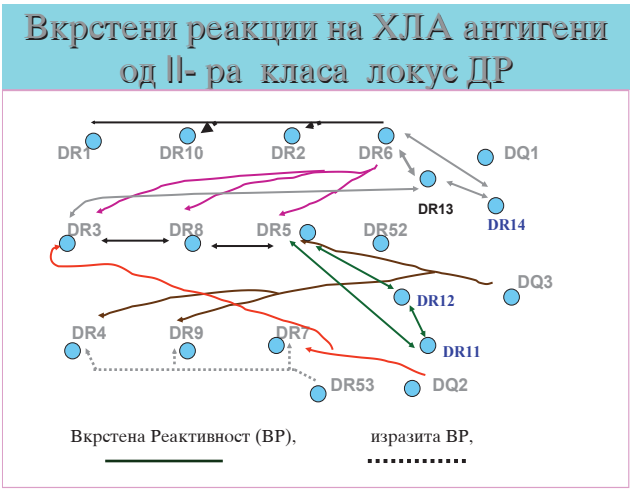
ХЛАалелите имаат посебни карактеристики во составот, што го прави многу сложен системот. Така, постојат основни специфичности, на пример, алела А2 има раздвоени и асоцирани алели, А203, А210 или А9 има А23, А24, А34, А66. Ова е присутно кај многу алели. Исто така, можни се замени на некои аминокиселини со други, па алелата добива свој полиморфизам. Постојат т.н. по структура долги алели и куси алели, каде што кусите алели може да бидат составен дел на долгите и антителата специфични за кусите алели да реагираат и со долгите алели. Тој феномен е наречен вкрстени реакции кои ги има во секоја класа посебно, поточно фамилии на вкрстена реактивност.

Вкрстени реакции на ХЛА антигени од I- ва класа локус А

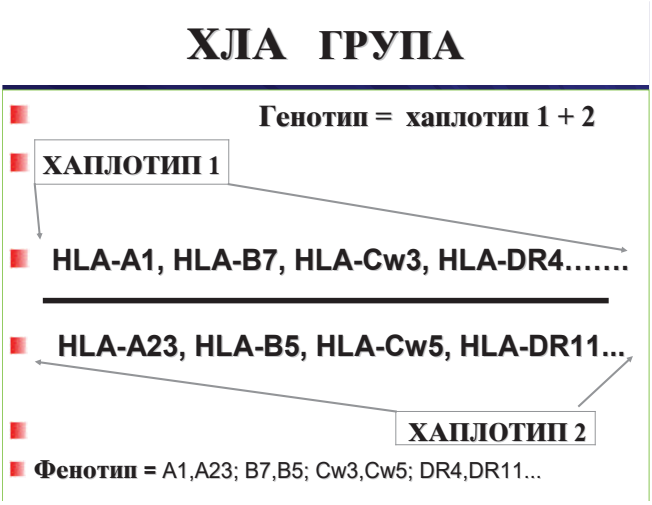


Вкрстени реакции на ХЛА антигени од I класа локус В





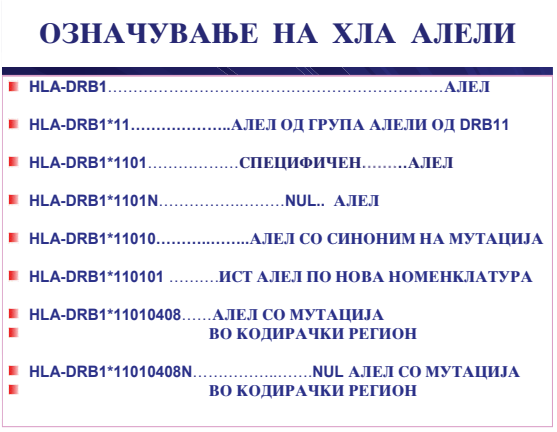
Определувањето на алелите претставува ХЛА-типизација, односно определување на антигените од локусите A, B, C, DR и DQ, па нивното фенотипско определување претставува ХЛА-група.



Инаку, тие се наследуваат во блокови – хаплотипно, и тоа по еден хаплотип од секој родител.

Во почетокот, алелите од прва и трета класа се определуваа серолошки –микролимфоцитотоксичен тест, фиксација на комплемент на тромбоцити, а од втора класа, со клетка – клетка реакција МЛР (мешана лимфоцитна реакција). Потоа се развија повеќе методи: олигонуклеотидно типизирање: RFLP (Restriction Fragment Length Polymorphism), SSOP (Sequence Specific Oligonukleotide Probes), SSP (Sequence Specific Primers), PCR (Polymerase Chain Reaction). Сега се користи PCR (полимераза верижна реакција).

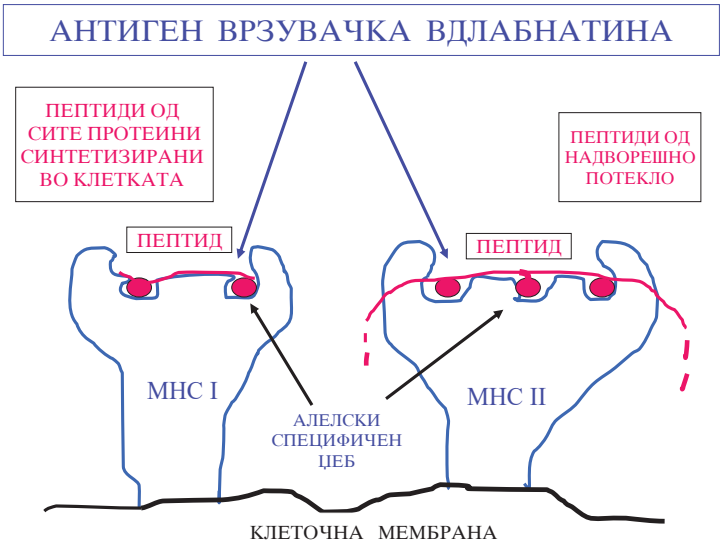
Означувањето на алелите е различно, на пр. HLA-DRB1.



Улога на ХЛА системот

Потребата од напредокот во трансплантацијата на органи и ткива овозможи испитување и добивање нови сознанија за структурата и улогата на ХЛА-системот. Негова главна употреба е во трансплантацијата на органи и ткива, а подоцна се покажа и во трансфузијата на крв и крвни деривати. Посебна важност има во репродуктивната имунологија, каде што односот помеѓу генетските структури на мајката, таткото и плодот, се наоѓаат во меѓусебни корелации. Породувањето претставува отфрлање на хаплоидентичен трансплантат.

Најважната функција на ХЛА е во **имунолошката одбрана** на организмот преку директно учество, и активација и синхронизација на многу имунолошки процеси, што овозможува добра имунолошка одбрана. Нобеловецот и откривачот на ХЛА-системот кај човекот, познатиот Жан Досе (Jean Dausset) истакнал дека ХЛА-системот е главниот штаб на имунолошката одбрана кај човекот и за неговото преживување. Уште во едукацијата на клетките – лимфоцитите во тимусот, се создава репертоар на селектирани лимфоцити, од кои едни носат класа на диференцијација CD8 и распознаваат молекули ХЛА – класа I, односно носат класа на диференцијација CD4 и распознаваат ХЛА-молекули од класа II. Тие препознаваат туѓо од свое, можат да го процесуираат или да го подготват и да го презентираат како интрацелуларни пептиди од ендогено потекло на молекулите од класа I, поврзани со CD8, додека презентацијата на пептидите од егзогено потекло оди со молекулите ХЛА од II класа со CD4-лимфоцити.



Молекулите од првата класа, преку крајните домени, во карактеристичната вдлабнатина формираат специфичен алелски пептиден џеб, и преку пептидна врска, специфично го врзуваат пептидот и го изнесуваат над клеточната мембрана (HLA-I-класата носат пептиди од сите протеини синтетизирани во клетките како ендоантигени, од вируси, малигноми, трансплантати), а од II класа носат пептиди од протеини кои биле разградени во ендозомите и лизозомите како егзоантигени (бактерии, вируси) и го презентираат на TCR од T(CD4), односно T(CD8). Пептидите за класа I имаат од 7 до 11 аминокиселини, а за класа II, од 18 до 25. Теоретски се генерираат 5 x10 на 11 нонамери пептиди, една молекула од I класа фиксира милион до 10 милиони. На мембраните на CPA се формираат 1.000 до 10.000 вакви комплекси. За активација на Tly треба 10 до 1.000 комплекси.

Компатибилноста во ХЛА-системот оди по редослед на локусите HLA-DR, B, A. Овде се зема предвид и бенефицирачкиот ефект на можните вкрстени реакции помеѓу алелите од дарителот и примателот.

ХЛА-типизацијата е извршена серолошки, со микролимфоцитотоксичниот тест за антигените од локусите A, B, Dr, C; клеточно – MLR за антигени од D-локусот. Вкрстена проба CM (Cross Match) меѓу лимфоцитите од дарителот и серумот од примателот е извршена со

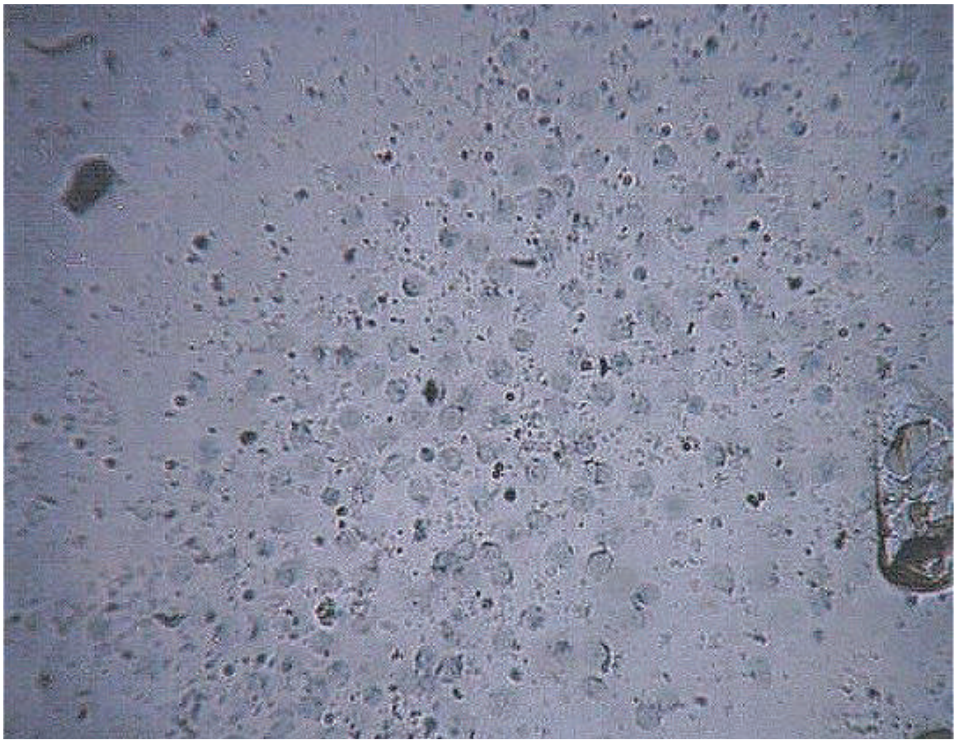
микролимоцитотоксичен тест (класична варијанта и со користење Coombs-серум).

Во 1977 г. е извршена првата трансплантација на бубрег кај болен примател, а дарител е негов, односно нејзиниот брат, со потполна ХЛА-компатибилност.

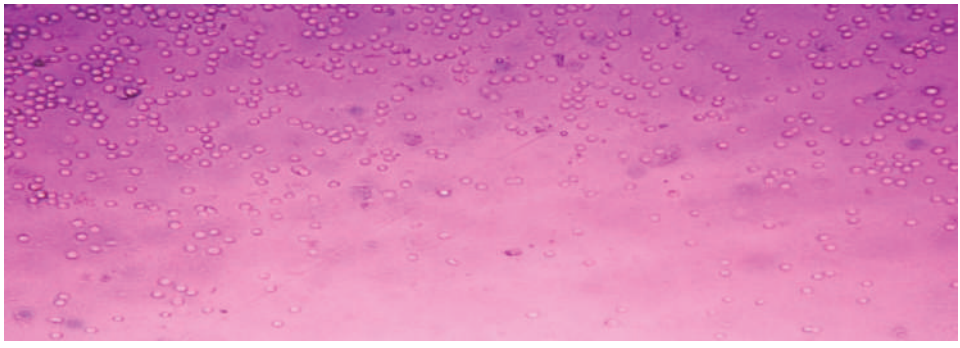
HLA-grupa bolna A9, A28/B12, BW35

ХЛА-група дарител A9, A28/B12, BW35

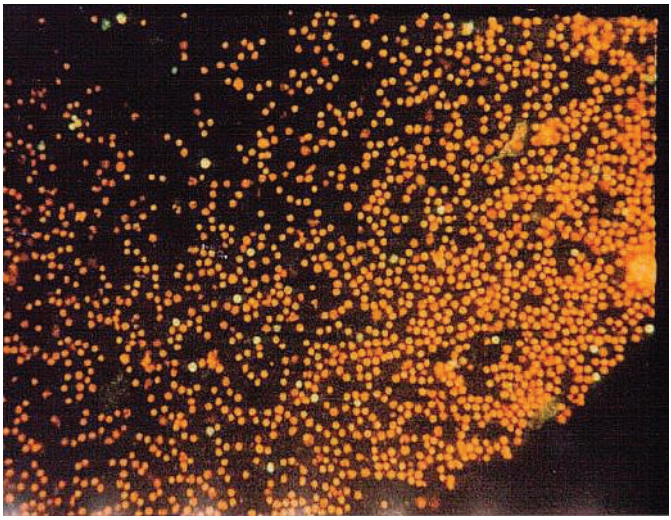
Понатаму се извршени над 300 трансплантации, од кои 15 се кадаверични. При изборот на најпогоден пар за трансплантација е проценувана имуната состојба кај примателот. Определувани се крвногрупните карактеристики и можната присутна ХЛА-алоимунизација. Неопходно е совпаѓање на крвните групи АВО бидејќи бубрегот е носител на повеќе крвногрупни антигени.



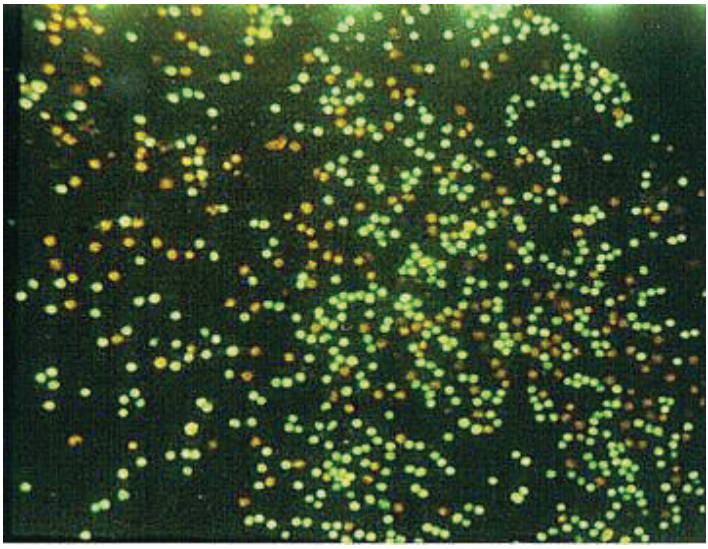
Позитивен микролимфоцитотоксичен тест



Негативен микролимфоцитотоксичен тест

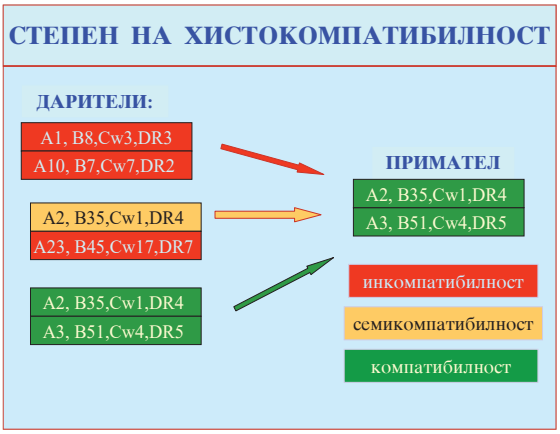


Позитивен СМ-тест со индиректна имунофлуоресценција (IIF-Indirect ImunoFluorescens)



Негативен СМ рест со IIF

Постојат 3 степени на хистокомпатибилност.



ХЛА-соодносите, односно бројот на инкомпатибилности според ХЛА-локусите меѓу паровите примател/дарител и преживувањето на трансплантатите, изразено во месеци кај една серија од 142 болни, се прикажани на следната табела.

ХЛА-инкомпатибилности и функционирање на трансплантирани бубрези

Број на инкомпат.	број на болни	преживување на трансплантатите по месеци 12	24	36	72	120	>120
0	3 (2,11 %)	1 (0,70 %)	0 -	0 -	0 -	0 -	2 (1,40 %)
1	14 (9,85 %)	3 (2,11 %)	0 -	5 (3,52 %)	1 (0,70 %)	2 (1,40 %)	3 (2,11 %)
2	117 (82,39 %)	43 (30,28 %)	18 (12,67 %)	15 (10,56 %)	37 (26,05 %)	2 (1,40 %)	2 (1,40 %)
3	4 (2,81 %)	1 (0,70 %)	0 -	2 (1,40 %)	1 (0,70 %)	0 -	0 -
4	4 (2,81 %)	1 (0,7 %)	2 (1,40 %)	1 (0,70 %)	0 -	0 -	0 -
Вкупно	142 (100 %)	49 (34,50 %)	20 (14,08 %)	23 (16,19 %)	39 (27,46 %)	4 (2,81 %)	7 (4,92 %)

Кај најголем број од трансплантираните болни (117 – 82, 39 %) помеѓу нив и дарителите постоеле 2 инкомпатибилности, односно постоела хаплоидентичност. Поголем број од нив имаат функционални бубрези по првата, односно третата година, а одреден број и над 10 години. Потоа следуваат болните со 1 инкомпатибилност, односно со 3 идентичности, кои имаат функционални бубрези подолго време, а 3 од нив имаат и над 10 години.

Општо земено, трансплантираните бубрези се наоѓаат во функција кај 92 (64,79 %) болни. Најголем процент од болните (50,70 %) со функционални пресадени органи се вбројуваат во групата со хаплоидентичност, од причини што тоа е најголема група со трансплантации.

Определувана е можна присутна ХЛА-алоимунизација – постоењето на преформирани цитотоксични антиХЛА-антитела создадени од поранешни трансфузии, бремености и трансплантации. Во овој систем нема природни антитела.

Покрај користењето на еритропоезната програма, има болни кои се трансфундирани и политрансфундирани и кои на таков начин се ХЛА-алоимунизирани. Иако тешко може да се уточни бројот на еритроцитни трансфузии, сепак тој битно влијае на појавата на алоимунизацијата и на нејзиниот моноспецифичен, биспецифичен и полиспецифичен карактер, како и на појавата на кризи на отфрлање.

Влијание на трансфузиите врз појавата на
ХЛА-алоимунизацијата и кризите на отфрлање

	Број на трансфузии				вкупен број болни
	без	до 5	до 10	>10	
број на болни	64 (45,77 %)	41 (28,87 %)	26 (18,32 %)	11 (7,04 %)	142 (100 %)
Болни со антитела	0 -	4 (2,81 %)	16 (11,26 %)	11 (7,74 %)	31 (21,83 %)
Болни со кризи на отфрлање	9 (6,33 %)	7 (4,93 %)	2 (1,40 %)	0 -	18 (12,67 %)

Трансфузии и ХЛА-алоимунизација

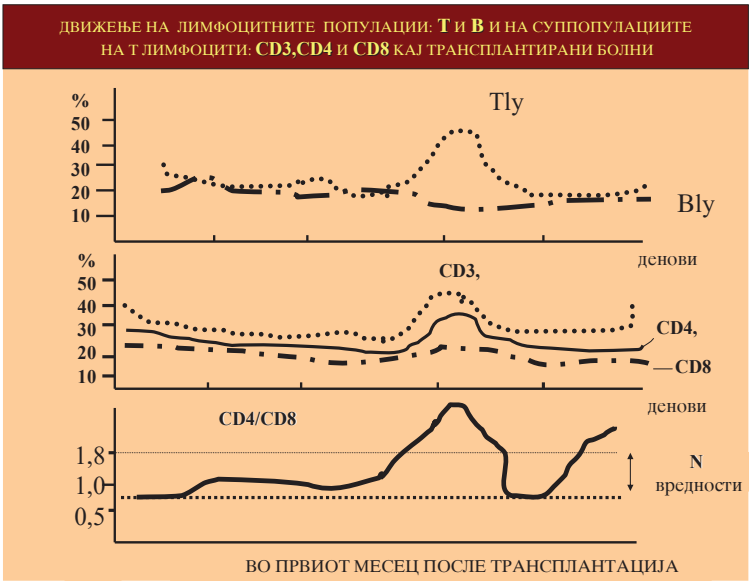
Најголем број од болните не примале трансфузии (64 – 45,77 %) или, пак, се во групата до 5 трансфузии. ХЛА-антителата почнуваат да се појавуваат кај болните во групата до 5 трансфузии, додека во групата болни до 10, односно над 10 трансфузии, значајно се присутни.

Меѓутоа, уште во 1973 г., Опелз покажа дека трансфузиите може да имаат бенефицирачки ефект врз прифаќањето на трансплантатот. Други автори покажаа постоење блокирачки имуноглобулини, како и макрофаги кои ги фагоцитираат алтерираните еритроцити и го попречуваат имунолошкиот контакт. Така, при алогената стимулација со трансплантација, трансфузија и бременост, се јавува и имуносупресија, односно имуномодулирачки ефект кој се должи на активноста на повеќе структури од имунолошката одбрана.

Докажано е дека алогените трансфузии може да бидат придружени со имуномодулирачки ефект и заради имуносупримирачкиот ефект да ја зголемат морбидноста кон карциноми, да се развијат метастази или, пак, да се појават инфекции, а при трансплантации да имаат погоден ефект во прифаќањето на трансплантатот.

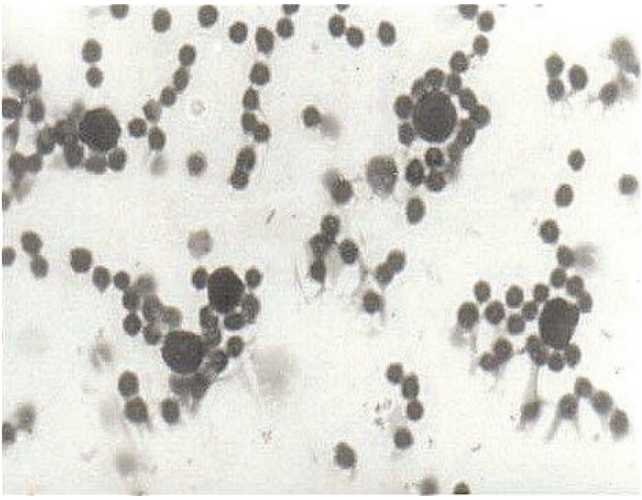
Следењето на прифаќањето се вршеше со определување на нивото на Tly, Bly, T ly (CD3), Thly(CD4) и Tc ly(CD8) со IIF-метода.

Кај оваа група болни нивото на имуните клетки покажува добра имуносупресија.

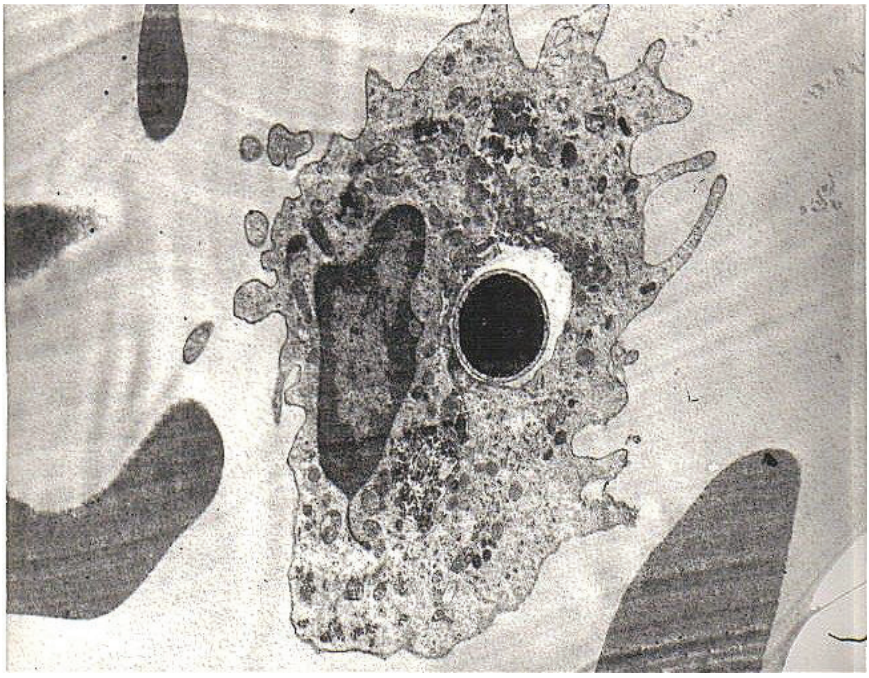


Имунолошкиот систем на човекот многу е моќен и го отфрла трансплантатот. Отфрлањето може да биде хиперакутно за неколку минути ако СМ е позитивен, забрзано за неколку дена поради постоење сензибилизирани Т Ly, акутно, со денови и недели – примарно активирани Т ly и хронично со месеци и години кога е контролирана активацијата на целиот имунолошки систем. ХЛА-структурите од CD8 Т-лимфоцитите од примателот директно ја препознаваат донорската клетка и го активираат целиот имунолошки систем, додека CD4-лимфоцитите од примателот, преку сопствените АРС, кои ги презентираат пептидите од дарителот, вршат индиректно препознавање и го активираат имунолошкиот систем за отфрлање. Во имуноспресијата, во почетокот е користен антилимоцитен серум, потоа антиТ-лимоцитен серум, па кортикостероиди imuran, cell cept, ciklosporin, FK-506, моноклонални антитела антиCD3 и во сегашно време хуманизирани моноклонални антитела (daclizumab, basiliximab и др.). Во основа на оваа терапија е блокирањето на функцијата на IL-2.

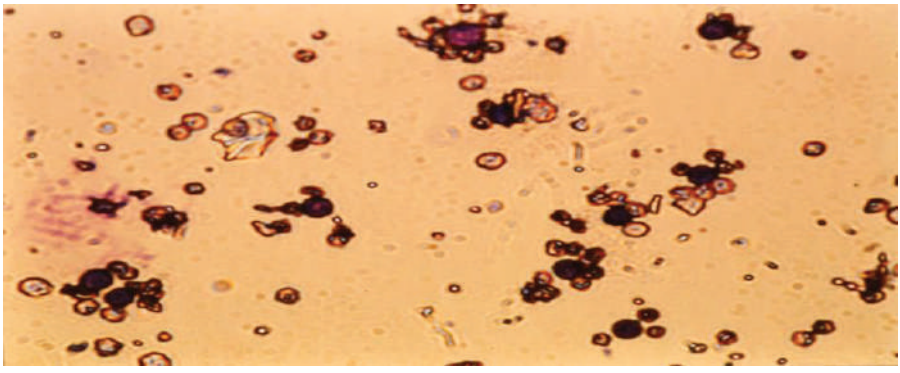
Направена е една трансплантација со различни крвни групи. Примател ќерка „Б“ група, донор мајка „АБ“, хаплоидентични со негативна вкрстена проба. По протокол правена плазмафереза, титарот алфа-аглутинини за 4 месеци сведен на 1:4. Извршена успешна транс-плантација.



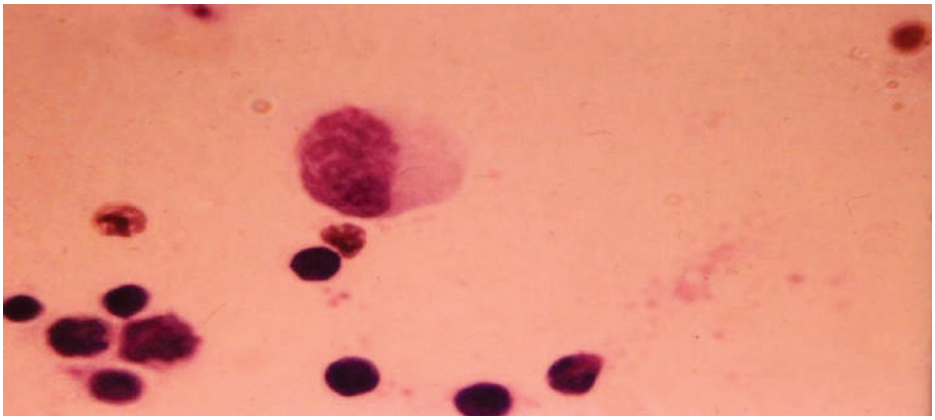
Возбудени активирани Т-лимфоцити



Активиран моноцит (електронски микроскоп).



Издвоени лимфоцити од седимент од урина – активирани во розети.
Доказ за акутно отфрлање.



Активирани клетки кај позитивна МЛР

Сознанијата за Human Leucocyte Antigen-Complex

Major **H**istocompatibility скромно се развиваат од 1930 до 1980 г. Посигурни податоци за неговата биолошка функција и полиморфизмот беа стекнати до 2000 г., а понатаму се утврди функцијата на презентацијата и улогата во интеракцијата **CMH-PEPTID-TCR,** во трансплантацијата и асоцијацијата на ХЛА и болестите. Во иднина, се очекуваат нови сознанија за процесот прифаќање/отфрлање на трансплантат и за одбраната од малигни болести.

ЛИТЕРАТУРА

1. Busson, M., Hors, J. *Le complexe HLA et les transplantation d'organes*. In: Dausset J., Pla M. eds. HLA complexe majeur d'histocompatibilité de l'homme. Ed.Flammarion Médicine-Sciences.1989: 317–337.
2. Colombani, J. *Immunologie des transplantations. Greffes d'organes*. In: Colombani J. eds. HLA – Fonctions immunitaires et applications médicales. Ed. John Libbey Eurotext. 1993: 205–217.
3. Colombani, J. *Fonction immunitaire du CMH: contrôle génètique de la réponse immunitaire*. In: Dausset J., PLa M., eds. HLA complexe majeur d'histocompatibilité de l'homme. Ed.Flammarion Médicine-Sciences.1989: 263–286.

4. Dausset, J. *L'acquis, le points d'ombre et l'avenir*. In: Dausset J., Pla M., . HLA complexe majeur d'histocompatibilité de l'homme. Ed. Flammarion Médicine-Sciences. 1989: 390–407.
5. Duquesnoy, R. J., Marrari, M. *Antibody spececficity analysis of sera from highly sensitized patients*. Identification of unacceptable HLA class I mismatches and its clinical relevance to transplant outcome. HLA-Genetic diversity of HLA, Functional and Medical Implication.Ed. by D. Charron, EDK. 1997, I: 453–459.
6. Elliot, T. *How do peptides associate with MHC class I molecules?* Immunol. Today. 1991, 12: 386–388.
7. Kaplan, C., Niaudet, P., Gagnadoux, M. F., Reznikoff, M. F., Muller, J. Y., Broyer, M. *Donor-Specific Blood Transfusion and Renal Graft Survival: A 3-Year Experience in Pediatrics. Histocompatibility Testing* 1984. Ed. by E. D. Albert, M. P. Baur, W. r. Mayr. 1984: 669–670.
8. Karuppan, S. S., Lindholm, A., Moller, E. *Fewer acute rejection episodes and improved outcome in kidney – transplanted patients with selection criteria based on crossmatching*. Transplantation 1992; 53: 666–673.
9. Opelz, G., Sengar, D. P. S., Mickey, M. R., Terasaki, P. I. *Effect of blood transfusions on subsequent kidney transplants*. Transpl. Proc. 1973, 5: 253.
10. Opelz, G., Wujciak, T., Mytilineos, J., Scherer, S. *Strategies and future of HLA matching in organ transplantation. HLA-Genetic diversity of HLA*, Functional and Medical Implication. Ed. by D. Charron, EDK. 1997, II: 80–84.
11. Terasaki, P. I., Cecka, J. M. *Clinical Transplants*. 1991. Los Angeles: UCLA Tissue Typing Laboratory 1992.
12. Histocompatibility Testing 1984. Ed. by E. D. Albert, M. P. Baur, W.r. Mayr. 1984: 669–670.
13. Karuppan, S. S., Lindholm, A., Moller, E. *Fewer acute rejection episodes and improved outcome in kidney – transplanted patients with selection criteria based on crossmatching*. Transplantation 1992; 53: 666–673.
14. Opelz, G., Sengar, D. P. S., Mickey M. R., Terasaki, P. I. *Effect of blood transfusions on subsequent kidney transplants*. Transpl. Proc. 1973, 5: 253.
15. Opelz, G., Wujciak, T., Mytilineos, J., Scherer, S. *Strategies and future of HLA matching in organ transplantation*. HLA-Genetic diversity of HLA, Functional and Medical Implication. Ed. by D. Charron, EDK. 1997, II: 80–84.
16. Terasaki, P. I., Cecka, J. M. *Clinical Transplants*. 1991. Los Angeles: UCLA Tissue Typing Laboratory 1992.

Perko KOLEVSKI, Ninoslav IVANOVSKI, Zivko POPOV

HLA IN KIDNEY TRANSPLANTATION

Summary

The current knowledge of the HLA system, its structure and role in the immune defense of the organism is able to achieve a broad process of transplantation of organs and tissues. Its huge polymorphism shows emphasis on the individuality of each person and requires histocompatibility between recipient and organ donor. Managing the processes of recognizing others, preparing other people's structures for presenting the immune system and activating the entire defense system indicate of the important role of the HLA system in the processes of graft acceptance.

In a series of 142 transplants HLA relations between the recipient-donor pairs and in the largest number of transplanted patients (117 - 82, 39%) were followed, Between them and the donors there were 2 incompatibilities, ie there was haploidentity. A large number of them have functional kidneys after the first or third year, and a certain number over 10 years. HLA alloimmunization resulting from previous transfusions or pregnancies and its effect on graft acceptance were monitored. The largest number of patients did not receive transfusions (64-45.77%) or were in the group of up to 5 transfusions. HLA antibodies begin to appear in patients in the group of up to 5 transfusions, while in the group of patients up to 10 or more than 10 transfusions. HLA antibodies were significantly present. The largest number of patients did not receive transfusions (64-45.77%) or were in the group of up to 5 transfusions. HLA antibodies begin to appear in patients in the group of up to 5 transfusions, while in the group of patients up to 10 or more than 10 transfusions are significantly present. The process of immunomodulation in the target transfusion protocols is discussed. The importance of the cross - test, the timely detection of the occurrence of rejection and the monitoring of the immunosuppression by determining the level of CD3, CD4 and CD8, which should be within normal limits, are emphasized.

doi: 10.20903/zpopov-2022-0014 UDK: 615.272:616.155.194]:616.61-008.64-78

Irena CAKALAROSKA[1], Lada TRAJCESKA[2], Ninoslav IVANOVSKI[2], Zivko POPOV[3,4], Koco CAKALAROSKI[2]

INFLUENCE OF CALCITRIOL ON ANAEMIA IN HAEMODIALYSIS PATIENTS

Abstract

36 haemodialysis patients were treated with oral calcitriol and were then analysed. The patients received from 23 to cca 518 μg calcitriol (total dose) with a daily regimen of 0.25 to 0.50 μg every second day. This was done to prevent metabolic renal osteopathy (known as a "renal osteodystrophy", ROD). In 30 of 36 (83.3%) patients, we found a significant increase in blood haemoglobin (Hgb) concentration (more than 10%) after treatment with calcitriol. All haemodialysis procedures, dietetic measures, and other medical treatments remained unchanged when compared to the pre-treatment period.

We have found that orally administered calcitriol is also effective in the therapy of uremic anaemia. More detailed studies on larger numbers of patients are needed to make a final conclusion.

Keywords: uremic anaemia, PTH, calcitriol

Introduction

Calcitriol [$1,25(OH)_2D_3$] is a hormonally active metabolite of vitamin D_3 and the basic regulator of bivalent ions homeostasis, in addition to

[1] Department of pediatrics, Faculty of Medicine, University "St. Cyrill and Methodius", Skopje,

[2] Department of nephrology, Faculty of Medicine, University "SS. Cyrill and Methodius", Skopje, North Macedonia

[3] Department of urology, Faculty of Medicine, University "SS. Cyrill and Methodius", Skopje

[4] Macedonian Academy of Sciences and Arts, Skopje, North Macedonia

having the effect of a parathyroid hormone (PTH) and calcitonin (CT). Calcitriol (C) is created in the mitochondria of epithelial cells in the upper renal tubules under the action of the complex enzyme 25(OH)-C 1α hydroxylase (a complex of reductase, rhenodoxine and cytochrome P-450 oxide/reductase). Calcitriolemia controls the creation of 1,25 $(OH)_2D_3$ by a feed-back mechanism, while PTH, CT and hypophosphatemia directly stimulate its synthesis. On the other hand, C is a very active substance which modulates the activity of some other hormones and affects the differentiation of certain tissues through specific receptors (known as the pleiomorphic effects of calcitriol).

C is also known that it supresses indirectly (through calcemia) and directly. It suppresses the synthesis of PTH, thus leading to a reduction of the serum alkaline phosphatase concentration (SAP) and the bone GLA-protein (BGP).

The suppression of secondary hyperparathyroidism (sHPT) would improve erythropoiesis, reduce haemolysis and correct myelofibrosis associated with renal osteodystrophy.

The aim of the study is to evaluate the influence of C on sc "uremic anaemia" in our patients and to rouse the interest for further study of the stated problem on a larger group of patients

Patients and methods

Out of 214 patients treated with chronically repeated haemodialysis (Department of Nephrology, Faculty of Medicine, Skopje) only 36 (16.8%) patients (19 males and 17 females) with chronic kidney disease who were undergoing haemodialysis (mean age of 45.5±13.1 years) were treated with calcitriol (0.25μg every day or 0.50-1.50 μg every second day at a minimum of three months before testing). The duration of the haemodialysis treatment was from 9 to 157 months (X = 54.2 ± 38.1, CV = 70.3%). Two female patients have an artificial hip implant due to the pathologic intracapsular fracture of the femoral neck, and three more patients had been previously parathyroidectomized and received a transplant. The data of the studied patients are shown in Table 1.

Table 1

Basic data for analysed patients

Patient	*Age (years)*	*Sex*	*Disease*	*HD-months*	*Total dose of Calcitriol (μg)*	*Comments*
AR	33	m	HTN/NaS	40	305	-
DN	66	f	PKD	52	350	-
KR	25	m	GN chr	22	175	
AA	22	m	GN RP	72	215	-
VV	40	m	T2DM	36	135	-
KS	46	m	IPN chr	**157**	**510**	-
AS	55	f	HTN/NaS	24	190	-
RD	55	m	PKD	30	83	-
JS	23	m	GN chr	15	56	-
NLj	44	f	TIN chr	18	135	-
IV	75	f	T2DM	10	38	-
RLj	61	f	T2DM	30	135	-
AV	58	f	HTN/NaS	22	112	-
MM	37	f	GN MP	122	45	PTHX/TR
EM	49	m	HTN/Nas	37	215	-
OV	40	m	ON	48	255	-
GM	56	f	ON	37	66	-
PN	61	f	TIN chr	90	105	F-ra coli fem.
PR	57	f	PKD	84	315	-
CA	50	f	GN chr	88	45	-
DD	55	m	HTN/Nas	28	147	-
NM	45	f	ON	21	158	-
MR	51	m	T2DM	42	98	-
LjF	29	f	GN chr	30	115	-
RD	49	m	PKD	30	83	-
SM	46	f	PKD	131	38	-
KS	40	m	IPN chr	147	410	PTHX/TR
SD	30	m	T2DM	45	45	-
ML	52	f	IPN chr	59	30	F-ra coli fem.
TS	51	f	HTN/NaS	65	45	-
RD	46	f	BEN	59	90	-
MV	50	m	BEN	83	255	-
GG	24	m	GN RP	**9**	31	-
BA	52	m	Gn RP	36	46	-
CE	43	m	GN chr	48	**16**	-
BJ	21	m	VUR bill	84	45	PTHX/TR

Table 1 shows that the duration of haemodialysis, as well as the total dose of consumed medicine (calcitriol) in the analysed study period, varies considerably (from 16 to 510 μg; X = 142.7± 118.7 μg, CV = 83.2%). C has been administered in doses which should provide the inorganic serum phosphorous (Pi) below 2 mmol/L (0.25-0.50 μg/daily or 1.0-1.5 μg every second day orally). Analysing the files of the aforementioned subjects, it was observed that the patients had improved their blood count tests after treatment with C. We have compared the mean values of haemoglobin (Hgb g/L), red blood cells (Er, n $x10^{12}$), haematocrit (Htc, percentage), calcium (Ca, mmol/L), Pi (mmol/L), and serum alkaline phosphatase (SAP,U/L) during the last three months prior the onset of treatment with C – with the respective values (average for the last three months) in the course of calcitriol therapy.

Blood samples were taken as described immediately before haemodialysis, and the measurements were made by routine laboratory methods (Clinical Biochemistry, Faculty of Medicine, Skopje). The haemodialysis procedure (including time of dialysis and type of membrane), dietetic regimen, and the other medicines were not changed in comparison with the pretreatment period. All patients were haemodialyzed on the same membrane three times weekly. Blood transfusions were not used during the follow-up.

We calculated the mean value of the analysed series of data before and after treatment with C and have determined the relationship between them as well as the statistical significance of the differences related to these values. The blood count values changed significantly after therapy with C, namely an increase in the red blood cells number (more than $0.5x10^{12}$/L), concentration of Hgb (about 10 g/L or change for more than 10%), and Htc (increase for about 0.05%). The values for total Ca (tCa; RV – 2.1-1.6 mmol/L), inorganic phosphorous (Pi; RV – 0.81-1.45 mmol/L) and alkaline phosphatase (RV-to 90 U/L) were measured as well.

The analysed group of patients also serves as a control. The patients have not been treated with despheroxamine, androgens, ultraviolet rays, different time of sun expositions, nor have additional ultrasonic investigations been made to find secondary (degenerative) cysts in the residual kidney parenchyma.

1,25 $(OH)_2D_3$ inhibits, indirectly (through ionic calcemia) and directly (through the cytosolic and nuclear receptors at the main parathyroid cells),

the production of PTH (pre/pro PTH sequence) and reduces its unfavourable effects on haematopoiesis (especially if the administration of calcitriol is done intravenously; for example, amp. Calcijex® (Abbot) à 1 μg/mL).

Calcitriol would influence the control of intra- and extramedullar haematopoiesis by the elimination of the peritrabecular fibrocystic myelofibrosis (blocking the PTH profibrotic effects), by reduction of the secondary hypersplenism and by stimulation of testosterone secretion, which stimulates the production of erythropoietin (EPO).

Results

Table 2 presents the findings of the study:

Table 2

Values of Er, Hgb, Htc, Ca, Pi, and SAP in the studied patients before and after treatment with Calcitriol

Patient	*RBC ($x10^{12}$/L) b/a*	*Hgb (g/L) b/a*	*Htc b/a*	*tCa (mmol/L) b/a*	*Pi (mmol/L) b/a*	*SAP (U/L) b/a*
AR	2.3/2.4	80/79	0.21/0.22	1.9/2.0	1.4/1.1	68/66
DN	2.4/3.2	88/101	0.24/0.31	1.8/1.9	2.1/1.8	116/120
KR	**1.6**/**2.0**	**51**/79	**0.07**/0.19	2.2/1.9	1.6/1.8	698/741
AA	2.6/2.9	91/87	0.26/0.26	**1.6**/**1.5**	2.4/2.1	**1000/1270**
VV	2.6/2.3	85/**74**	0.23/0.22	2.2/2.3	1.4/1.2	343/108
KS	2.5/2.9	89/89	0.26/0.28	2.3/2.5	1.3/1.2	233/257
AS	2.6/3.0	77/82	0.26/0.28	1.8/2.0	0.9/**0.7**	120/110
RD	2.9/3.4	96/89	0.29/0.31	2.3/2.5	1.3/1.2	261/223
JS	2.2/2.8	78/94	0.21/0.28	1.7/1.9	**3.1**/2.9	172/129
NLj	2.6/3.1	88/102	0.24/0.32	1.8/1.9	1.6/1.8	425/341
IV	2.6/2.2	88/102	0.24/0.32	2.3/2.2	1.4/1.2	101/87
RLj	2.9/3.8	77/99	0.22/0.31	2.4/2.1	1.5/1.4	235/190
AV	2.8/3.0	95/104	0.25/0.28	2.0/1.7	1.6/1.6	369/328
MM	3.0/**4.5**	68/**127**	0.29/**0.44**	2.3/2.2	2.3/2.5	93/99
EM	3.4/3.2	95/92	0.29/0.29	2.2/2.0	1.7/1.8	205/174

OV	3.4/3.5	85/86	0.26/0.27	1.9/2.3	1.2/1.1	432/432
GM	4.1/**4.5**	**113**/117	**0.37**/0.41	2.0/2.0	1.3/1.5	132/147
PN	3.2/4.0	98/139	0.33/0.49	**1.6**/2.1	1.6/2.2	81/74
PR	2.9/3.0	101/101	0.20/0.30	1.7/1.9	1.7/1.6	78/138
CA	2.1/2.8	79/96	0.23/0.28	2.1/2.0	1.6/1.6	251/269
DD	2.1/2.4	82/105	0.19/0.21	2.4/2.4	1.5/1.2	132/1304
NM	3.1/3.6	87/112	0.24/0.32	2.0/2.1	1.8/1.9	462/204
MR	**4.3**/**4.5**	109/114	0.36/0.39	2.2/2.2	1.6/1.7	608/543
LjF	2.8/4.2	68/118	0.19/0.35	2.0/1.9	2.4/1.5	970/254
RD	3.2/3.4	94/111	0.33/0.34	1.6/2.3	1.9/1.7	116/79
SM	2.6/2.5	68/67	0.23/**0.20**	1.7/2.0	1.5/**3.1**	91/99
KS	2.6/3.5	90/**127**	0.28/0.42	1.7/1.9	3.0/2.7	141/408
SD	3.2/4.0	98/117	0.34/0.39	2.3/2.3	2.0/1.5	67/53
ML	2.0/2.2	69/76	0.21/0.22	**1.6**/**2.6**	2.5/2.1	114/191
TS	2.0/3.9	80/110	0.20/0.35	2.0/2.4	1.9/2.1	160/278
RD	1.8/3.7	61/125	0.15/0.40	**2.5**/2.0	**0.7**/0.9	179/144
MV	2.3/2.4	72/71	0.20/0.26	2.4/1.9	1.9/1.6	104/101
GG	2.2/2.4	80/67	0.20/0.20	2.3/2.0	2.8/2.5	**35**/**50**
BA	2.1/2.7	83/100	0.23/0.26	2.1/2.0	1.8/2.4	68/56
CE	2.2/2.3	84/87	021/0.22	1.7/1.8	2.8/2.7	68/112
BJ	1.8/3.5	57/98	0.16/0.29	1.8/2.0	1.6/1.3	812/113
X ± SD Coefficient of Variation (%)	2.6 ± 0.6 (23.2) / 3.2 ± 0.7 (22.5)	83.4 ± 13.6 (16.3) / 98.4 ± 18.1 (18.3)	0.24 ± 0.1 (25.0) / 0.30 ± 0.1 (23.3)	2.0 ± 0.3 (13.9) / 2.1 ± 0.2 (11.5)	1.8 ± 0.6 (31.1) / 1.8 ± 0.6 (33.0)	265.0 ± 257.1 (97.0) / 258.1 ± 293.4 (113.7)
σ_{X1-X2} **(p)**	< 0.03	< 0.02	< 0.03	NS	NS	NS

∗-the extremes are bolded

From the results presented in table 2, it is obvious that the statistical difference in RBC (red blood cells), Hgb (Haemoglobin) and Htc (Haematocrit) values before and after treatment with C is significant and clinically acceptable. This is not, however, also the case for tCa, Pi and SAP. The

distribution of the statistical units and data around the average value (X) ranges between 11.5% to 33.0% for RBC, Hgb, Htc, tCa, and Pi (values before and after treatment with C) and with much more scatter (CV = 97.0% - 113.7%) for SAP, the changes in the serum concentration of calcitriol and Pi as well as the activity of SAP.

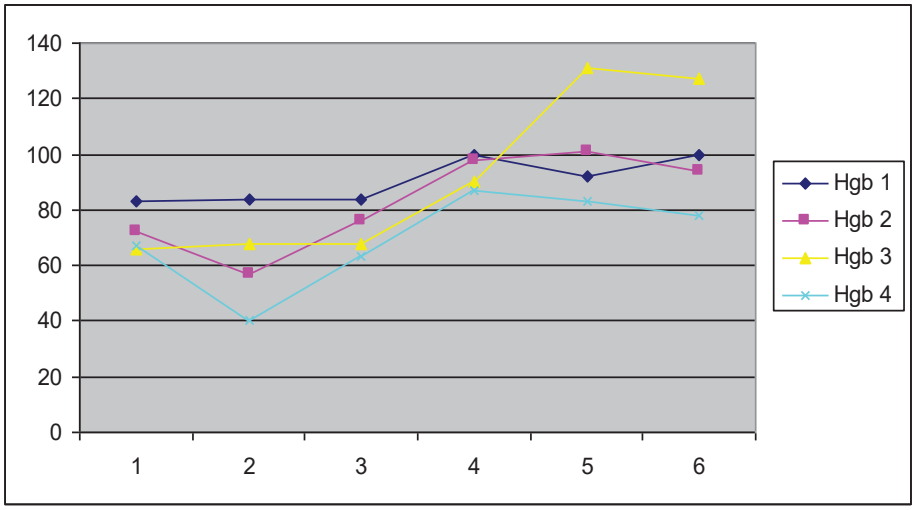


Figure 1 – Haemoglobin variations after three months treatment with calcitriol in four of our patients

From the fig 1 one could conclude that the concentrations of Hgb generally rise about three (3) months after the initiation of the C therapy.

In order to evaluate the direct association between the antianemic action of C and possible suppression of PTH secretion (indirectly, by the reduction of the total activity of SAP), we associated the percentage of change of SAP with the percentage variation of Hgb-emia in the group of analysed patients. We have found a weak but negative linear correlation ($r = -0.15$, $y = -0.25x + 20.1$). This finding leads to the conclusion that even a lesser degree of SAP activity diminution corresponds to a higher concentration of Hgb in the blood (mean percentage change for SAP = $11.6 \pm 59.6\%$; corresponding variation for Hgb = $33.6 \pm 34\%$).

Discussion

Anaemia in patients with end-stage kidney disease is a nosocomial problem with multifactorial etiopathogenesis.(1),(2),(3)

Associating uremic anaemia with sHPT has been a subject of discussion over the past forty years, despite being very complex and controversial. PTH, the potential uremic toxin, is related with the inhibition of erythropoiesis (especially at the level of BFU-erythroid precursors).(4)

Because the failure of the function of Na^+/K^+-ATP-ase with reduced utilization of carbohydrates in the presence of PTH, the fragility of osmotic erythrocytes increases, and there is a tendency to haemolysis. With the reduction of the thrombocytes' function (mechanism dependent of calcium), there is an increase in the inclination of gastroduodenal haemorrhage, resulting in iron-deficiency anaemia. Considering the positive correlation between the serum PTH and stomach hyperchlorhydria, which is associated with ulcerations and/or bleeding of the upper digestive tract, microcytic iron-deficiency anaemia is quite acceptable. (5)

PTH stimulates general protein catabolism, thus reducing the globin synthesis in the haemoglobin structure, although there are a lot of contradictions when comparing the laboratory and clinical findings.

When PTH and sHPT process the fibrocystic osteodysplasia the bone marrow undergoes myelofibrosis and calcification. It certainly reduces the erythroid potential of the bone marrow and precedes hypo or aregenerative anaemia. The favourable effect of PTHX-ia on uremic anaemia supports the previous position. The hypocalcemic /hypophosphatemic shape of sHPT (hypomineralizing hyperosteoidosis) develops a strong fibrous reaction in the bone marrow, which is especially suitable for C treatment.(6)

1,25 $(OH)_2$ D_3, indirectly (through ionic calcemia) and directly (through the cytosolic and nuclear receptors at the main parathyroid cells), inhibits production and incretion of PTH and reduces its unfavourable effects on haematopoiesis (especially if administered intravenously). (7)

C further influences uremic anaemia favourably by controlling the intramedullary haematopoiesis and spleen RBC sequestration. This is done by the elimination of fibrocystic myelofibrosis, stimulation of testosterone secretion in men (which in turn stimulated the EPO production), and the reduction of secondary hypersplenism. (8)

In addition to the classic effects of C (synthesis of the vitamin D-dependent calcium-binding protein s.c.Ca-BPs and stimulation of Ca-absorption), C increase the absorption of Pi in the distal kidney tubules (calcitriol receptors for the C_1-OH group).(9),(10).

Using the techniques of monoclonal antibodies, the presence of specific calcitriol receptors is described in various tissues and organs (fibroblast, hypophysis, beta-cells of the pancreas islets, kidneys, gonads, skeleton muscles, parathyroid glands, gastro-intestinal tract, heart, thymocytes, haematolymphoid, and malignant tissues).(11)

C stimulates the transformation of blood monocytes in tissue macrophages, inhibits the proliferation of renal epithelioma cells, modulates the reaction of target cells according to the activity of specific hormones (influence on cAMP synthesis), induces expression of prolactin gen, and stimulates the production of thrombocytic thromboxane (TxA_2).(12),(13). C reduces the sensitivity of osteoblasts to the influence of PTH and indirectly reduces the synthesis of BGP (bone GLa protein) and SAP.(14)

C influences insulin secretion by a feedback link increasing the concentration of hepatocyte cytosolic Ca and regulates, or rather, reduces the production of $25(OH)D_3$ (calcifediol/calcidiol, as a circulating reserve for calcitriol).(15) There is a great deal of information about the effect of C on the biotransformation of androstenedione in estron, possessing immunosuppressive properties. There is also data that show how it is also efficient in the treatment of idiopathic myelofibrosis (re-expansion of the bone marrow).(16),(17),(18)

Taking the above statements into consideration, for the present time still questionable, the participation of other, we can purport other PTH non-associated effects of C on uremic anaemia can be also supposed. (19), (20), (21)

REFERENCES

1. Wills MR. Uremic Toxins, and Their Effect on Intermediary Metabolism Clin Chem. 1985; 31/1: 5-13.
2. Eschbach JW,Adamson JW. Anaemia of end-stage renal disease. Kidney Intern. 1985; 26: 1-5.

3. Zappacosta AR,Caro J,Erlev A. Normalization of haematocrit in patients with end-stage renal disease on CAPD. Am J Med. 1982; 72: 53-57.
4. Drüeke TB,Eckardt KU. Role of secondary hyperparathyroidism in erythropoietin resistance of chronic renal failure patients. Nephrol Dial Transplant. 2002; 17 Suppl 5: 28-31.
5. Bogin E,Massry SG,Levi j et al. Effects of PTH on osmotic fragility of human erythrocytes. J Clin Invest. 1982; 69: 1017.
6. Barbour GL. Effect of parathyroidectomy on anaemia in chronic renal failure. Arch Intern Med. 1979 Aug; 139(8): 889-91.
7. Hasnain M, Hauncher C, Pegoraro AA, Arruda JA, Dunea G. Suppression of hyperparathyroidism by calcitriol therapy. ASAIO J. 1999 Sep-Oct; 45(5): 424-7.
8. Nomura S, Ogawa Y, Osawa G, Katagiri M, Harada T, Nagahana H. Myelo-fibrosis secondary to renal osteodystrophy. Nephron. 1996; 72(4): 683-687.
9. Bikle DD. Vitamin D Metabolism, Mechanism of Action, and Clinical Applications. Chemistry&Biology. 2014; 21: 319-329.
10. Merke J,Ritz E,Roland R. Are recent findings on 1.25-dihydroxycholecalciferol metabolism relevant for the pathogenesis of uremia? Nephron. 1986; 42: 277-284.
11. Minghetti PP, Norman AW. 1,25(OH)2-vitamin D3 receptors: gene regulation and genetic circuitry. FASEB J. 1988 Dec; 2(15): 3043-53.
12. Sperschneider H, Humbsch K, Abendroth K.Oral calcitriol pulse therapy in hemodialysis patients. Effects on histomorphometry of bone in renal hyperparathyroidism. Med Klin (Munich). 1997; 92(10): 597-6.
13. Kassi E,Adamopoulos Ch,Basdra EK,Papavassiliou AG. Contemporary Reviews in Cardiovascular Medicine: Role of Vitamin D in Atherosclerosis. Circulation. 2013; 128: 2517-2531.
14. Motlagh BM, Ahangaran NA, Froushani SMA. Calcitriol modulates the effects of bone marrow-derived mesenchymal stem cells on macrophage functions. Iran J Basic Med Sci. 2015; 18: 672–676.
15. Inoue D, Matsumoto T, Ogata E, Ikeda K.22-Oxacalcitriol, a noncalcemic analogue of calcitriol, suppresses both cell proliferation and parathyroid hormone-related peptide gene expression in human T cell lymphotropic virus, type I-infected T cells. J Biol Chem. 1993 Aug 5; 268(22): 16730-6.
16. Patel S, Simpson RU, Hsu CH. Effect of vitamin D metabolites on calcitriol metabolism in experimental renal failure. Kidney Int. 1989; 36(2): 234-9.

17. Kerr DNS. Hypercalcemia and metastatic calcification. Cardiovascular Res-earch. 1997; 36: 293-297.
18. Andress DL, Norris KC, Coburn JW, Slatopolsky EA, Sherrard DJ. Intravenous calcitriol in the treatment of refractory osteitis fibrosa of chronic renal failure. N Engl J Med. 1989 Aug 3; 321(5): 274-279.
19. Muscogiuri G. et al. Mechanisms in endocrinology: Vitamin D as a potential-al contributor in endocrine health and disease. European Journal of Endo-crinology. 2014; 171: 101–110.
20. Erman A, Raz A. Effects of bivalent cations on prostaglandin biosynthesis and phospholipase A2 activation in rabbit kidney medulla slices. Biochem J. 1979; 182: 821-825.
21. Panichi V, Andreini B, De Pietro S, Migliori M, Taccola D, Giovannini L, Ferdeghini M, Palla R. Calcitriol oral therapy for the prevention of secondary hyperparathyroidism in patients with predialytic renal failure. Clin Nephrol. 1998; 49: 245-250.

Ирена ЧАКАЛАРОСКА, Лада ТРАЈЧЕСКА, Нинослав ИВАНОВСКИ, Живко ПОПОВ, Кочо ЧАКАЛАРОСКИ

ВЛИЈАНИЕ НА КАЛЦИТРИОЛ ВРЗ АНЕМИЈА КАЈ ХЕМОДИЈАЛИЗНИ ПАЦИЕНТИ

Резиме

Анализирани се 36 хемодијализни пациенти третирани со орален калцитриол. Пациентите примале од 23 до сса 518 μg калцитриол (вкупна доза) со дневна nozologija од 0,25 до 0,50 μg секој втор ден, со цел да се спречи метаболичката бубрежна остеопатија (porano позната како „бубрежна остеодистрофија“, ROD). Во 30 / 36 (83,3%) пациенти, откривме значително зголемување на концентрацијата на хемоглобин во крвта (Hgb) (повеќе од 10%) по третманот со калцитриол. Сите постапки asocirani so хемодијализа, диететски мерки и други медицински третмани останаа непроменети во споредба со периодот на пред-третман.

Откривме дека орално администрираниот калцитриол е исто така ефикасен во терапијата на уремична анемија. Потребни се подетални студии so поголем број пациенти за да се donese конечен заклучок.

Клучни зборови: уремична анемија, PTH, калцитриол.

doi: 10.20903/zpopov-2022-0015 UDK: 616.61-089.843:616.61-089.819(497.7)"2018/2020"

Сотир СТАВРИДИС[1], Маја МОЈСОВА-МИЈОВСКА[2],
Оливер СТАНКОВ[1], Александра ПАНОВСКА ПЕТРУШЕВА[2],
Јосиф ЈАНЧУЛЕВ[1], Александра ГАВРИЛОВСКА-БРЗАНОВ[2],
Виктор СТАНКОВ[1], Владимир ЛОЗАНОВСКИ[1], Сашо ДОХЧЕВ[1]

ЛАПАРОСКОПСКА НЕФРЕКТОМИЈА „HAND-ASSISTED", КАЈ ТРАНСПЛАНТАЦИЈА ОД ЖИВ ДОНОР ИНИЦИЈАЛНИ РЕЗУЛТАТИ

Вовед

„Hand-assisted"-донорската нефректомија е често користена метода кај трансплантацијата од жив донор. Првите лапароскопски донорски нефректомии во РСМ беа направени во 2004 година. Со оваа евалуациска студија ги претставуваме иницијалните искуства на нашата клиника со оваа метода.

Материјал и методи: Од ноември 2018 до мај 2020 година, направени се вкупно 17 лапароскопски трансперитонеални нефректомии од жив донор. Кај еден пациент е направена конверзија. Кај 13 пациенти е направена лева, додека кај 3 пациенти, десна нефректомија. Еден од пациентите имал анамнеза за претходно изведена, отворена, хируршка процедура. Беа иследувани следните параметри: епидемиолошките карактеристики, возраста, тежината и висината, физиолошката класификација по системи на Американската асоцијација на анестезиолози, BMI, квалитетот на графтот, времето до добивање урина, раните компликации и вредностите на гломеруларната филтрациона рата. Беше одредуван и

[1] Универзитетска клиника за урологија, Медицински факултет, Универзитет „Св. Кирил и Методиј", Скопје, РС Македонија

[2] Универзитетски клиники за трауматологија, ортопедија, анестезија, реанимација и интензивно лекување и Ургентен центар, Медицински факултет, Универзитет „Св. Кирил и Методиј", Скопје, РС Македонија

квалитетот на оперативната техника преку времето на топла исхемија, крвозагубата, траењето на оперативниот зафат, времетраењето на хоспитализацијата.

Резултати: Во период од 18 месеци, беа оперирани 17 пациенти со користење на *hand assisted*-техниката. Кај еден пациент беше направена конверзија поради екстремна дебелина. Сите операции беа изведени од еден хирург. Соодносот жени/мажи беше 62,5 % – 37,5 %. Просечната возраст на пациентите беше 57±12,3. Според АСА-класификацијата, 8 од нив беа класифицирани како АСА 1, 7 пациенти беа класифицирани како АСА 2 и еден пациент – АСА 3. Според БМИ, 43 % од пациентите имаа прекумерна тежина, а 43 % од пациентите имаа нормална тежина. Просечното време до добивањето на првата диуреза беше 148±102 сек. Просечната диуреза на крајот на операцијата изнесуваше 499±379 мл. Вредностите на креатининот, 48 часа по операцијата, споредени со предоперативните вредности, беа намалени за 32 % (376±95umol/L vs. 265±167 umol/L), додека вредностите на уреата беа зголемени за 50 % поради изведена хемодијализа предоперативно. Просечниот период на хемодијализа изнесуваше 32±67 месеци. Левостраните нефректомии имаа пократка топла исхемија во споредба со десностраните (1,86 ± 0,5 min vs. 2,6 ± 0,5 min).

Заклучок: Оваа студија претставува наше иницијално искуство со трансперитонеална лапароскопска *hand assisted*-донорска нефректомија. Таа е ефикасна и безбедна метода за донорот на бубрег и, во исто време, обезбедува добар и функционален графт. По иницијалните добри резултати и беспрекорната безбедност на методата, ја воведовме како рутинска на Универзитетската клиника за урологија.

Клучни зборови: трансплантација, донор, бубрег, лапароскопија.

ВОВЕД

Бубрежната трансплантација претставува третман од избор и единствена опција за нормален живот и добар квалитет на живот кај пациентите со хронична бубрежна болест во терминален стадиум [1].

Обезбедувањето нов бубрег за секој пациент кој претставува дел од Програмата на хемодијализа претставува почеток на нов живот

и шанса за враќање кон нормално извршување на секојдневните животни активности.

Сепак, денес, условно, на почетокот на 21-от век, сè потешко се доаѓа до соодветен орган, и листите на чекање за органи за трансплантација се сè поголеми.

Донирањето органи, особено органи од жив донор, секогаш претставувало највисок чин на човекољубие. Бубрезите од жив донор не само што го прошируваат кругот на донори, но нудат и подобра функција и подолго преживување на графтот во споредба со бубрезите од починат донор [2]. Сепак, живите донори се здрави индивидуи и од најголема важност е да се обезбеди нивна сигурност, со што би можеле да продолжат со нивните вообичаени активности колку што е можно побрзо [2].

Бубрежната трансплантација е веќе општоприфатен начин за третман на хроничната бубрежна болест и начинот и техниките поврзани со начинот на трансплантирање се добро познати. Сепак, со напредокот на технологијата, усовршувањето на хируршките техники и вештини, тенденција е начинот на добивање бубрег од жив донор да биде поврзан со што помала траума за донорот, како психолошка така и физичка, и побрзо враќање назад, на извршување на секојдневните животни и работни обврски [3].

Токму поради тоа, сè повеќе се практикува минимално инвазивен пристап за изведување на донорската нефректомија.

Трансплантацијата на бубрези има долга традиција во Република Македонија, со корени кон крајот на седумдесеттите години на 20-тиот век, со премин во редовна програма на трансплантација на бубрези во почетокот на 90-тите години од минатиот век [4, 5].

Со воведувањето на лапароскопијата, во Република Македонија се прават напори за следење на светските трендови и користење на минимално инвазивните процедури за добивање орган за трансплантација од жив донор, секако, со почитување на сите правила за обезбедување добар, витален орган и безбедна операција за донорот на органот.

Првите четири лапароскопски нефркетомии со жив донор (*living donor* нефркетомии) се направени уште на почетокот на 21-от век од

акад. проф. Попов, со помош на проф. Доминик Шопен и проф. Ајалон. Беа направени четири (4) лапароскопски нефректомии и, последователно, четири (4) успешни трансплантации. Биле користени различни пристапи во текот на нефректомијата, два трансперитонеални и два ретроперитонеални [6]. Сепак, овие пионерски зафати не продолжија сè до почетокот на 20-тите години од овој век, кога заедно со комплетното прифаќање на лапароскопската техника во изведувањето на сите уролошки процедури, на Универзитетската клиника за урологија почнува редовна и рутинска примена на лапароскопската, т.н. *hand assisted* нефректомија за обезбедување донорски бубрег за трансплантација.

Според препораките на Европската уролошка асоцијација, токму минимално инвазивниот, лапароскопски пристап е начинот на избор за изведување на нефректомиите од жив донор (7).

Лапароскопската нефректомија може да се изведе на неколку начини, ретроперитонеална или трансперитонеална, комплетно лапароскопска, или помогната со рака, т.н. *hand-assisted* нефректомија, лапароендоскопска хирургија преку еден отвор и лапароскопска хирургија преку природни отвори, како и роботски асистирана лапароскопска техника.

Првата *hand-assisted* лапароскопска нефректомија на жив донор (HALLDN) била направена во 1995 година, од страна на Кавуси и Ратнер, и беше вовед во ново поглавје во минимално инвазивната донорска нефректомија [8]. HALLDN ја прави хируршката дисекција поефикасна поради можноста за употреба на рацете и инструментите на повеќе различни начини, сигнификантно зголемувајќи го техничкиот капацитет, резултира со целосна предност во споредба со OLDN. Во 2002 г., Хорган (Horgan) прв пријавил роботски асистирана лапароскопска нефректомија на жив донор (RLDN) [9]. Споредбено со стандардната лапароскопска хирургија, овој роботски систем нуди тридимензионален вид со зголемена прецизност, а со тоа и зголемување на способноста на хирурзите за извршување комплексни зафати во лапароскопска средина [10]. Секоја од овие модификации на лапароскопската хирургија има свои специфични технички предности.

ЦЕЛ

Со оваа студија сакаме да ги прикажеме нашите иницијални искуства со т.н. *hand assisted* донорска нефректомија, изведена од страна на еден хирург.

МАТЕРИЈАЛ И МЕТОДИ

Спроведена е евалуација на пациентите кои подлегнале на лапароскопска трансперитонеална нефректомија од жив донор, во периодот меѓу ноември 2018-тата до мај 2020 година, на Универзитетската клиника за урологија во Скопје. Студијата се спроведуваше во единствениот центар во нашата Република каде што се работат ваков тип на интервенции. Сите пациенти кои подлегнале на лапароскопска трансперитонеална нефректомија од жив донор беа вклучени во евалуцијата. Сите пациенти кои биле подложени на отворена нефректомија од жив донор беа исклучени од евалуацијата, како и еден пациент каде што била направена конверзија од лапароскопска во отворена интервенција. Кај сите пациенти вклучени во евалуацијата беа иследувани следните параметри: епидемиолошките карактеристики на донорите, возраста, телесната тежина и висината, физиолошката класификација по системи на Американската асоцијација на анестезиолози (ACA), BMI, квалитетот на графтот, времето до добивање урина, раните компликации и вредностите на гломеруларната филтрациона рата (GFR). Беше одредуван и квалитетот на оперативната техника преку времето на топла исхемија, крвозагубата, траењето на оперативниот зафат, времетраењето на хоспитализацијата.

Од ноември 2018 год. до мај 2020 год., во нашиот центар беа направени 17 лапароскопски донор-нефректомии, од кои 14 беа леви, а 3 десностранн нефректомии. Десниот бубрег беше избран поради присуството на повеќе ренални вени на спротивната страна или поради подобар лев бубрег кај донорот. Кај 16 пациенти, графтот беше извaден со коса Гибсон-инцизија а кај еден пациент е направена конверзија поради екстремната дебелина и невозможноста за безбедно вадење на графтот.

Оперативна техника

Пнеумоперитонеум постигнуваме со користење на Верес игла и поставување на првиот 10 мм троакар параректално, на неколку сантиметри кранијално од умбиликусот, преку кој подоцна се поставува камерата, т.н. оптички порт. Дополнително поставуваме уште три троакари, два 10 мм троакари, еден на 5 до 7 см параректално и кранијално од оптичкиот пост, втор 10 мм троакар, на предната аксиларна линија, латерално и каудално од оптичкиот пост. Последниот порт е 5 мм, кој се поставува латерално и во линија со оптичкиот пост, на задната аксиларна линија, и го користи асистентот во иницијалната фаза на операцијата. Кај сите 16 пациенти користена е аголна 30 ^{o}-оптика.

Кај левата страна, иницијално се прави спуштање на десценцендентниот колон медијално, и мобилизација на лиеналната флексура, додека од десната страна се изведува т.н. Кохеров маневар, ослободување на дуоденумот и прикажување на долната шуплива вена, а потоа и спуштање на асцендентниот колон медијално. По спуштањето на соодветниот колон медијално, се преминува кон тапа и остра препарација на уретерот и негова делиберација до ниво на соодветната заедничка илијакална артерија. Кај десностраните нефректомии се поставува уште еден 5 мм троакар, епигастрично, во средишна линија, за подигање на црниот дроб кранијално. Потоа следува визуализација на соодветната артерија и нејзино елиберирање од околните ткива и реналната вена.

Следува максимално ослободување на бубрегот од перирениалното масно ткиво и надбубрежната жлезда. На левата страна, при препарирањето на реналната вена се изолираат и се одделуваат гонадната и надбубрежната вена, со 5 или 10 мм титаниумски клипси. Пред да се почне со поголемо манипулирање со крвните садови на бубрегот, интравенски се администира манитол 1мг/кг ТТ. Потоа, следува комплетно мобилизирање на хилусот на бубрегот од сите страни и комплетна дисекција на реналната артерија и вена постериорно. На десната страна се изведува подигнување на долната шуплива вена заради постигнување максимална должина на реналната артерија.

По постигнувањето на максималното ослободување на бубрегот од околните структури, се пристапува кон правење модифицирана коса

Гибсон-инцизија, во должина од 7 см која почнува во висина на tuberculum pubicum ant. sup.

Нашата техника се состои од воведување на десна рака при лева нефректомија, односно лева рака при десна нефректомија, не земајќи ја доминантната рака на операторот поради неговата амбидекстерност.

По воведувањето на раката, камерата се воведува на кранијалниот 10 мм порт, дополнително се ослободуваат крвните садови и се прави подготовка за клемување. Се пресекува уретерот и се поставува на видливо место, на самиот бубрег заради негова безбедност. Се воведува вреќичка за екстракција на препаратот и се пристапува кон клемување и пресекување на крвните садови. Користиме Hem-O-Lock клипси, по две на делот на дисталните крвни садови, со тенденција кон нивна максимална должина. Кај десностраните нефректомии, реналната вена е пократка, и дополнително користиме т.н. *bench*-хирургија, за добивање поголема должина. По екстракција на препаратот, тој се поставува на перфузија, додека се прави ревизија на хемостазата, поставување дрен и примарно затворање на инцизијата по слоеви.

РЕЗУЛТАТИ

Во периодот од 18 месеци, 17 пациенти беа подложени на лапароскопска трансперитонеална нефректомија од жив дарител за трансплантација на бубрег. Сите 17 пациенти беа оперирани со користење *hand assisted*-техниката, и кај еден пациент беше направена конверзија во отворена техника поради екстремна дебелина и неможност за безбедно клипување и вадење на графтот. Сите операции беа изведени од еден хирург.

Основните демографски карактеристики беа слични во однос на пол, возраст, тежина, висина, физиолошка класификација на системите по Американската асоцијација на анестезиолози (ASA), индекс на телесната маса (БМИ). Демографските и клиничките карактеристики на пациентите се прикажани во табела 1-4.

Од сите пациенти во студијата, бројот на жени дарители беше повисок во однос на мажите. Де факто, 10 (62,5 %) беа жени и 6 (37,5 %) беа мажи (табела 1). Најголемиот дел од донорите беа роднини до

прво колено, а во три случаи стануваше збор за брачни партнери. Просечната возраст на пациентите беше 57±12,3, со најмладиот 40 год., најстариот 77 год. (табела 2). Просечната висина на пациентите беше 166±10, со најнискиот 148 см, а највисокиот 181 см (табела 2). Просечната тежина на пациентите беше 69±12,4 кг, со најслабиот 52 кг, а најтешкиот 95 кг (табела 2). Според АСА-класификацијата, 8 (50 %) од нив беа класифицирани како АСА 1, 7 (43 %) пациенти беа АСА 2 и еден пациент (6,25 %) беше АСА 3 (табела 3). Според БМИ-класификацијата, еден пациент (6,25 %) беше дебел (обезен), 7 (43 %) беа со прекумерна тежина, 7 (43 %) пациенти беа со нормална тежина и еден пациент (6,25 %) беше со премала тежина (табела 4).

Табела 1

Дистрибуција на пациентите по пол

Пол	**Број на пациенти**	**%**
Жени	10	62,5
Мажи	6	37.5

Табела 2

Дистрибуција на пациентите по возраст, тежина и висина

Параметар	**Просек**	**±Стандардна девијација**
Години	57 години	12,3
Висина	166 см	10
Тежина	69 кг	12,4

Табела 3

Дистрибуција на пациенти по физиолошка класификација
на системи по Американската асоцијација на анестезиолози (ASA)

ASA	**Број на пациенти**	**%**
1	8	50
2	7	43
3	1	6,25

Табела 4

Дистрибуција на пациенти по БМИ

БМИ	**Број на пациенти**	**%**
Недоволна тежина <18,5	1	6,25
Нормална тежина 18,5 – 24,9	7	43
Прекумерна тежина 25 – 29,9	7	43
Обезност >30	1	6,25

Кај сите трансплантирани бубрези, веднаш по трансплантацијата се доби диуреза. Просечното време до добивање на првата диуреза беше 148±102 сек. Со исклучок на два пациента, кај останатите беше стимулирана диуреза со интравенска апликација на фуросемид во болус. Кај останатите два пациента беше почнато со терапија со допамин во ренални дози (2μg/kg/h) со времетраење од 12 ч кај едниот пациент и 24 ч кај другиот пациент. Просечната диуреза на крајот на операцијата беше 499±379 мл. Вредностите на креатининот, 48 ч по операцијата, споредени со предоперативните вредности, беа намалени за 32 % (376±95 umol/L vs. 265±167 umol/L), додека вредностите на уреата беа зголемени за 50 % затоа што сите пациенти беа подложени на хемодијализа пред операцијата (8.1±6.9 mmol/L vs. 14±6.6 mmol/L). Просечен период на хемодијализа беше 32±67 месеци.

Во табелите 5 и 6 се прикажани карактеристиките на експлантираните бубрези и операциите. Поголемиот број пациенти беа подло-жени на левострана нефкретомија n=13 (81,2 %). Само три пациента имаа десностранa нефректомија n=3 (18,7 %). Споредувајќи ја топлата исхемија по страни, левостраните нефректомии имаа пократка топла исхемија во однос на десностраните нефректомии (1,86±0,5 min vs. 2,6±0,5 min).

Ниту еден пациент немаше големи анестезиолошки и хируршки компликации во оперативниот и постоперативниот период.

Кај реципиентите не беше забележена артериска или венска тромбоза. Престојот во болница беше без особености, и средниот период на хоспитализацијата беше 5±1,03 дена. Сите пациенти беа испуштени од болница до седмиот постоперативен ден.

Табела 5

Карактеристики на експлантираните бубрези

Параметар	**Број на пациенти**	**%**
Лев графт	13	81
Десен графт	3	18
Артерии		
1	14	87,5
2	2	12,5

Табела 6

Карактеристики на операцијата

Параметар	**Просек**	**±Стандардна девијација**
Времетраење на операција	180 мин	28,5
Âðåìåòðàåœå íà àíåñòåçè¼à	250 мин	23,9
Време до прва диуреза	148 сек	102
Должина на болнички престој	5 дена	1,03
Време на топла исхемија (ВТИ)	2,43 мин	0,6
ВТИ на левостраните графтови	1,86 мин	0,5
ВТИ на десностраните графтови	2,6 мин	0,5

ДИСКУСИЈА

Лапароскопската донорска нефректомија, изведена само лапароскопски или со *hand assisted*-техниката, претставува комплексна процедура која изискува искусен медицински и хируршки тим, со одлични лапароскопски вештини. Според Хишихара и Сикуера, потребни се 30 лапароскопски нефректомии за да се надмине кривата на учење и два искусни лапароскописти во тимот за експлантација, за да почне програмата за лапароскопска донорска нефректомија [11, 12]. Левата донорска нефректомија е секогаш прв избор кај трансплантација од жив донор, пред сè, поради подолгата лева ренална вена и полесен пристап до хилусот на левиот бубрег. За разлика од лево, кај отворената десна донорска нефректомија, должината на десната ренална вена секогаш се зголемува со отстранување и на дел од долната шуплива вена заедно со

графтот. Поради ова се прават сè повеќе технички модификации во обид да се надмине овој проблем. Во нашата серија кај 3 (18,75 %) пациенти беше направена десна нефректомија, но поради користење на hem-o-lock клипси, секогаш по 2 на страната на донорот, изгубивме 7 до 10 мм, во споредба со отворената техника. Користевме мануелна елонгација на вената и колку што е можно подистално пласирање на клипсите, кон утоката во долната шуплива вена. Ова е опишано од Ратнер и коавторите, во нивните истражувања, според кои 10-15 мм од должината на реналната вена се губат, во споредба со отворената техника [13]. Користењето на овие композитни клипси во периодот на нивното воведување беше поврзано со контроверзии поради неколку смртни случаи поврзани со процесот на нивното користење [14]. Сепак, утврдено е дека доколку се користат по две hem-o-lock-клипси на страната на долната шуплива вена и на аортата, тие се апсолутно безбедни [15–17].

Тимот на Џон Хопкингз (John Hopkings) предлага терминален субкостален отворен пристап за ренална вена која е пократка од три сантиметри во должина. Се поставува вообичаена Сатински-клема (Satinsky-клема) преку долната шуплива вена, вената се разделува заедно со манжетната од долната вена кава, и со оваа инцизија се поправа и кавомијата. Како и да е, полуотворениот пристап ги компромитира придобивките од целосниот лапароскопски пристап [18].

Тимот на Клиниката „Кливленд“ (Cleveland Clinic) користеше ретроперитонеоскопски пристап за десна донор-нефректомија. Овој тим предлага модифицирана употреба на зглобувачки *Endo GIA*-степлер со негово поставување во десниот долен квадрант, со настојување да се максимира должината на реналната вена. Тие увиделе дека, во оваа позиција, *Endo GIA*-степлерот може да се постави преку реналната вена до вена кава [19]. Кратките крвни садови на графтот често се причина за отежната анастомоза со крвните садови на реципиентот, што може да доведе до васкуларни компликации и губење на графтот [20].

Ретроспективната мултиинституционална анализа, направена од Буел и соработниците, анализира 97 десностранн лапароскопски донорски нефректомии, и во причините за изборот ги поставува истите индикации како во нашата серија, послаб и помал десен бубрег, циста во десниот бубрег, повеќе артериски крвни садови на левата страна.

Дополнително, како и во серијата на Буел, причината за единствениот случај на конверзија во нашата серија беше екстремна дебелина на пациентот и висок БМИ 35,9 (179 см/115кг) [21]. Иако во минатото постоеја поделени мислења околу должината на уретерот која се добива при лапароскопска донор-нефректомија, ние немавме разлика анализирано според страната на операцијата како во студијата на Берендс, каде што се укажува на слични резултати [22].

Времето на топла исхемија (ВТИ) е еден од показателите на оперативната техника, вештината и искуството на операторот. Поради подолгото време на вадење на графтот, ВТИ се смета за една од најголемите негативности на лапароскопската техника [23].

Подолгото ВТИ доведува до полош резултат на трансплантацијата и одложено време на промокрување, Ролинс и Волф, во различни студии на повеќе од 100 случаи, го потврдуваат спротивното, односно отсуство на големи разлики при времиња на топла исхемија од 95 до 300 секунди [24, 25].

Историски, ВТИ е намалено на 75 до 105 секунди со следењето на кривата на учење, и дополнително, ВТИ кај левостраната нефректомија е подолно, во споредба со десностраните операции. Сепак, не е најдена корелација на подолго ВТИ и повисоки вредности на деградациските продукти три месеци, постоперативно [26]. Ова се поклопува со нашите резултати за општо ВТИ од 2,63±0,6 SD и (лева страна 1,86±0,5 мин; десна страна 2,6±0,5 мин). Овие времиња се споредливи со оние опишани од Јакобс, во серијата на Универзитетот во Мериленд и повеќе од 703 случаи [27] Јакобс, во студијата ги компарирал ВТИ <3 min со ВТИ >3 min, и ВТИ <5min, ВТИ 5-10 min, и ВТИ >10 min утврдил дека пролонгираното ВТИ не влијае на серумскиот креатинин и функцијата на графтот во првите три месеци по трансплантацијата. Кога се евалуирало влијанието на пролонгираното ВТИ на долг временски рок, утврдено е дека повторно нема разлика и влијание на серумскиот креатинин и функцијата на графтот [28]. Во систематскиот преглед на Хандшин и сор., утврдено е дека нема значајна разлика во процентот на отфрлање на графтот, кога се споредува лапароскопската со отворената нефректомија, и ја потврдува безбедноста на лапароскопската техника кон донорот, реципиентот и графот [29]. Слични се

и резултатите на Јакобс од серија на над 730 случаи [27]. Во нашата серија немаше забележано акутно отфрлање на графтот.

Денес постојат многу студии кои укажуваат на бенефитот на лапароскопската техника споредено со отворената, во повеќе аспекти на процедурата, како намалена крвозагуба, пократок болнички престој, побрзо почнување со исхрана, враќање кон нормалните социјални и работни активности. Дополнително, намалената потреба од аналгетици и подобриот естетски исход кај пациентите оперирани со лапароскопски метод, дефинитивно ја стават оваа техника повисоко на пиедесталот [30].

Во последните години, сè поголем замав земаат ретроперитонеалната и LESS (laparoendoscopic single site surgery) хирургија преку еден отвор, пред сè поради слични или идентични резултати за графтот, а помал морбидитет и подобар естетски исход за донорот на бубрегот [31].

ЗАКЛУЧОК

Оваа мала студија претставува наше првично искуство со hand assisted донор-нефректомија преку трансперитонеален пристап. Постојат силни докази дека лапароскопската техника за донорска нефректомија е ефикасна и безбедна метода која е слична на отворената техника од аспект на функција на графтот, уролошки компликации и преживување на графтот. Употребата на аналгетици, времето на хоспитализација и времето на топла исхемија, како и побрзата мобилизација и вертикализација на донорот, одат во прилог на лапароскопската техника.

ЛИТЕРАТУРА

1. Timsit, M. O., Kleinclauss, F., Thuret, R. *History of kidney transplantation surgery*. Prog Urol. 2016 Nov; 26 (15): 874–881.
2. Bellini, M. I.; Courtney, A. E.; McCaughan, J. A. *Living donor kidney transplantation improves graft and recipient survival in patients with multiple kidney transplants*. J. Clin. Med. 2020, 9, 2118.

3. Antcliffe Det al. *A meta-analysis of mini-open versus standard open and laparoscopic living donor nephrectomy*. TransplInt, 2009. 22: 463.
4. Popov, Z., Ivanovski, N., Kolevski, P. *Transplantation rénale en Macédoine* [Kidney transplantation in Macedonia]. Ann Urol (Paris). 2000 Oct; 34(5): 294–301.
5. Spasovski, G., Dohcev, S., Stankov, O., Stavridis, S., Saidi, S., Josifov, A., Masin-Spasovska, J. *The improvement in kidney transplant program in R. Macedonia--what are the clues?* Pril (Makedon Akad Nauk Umet Odd Med Nauki). 2013; 34(2): 9–13.
6. Popov, Z. et al. *Prelevement renal coelioscopique chez les donneurs vivant – les premiers quatre case dans la Republique de Macedoine*. ProgUrol (2005), 15. Supp. No1, 1227–1228
7. Lennerling, A., Lovén, C., Dor, F. J., Ambagtsheer, F., Duerinckx, N., Frunza, M., Pascalev, A., Zuidema, W., Weimar, W., Dobbels, F. *Living organ donation practices in Europe – results from an online survey*. Transpl Int. 2013 Feb; 26(2): 145–53. doi: 10.1111/tri.12012. Epub 2012 Dec 1.
8. Ratner, L. E., Ciseck, L. J., Moore, R. G., Cigarroa, F. G., Kaufman, H. S., Kavoussi, L. R. *Laparoscopic live donor nephrectomy*. Transplantation. 1995 Nov 15; 60(9): 1047.
9. Horgan, S., Vanuno, D., Benedetti, E. *Early experience with robotically assisted laparoscopic donor nephrectomy*. Surg Laparosc Endosc Percutan Tech. 2002 Feb; 12(1): 64–70. doi: 10.1097/00129689-200202000-00011.
10. Horgan, S., Vanuno, D., Sileri, P., Cicalese, L., Benedetti, E. *Robotic-assisted laparoscopic donor nephrectomy for kidney transplantation*. Transplantation. 2002 May 15; 73(9): 1474-9. doi: 10.1097/00007890-200205150-00018.
11. Higashihara, E., Baba, S., Nakagawa, K., Murai, M., Go, H., Takeda, M. et al. *Learning curve and conversion to open surgery in cases of laparoscopic adrenalectomy and nephrectomy*. J Urol. 1998; 159: 650-3.
12. Siqueira, T. M. Jr., Gardner, T. A., Kuo, R. L., Paterson, R. F., Stevens, L. H., Lingeman, J. E., et al. *One versus two proficient laparoscopic surgeons for laparoscopic live donor nephrectomy*. Urology. 2002; 60: 406-9; discussion 409-10.
13. Ratner, L. E., Kavoussi, L. R., Chavin, K. D. et al. *Laparoscopic live donor nephrectomy: technical consideration and allograft vascular length (letter to editor)*. Transplantation 1998; 65: 1657
14. Hsi, R. S. et al. *Mechanisms of hemostatic failure during laparoscopic nephrectomy: review of Food and Drug Administration database*. Urology, 2007. 70: 888.

15. *Ay, N., Dinc, B., Dinckan, A., Yilmaz, V. T., Erdogan, O., Gurkan, A. The safety of hem-o-lock clips at donor nephrectomies*. Ann Transplant. 2010 Jan-Mar; 15(1): 36-9.
16. Ponsky, L. et al. *The Hem-o-lok clip is safe for laparoscopic nephrectomy: a multi-institutional review.* Urology, 2008. 71: 593.
17. Simforoosh, N., Tabibi, A., Soltani, M. H., Zare, S., Yahyazadeh, S. R., Abadpoor, B. *Long-Term Follow-up After Right Laparoscopic Donor Nephrectomy and Inverted Kidney Transplant*. Exp Clin Transplant. 2016 Feb; 14(1): 27–31.
18. Mandel, A. K., Cohen, C., Montgomery, R. A., Kavoussi, L. R., Ratner, L. E. *Should the indication of the right kidney be the same as for the open procedure: Anomalous left renal vasculature is not a contraindication to laparoscopic donor nephrectomy*. Transplantation 2001; 71: 600-64.
19. Gill, I. S., Uzzo, R., Hobart, M. G. et al. *Laparoscopic retroperitoneal live donor right nephrectomy for purposes of allotransplantation and auto-transplantation*. J Urol 2000; 644: 1500.
20. Kim, F., Ratner, L. E., Kavoussi, L. R. *Renal transplantation: Laparo-scopic live donor nephrectomy*. UrolClin North Am 2000; 27: 777.
21. Buell, J. F., Edye, M., Johnson, M. et al. *Are concerns over right laparoscopic donor nephrectomy unwarranted?* Ann Surg 2001; 233: 645.
22. Berends, F. J., Den Hoed, P. T., Bonjer, H. J., Kazemier, G., Van Riemsdijk, I., IJzermans, J. N. et al. *Technical considerations and pitfalls in laparoscopic live donor nephrectomy*. SurgEndosc. 2002; 16: 893–8.
23. Srivastava, A., Gupta, N., Kumar, A., Kapoor, R. and Dubey, D. *Tran-speritoneal laparoscopic live donor nephrectomy: Current status*. Indian J Urol 2007; 23(3): 294–298.
24. Rawlins, M. C., Hefty, T. L., Brown, S. L., Biehl, T. R. *Learning lapa-roscopic donor nephrectomy safely: A report on 100 cases*. Arch Surg. 2002; 137: 534–5.
25. Wolf, J. S., Marcovich, R., Merion, M., Konnak, J. W. *Prospective case matched comparison of hand assisted laparoscopic and open surgical live donor nephrectomy*. J Urol. 2000; 163: 1650–3.
26. Hazebroek, E. J., Gommers, D., Schreve, M. A., Van Gelder, T., Roodnat, J. I., Weimar, W. et al. *Impact of intraoperative donor management on short term renal function after laparoscopic donor nephrectomy*. Ann Surg. 2002; 236: 127–32.
27. Jacobs, S. C., Cho, E., Foster, C., Liao, P., Bartlett, T. S. *Laparoscopic live donor nephrectomy: The University of Maryland 6-year experience*. J Urol. 2004; 171: 47–51.

28. Simforoosh, N., Bassiri, A., Ziaee, S. A., Maghsoodi, R., Salim, N. S., Shafi, H. et al. *Laparoscopic versus open live donor nephrectomy: The first randomized clinical trial*. Transplant Proc. 2003; 35: 2553–4.
29. Handschin, A. E., Weber, M., Demartines, N., Clavien, P. A. *Laparoscopic donor nephrectomy*. Br J Surg. 2003; 90: 1323–32.
30. Yuan, H. et al. *The safety and efficacy of laparoscopic donor nephrectomy for renal transplantation: an updated meta-analysis*. Transplant Proc, 2013. 45: 65.
31. Elmaraezy, A. et al. *Should hand-assisted retroperitoneoscopic nephrectomy replace the standard laparoscopic technique for living donor nephrectomy?* A meta-analysis. Int J Surg, 2017. 40: 83.

Sotir STAVRIDIS, Maja MOJSOVA-MIJOVSKA, Oliver STANKOV,
Aleksandra PANOVSKA PETRUSHEVA, Josif JANCHULEV,
Aleksandra GAVRILOVSKA-BRZANOV, Viktor STANKOV,
Vladimir LOZANOVSKI, Sasho DOHCHEV

HAND-ASSISTED LAPAROSCOPIC NEPHRECTOMY IN LIVING DONOR TRANSPLANTATION
INITIAL RESULTS

Abstract

Introduction: Hand-assisted donor nephrectomy is a commonly used method in living donor transplantations. First laparoscopic living donor nephrectomies (LDN) in RSM were made in 2004. In this evaluative study we present the initial results in our clinic with this method.

Materials and methods: Seventeen laparoscopic transperitoneal LDN were performed between november 2018 and may 2020. Conversion was made in one patient. There were 13 left and 3 right nephrectomies. One of the patients had positive anamnesis for previous open surgery. In this study we determined the epidemiological features of the donors, sex, weight, height, physiological classification of The American Society of Anesthesiologists, BMI, quality of renal graft, time to first diuresis, early complications and glomerular filtration rate. The quality of operative technique was determined through time of warm ischemia, blood loss, duration of surgery, and hospital stay.

Results: In a period over 18 months, 17 patients underwent hand assisted laparoscopic nephrectomy. One patient had conversion to open surgery due to extreme obesity. All surgeries were made by a single surgeon. Ratio of female/male donors was 62.5%/37.5%. The average age of patients was 57±12.3. 8 of the patients were classified ASA 1, 7 patients were ASA 2 and one patient was ASA 3. According to BMI, 43% of the patients were overweight and 43% of the patients were with normal weight. Average time until first diuresis was 148±102 seconds. Average diuresis at the end of surgery was 499±379 ml. Creatinine level 48 hours after surgery compared to preoperative results, were lowered by 32% (376±95umol/L vs. 265±167 umol/L), while urea levels in serum were increased by 50% due to hemodialysis done before surgery. Average length of hemodialysis was 32±67 months. Left sided nephrectomies had shorter worm ischemia compared to right sided ones (1.86±0.5 min vs. 2.6±0.5 min).

Conclusion: This study represents our initial experience with transperitoneal laparoscopic hand assisted living donor nephrectomy. It is an efficient and safe method for kidney donors and at same time it provides well functioning kidney graft. After the initial good results and flawless safety, we implemented this procedure as routine at University clinic of Urology.

Keywards: transplantaion, donor, kidney, laparoscopy.

doi: 10.20903/zpopov-2022-0016 UDK: 616.61-089.843-06-073.432.19

Соња ЏИКОВА[1], Живко ПОПОВ[2,3], Нинослав ИВАНОВСКИ[1]

УЛТРАЗВУК, КОЛОР ДУПЛЕКС ДОПЛЕР И ИНТЕРВЕНТНА ЕХОСОНОГРАФИЈА ВО ДИЈАГНОСТИКА НА ПРОМЕНИ И КОМПЛИКАЦИИ КАЈ ТРАНСПЛАНТИРАН БУБРЕГ

Апстракт

Историјат: Примена на ултразвукот во дијагностика на морфологијата на различни паренхимски органи зема значаен подем од 1980-тите години, со појавата на *real-time* сонографијата. Со внесувањето на многу новини во електрониката и компјутерските програми во апаратурата беше постигната уште поголема прецизност во приказот на промените на паренхимските органи.

Цел: Целта на студијата е да се види корисноста на ултразвучните испитувања кај трансплантираните бубрези за да се овозможи брза и неинвазивна дијагностика.

Методи: Направена е студија на 126 трансплантирани болни од кои 20 со кадаверична трансплантација, а 106 живи дарители во блиско сродство со пациентот. Сите се испитувани со ултразвучни методи и со колор дуплекс-сонографија. Кај 40 пациенти е направена ренална биопсија на графтот, водена под ултразвук.

Резултати: Во акутните испитувања, веднаш по трансплантацијата, најдени се промени кај 16 % од пациентите, а кај хроничните следења на 3 и 6 месеци во текот на пет години, кај 27,7 %.

[1] Универзитетска клиника за нефрологија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, РС Македонија

[2] Универзитетска клиника за урологија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, РС Македонија

[3] Македонска академија на науките и уметностите, Скопје, РС Македонија

Заклучок: Според добиените резултати, студијата покажа дека примената на ултрасонографската дијагностика е брза, неинвазивна, безболна и точна техника за утврдување на промени на графтот во раниот и долгорочниот период на следење.

Клучни зборови: ултразвук, колор дуплекс-доплер, трансплантиран бубрег.

ВОВЕД

Примената на ултразвукот во дијагностиката на морфологијата на различни паренхимски органи зема значаен подем од 1980-тите години, со појавата на *real-time* сонографијата. Со внесување на многу новини во електрониката и компјутерските програми во апаратурата беше постигната уште поголема прецизност во приказот на промените на паренхимските органи. Со усовршување, пак, на доплер-модулите, особено на колор дуплекс-доплерот, кон крајот на осумдесеттите и во почетокот на деведесеттите години на минатиот век, се доби можност за приказ на големите крвни садови во абдоменот, нивниот sид, на брзината на протокот на крвта низ нив, а понатаму се овозможи и согледување на циркулацијата и проточната брзина на крвта низ најмалите артерии и вени во паренхимот на органите. Ова овозможи значаен напредок на неинвазивната дијагностика и во нефрологијата и урологијата.

МАТЕРИЈАЛ И МЕТОДИ

Направена е студија со ултрасонографски преглед на сите трансплантирани бубрежни болни на Универзитетската клиника за урологија, Медицинскиот факултет, Универзитетот „Св. Кирил и Методиј“, Скопје, во првите 2 до 7 дена по операцијата, и 20-30 дена потоа. Понатаму, пациентите се следени во текот на 5 години, на 3 и 6 месеци, на Универзитетската клиника за нефрологија, Медицинскиот факултет, Универзитетот „Св. Кирил и Методиј“, Скопје, според одредени протоколи. Кај 40 пациенти, според индикацијата и клиничката слика, е направена ренална биопсија водена под ултразвук, со примена

на *true-cut* игла 14 G со автоматски „пиштол“. Биопсијата е направена кај оние пациенти каде што се забележени паренхимски промени на ултразвук и лабораториски промени на рутинските контроли. Кај овие пациенти, според хисто-патолошкиот наод, напра-вено е дополнување или промена во имуносупресивната терапија. Прег-ледани и следени се вкупно 126 пациенти со трансплантација на бубрег до периодот 1986 – 1996 година, и тоа 20 со кадаверична трансплантација и 106 пациенти кај кои дарители биле живи блиски роднини. Средна возраст на дарителите е 59±4 години, а кај примателите 34±6 години.

РЕЗУЛТАТИ

Во табела 1 се прикажани наодите од раните контроли на транс-плантираните пациенти кај кои се најдени промени на графтот од 2-от до 7-от ден постоперативно. По индикација, кај дел од нив се напра-вени компјутеризирана томографија, магнетна резонанца и ренална ангиографија. Кај васкуларните промени на реналните артерии, кај 1 пациент е поставен стент, а кај 2 пациента е направена балон-дилата-ција со добар исход на крвниот проток. Кај 2 пациенти со хидронеф-роза, беа поставени перкутани нефростоми, каде што интервенцијата беше водена под ултразвук, а кај третиот пациент пречката спонтано се разреши со елиминација на калкул. Кај пациентите каде што имаше акутно отфрлање, направена е корекција на имуно-супресивната тера-пија. Кај сите пациенти, каде што имаше промени, постигнато е зна-чајно подобрување на состојбата и сите беа испишани на домашно лекување. Кај останатите пациенти немаше промени. Текот на хоспи-талното лекување беше уреден.

Табела 1

Рани промени и наоди по трансплантација

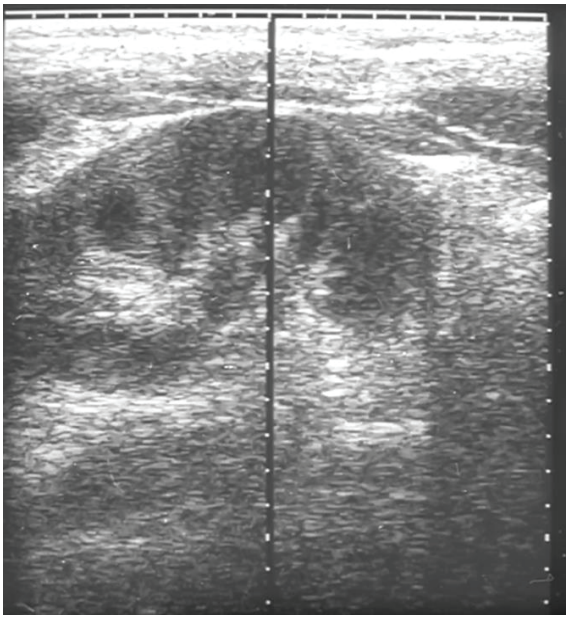
Рани промени и наоди по трансплантација	**Број на пациенти**	**Процентуална застапеност**
Тромбоза на v. renalis	1	0,79 %
Стеноза на a. renalis	3	2,3 %
Крвавење од мала гранка на v. renalis	1	0,79 %

Руптура на капсула на графт	2	1,5 %
Акутно отфрлање на графт	5	3,9 %
Хидронефроза	3	2,3 %
Перинефрална колекција	6	4,7 %
Вкупно	**21**	**16 %**

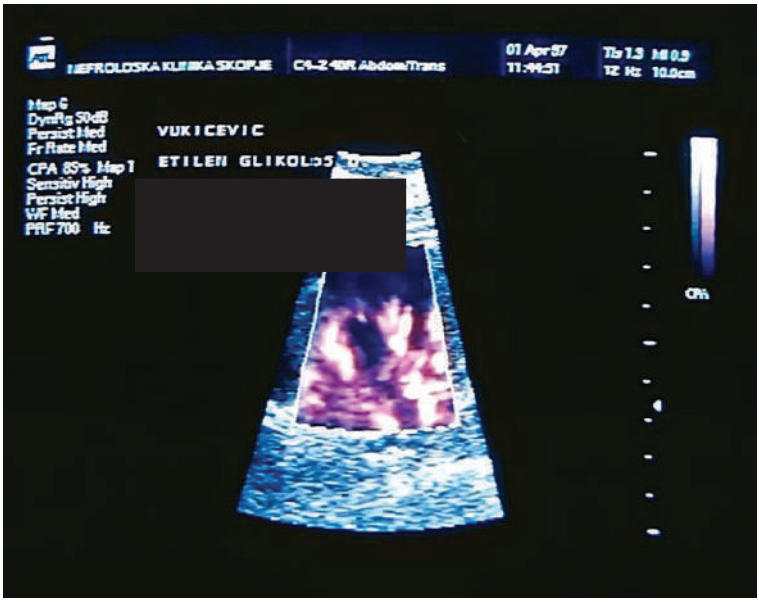
Табела 2
Резултати од следењето на пациентите во текот на 5 години, со периодични контроли на 3 и 6 месеци

Долгорочни контроли во текот на 5 години	**Број на пациенти**	**Процентуална застапеност**
Хронична алографт-нефропатија	6	4,7 %
Малигном на графт	2	1,58 %
Рекурентен гломерулонефрит	4	3,17 %
Borderline хронични промени	23	18,2 %
Вкупно со промени	**35**	**27,7 %**
Без промени	**91**	**72,7 %**

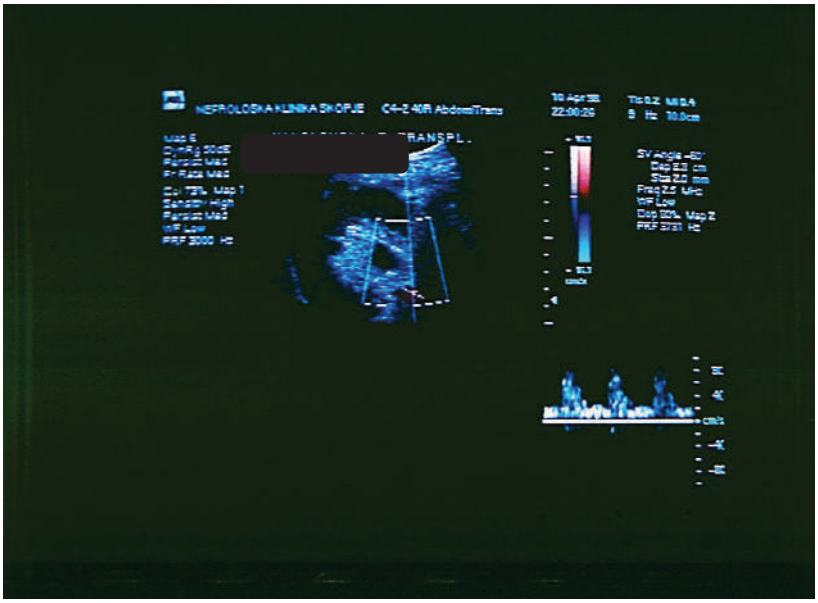
Во табела 2 се прикажани резултатите од следењето на пациентите во текот на 5 години, со периодични контроли на 3 и 6 месеци. Кај 27,7 %, се констатирани паренхимски промени, и кај нив се направи ренална биопсија водена под ултразвук на графтот. Компликации по изведената биопсија на графтот не беа забележени кај ниеден пациент. Кај 72,7 % од пациентите немаше значајни промени ниту лабораториски, ниту на ултразвук. Кај пациентите со рекурентен гломерулонефрит, направено е дополнување на имуно-супресивната терапија, а кај пациентите со хронична алографт-нефропатија, направена е измена на имуно-супресивната терапија. Кај едната пациентка со малигном на графт направена е нефректомија, а кај втората, каде што немаше јасна ограниченост на туморот, спроведена е онколошка конзервативна терапија. Кај пациентите со гранична (borderline) нефропатија, не е дополнувана терапијата, туку тие беа оставени на следење.



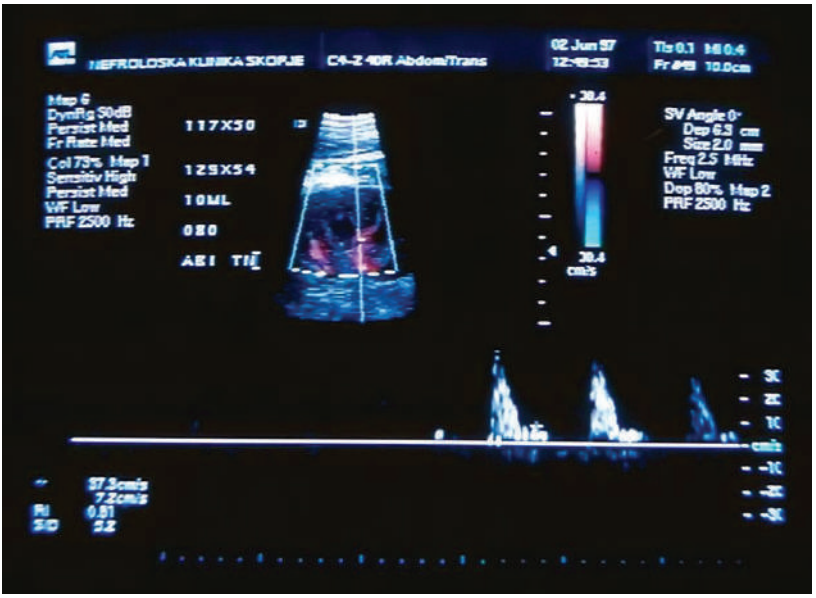
Слика 1 – Акутно отфрлање на графт



Слика 2 – Акутна тубуларна некроза



Слика 3 – Нормален проток низ a. renalis на графт



Слика 4 – Оштетување на микроциркулацијата на паренхимот
со можна тромбоза на аркуатните артерии

ДИСКУСИЈА

Ултразвучната неинвазивна дијагностика кај трансплантирани бубрежноболни има голема важност за брзо и точно утврдување на морфолошките промени на графтот, не само на глобалната слика туку и на деталните ткивни и васкуларни промени на паренхимот [1]. Исто така е многу битно откривањето на причините за акутни збиднувања како што се: нагло настанување на анурија, акутен пораст на дегрaдациските продукти во крвта, локална болка и слично.

Со ехосонографијата се утврдува промената на големината на графтот, појавата на едем, циркулаторните промени на бубрегот на ниво на арјкуатните артерии или основната ренална артерија и вена [2]. Исто така, причина за олигоанурија може да биде пречка во уроодводниот систем, што лесно се утврдува со помош на ултразвучната дијагностика. Ехосонографски може да се утврди и местото на настанатата промена (на пр., на ниво на анастомоза на артерија или вена [3, 4] или на местото на имплантација на уретерот), а со тоа во најкусо време да се преземат мерки за надминување на соодветната компликација.

Наод на високи вредности на RI на ниво на одредени крвни садови, како и прекин на континуитетот на систоло-дијастолната линија, при следење на крвотокот (особено на ниво на аркуатните артерии), укажува на паренхимско страдање и можно отфрлање на графтот [5, 6, 7]. Овие промени точно се утврдуваат со користење на колор дуплекс-доплерот. Кај поставено сомнение за отфрлање на графтот, хронична алографт-нефропатија или рекурентен гломерулонефрит, индицирано е биопсија на трансплантираниот бубрег [8, 9]. Ние ја изведуваме со помош на *true-cut* игла, со „пиштол“, водена со ултразвук. На овој начин, пациентот е заштитен од несакани можни компликации при интервенцијата, а истовремено е обезбедена прецизност на нејзиното изведување.

Со примената на ехосонографијата и колор дуплекс-доплерот во дијагностиката на промени на трансплантиран бубрег, се намали потребата од правење компјутеризирана томографија, ангиографија или магнетна резонанца. Така, се утврдија точните индикации за изведување дополнителни, поскапи и поинвазивни морфолошки испиту-

вања [10]. Ултрасонографските прегледи може да се изведуваат повеќепати без ризик за пациентот, а апаратурата е мобилна и прегледите може да се вршат додека пациентот лежи во својот кревет. Кај оваа група пациенти се покажаа рани компликации кај 16 % од нив, а во следење од 5 години, промени се најдени кај 27,7 %. Ова помогна во навремена корекција на состојбата и дополнување на терапијата.

ЗАКЛУЧОК

Студијата покажа дека примената на неинвазивната ултрасонографска дијагностика е брза, безболна и точна имиџинг-техника за утврдување на наодите и промените на графтот. Со примена на доплерските техники, покрај следењето на циркулацијата, може индиректно да се укаже на функционалните промени на графтот, особено ако редовно се следи состојбата на пациентот.

Техничките усовршувања на апаратурата придонесуваат за уште поточни наоди во 2Д или 3Д-техники, како и на ткивните промени на графтот. Во последните години се применува и ултразвучната еластографија која укажува на квалитетот на ткивните промени на графтот. Со напредокот на науката и техниката, можностите на ехосонографијата се зголемуваат, а со тоа и точноста на утврдувањето на промените, сè со цел да се постигне прецизен и навремен тераписки пристап.

РЕФЕРЕНЦИИ

1. Kolofousi, C., Stefanidis, K., Cokkinos, D. D., Karakitsos, D., Antypa, E., Piperopoulos, P. *Ultrasonographic Features of Kidney Transplants and Their Complications: An Imaging Review*. ISRN Radiology. 2013 Dec 2; 2013: 1–12.
2. Ammi, M., Daligault, M., Sayegh, J., Abraham, P., Papon, X., Enon, B. et al. *Evaluation of the Vascular Surgical Complications of Renal Transplantation. Annals of Vascular Surgery*. 2016 May; 33: 23–30.
3. Fananapazir, G., McGahan, J. P., Corwin, M. T., Stewart, S. L., Vu, C. T., Wright, L. et al. *Screening for Transplant Renal Artery Stenosis: Ultrasound-Based Stenosis Probability Stratification*. American Journal of Roentgenology. 2017 Nov;209(5): 1064–73.

4. Horrow, M. M., Parsikia, A., Zaki, R., Ortiz, J. *Immediate Postoperative Sonography of Renal Transplants: Vascular Findings and Outcomes. American Journal of Roentgenology*. 2013 Sep.; 201(3): W479–W486.
5. Leong, K. G., Coombs, P., Kanellis, J. *Renal transplant ultrasound: The nephrologist's perspective*. Australasian Journal of Ultrasound in Medicine. 2015 Nov;18(4): 134–42.
6. Galgano, S. J., Lockhart, M. E., Fananapazir, G., Sanyal, R. *Optimizing renal transplant Doppler ultrasound*. Abdominal Radiology. 2018 Aug 18; 43(10): 2564–73.
7. Granata, A., Clementi, S., Londrino, F., Romano, G., Veroux, M., Fiorini, F. et al. *Renal transplant vascular complications: the role of Doppler ultrasound*. Journal of Ultrasound. 2014 Apr 11;18(2): 101–7.
8. Patel, M. D., Young, S. W., Scott Kriegshauser, J., Dahiya, N. *Ultrasound-guided renal transplant biopsy: practical and pragmatic considerations.* Abdominal Radiology. 2018 Feb 9;43(10): 2597–603.
9. Uppot R. N., Harisinghani, M. G., Gervais, D. A. *Imaging-Guided Percutaneous Renal Biopsy: Rationale and Approach. American Journal of Roentgenology.* 2010 Jun;194(6): 1443–9.
10. Jehn, U., Schuette-Nuetgen, K., Kentrup, D., Hoerr, V., Reuter, S. *Renal Allograft Rejection: Noninvasive Ultrasound- and MRI-Based Diagnostics.* Contrast Media & Molecular Imaging. 2019 Apr 10; 2019: 1–9.

Sonja DZIKOVA, Zivko POPOV, Ninoslav IVANOVSKI

ULTRASOUND, COLOR DUPLEX DOPLER AND INTERVENTIONAL ECHOSONOGRAPHY IN DIAGNOSTICS OF CHANGES AND COMPLICATIONS IN TRANSPLANTED KIDNEY

Summary

History: The application of ultrasound in the diagnostics of the morphology of various parenchymal organs has taken a significant rise since the 1980s with the advent of real-time sonography. With the introduction of many innovations in electronics and computer programs in the apparatus, even more precision was achieved in the display of the changes of the parenchymal organs.

Aim: The aim of the study is to see the usefulness of ultrasound examinations in transplanted kidneys to enable rapid and non-invasive diagnosis.

Methods: A study was performed on 126 transplant patients, 20 of whom had cadaveric transplants and 106 had living donors in close kinship with the patient. All were examined by ultrasound methods and color duplex sonography. In 40 patients, an ultrasound-guided renal graft was performed.

Results: In the acute examinations immediately after the transplant, changes were found in 16% of the patients, and in the chronic follow-ups at 3 and 6 months during five years, in 27.7%.

Conclusion: According to the obtained results, the study showed that the application of ultrasonographic diagnostics is a fast, non-invasive, painless and accurate technique for determining graft changes in the early and long-term follow-up period.

Keywords: Ultrasound, color duplex Doppler, kidney transplant.

doi: 10.20903/zpopov-2022-0017 UDK: 616.71-018.46-089.843(497-15)"2021"

Emirjeta BAJRAMI[1], Zivko POPOV[2,34],
Ana VUCUREVIC[4], Mirko SPIROSKI[3,4]

NON-GOVERNMENTAL WORLD MARROW DONOR REGISTRIES AND WESTERN BALKAN MARROW DONOR REGISTRIES (2021 YEAR)

Abstract

Background: The Western Balkan countries include Albania, Bosnia and Herzegovina, Kosovo, Montenegro, Republic of North Macedonia, and Serbia. The Western Balkan region is the last region to be fully integrated into European Union and many of the indicators classify these countries near the bottom of European countries. From the six countries of the Western Balkan region, only two marrow donor registries are included in the World Marrow Donor Registry (North Macedonia and Serbia), and one is in preparation (North Macedonia).

Aim: We aimed to investigate the current non-governmental world marrow donor registries and marrow donor registries in the Western Balkan countries as of 2021.

Methods: We visited all of the websites at World Marrow Donor Association looking for any information about the organizational structure as of June 2021. The Western Balkan marrow donor registries were identified in the register of the World Marrow Donor Association as of July 2021.

Results: We identified 196 marrow donor registries in total, from which 140 (71%) were governmental and 56 (29%) were non-governmental.

[1] University Clinical Centre, Neonatology Clinic, Prishtina, Kosovo;

[2] Macedonian Academy of Sciences and Arts, Skopje, RN Macedonia;

[3] Scientific Foundation SPIROSKI, Skopje, RN Macedonia.

[4] Faculty of Medicine, University "SS. Cyrill and Methodius", Skopje, North Macedonia

The number of registries in several countries are 100% non-governmental, in several countries have more than half of the registries are non-governmental or are equal in number between non-governmental and governmental registries, and in few countries there are equal or smaller amounts of non-governmental than governmental registries. The rest of the countries have only governmental registries. There are two functional marrow donor registries included in World Marrow Donor Registries from the Western Balkan: MBMDR - Macedonian Bone Marrow Donor Registry from North Macedonia and Serbian Bone Marrow Donor Registry from Serbia. There is one more marrow donor registry in preparation from Scientific Foundation SPIROSKI as a part of the non-governmental sector in North Macedonia. Currently there are no marrow donor registries in Albania, Bosnia and Herzegovina, Kosovo, or Montenegro.

Conclusion: We can conclude that in most of the Western Balkan countries (Albania, Bosnia and Herzegovina, Kosovo, and Montenegro) there are no marrow donor registries, and only in North Macedonia and Serbia there are governmental marrow donor registries. There is a need to develop more non-governmental and governmental marrow donor registries in the Western Balkan countries.

Keywords: Marrow donor registry; Western Balkan; nongovernmental; governmental.

Introduction

The Western Balkan Countries include Albania, Bosnia and Herzegovina, Kosovo, Montenegro, Republic of North Macedonia, and Serbia. The European Union (EU) has developed a policy to support the gradual integration of the Western Balkan countries into the European Union. Montenegro, Serbia, the Republic of North Macedonia, and Albania are official candidates. Accession negotiations and chapters have been opened with Montenegro and Serbia, and Bosnia and Herzegovina and Kosovo are potential candidate countries [1].

The Western Balkan region is the last area to be fully integrated into the European Union, and many of the indicators classify these countries as near the bottom.

World Marrow Donor Association (WMDA) is made up of organisations and individuals, promoting global collaboration and best practices for the benefit of stem cell donors and transplant patients. WMDA was informally initiated in 1988 by three pioneers in the field of transplantation: John Goldman (United Kingdom), E. Donnall Thomas (United States), and Jon J. van Rood (the Netherlands). This led to the foundation of WMDA in 1994. In 2007, the WMDA became one of the founding members of the Worldwide Network for Blood and Marrow Transplantation (WBMT). WBMT is a non-profit scientific organisation which aims to promote excellence in stem cell transplantation, donation, and cellular therapy. The WBMT has an official relationship with the World Health Organization. The other three founding organisations of the WBMT are the European Society for Blood and Marrow Transplantation (EBMT), the Center for International Blood & Marrow Transplant Research (CIBMTR), and the Asian Pacific Blood and Marrow Transplantation Group (APBMT). In 2017, WMDA took over the activities of Bone Marrow Donors Worldwide (BMDW) and the NetCord Foundation. WMDA is led by the WMDA Board and committees, whose members are all experts in their fields. Day-to-day activities are carried out by the WMDA office by a team of professionals based in Leiden, the Netherlands [2].

Of the six countries in the Western Balkans, only two marrow donor registries are included in World Marrow Donor Registry (North Macedonia and Serbia), and one is in preparation (North Macedonia) [2].

We aimed to investigate current non-governmental world marrow donor registries and marrow donor registries in the Western Balkan countries (2021 Year).

Methods

We visited all websites at World Marrow Donor Association (WMDA), looking for any information about the organizational structure and found many different approaches. Some of the websites are without any information about their organizational structure, some of the websites are only in the local language, some of the websites declare mixed organizational structure (governmental and non-governmental), some websites have traditional non-

governmental organizations (i.e., Red Cross), and are also defined as governmental organizations, and some of the websites state that they have a charitable structure (which was classified as non-governmental organization). We categorized all websites with a clear definition of foundation and/or charity organization as foundations.

Western Balkan marrow donor registries were identified from the register of the World Marrow Donor Association (WMDA) [3] in July 2021.

Results

We identify 196 marrow donor registries in total, from which 140 were governmental and 56 were nongovernmental (Table 1). The number of registries in several countries are 100% non-governmental (Armenia, Lithuania, Luxembourg, Nigeria, United Kingdom, and the United States of America). Several countries have more than half non-governmental registries (India, Mexico, and South Africa). Equal number of non-governmental and governmental registries are in several countries (Australia, Austria, Chile, North Macedonia, Slovakia, Thailand, and The Netherlands). The group of countries with fewer non-governmental than governmental registries are Cyprus, Czech Republic, Germany, Hong Kong, and Spain. The rest of the countries have only governmental registries (Table 1).

Table 1

Current structure of foundations and charity organization (in alphabetical order) of the World Marrow Donor Association (WMDA) (July, 2021)

Country	Governmental	Foundation (Charitable)	Website of the Foundation (Charity) [reference]	N
Andorra	1	0		1
Argentina	2	0		2
Armenia	0	1 (100%)	Armenian Bone Marrow Donor Registry Charitable Trust [4]	1

Australia	2	2 (50.0%)	ABMDR is a public company limited by guarantee under the Australian Corporations Law and a registered charity under the Australian Charities and Not-for-profits Commission Act 2012 (Cth) [5]; Mater Foundation is registered as a charity with the Australian Charities and Not-for-profits Commission ABN 96723184640 [6]	4
Austria	2	2 (50.0%)	Red Cross Blood Transfusion Service of Upper Austria [7]; Geben für Leben - Leukämiehilfe Österreich [8]	4
Belgium	6	0		6
Brazil	8	0		8
Bulgaria	2	0		2
Canada	0	5	Canadian Blood Services Stem Cell Registry. Canadian Blood Services (Charitable Registration No. 870 157 641 RR0001) [9]; Canadian Blood Services' Cord Blood Bank (Charitable Registration No. 870 157 641 RR0001) [10]; Hema-Quebec Public Cord Blood BankHéma-Québec is a non-profit organization that supplies blood and other biological products of human origin to hospitals for the Canadian province of Quebec. [11]; Hema-Quebec Stem Cell Donor Registry [12]; Victoria Angel Public Cord Blood Bank [13]	5
Chile	1	1 (50.0%)	Fundación de Beneficencia Pública DKMS [14]	2
China	4	2 (33.4%)	The Data Bank of Chinese Hematopoietic Stem Cell Donors, also known as the China Marrow Donor Program (CMDP), is a non-profit organization under the umbrella of the Red Cross Society of China (RCSC). [15]; Beijing New Sunshine Charity Foundation [16]	6
Colombia	2	0		2
Croatia	2	0		2
Cyprus	2	1 (33.4%)	Karaiskakio Foundation is a non – profit organization established with the sole purpose of organizing a volunteer Bone Marrow Donor Registry. [17]	3

Czech Republic	2	1 (33.4%)	The Umbilical Cord Blood Bank of the Czech Republic was established as a non-profit donor project of the Institute of Hematology and Blood Transfusion in Prague. Its mission is to ensure the collection, processing, examination and freezing of donated umbilical cord blood. [18]	3
Denmark	2	0		2
Finland	0	2 (100%)	The Blood Service is a part of the Finnish Red Cross. We are a not-for-profit organisation, and our prime concern is what is best for the patient. [19]; Stem Cell Registry [20]	2
France	4	0		4
Germany	8	2 (20.0%)	The DKMS Stiftung Leben Spenden (Foundation for Giving Life) exclusively and directly pursues non-profit and charitable purposes. [21]; DKMS Registry gGmbH [22]	10
Greece	6	0		6
Hong Kong	2	1 (33.4%)	The Hong Kong Red Cross began its voluntary, non-remunerated blood donation programme in 1952. [23]	3
Hungary	1	0		1
India	1	7 (87.5%)	Be The Cure Registry by Jeevan Stem Cell Foundation [24]; DATRI is a Not-for-Profit organization that was founded in 2009 with a mission to save lives of those suffering from life threatening fatal blood disorders like Blood Cancer, Thalassemia, Leukaemia, Aplastic Anaemia, Sickle Cell Anaemia etc. [25]; DKMS BMST Foundation India [26]; GENEBANDHU is a not for profit Non-Governmental Organization (NGO) located in New Delhi, India. [27]; Jeevan Stem Cell Foundation [28]; The MDR(I) is India's first NGO which maintains computerised database of voluntary, -unrelated stem cell donors and facilitates blood Stem Cell transplants for patients with life-threatening blood diseases. [29]; The Arjan Vir Foundation [30]	8
Iran	5	0		5
Ireland	1	0		1
Israel	6	0		6
Italy	19	0		19
Japan	13	0	In preparation	13

Korea	3	0		3
Lithuania	0	1 (100%)	Since 2002 a non-profit, non-government organization COHP 'Kraujas' unites patients with oncohematological diseases, their relatives, medicine specialists and everyone who supports the ideas of organisation's activities, volunteering as well as bone marrow and blood donation [31]	1
Luxembourg	0	1 (100%)	The Stefan Morsch Foundation, based in Birkenfeld in Rhineland-Palatinate, is Germany's first stem cell donor center. It is named after Stefan Morsch, son of the two founders Hiltrud and Emil Morsch, who was the first European to have a stem cell transplant with a non-related donor. [32]	1
Malaysia	1	0		1
Mexico	2	3 (60%)	Be The Match®, operated by the National Marrow Donor Program® (NMDP), is a non-profit organization that's dedicated to helping every patient get the life-saving transplant they need. [33]; Mexican BMDR – DONORMO [34]; Mexican BMDR - DONORMO (CORD) – BACECU [35]	5
New Zealand	1	0	New Zealand Bone Marrow Donor Registry [36]	1
Nigeria	0	1 (100%)	The Bone Marrow Registry in Nigeria ("BMRN") is a not-for-profit organization based at the College of Medicine, University of Nigeria Teaching Hospital, Enugu. [37]	1
North Macedonia	1	1 (50%)	Scientific Foundation SPIROSKI, in preparation [38]	2
Norway	1	0		1
Paraguay	1	0		1
Peru	1	0		1
Poland	3	2 (66.7%)	Against Leukemia Foundation (ALF), Warsaw [39]; Fundacja DKMS [40]	5
Portugal	1	0		1
Qatar	1	0		1
Romania	1	0		1
Russian Federation	7	0		7
Saudi Arabia	4	0		4

Serbia	1	0		1
Singapore	2	0		2
Slovakia	1	1 (50%)	It was founded in 1997 as a civic association. Based on the permission of the Ministry of Health of the Slovak Republic, it operates laboratories authorized to process and store stem cells from umbilical cord blood, umbilical cord tissue and bone marrow. [41]	2
Slovenia	2	0		2
South Africa	1	2 (66.7%)	Registered as a non-profit company and a Section 18A Public Benefit Organisation (Reg No 2013/152553/08, 004-003 NPO), the SABMR is governed by a dedicated Board of specialist professionals. [42]; DKMS Africa is the trading name of DKMS Foundation NPC registered in South Africa. Reg. No: 2000/008979/08; VAT Reg. No: 4350236958; Non-Profit Organisation Reg. No: 020-728-NPO; Public Benefit Organisation (section 18A) Ref. No: 130000158 in Terms of Section 18A (2) of the Income Tax Act, No. 58 of 1962. [43]	3
Spain	6	2 (33.4%)	Andalucia Cord Blood Bank (Malaga). Registered at the Registro de Fundaciones Privadas de la Generalitat de Catalunya with number 424. Classified by Order of the Justice Counselor on 10th April 1989. NIF: G-58734070 [44]; Spanish Bone Marrow Donors Registry (REDMO). Registered at the Registro de Fundaciones Privadas de la Generalitat de Catalunya with number 424. Classified by Order of the Justice Counselor on 10th April 1989. NIF: G-58734070 [45]	8
Sweden	2	0		2
Switzerland	4	0		4
Taiwan	7	0		7
Thailand	1	1 (50%)	Thai National Stem Cell Donor Registry [46]	2
The Netherlands	1	1 (50%)	Matchis is a Dutch foundation that enables patients with leukaemia and other severe blood disorders to receive stem cell transplantation, by finding a matching donor as quickly as possible. [47]	2
Turkey	4	0		4

Ukraine	1	1 (50%)	Charitable Foundation "Ukrainian Register of Bone Marrow Donors" - created to help find a non-family donor for patients with leukemia and other serious blood diseases that require bone marrow transplantation. [48]	2
United Kingdom	3	3 (100%)	Anthony Nolan. Anthony Nolan is a registered charity no 803716/SC038827 and a registered company no 2379280. Registered address: Royal Free Hospital, Pond Street, Hampstead, NW3 2QG [49]; Anthony Nolan Cord Blood Bank [50]; DKMS United Kingdom [51]	6
United States of America	14	14 (100%)	Bloodworks NW ARC unit. We're an independent, non-profit organization harnessing donor gifts to provide a safe, lifesaving blood supply to 95% of hospitals in the pacific northwest. [52]; Bloodworks NW Cord Blood Services [53]; The C.W. Bill Young Department of Defense Marrow Donor Recruitment and Research Program, also known as Salute to Life [54]; Caribbean Bone Marrow Registry [55]; Supported by The Abraham J. and Phyllis Katz Foundation and Dr. Donald J. and Ruth Weber Goodman Philanthropic Fund. [56]; DKMS United States of America [57]; The Carolinas Cord Blood Bank (CCBB) [58]; ITxM Clinical Services Cord Blood Lab. The Vitalant Cord Blood Services is a not-for-profit umbilical cord blood donor bank serving Pennsylvania and New Jersey area hospitals. [59]; The J.P. McCarthy Cord Stem Cell Bank at the Karmanos Cancer Institute is a public, non-profit stem cell bank with over 1,200 umbilical cord blood units in its inventory. [60]; LifeCord/LifeSouth Community Blood Centers. Our parent organization, LifeSouth Community Blood Centers, Inc. was founded in 1974. LifeSouth is a 501(c)(3) non-profit community blood supplier for hospitals in Alabama, Florida and Georgia. [61]; New York Blood Center (NYBC) is one of the largest independent, community-based, non-profit blood centers in the United States. [62]; NMDP-National Marrow Donor Program/Be The Match [63]; San Diego Blood Bank is an independent, 501(c)(3) non-profit that serves hospitals in San Diego, Orange, Imperial and Los Angeles counties with blood transfusion products and reference laboratory services. [64]; St. Louis Cord Blood Bank [65]	28
Uruguay	1	0		1
Vietnam	1	0		1
WMDA	140	56		196

The total number of non-governmental registries included in the World Marrow Donor Association is 56 (29%) and the other 140 (71%) registries are governmental (Figure 1).

There are two functional marrow donor registries included in World Marrow Donor Registries (WMDA) from the Western Balkans: MBMDR - Macedonian Bone Marrow Donor Registry from North Macedonia and the Serbian Bone Marrow Donor Registry from Serbia. There is one more marrow donor registry in preparation from Scientific Foundation SPIROSKI as a part of the non-governmental sector in North Macedonia. Currently, there are no marrow donor registries in Albania, Bosnia and Herzegovina, Montenegro, or Kosovo (Table 2).

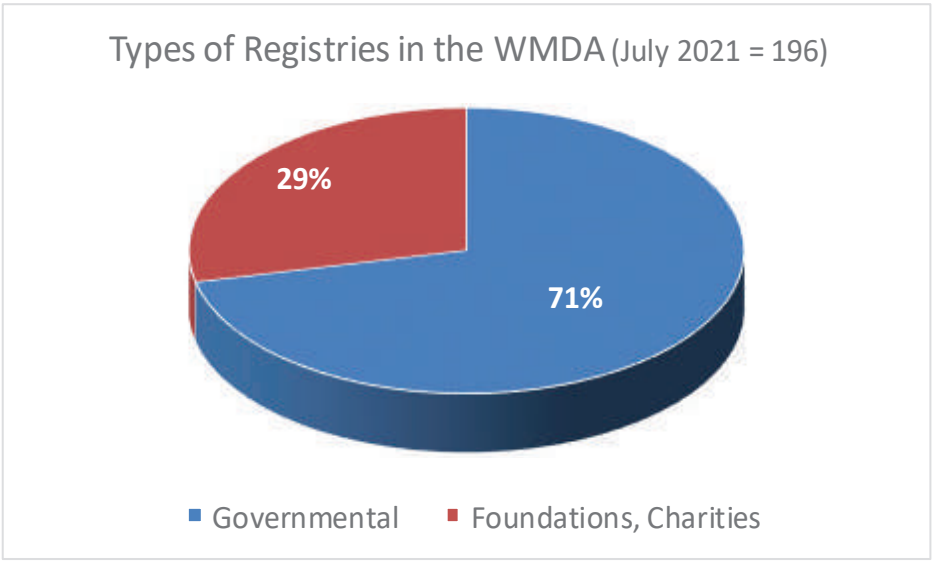


Figure 1 – Organizational structure of the marrow donor registries included in the World Marrow Donor Association (WMDA) (July 2021). From the total of 196 registries (71%), 56 registries are in the group of foundations and/or charities (29%).

The total number of marrow donors in MBMDR - Macedonian Bone Marrow Donor Registry is 2349 with 99.5% ABDR typed samples. In the Serbian Bone Marrow Donor Registry there are 6923 marrow donors with 99% ABDR typed samples (Table 2).

Table 2

Marrow Donor Registries in the Western Balkan countries (in alphabetical order) included in the World Marrow Donor Registries (WMDA), July 2021

Western Balkan Country	Marrow Donor Organization	WO	ION	Total	ABDR	ABDR% Typed	DNA Class I	DNA Class II
Albania	No							
Bosnia and Herzegovina	No							
Kosovo	No							
Montenegro	No							
North Macedonia	MBMDR - Macedonian Bone Marrow Donor Registry	1252	4307	2349	2338	99.5	2349	2344
	Scientific Foundation SPIROSKI – Marrow Donor Registry, in preparation	2117						
Serbia	Serbian Bone Marrow Donor Registry	1078	4650	6923	6852	99	6923	6854

Discussion

In this paper we present the current status of non-governmental vs. governmental marrow donor registries in the World Marrow Donor Association (WMDA). Most of the registries are governmental (71%), but a significant number of registries are found in the non-governmental sector (29%). It should be mentioned that the WMDA is a non-governmental organization and, as one of the founding members of the Worldwide Network for Blood and Marrow Transplantation (WBMT), aims to promote excellence in stem cell transplantation, donation, and cellular therapy.

The Macedonian Donor Registry Foundation (MKDR) was established on 03.02.2002 as part of the ICGEB project (CRP/MAC03-01) "Ambiguities resolution of HLA genotypes in Macedonian population" [66]. The

Macedonian Bone Marrow Donor Registry (MBMDR) was jointly established on February 28, 2013 by the Institute of Immunobiology and Human Genetics (IIBHG) and the Macedonian Donor Registry foundation as a combination of a governmental institution and a non-governmental foundation. The division of activities was such that blood was taken by the IIBHG (DNA was isolated and HLA-DNA genotyped), and the MKDR foundation created the Marrow Donor Registry, organized lectures, organized donation activities, and established international communications. In September 2013, the functions performed by MKDR foundation were undertaken by the IIBHG and, to date, the registry functions only as a governmental bone marrow donation registry of IIBHG, without cooperation with the MKDR Foundation [67].

On July 30, 2018, the Macedonian Donor Registry Foundation (MKDR) expanded its activities and changed its name to the Scientific Foundation SPIROSKI. Most of the work of the foundation involves publishing scientific journals, the most famous and extensive of which is the scientific journal *Open Access Macedonian Journal of Medical Sciences* [68]. Following the name change, activities began in order to establish a non-governmental Marrow Donor Registry (in addition to the existing state Bone Marrow Donor Registry of the IIBHG) as an additional potential for treating patients with blood cancer with marrow transplantations.

The Serbian Bone Marrow Donor Registry was created on January 31, 2012, as governmental institution at the Blood Transfusion Institute of Serbia in Belgrade, Serbia. The Blood Transfusion Institute of Serbia (BTIS) was founded on the 24th of October, 1944. BTIS started as a Federal military establishment on the 24th of October 1944, and later became a Federal Civilian institution in October 1945, and in March 1946, it became a civilian institution of the Republic of Serbia. The HLA Tissue Typing Laboratory was founded in 1972 and the PCR Laboratory was founded within the HLA Tissue Typing Department in 2003. The current medical director of BTIS is Glorija Miletic. The organisation's top administrator, CEO/director regular or provisional member organization, and WSMS Registry user is Zorana Andric [68].

The absence of marrow donor registries in the most of the Western Balkan countries (Albania, Bosnia and Herzegovina, Kosovo, and Montenegro) is a consequence of low development and has hindered many

patients who need stem cell transplantation to be cured. There are several alternatives that could be used to include citizens from these countries so that they will be included in the World Marrow Donor Association. The best approach is to create marrow donor registries in countries, which is a slow and expensive process, but including citizens in the neighboring countries could be a more efficient approach.

In the last 5 years, the number of registered potential hematopoietic stem cells donors in Poland increased by more than 4 times, from about 146,000 to over 750,000. During the same period, the number of patients qualified for hematopoietic stem cell transplantation from unrelated donors increased from 557 in 2010 to 817 in 2014. A striking change in the percentage of transplantations performed in Polish centers was observed. The material that was collected from national donors jumped from 24% to 60%. This shift was also evident in the number of search procedures closed with acceptance of Polish donors – from 27% in 2010 to 58% in 2014. Another consequence of the Polish registry growth is the increasing number of donations from Polish donors for international patients. Between 2010 and 2014, the percent of donations for non-national patients increased from 33% to 76%, placing Poland in 6th place in the ranking of the HSC "exporters" worldwide [69].

Authorities involved with an HCT donor registry establishment will have to balance the advantages and costs of such a project and accommodate the emerging alternatives, such as cord blood or related haploidentical transplants. Miscalculations and an incomplete understanding of the various aspects of the process can have a tremendous impact on the optimization of a HCT donor registry, especially in developing countries [70].

The main limitation of this study is the classification of the marrow donor registries. We suppose an underestimated number of non-governmental organizations in this paper, and we expect more organizations to be included going forward.

We can conclude that in most of the Western Balkan countries (Albania, Bosnia and Herzegovina, Kosovo, and Montenegro) there are no marrow donor registries and only in North Macedonia and Serbia there exist governmental marrow donor registries. There is a great need to develop more non-governmental and governmental marrow donor registries in the Western Balkan countries.

Acknowledgements

This study was partially supported by the project "SFS – Marrow Donor Promotion Program" Support for Actions – 2021, Project number: 21 11 PCM 202, Civica Mobilitas, Skopje, North Macedonia.

REFERENCES

1. Cellab.com. 2021. [online] Available at: <https://www.cellab.com/> [Accessed 9 July 2021].
2. Europarl.europa.eu. 2021. The Western Balkans | Fact Sheets on the European Union | European Parliament. [online] Available at: <https://www.europarl.europa.eu/factsheets/en/sheet/168/the-western-balkans> [Accessed 9 July 2021].
3. Share.wmda.info. 2021. Database - Organisation data (Public Access) - Share. [online] Available at: <https://share.wmda.info/display/WMDAREG/Database> [Accessed 9 July 2021].
4. Abmdr.am. 2021. ABMDR: Armenian Bone Marrow Donor Registry | Armenian Bone Marrow Donor Registry. [online] Available at: <https://www.abmdr.am/> [Accessed 9 July 2021].
5. ABMDR. 2021. About us | ABMDR. [online] Available at: <https://www.abmdr.org.au/abmdr/organisation/> [Accessed 9 July 2021].
6. Mater.org.au. 2021. Group. [online] Available at: <https://www.mater.org.au/> [Accessed 9 July 2021].
7. Roteskreuz.at. 2021. Das Österreichische Rote Kreuz. [online] Available at: <https://www.roteskreuz.at/home> [Accessed 9 July 2021].
8. Österreich, V., 2021. Geben für Leben - Nachrichten. [online] Gebenfuerleben.at. Available at: <https://www.gebenfuerleben.at/> [Accessed 9 July 2021].
9. Blood.ca. 2021. Canadian Blood Services Stem Cells for Life. [online] Available at: <https://blood.ca/en/stem-cells> [Accessed 9 July 2021].
10. Blood.ca. 2021. How donating cord blood helps. [online] Available at: <http://www.blood.ca/cordblood> [Accessed 9 July 2021].
11. Hema-Quebec. 2021. Accueil Héma-Québec. [online] Available at: <http://www.hema-quebec.qc.ca> [Accessed 9 July 2021].
12. Hema-Quebec. 2021. Homepage. [online] Available at: <http://www.hema-quebec.qc.ca/index.en.html> [Accessed 9 July 2021].

13. Victoria Angel. 2021. Home - Victoria Angel. [online] Available at: <https://victoriaangel.org/> [Accessed 9 July 2021].
14. Professional.dkms.org. 2021. [online] Available at: <https://professional.dkms.org/> [Accessed 9 July 2021].
15. Cmdp.org.cn. 2021. 中华骨髓库. [online] Available at: <http://www.cmdp.org.cn/> [Accessed 9 July 2021].
16. 2021. [online] Available at: <https://www.isun.org/> [Accessed 9 July 2021].
17. Karaiskakeio. 2021. HOME - Karaiskakeio. [online] Available at: <https://karaiskakio.org.cy/en/> [Accessed 9 July 2021].
18. inclusive, N., 2021. Banka pupečníkové krve České republiky. [online] Bpk.cz. Available at: <http://www.bpk.cz/> [Accessed 9 July 2021].
19. Bloodservice.fi. 2021. www.veripalvelu.fi. [online] Available at: <http://www.bloodservice.fi/> [Accessed 9 July 2021].
20. Bloodservice.fi. 2021. www.veripalvelu.fi. [online] Available at: <https://www.bloodservice.fi/stem-cell-registry> [Accessed 9 July 2021].
21. Professional.dkms.org. 2021. [online] Available at: <https://professional.dkms.org/> [Accessed 9 July 2021].
22. Dkmsregistry.org. 2021. [online] Available at: <https://dkmsregistry.org/> [Accessed 9 July 2021].
23. Www5.ha.org.hk. 2021. 香港紅十字會輸血服務中心. [online] Available at: <https://www5.ha.org.hk/rcbts> [Accessed 9 July 2021].
24. Jeevan.org. 2021. Jeevan | Cord Blood Donation FAQ's. [online] Available at: <http://www.jeevan.org/stemcell/> [Accessed 9 July 2021].
25. DATRI - India's Largest Blood Stem Cell Donors Registry. 2021. Blood Cancer is Curable. Be the Cure. Sign in Now. [online] Available at: <https://datri.org/> [Accessed 9 July 2021].
26. Professional.dkms.org. 2021. [online] Available at: <https://professional.dkms.org/> [Accessed 9 July 2021].
27. Dev.genebandhu.in. 2021. My CMS. [online] Available at: <http://dev.genebandhu.in/wordpress/> [Accessed 9 July 2021].
28. Jeevan.org. 2021. Jeevan | Cord Blood Donation FAQ's. [online] Available at: <http://www.jeevan.org/stemcell/> [Accessed 9 July 2021].
29. Mdrindia.org. 2021. Home. [online] Available at: <http://mdrindia.org/index.htm> [Accessed 9 July 2021].
30. Arjanvirfoundation.org. 2021. AVSF. [online] Available at: <https://www.arjanvirfoundation.org/> [Accessed 9 July 2021].
31. Bukgeras.lt. 2021. Būk geras. [online] Available at: <https://bukgeras.lt/> [Accessed 9 July 2021].

32. Stefan Morsch Stiftung. 2021. Die Stefan-Morsch-Stiftung: gemeinsam gegen Blutkrebs. [online] Available at: <https://stefan-morsch-stiftung.com/> [Accessed 9 July 2021].
33. Bethematch.org. 2021. Donate Marrow or Blood Stem Cells | Be The Match. [online] Available at: <https://bethematch.org/> [Accessed 9 July 2021].
34. Fundación Comparte Vida. 2021. Dona Médula - Fundación Comparte Vida. [online] Available at: <https://fundacioncompartevida.org.mx/dona-medula/> [Accessed 9 July 2021].
35. Fundación Comparte Vida. 2021. Dona Cordón - Fundación Comparte Vida. [online] Available at: <https://fundacioncompartevida.org.mx/dona-cordon/> [Accessed 9 July 2021].
36. Bonemarrow.org.nz. 2021. NZBMDR | New Zealand Bone Marrow Donor Registry | Information and resources for donors, patients and physicians about bone marrow donation and stem cell transplants, NZ Bone Marrow Donor Registry. [online] Available at: <https://www.bonemarrow.org.nz/> [Accessed 9 July 2021].
37. Bonemarrownigeria.org. 2021. | Bone Marrow Registry in Nigeria. [online] Available at: <http://www.bonemarrownigeria.org/> [Accessed 9 July 2021].
38. Sfsmdr.eu. 2021. SFSMDR.eu. [online] Available at: <https://www.sfsmdr.eu/> [Accessed 9 July 2021].
39. Leukemia.pl. 2021. AfterMarket.pl :: domena leukemia.pl. [online] Available at: <http://www.leukemia.pl/> [Accessed 9 July 2021].
40. Professional.dkms.org. 2021. [online] Available at: <https://professional.dkms.org/> [Accessed 9 July 2021].
41. Eurocord-Slovakia. 2021. Domov - Eurocord-Slovakia. [online] Available at: <https://eurocord.sk/> [Accessed 9 July 2021].
42. 8638, C., 2021. South African Bone Marrow Registry -. [online] South African Bone Marrow Registry. Available at: <https://sabmr.co.za/> [Accessed 9 July 2021].
43. DKMS Africa. 2021. The Sunflower Fund | non-profit organisation | stem cell donors | blood disease. [online] Available at: <https://dkms-africa.org/> [Accessed 9 July 2021].
44. José Carreras Leukaemia Foundation. 2021. Umbilical Cord Blood Donation. [online] Available at: <https://www.fcarreras.org/en/scu> [Accessed 9 July 2021].
45. José Carreras Leukaemia Foundation. 2021. Spanish Bone Marrow Donors Registry (REDMO). [online] Available at: <https://www.fcarreras.org/en/redmo> [Accessed 9 July 2021].

46. Stemcellthairedcross.nbc.in.th. 2021. Home – Thai National Stem Cell Donor Registry. [online] Available at: <https://stemcellthairedcross.nbc.in.th/en/home/> [Accessed 9 July 2021].
47. Stichting Matchis (English). 2021. Home- Are you the perfect match?. [online] Available at: <https://www.matchis.nl/en/> [Accessed 9 July 2021].
48. Ubmdr.org. 2021. УКРАЇНСЬКИЙ РЕЄСТР ДОНОРІВ КІСТКОВОГО МОЗКУ. [online] Available at: <https://www.ubmdr.org/> [Accessed 9 July 2021].
49. Anthonynolan.org. 2021. Home | Anthony Nolan. [online] Available at: <https://www.anthonynolan.org/> [Accessed 9 July 2021].
50. Anthonynolan.org. 2021. People | Anthony Nolan. [online] Available at: <https://www.anthonynolan.org/clinicians-and-researchers/services-transplant-centres/people> [Accessed 9 July 2021].
51. Professional.dkms.org. 2021. [online] Available at: <https://professional.dkms.org/> [Accessed 9 July 2021].
52. Bloodworks Northwest. 2021. Bloodworks Northwest | Donate Blood. [online] Available at: <https://www.bloodworksnw.org/> [Accessed 9 July 2021].
53. Bloodworks Northwest. 2021. Bloodworks Northwest | Donate Blood. [online] Available at: <http://www.bloodworksnw.org/> [Accessed 9 July 2021].
54. Salute to Life. 2021. Salute to Life. [online] Available at: <https://www.salutetolife.org/> [Accessed 9 July 2021].
55. Caribmarrowreg.com. 2021. Caribbean Bone Marrow Registry. [online] Available at: <http://caribmarrowreg.com/> [Accessed 9 July 2021].
56. Cleveland Cord Blood. 2021. Home. [online] Available at: <https://clevelandcordblood.org/> [Accessed 9 July 2021].
57. Dkms.org. 2021. [online] Available at: <http://dkms.org/> [Accessed 9 July 2021].
58. Sites.duke.edu. 2021. Carolinas Cord Blood Bank – A Not-for-Profit Public Cord Blood Bank. [online] Available at: <https://sites.duke.edu/ccbb/> [Accessed 9 July 2021].
59. Givecord.org. 2021. Biotherapies Lab. [online] Available at: <http://www.givecord.org/> [Accessed 9 July 2021].
60. Karmanos.org. 2021. Cord Blood Program. [online] Available at: <https://www.karmanos.org/karmanos/cord-blood-program> [Accessed 9 July 2021].

61. LifeSouth Cord Blood Bank. 2021. LifeSouth Cord Blood Bank. [online] Available at:
<http://www.lifesouthcord.org/> [Accessed 9 July 2021].
62. Nybloodcenter.org. 2021. Home | New York Blood Center. [online] Available at:
<https://nybloodcenter.org/> [Accessed 9 July 2021].
63. Bethematch.org. 2021. Donate Marrow or Blood Stem Cells | Be The Match. [online] Available at:
<https://bethematch.org/> [Accessed 9 July 2021].
64. San Diego Blood Bank. 2021. San Diego Blood Bank. [online] Available at:
<http://www.sandiegobloodbank.org/> [Accessed 9 July 2021].
65. 2021. [online] Available at:
<http://www.iibhg.ukim.edu.mk/mkbmdr/> [Accessed 9 July 2021].
66. Spiroski M, Petlichkovski A, Strezova A. Ambiguities Resolution of HLA Genotypes in Macedonian Population. Macedonian Journal of Medical Sciences. 2008 Dec 15; 1(2).
67. 2021. [online] Available at:
<http://www.iibhg.ukim.edu.mk/mkbmdr/> [Accessed 9 July 2021].
68. Nbti.org.rs. 2021. History - About us. [online] Available at:
<https://nbti.org.rs/NBTI/1325/History.shtml> [Accessed 9 July 2021].
69. Filipiak J, Dudkiewicz M, Czerwiński J, Kosmala K, Łęczycka A, Malanowski P, Żalikowska-Hołoweńko J, Małkowski P, Danielewicz R. Organization and development of bone marrow donation and transplantation in Poland. Annals of transplantation. 2015 Oct 1; 20: 588-95.
70. Aljurf M, Weisdorf D, Alfraih F, Szer J, Müller C, Confer D, Hashmi S, Kröger N, Shaw BE, Greinix H, Kharfan-Dabaja MA. Worldwide Network for Blood & Marrow Transplantation (WBMT) special article, challenges facing emerging alternate donor registries. Bone marrow transplantation. 2019 Aug; 54(8): 1179-88.

ПОГЛАВЈЕ IV

СОВРЕМЕНИ МЕТОДИ ВО УРОЛОШКАТАХИРУРГИЈА И МЕДИЦИНАТА

doi: 10.20903/zpopov-2022-0018 UDK: 616.6-089.844:616.311(497.311)"200/2021"

Prof. Dr. Chavdar SLAVOV[1], MD, Academician of BAS

ORAL MUCOSA AS GRAFT MATERIAL FOR THE PURPOSES OF RECONSTRUCTIVE UROLOGY

Abstract

Introduction: Despite the significant technological advances in recent years, numerous challenges still face the field of reconstructive urology. One of the main issues is the lack of universal graft material.

Aim: The present study aims to conduct a retrospective analysis of the functional results following the use of free mucosal graft harvested from the oral cavity.

Material and methods: For a period of twenty years (2000-2021) a total of 521 cases of reconstructive surgery with the use of oral mucosa were performed. They are divided into 5 main groups, based on the organ involved:

I. Urethroplasty in urethral strictures – 427 (82%)

II. Urethroplasty in complicated (crippled) hypospadias - 70 (13.4%)

III. Ureteroplasty in ureteral stricture - 1 (0.2%)

IV. Substitute corporoplasty for Peyronie's disease - 15 (2.8%)

V. Organ - preserving operations in carcinoma of the penis – 8 (1.5%)

Results: Depending on the source of the substitute material harvested:

1. Buccal mucosa (BMG) - 368 (70.6%)
2. Lingual mucosa (LMG) - 135 (25.9%)
3. Lower lip mucosa- 18 (3.5%)

No major complications resulting from graft harvesting were observed. The main type of operations were:

[1] Clinic of Urology and Andrology, University Hospital "Queen Joanna - ISUL", Medical University, Sofia; Department of Medical Sciences - Bulgarian Academy of Sciences, Sofia, Bulgaria

- one-act operative techniques 460 (88.3%)
- two-act operations 38 (7.3%)
- operations on the penis 23 (4.3%)

Functional results were reported according to the type of operation. In the largest group – urethroplasty, a success rate of 84.29% was achieved.

Conclusion: Currently, reconstructive surgery of the urinary tract and penis using free oral mucosa graft is the most widely used surgical technique in modern urology. This operative technique requires strict patient selection and substantial professional experience.

Keywords: oral mucosa graft (BMG, LMG), urethroplasty, ureteroplasty, corporoplasty, organ-sparing operations for penile cancer

Despite the significant technological advances in recent years, numerous challenges still remain in the field of reconstructive urology. One of the main issues is the lack of universal graft material. Reconstructive surgeries involving the male urethra (the majority of cases), ureter, and penis require the development of easily accessible and adaptive graft material. In the last 20-25 years, free tissue grafts from oral mucosa have been established as such [1, 2, 3, 4, 5, 6]. Buccal mucosa has become the gold standard for reconstructive urethroplasty [7, 8]. An increasing number of urological centers around the world are adopting this approach, which is facilitated by regular specialized sessions at European and World Forums, as well as annual independent congresses on this topic. Many retrospective, extended, long-term analyses and articles on the topic appearing in specialized literature support the effectiveness of this method [9, 10, 11, 12]. Our experience in this field spans over 25 years, allowing us to draw reliable scientific and practical conclusions [13, 14, 15, 16, 17, 18].

In this article, we present a retrospective study of the use of oral mucosa as graft material in reconstructive urology.

Material and methods.

For a period of twenty years (2000-2021) a total of 521 cases of reconstructive surgery with the use of oral mucosa were performed.

They are divided into 5 main groups, based on the organ involved:

I. Urethroplasty in urethral strictures – 427(82%).
II. Urethroplasty in complicated (crippled) hypospadias - 70 (13.4%).
III. Ureteroplasty in ureteral stricture - 1 (0.2%).
IV. Substitute corporoplasty for Peyronie's disease -15 (2.8%).
V. Organ - preserving operations in penile cancer – 8 (1.5%).

Depending on the source of the graft material:
I. Buccal mucosa (BMG) - 368 (70.6%).
II. Lingual mucosa (LMG) - 135 (25.9%)
III. Mucosa of the lower lip - 18 (3.5%).

The specific technique of sourcing each graft is described in the respective chapters. (Fig 9, Fig 10)

According to the type of operative technique, the cases can be divided into:
I. One-stage techniques - 460 (88.3%).
1. Urethroplasty in urethral strictures.
1.1. Onlay augmentation techniques.
1.1.1. Dorsal onlay technique - 262 (59.9%). (Fig 1)
1.1.2. Ventral onlay technique - 58 (12.6%).(Fig 2)
1.1.3. Combined onlay-inlay technique - 8 (1.7%).
1.2. Augmentation-anastomotic urethroplasty - 97 (21.1%). (Fig 4)
1.3. One-stage operations in crippled hypospadias - 38 (8.3%). (Fig 3)

II. Two-stage operative techniques - 38 (7.3%).
1. Local fixation of the graft - 12 (31.5%)
2. Perineal fixation of the graft - 7 (18.4%).(Fig 5)
3. Two-stage operations in crippled hypospadias - 16 (42.1%).

III. Operations involving other localizations - 4.6%
1. Ureteroplasty - 1 (0.3%). (Fig 8)
2. Replacement corporoplasty - 15 (2.8%). (Fig 6)
3. Organ-sparing operations for penile cancer - 8 (1.5.5). (Fig 8)

The following methods were used in the retrospective analysis:

1. Diagnostic methods.

Along with the generally accepted methods, anamnesis, physical examination, interventional ultrasound, specialized imaging, and examination methods were used: retrograde and voiding cystourethrography; cavernosography; CT- urography; MRI; uroflowmetry.

2. Surgical methods - the basic operating techniqu s used are described and illustrated briefly:

2.1. Surgical techniques for urethral strictures.

2.1.1. Ventral onlay urethroplasty. (Fig 2)

2.1.2. Dorsal onlay urethroplasty. (Fig 1)

- graft preparation (Fig 9, Fig 10)
- urethroplasty

2.1.3. Augmentation-anastomotic urethroplasty. (Fig 4)

2.2. Combined urethroplasty in crippled hypospadias (Fig 3)

2.3. Reconstructive operations in cases of absent part of the urethra. (Fig 5)

2.4. Reconstructive surgery for Peyronie's disease (Fig 6)
(replacement corporoplasty)

2.5. Organ - preserving operations in localized penile cancer (Fig 7)

2.6. Reconstructive operations for ureteral stricture. (Fig 8)

3. Methods for evaluation of therapeutic results:

3.1. Methods for assessing the patency of the urethra (ureter) and the degree of urine flow:

3.1.1. Uroflowmetry.

3.1.2. Retrograde and voiding cystourethrography.

3.1.3. CT urography.

3.1.4. MRI

3.2. Methods for assessing erectile function after reconstructive surgery of the male urethra and penis.

We used a modified questionnaire [19] for erectile function after reconstructive surgery, as well as some physical tests - papaverine test; Morales test; Doppler of blood vessels.

Dorsal onlay urethroplasty

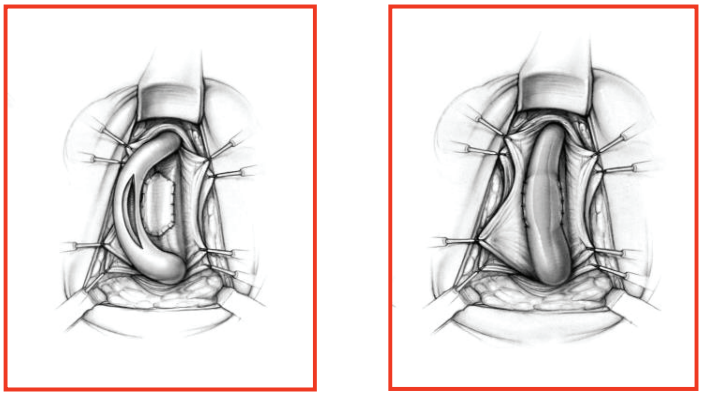


Fig 1.1 – Schematic drawing of dorsal onlay urethroplasty A. Liberation of the bulbar urethra, its` rotation and dorsal longitudinal incision of the stricture until reaching healthy tissue, fixation of the graft on the ventral surface of corpora cavernosa B. Fixation of the graft along the incised strictured segment and reconstitution of the normal anatomical position of corpus spongiosum

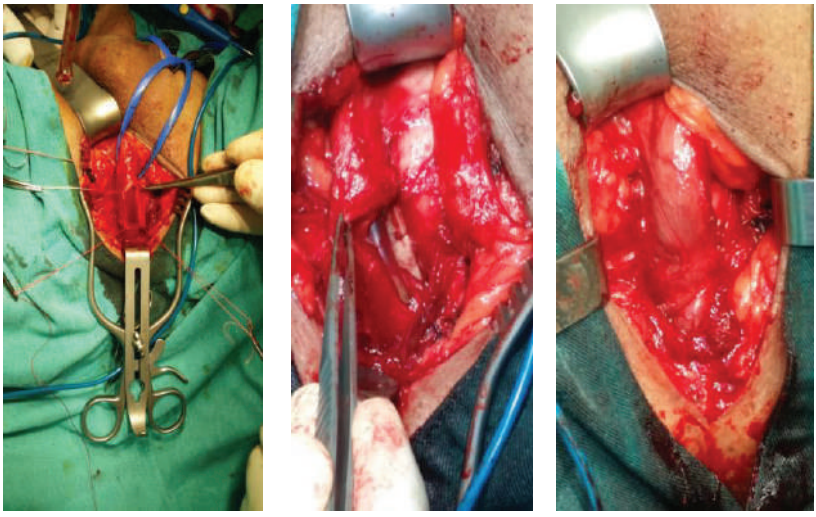


Fig 1.2 – Intraoperative images of ventral onlay urethroplasty A.Liberation of the bulbar urethra, its` rotation and dorsal longitudinal incision of the stricture until reaching healthy tissue. B fixation of the graft on the ventral surface of corpora cavernosa C. Fixation of the graft along the incised strictured segment and reconstitution of the normal anatomical position of corpus spongiosum

Ventral onlay urethroplasty

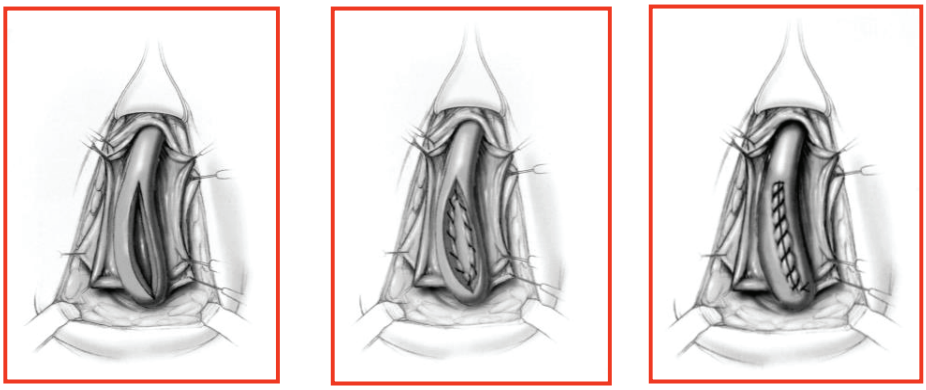


Fig 2.1 – Schematic drawing of ventral onlay urethroplasty A.Ventral longitudinal incision of the stricture until reaching healthy tissue. B. Ventral fixation of oral mucosa graft C. Second layer of the urethroplasty from corpus spongiosum

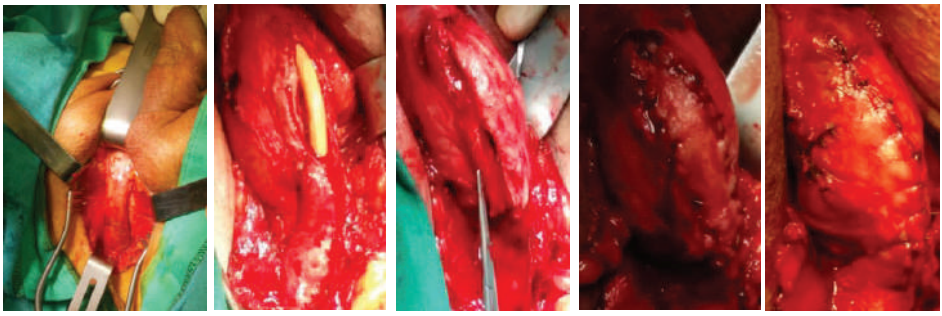


Fig 2.2 – Intraoperative images of ventral onlay urethroplasty A.Transperineal approach to the stricture. B. Ventral longitudinal incision of the stricture until reaching healthy tissue and assessment of the length of the needed graft. C. Ventral fixation of oral mucosa graft – initial sutures in the proximal and dorsal ends of the graft. D. Ventral fixation of oral mucosa graft – fixation with interrupted sutures along the circumference of the graft E. Second layer of the urethroplasty from corpus spongiosum

Combined urethroplasty

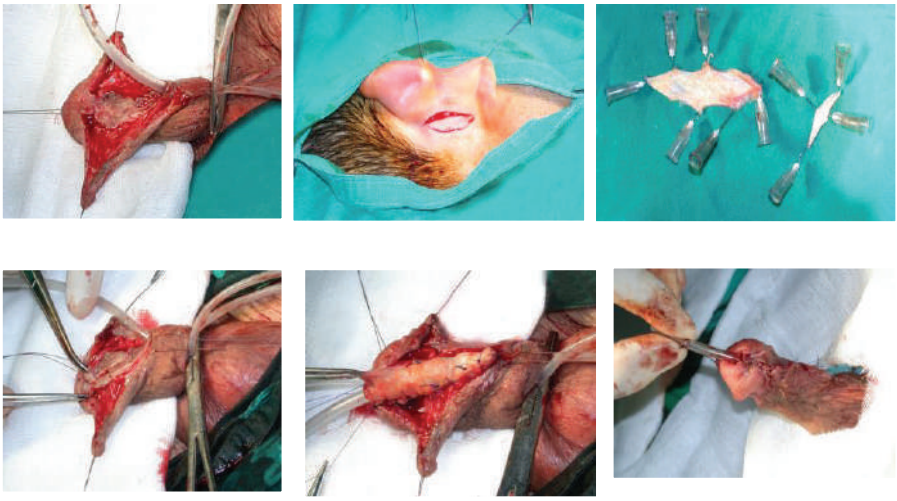


Fig 3 – Intraoperative images of combined urethroplasty A. Initial incision and preparation of the urethral plate in case of crippled hypospadia B.Harvesting of graft of retroauricular skin C. grafts on completion D. incision of the urethral plate –type Snodgrass (TIP, "Snodgraft" with inlay retroauricular skin graft)
E.Onlay fixation of BMG F. Complete reconstruction

Anastomotic-augmentation urethroplasty

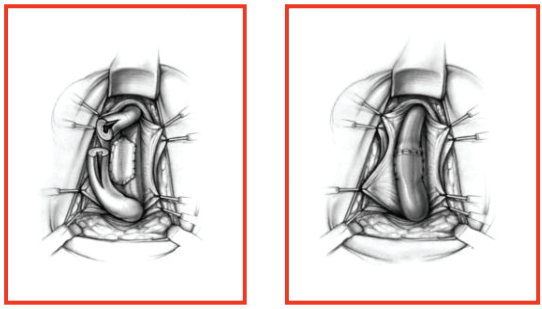


Fig 4.1 – Schematic drawing of augmentation urethroplasty A. Complete transection of the urethra in the strictured segment with eventual excision of obliterated part of the urethra and spatulating of theproximal and distal ends, followed by fixation of the graft on the ventral surface of corpora cavernosa B. Completion of the urethroplasty with both anastomotic and augmentation suture lines

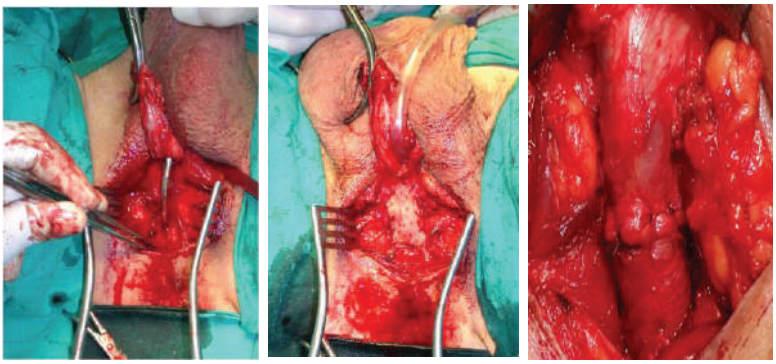


Fig 4.2 – Intraoperative images of ventral onlay urethroplasty A. Complete transection of the urethra in the strictured segment with eventual excision of obliterated part of the urethra and spatulating of theproximal and distal ends B. fixation of the graft on the ventral surface of corpora cavernosa C. Completion of the urethroplasty with both anastomotic and augmentation suture lines

Reconstructive surgery in long missing part of the urethra

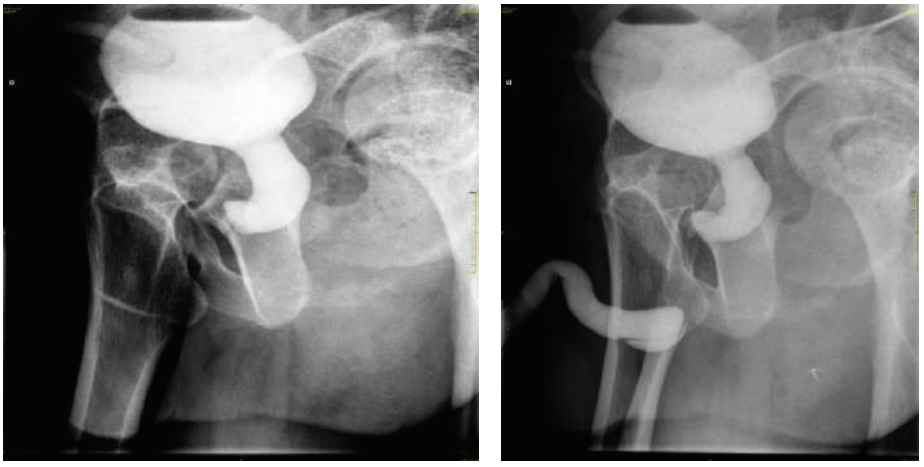


Fig 5.1 – preoperative urethrography in case of missing part of the urethra A. Antegrade cystography through previously fixed cystostomy tube, delineating the proximal end of the defect B.Simultaneous retrograde urethrography, delineating distal end of the defect and its overall lenght

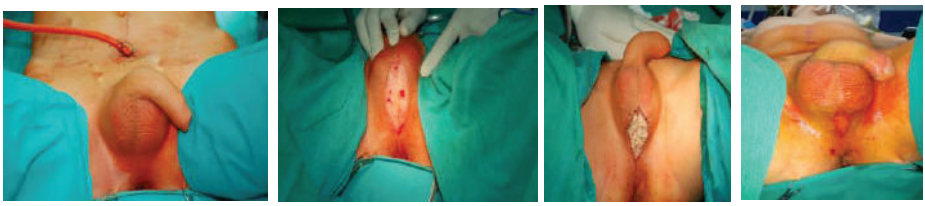


Fig 5.2 – First stage of the urethroplasty in case of missing part of the urethra A.Previously fixed cystotomy tube B.transperineal approach to the defect уретрата C. fixation of the graft along the axis of the missing segment of the urethra D. Graft appearance after 6 months with good vascularization

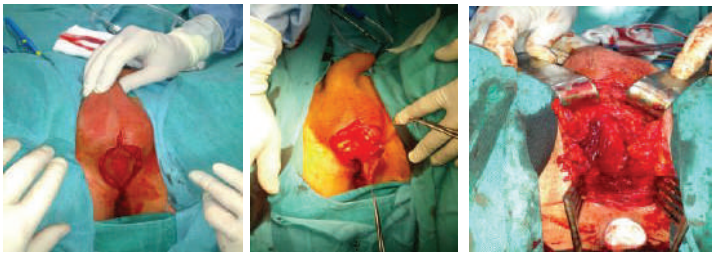


Fig 5.3 – Second stage of the urethroplasty in case of missing part of the urethra A. mobilization of the BMG B.Formation of a perineal based flap C.reconstruciton of the missing part of the urethra through tubularization of the flap

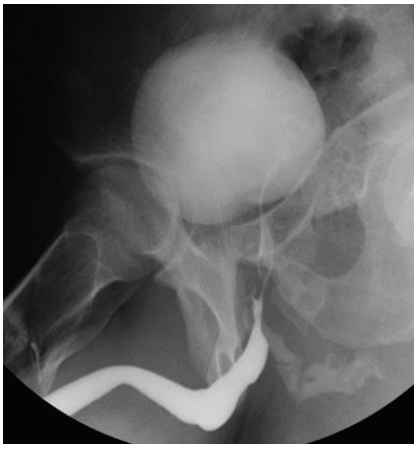


Fig 5.4 – Urethrography in case of missing part of the urethra 6 months after second stage – full patency of the reconstructed segment

Reconstructive surgery in Peyronie`s disease

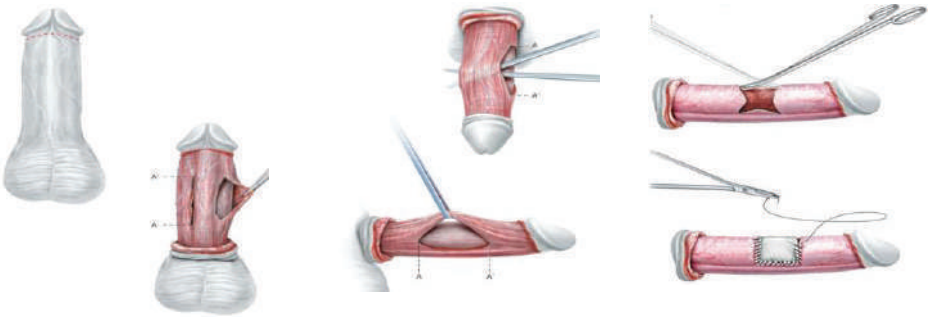


Fig 6.1 – Schematic drawing of plaque excision and corporoplasty with oral mucosa in Peyronie`s disease A.Degloving of the penis in incision of Buck`s fascia B. Dissection of dorsal neuro-vascular bundle of the penis C.excision of the plaque and covering of the defect with BMG

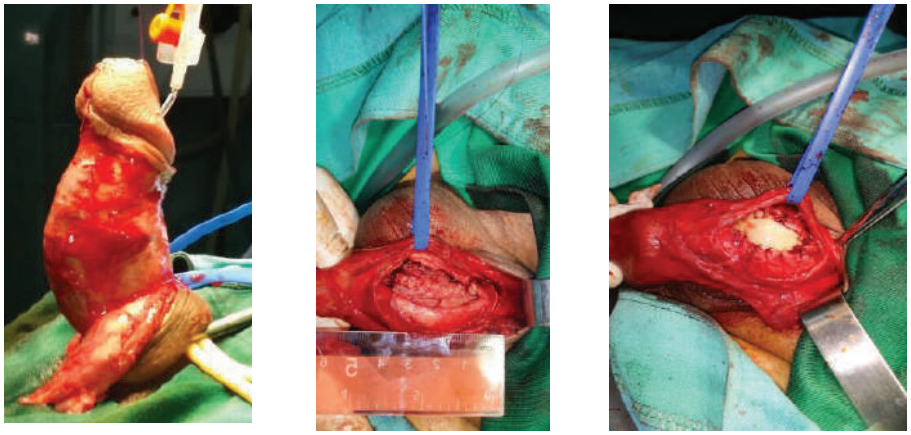


Fig 6.2 – intraoperative images of reconstructive surgery in Induration penis plastica A.degloving of the penis and artificial erection for assessment of the plaque and curvature degree B. Dissection of dorsal neuro-vascular bundle of the penis and excision of the plaque C. covering of the defect in tunica albuginea with BMG

Organ preserving surgery in penile cancer/melanoma

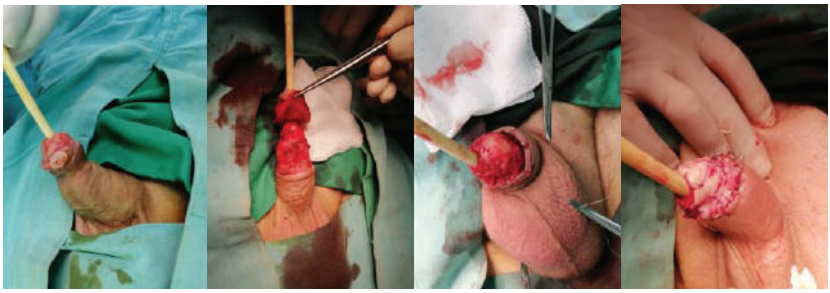


Fig 7 – Organ preserving surgery in penile cancer A. penile cancer near sulcus glandis penis B. Glansectomy along Buck`s fascia C.formation of neo-glans from the tips of corpora cavernosa D. Covering of the defect with BMG

Ureteroplasty

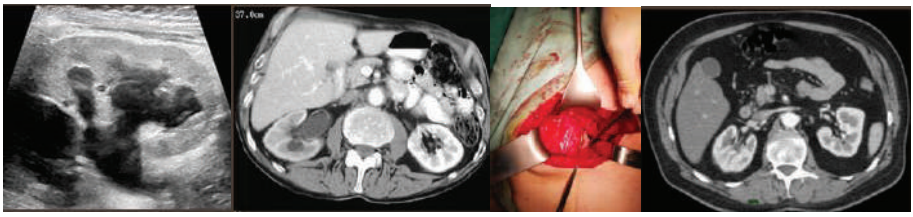


Fig 8 – Ureteroplasty in case of recurrent ureteral stricture – A. preoperative US and B. CT-KUB of hydronephrosis in a case of recurrent iatrogenic ureteral stricture C. Onlay fixation of BMG along DJ-stent protection in the strictured area D. postoperative CT-KUB one month after DJ extraction with absence of hydronephrosis

Oral mucosa graft harvesting

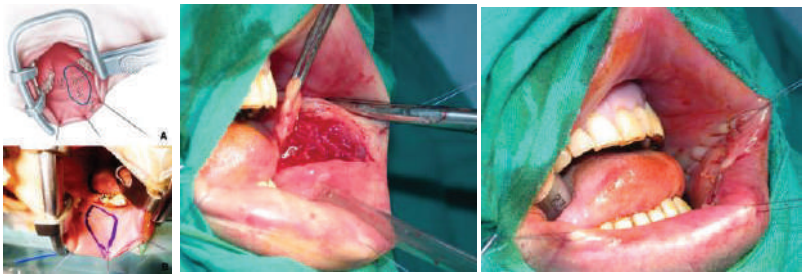


Fig 9 – Buccal mucosa graft harvesting – A. Schematic drawing B. Intraoperative marking of the graft. C Excision of the graft. D. Restoration of the graft site

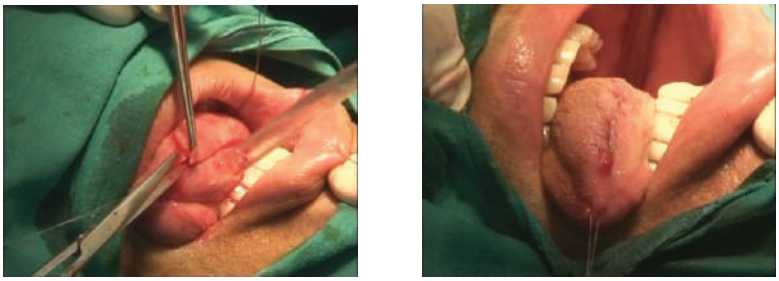


Fig 10. Lingual mucosa graft harvesting – A. Intraoperative marking and excision of the graft onto dorso-lateral surface of the tongue B. Restoration of the graft site

Operative technique

The Kilner-Doughty or other type of mouth retractor is used or stay sutures; three of which are placed along the edge of the mouth to stretch the oral mucosaThe Stensen duct is mandatory to be identified in proximity of the second molar Solution, containing local anesthetic (Lidocain, Bipuvacain) and Adrenaline 1/20.000, is injected at the donor site to achieve hemostasis and hydro-dissection. The dissection plane of the graft is between the mucosa and the muscle. The donor site is closed with running 5-0 resorbable sutures.

Results and discussion.

Figure 11 shows the age distribution of patients with urethral strictures:

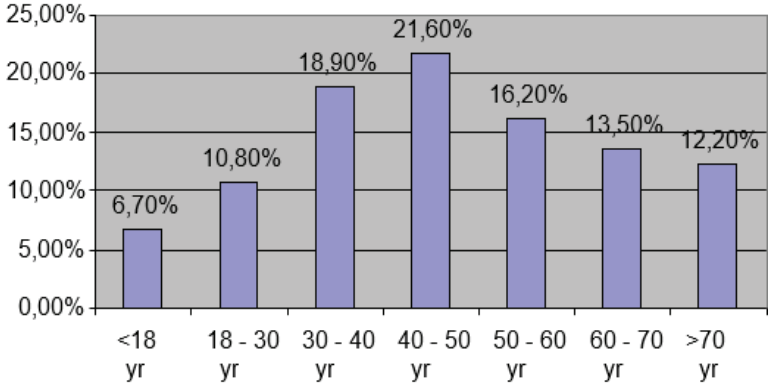


Fig 11 – Incidence rate of strictures based on age

Fig 12 – demonstrates the etiologic distribution:

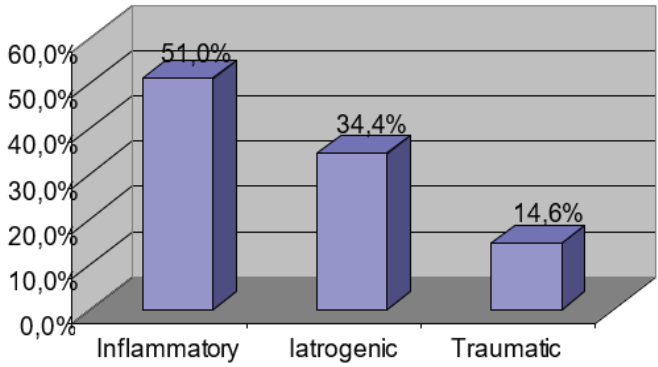


Fig 12 – Incidence rate based on etiology

In reconstructive operations on the male urethra, the recovery of patency was noted in 84.29%. Recurrence was found in 10%, with only 4.6% requiring re-urethroplasty. According to the erectile function questionnaire, there was an improvement of 9.4% compared to the questionnaire prior to surgery.

The age distribution in our patients varies widely (from 14 to 88 years). The majority was in the range between 30 and 60 years of age. This is largely due to the fact that some of the most common etiological factors (inflammatory and traumatic) mainly involve this age group [20].

The obtained results show that age is not a significant factor in influencing the harvesting of the graft material and its revascularization after urethroplasty. About 40% of cases are of idiopathic etiology [21]. The widespread use of endoscopic operations and procedures in urology is one of the most common causes of iatrogenic trauma to the urethra. It is now known that urethral strictures are found in 300 per 100,000 men and are a significant challenge in urological pathology [22]. Their treatment is one of the main issues faced. Excision of the stricture with subsequent urethro-anastomosis gives the best long-term results - over 90% [23]. However, the application of this approach is limited to short strictures located mainly in the bulbar urethra. That is why the introduction of urethroplasty with the oral mucosa flap is considered revolutionary. In 1996, Barbagli, G. outlined

the technique of dorsal "onlay" urethroplasty with a free graft of buccal mucosa, and since then this approach has been adopted in many countries around the world [24]. The use of different modifications of this technique (dorsal inlay, dorso-lateral onlay, ventral onlay) is a matter of preference for the surgical team [9, 13, 25]. The success of augmentation anterior urethroplasty in the long-term ranges from 73 to 90% [1, 6, 26]. A comparative analysis between onlay and inlay techniques shows almost identical results in long-term outcomes - 88% vs. 86.4% [27].

Augmentation anastomotic urethroplasty is mostly used in the bulbar and posterior urethra [4, 28].

The morphometric evaluation of the free buccal mucosa graft shows a high degree of vascularization - 4.9%. Maximal excision of stricture tissues, well into the healthy tissue, is an important factor for the success of urethroplasty [29].

Currently, oral mucosa is typically harvested from the cheek, the tongue, and the lower lip [30]. Buccal mucosa has been the main donor site for years, due to the ease of access and possibility of harvesting a wider graft[7]. Despite these advantages, BMG is associated with a series of inconveniences and discomfort for the patients: perioral numbness, difficulty with opening of the mouth, oral cavity dryness, etc [8].

Using the tongue as a donor site – lingual mucosa graft (LMG) was first been proposed in 2006[31]. Subsequently, this technique has gained popularity and has further been developed and perfected.

In the recent years (2006-2017) there have been over 20 publications in the English literature regarding LMG[5, 10, 11, 17, 32]. The usual technique of graft harvesting is from the lateral and ventrolateral side of the tongue, between the papillae on the dorsal and sublingual mucosa. This approach allows harvesting longer grafts – over 7 centimeters[11]. Using LMG for anterior urethroplasty shows highly positive functional results, with very few complications and patient inconvenience. This is supported by our observations[17, 32]. Additionally, using LMG allows tabularization for a wider urethral lumen. In this technique the graft is sutured horizontally, forming a wide luminal diameter and outstanding functional results – 91.6% effectiveness[10]. Results of reconstructive urethroplasty with LMG are comparable to those with buccal grafting in addition to the smaller risk of complications associated with the procedure[33].

Other substantial challenges in reconstructive urology are the long and complicated urethral strictures, as well as the treatment of recurrence [34, 35]. Even in these cases, oral mucosa grafting is associated with a high success rate – over 81%[2]. In most cases, such complicated strictures are reconstructed in two stages[36], with the second stage being after six to nine months.

In high-degree strictures and especially when part of the urethra is missing, following combined trauma, we developed and introduced a two-stage surgical technique utilizing buccal mucosa[18]. Our results in the treatment of seven patients give us reason to regard this approach as an alternative in this serious pathology.

Few studies demonstrate the results of the so-called Redo-urethroplasty in recurring strictures. Even here, the use of oral mucosa is a preferred technique, mainly with a buccal graft[12].

An important aspect in male urethral reconstructive surgery is vascular, nerve, and muscle sparing, which is associated with better functional results and fewer complications [3].

Ureteroplasty.

Using oral mucosa as graft material in reconstructive surgery of the ureter has been gaining popularity[37, 38] because of the high risk of complications associated with using an intestinal segment or auto transplantation.

Acquired ureteral strictures are relatively rare and usually due to:

1 complications of ureterorenoscopy (1%);

2 impacted ureteric stone (5-24%);

3 radiotherapy for an adjacent neoplasm (2.3%) [39.]

Other, more rare causes are trauma, retroperitoneal fibrosis, endometriosis.

Treatment of ureteral strictures is a serious challenge in reconstructive urology. Long and proximal strictures, as well as those involving the mid-third of the ureter, are especially challenging. The use of endoscopic methods is on the decline because of the unsatisfactory results and the necessity of follow-up surgeries and procedures[40]. Using buccal or lingual

mucosa is emerging as an alternative for ureteroplasty. In this study we put forward a personal observation in a patient with a mid-third ureteric stricture, resulting from several endoscopic procedures for an impacted stone. We used a 7 centimeter segment of lingual mucosa, grafted with the "onlay" technique. One year later results show no evidence of hydronephrosis.

Literature review regarding this approach demonstrates high success rates for ureteroplasty with oral mucosal graft and low rates of complications with both types of surgery – open or laparoscopic. Unfortunately, there are only 72 cases reported in the accessible literature, and more reports and later results have yet to be published.

Corporoplasty with buccal mucosal graft.

Another serious challenge in urologic practice is the presence of abnormal curvature of the penis (Peyronie's disease).

Peyronie's disease (PD) is an acquired condition, characterized by fibrosis of the tunica albuginea and leads to abnormal penile curvature with erectile dysfunction (ED) as a result.

PD affects 3.2 to 13% of men, and it is seen in men ranging from 50 to 60 years old[41, 42].

Predisposing etiological factors are diabetes mellitus, previous prostatectomy, hyperlipidemia, arterial hypertension, and tobacco smoking. Nevertheless, this disease's pathophysiology still is not completely clear. Recently it is thought that penile microtrauma plays a central role, causing fibroblastic proliferation and abnormal collagen deposition in the tunica albuginea[43]. PD's course is biphasic: acute and chronic, and is characterized by pain and inflammatory elements, after which permanent curvature of the penis develops. Surgical therapy is indicated in cases with difficult or impossible penetration during intercourse and is subdivided into three categories based on the angle of deviation[44]:

1. shortening of the opposite side of the penis (plication technique);

2. straightening by plaque excision and grafting of the defect with suitable material

3. penile implant placement

The second category includes multiple options for grafting material after plaque excision:

1. derma[45]
2. venous graft[46]
3. cadaveric or animal pericardium[47]
4. dura mater[48]
5. synthetic materials[49]
6. intestinal submucosa[50]
7. tissue-engineered graft[51].

Over time, all these techniques could not establish themselves as methods of choice, and the results are variable. Buccal mucosa as a graft material was first proposed in 2005[52]. This approach has been developed and there are currently publications on using lingual mucosa for the same purposes[53, 54].

In our analysis, substitution corporoplasty in PD has been carried out in 15 (2.8%) patients. For all of them, the following have been assessed:

1. IIEF
2. curvature angle measurement with follow-up for 9 to 18 months.

Regarding the first index, 86% of cases have achieved satisfactory coitus, and, in 72%, penis straightening has been observed[14].

Oral mucosal tissue with its elasticity and autology is an optimal alternative to the many proposed options for graft material in PD treatment – with highly positive cosmetic and functional effects.

Organ-sparing surgery for carcinoma of the penis.

Carcinoma of the penis is a rare neoplasm affecting around 1% of the male population in the USA[55] and up to 10% in developing countries[56].

Despite this, traditional treatment methods – partial or total penectomy – impose a serious and dramatic effect on the quality of life and psychological condition of patients.

In some cases, there is a feasible alternative to total or partial penectomy, which still effectively eliminates the tumor and preserves the sexual function with a maximal cosmetic effect[57].

Most tumors involve the glans penis and preputium, which allows for such type of surgery. Despite this, patients should be carefully selected, taking into account the anatomical specifics – during cavernous body resection the borders have to be clear of tumor invasion[58].

Tumor excision and glansectomy are part of the organ-sparing techniques for carcinoma of the penis. The problem of what grafting material is to be used arises. Most often this is a skin graft – the "split-thickness" approach[59, 60].

Buccal mucosa as a substitute material is seldom used in urologic practice and dermatological surgery[61]. We have carried out 8 (1.5%) such procedures with very good functional and oncologic results[62]. In one patient with malignant melanoma, 18 months after surgery, generalization of the disease was found. In this particular case, organ-sparing surgery was performed despite inguinal nodal invasion, due to the patient's exclusive demand[61].

Conclusion:

Oral mucosa has recently been proven to be superior as a graft material in reconstructive urology. Its advantages are

1. high accessibility and ease of harvest
2. low rate of infection
3. compatibility with the permanent contact with urine
4. thick epithelium and thin lamina propria
5. early revascularization and tissue adhesion.

Reconstructive surgery using free oral mucosal graft demands strict patient selection, possession of more operative techniques, and specialized professional experience. Oral mucosa as grafting material is the most widely used approach in modern urology because of the positive results of its application.

BIBLIOGRAPHY

1. Barbagli, G.Kulkarni, S., Fossati, N. et al. “Long term follow-up and deterioration rate of auterior substitution urethroplasty”, J. Urol., 2014; 192: 808-813;
2. Spilotros, M., Sihra, N., Malde, S. et al. “Buccal mucosal graft urethraplasty in men – risk factors for recurrence and complications: a third referral centre experience in anterior urethroplasti using buccal mucosal graft; Transl. Androl. Urol., 2017; 6(3): 510-516;
3. Khalil, M. I., Tourchi, A., Davis, R., Eltahawy, E. “Vessel-and Muscle – Sparing Technique in Bulbous urethroplasty: A review”, Urology Practice, 2019; DOI: 10/1097/UPJ.0000000000000085; Ref.: UP-19-57;
4. Chapple, C., Andrich, D., Atala, A. et al. “SIU/ICUD Consultation on Uretheral Strictures: The management of anterior urethral stricture disease using substitution urethroplasty”, Urology, 2014; 83: S31-47;
5. Abrate, A., Gregori, A., Simonato, A. “Lingual mucosal graft urethroplasty 12 years later: Systematic review and meta-analysis”, Asian. J., 2019; 6: 230-241;
6. Slavov, C., Popov, E., Sabriev, V. et al. “Reconstructive surgery of male urethra – 10-years' experience and long-term result”, Urology, Endourology, 2012; 18(2): 34-39;
7. Bhargava, S., Chapple, C. “Buccal mucosal urethroplasty is it the new gold standard?”; BJU Int., 2004; 93: 1191-1193;
8. Wood, D., Allen, S., Andrich, D. et al. “The morbidity of buccal mucosal graft harvest for urethroplasty and the effect of nonclosure of the graft harvest site on postoperative pain”, J. Urol., 2004; 172: 580-583;
9. Kulkarni, S., Barbagle, G., Sansalonne, S. et al. “One – sided anterior urethroplasty: a new dorsal onlay graft technique”, BJU nt., 2009; 104: 1150-1155;
10. Sharma, U., Yadav, S., Tomar, V. et al. “Single stage circumferential lingual mucosal graft urethroplasty in near obliterative bulbar urethra stricture: a novel technique”, Urol. Ann., 2016; 8: 146-150;
11. Zhang, K., Zhou, S., Zhang, Y. et al. “Anterior urethra reconstruction with lateral lingual mucosa harvesting technique”, Urology, 2016; 90: 208-212;
12. Jaslonowska, S., Brunckhorst, O., Rees, R. et al. “Redo-urethroplasty for the management of recurrent urethral strictures in males: a systematic review”, World J. of Urol., 2019; March: https://doi.org/10.1007/s 00345;

13. Slavov, C., Tchukov, I. and Pantchev, P. “A comparative assessment of some urethroplasties in strictures of the urethra”, BJU Int., 2004; 94(SR), 117;
14. Slavov, C., Venkov, G., Tchukov, I., Popov, E. “Using of buccal mucosal graft in Penile surgery’’, IMORU, 2006; Abstracts from 4th International Meeting on Reconstructive Urology”;
15. Slavov, C., Donkov, I., Popov, E. “Case of Duplication of the Urethra in an Adult Male, presenting with symptom of Bladder Outlet Obstruction”, Eur. Urol., 2007; 52(4):1249-1251 (Part 1);
16. Slavov, C., Popov, E., Venkov, G. et al. “Complex Urethroplasties using combination of Free tissue graft”, Urology, 2009; 74(S4A): S91;
17. Slavov, C., Popov, E., Venkov, G. et al. “Urethroplasty with usage of Lingual Mucosa free graft: Single Center Initial Experience and Early Results”, Urology, 2009; 74(S4A): S91;
18. Slavov, Ch. “New approach for reconstruction of missing part of the urethra”, Andrologiia, 2013; 22(4):
19. Morey, A. and Kirer, W. “Proximal bulbar urethroplasty via extended anastomotic approach – what are the limits?”, J. Urol., 2006; 175: 2145-2149.
20. Santucci, UR., McAninch,J., Mario, L. et al. “Urethroplasty in patient older than 65 years: indications, results, outcomes and suggested treatment modifications”, J. Urol., 2004; 172: 201-203.
21. Lumen, N., oebeke, P., Willemsen, P. et al. “Etiology of urethral stricture disease in the 21st century”, J. Urol., 2009; 182: 983.
22. Stein, D., Thum, D., Barbagli,G. et al. “A geographic of male urethral stricture etiology and location”, BJU Int, 2013; 112: 830-834.
23. Morey, A., Watkin, N., Shenfeld, O. et al. “SIU/ICUD Consultation on Urethral strictures: Anterior urethra-primary anastomosis”. Urology, 2014; 83-S23.
24. Barbagli, G., Sellr, C., Tosto,A. et al. “Dorsal free graft urethroplasty”, J. Urol., 1996; 155: 123-126.
25. Meeks, J., Erickson, B., Granieri, M. et al. “Stricture recurrence after urethroplasty: a systematic review”, J. Urol., 2009; 182: 1266-70.
26. Benson, C., Li Gen, Brandes, S. “Long term outcomes of one-stage augmentation anterior “urethroplasty: a systematic review and meta-analysis”, BJU Int., 2021; 47/2/: 237-250.
27. Aldaqadossi, H., El Gamal, S., El-Nadey, M. et al. “Dorsal onlay (Barbagli technique) versus dorsal inlay (Asopa technique) buccal mucosal graft urethroplasty for anterior urethral stricture: a prospective randomized study”, Int. J. Urol., 2014; 21: 185-188.

28. Slavov, Ch. “Urethroplasty in strictures of the posterior urethra with buccal mucosal graft”, Khirurgia (Sofia); 2003; 59(6): 25-7, Bulgarian, PubMed PMID: 15641557.
29. Mateev, D., Belovodov, V., Slavov, C. et al. “Morphometric Assessment of tissues in reconstructive surgery of strictures of the male urethra”, Eur. Urol., 2008; 7(3): 114.
30. Simonato, A., Gregori, A. “Lingual mucosal graft for anterior urethraplasti: a review”, BJU Int., 2010; 105: 132-133.
31. Simonato, A., Gregori, A., Lissiani, A. et al. “The tongue as an alternative donor site for graft urethroplasti: a pilot study”, J. Urol., 2006; 175: 589-592.
32. Popov, E., Slavov, C. “Urethroplasty with Lingual Mucosa graft – Long term results and operative techniques used”, Urology, 2011; 78 (Suppl. 3A): S389.
33. Lumen, N., Vierstraete – Vertinde, S., Oosterling, W. et al. “Buccal versus lingual mucosa graft in anterior urethroplasty: a prospective comparison of surgical outcome and donor site morbidity”, J. Urol., 2016; 195: 112-117.
34. Slavov, C. “Urethroplasty in Long strictures of the anterior urethra”, Khirurgia (Sofia); 2006; LX (2): 19-23.
35. Andrich, D., Mundy, A. “What is the best technique for urethroplasty?”, Eur. J. Urol., 2008; 54 (5): 1031-1041.
36. Salim, M., Salem, Sh., Elsherif, E. et al. “Outcome of staged buccal mucosal graft for repair of long segment anterior urethral stricture”, BMC Urology, 2019; 19: 38.
37. Heijkoop, B. and Kahokehr, A. “Buccal mucosal graft ureteroplasty for the management of ureteric strictures: A systematic review of the literature”, Int.J. of Urol., 2021; 02: 1-7.
38. Yong Kinlin, Fan, Sh., Li, Z. et al. “Lingual mucosa graft ureteroplasty for ureteral stricture: a narrative review of the current literature”, Ann.Palliat. Med., 2021/http//dx.doi. org./10.21037.
39. Lucas, J., Ghirardi, E., Ellis, J. et al. “Endoscopic management of ureteral stricture: an update”, Curr. Urol. Rep., 2018; 19:24.
40. Reus, C., Brehmer, M. “Minimally invasive management of ureteral strictures a 5-years retrospective study”, World J. Urol., 2019; 37: 1733-1738.
41. Schwaraer, U., Sommer, F., Klotz, T. et al. “The prevalence of Peyronie's disease: results of a large survey”, BJU Int., 2001; 88: 727-730.
42. Dibenedetti, D., Nguyen, D., Zografos, L. et al. “A population based study of PD: Prevalence and treatment”, in USA, Adv. Urol., 2011; 1: 1-9.

43. Devine, C., Somers, K., Jordan, G. et al. “Proposal: trauma as the cause of the Peyronie’s disease in yang men”, J. Urol., 1997; 157: 285-290.
44. Ralph, D., Gonzales-Cadavid, N., Mirone, B. et al. “The management of Peyronie’s disease: evidence based 2010 guidelines”, J. Sex. Med., 2010; 7: 2359-2374.
45. Devine, C., Horton, C. “Surgical treatment of Peyronie’s disease with a dermal graft”, J. Urol., 1974; 111: 44-49.
46. Adeniyi, A., Goomey, S., Pryor, J. et al. “The Lue procedure: an analysis of the outcome in Peyronie’s disease”, BJU Int., 2002; 89: 404-408.
47. Egudio, P., Lucon, A., Arap, S. “Treatment of Peyronie’s disease by incomplete circumferential incision of the tunica albuginea and plaque with bovine pericardium graft”, Urology, 2002; 59: 570-574.
48. Sampaio, J., Fonseca, J., Passarinho, A. et al. “Peyronie’s disease: surgical correction of 40 patients with dura mater graft”, Eur. Urol., 2002; 41: 551-555.
49. Licht, M., Lewis, R. “Modified Nesbit procedure for the treatment of Peyronie’s disease a comparative outcome analysis”, J. Urol., 1997; 159: 460-463.
50. Knoll, L.D. “Use of porcine small intestinal submucosal graft in the surgical treatment of Peyronie’s disease”, Urology, 2001; 57: 753-757.
51. Imbeault, A., Bernard, G., Queller, G. et al. “Surgical option for the correction of Peyronie’s disease an autologous tissue – engineered endothelized graft”, J. Sex. Med., 2011; 8: 3227-3235.
52. Shioshvili, T., Kakonashivili, A. “The surgical treatment of Peyronie’s disease: replacement of plaque by free autograft of buccal mucosa”, Eur. Urol., 2005; 48: 129-135.
53. Salem, E., Elkadi, E., Sakr, A. et al. “Lingual mucosal graft in treatment of Peyronie’s disease”, Urology, 2014; 84: 1374-1377.
54. Fabiani, A., Servi, L., Fioretti, F. et al. “Buccal mucosa is a promising graft in Peyronie’s disease surgery. Our experience and a brief literature review on autologous grafting material”, Arch. Ital. di Urol. E Andr., 2016; 88; 2: 115-121.
55. Siegler, R., Miller, K. and Jemal, A. “Cancer statistics”, Cancer J. Clin., 2019; 69 (1): 7-34.
56. Bray, F., Ferlay, J., Laversanne, M. et al. “Cancer incidence in five continents”, Int. J. Cancer, 2015; 137 (9): 2060-2071.
57. Hakenberg, O.W., Minhas, S. et al. “EAU Guidelines on Penile Cancer”, 2020, June, 3, available from: https://uroweb. Org./guidelines.

58. Philippou, P., Shabbir, M., Malone, P. et al. “Conservative surgery for squamous cell carcinoma of the penis: resection margins and long term oncologic control”, J. Urol., 2012; 188 (3): 803-808.
59. Burnett, A. “Penile preserving and reconstructive surgery in the management of penile cancer”, Nat.Rev. Urol., 2016; 13 (5): 249-257.
60. Ferguson, J. and Fang, A. “Penile sparing techniques for penile cancer”, Postgr. Med., 2020; DOI: 10.1080/00325481.
61. Slavov, C., Venkov, G., Velikova, K. et al. “Malignant Melanoma of glans penis and prepuce treated with organ-preserving surgical procedure”, Acta Derm. Croat., 2009; 17 (2): 131-133.
62. Venkov, G. and Slavov, C. “New Method for Recovering of defects on Glans Penis after partial or total glansectomy with transplant of oral mucosa”, Surgery, 2006; LIX (3): 23-27.

Проф. д-р Чавдар СЛАВОВ, МД, академик од БАН

ЛИГАВИЦА ОТ УСТНАТА КУХИНА КАТО ПЛАСТИЧЕН МАТЕРИАЛ В РЕКОНСТРУКТИВНАТА УРОЛОГИЯ

Абстракт

Увод.

Въпреки големият технологичен напредък в медицината, остават сериозни предизвикателства пред реконструктивната хирургия в урологичната практика. Липсата на универсален пластичен материал при заместващите операции на уро-гениталната система при мъжа е един от основните проблеми.

Цел.

Целта на настоящата студия е да се направи ретроспективен анализ на функционалните резултати от използването на свободни лигавични ламба от устната кухина.

Материал и методи.

За период от 20 години (2000-2021) са проследени 521 пациенти, при които е извършена реконструктивна заместваща операция с използване на свободни лигавични ламба от устната кухина. Те са

разпределени в 5 основни групи, според органа върху който е извършена реконструктивната операция: 1/ Уретропластика при стриктури на уретрата – 427 (82%); 2/ Уретропластики при осакатена хипоспадия – 70 (13.4%); 3/ Уретеропластика при стриктури на уретера – 1 (0.2%); 4/ Заместителна корпоропластика при болестта на Пейрони – 15 (2.8%); 5/ Органсъхраняващи операции при карцином на пениса – 8 (1.5%).

Резултати.

Като заместителен (пластичен) материал от зоната на устната кухина са използвани три места:

1. Букална лигавица (BMG) - 368 (70.6%)
2. Лингвална лигавица (LMG) - 135 (25.9%)
3. Лигавица от долната устна - 18 (3.5%)

Не са отбелязани по-сериозни усложнения от добиването на графта.

Основен вид операции са били: едноактни оперативни техники 460 (88.3%), следвани от двуактните операции – 38 (7.3%) и операции върху половия член – 23 (4.3%).

Функционалните резултати са отчетени според вида на операцията, като в най-голямата група – уретропластиките е постигнат успех в 84.29%

Заключение.

Понастоящем реконструктивната хирургия върху пикочните пътища и половия член при мъжа с използване на свободни ламба от лигавицата на устната кухина е най-застъпената оперативна техника в модерната урология. Този вид дейност изисква стриктен подбор на пациентите и сериозен професионален опит.

Ключови думи. лигавица от устната кухина (BMG, LMG), уретропластика, уретеропластика, корпоропластика, органсъхраняващи операции при Са на пениса.

doi: 10.20903/zpopov-2022-0019 UDK: 616.65-007.61-085.849.19

Dragoslav BAŠIĆ[2]

LASER THERAPY FOR BENIGN PROSTATIC HYPERPLASIA: PHYSICAL BASICS AND CURRENT STATUS

Abstract

Introduction: Contemporary laser technologies in urology are becoming a new gold standard and a necessary tool for minimally invasive treatment of benign prostatic hyperplasia (BPH). Advances in laser technology applied to the treatment of BPH have been inspired primarily by the need to find a comparable alternative therapeutic option to transurethral resection of the prostate (TURP) and open prostatectomy (OP).

Material and methods: Laser procedures for BPH encompasses a variety of laser types and operative techniques, including visual laser ablation of the prostate (VLAP), transurethral ultrasound guided laser incision prostatectomy (TULIP), potassium titanyl phosphate (KTP) laser, contact laser vaporization of the prostate (CLV), interstitial laser coagulation (ILC), holmium laser resection of the prostate (HoLRP), holmium laser enucleation of the prostate (HoLEP), holmium laser ablation of the prostate (HoLAP) and thulium laser (vapo)enucleation or resection. Each of these techniques has certain advantages and disadvantages, both when compared with each other, and when compared to TURP.

Results: Modern lasers have far greater energy power compared with the older generations of lasers, and these modern lasers are used in surgery for precise cutting, evaporation and tissue coagulation. The effect of the laser depends on the wavelength as well as on the ability of the target tissue to absorb radiation energy. Thus, to choose the best laser for a given type of surgery, one should keep in mind the differences between lasers, the

[2] Clinic of Urology, University Clinical Center Niš, Niš, Serbia Faculty of Medicine, University of Niš, Niš, Serbia; basicdr@gmail.com

variability of their use, and the factors that determine how the laser affects the tissue. The aspirations of this study were aimed at creating innovative procedures, which would not have limitations in terms of prostate volume and whose clinical results related to perioperative morbidity, hemorrhage and hospitalization period would be at least identical to the clinical results achieved by the TURP and OP.

Conclusions: Traditional operating techniques are slowly becoming a thing of the past, and classical methods are being replaced by technological innovations, primarily advanced lasers. Among the many types of lasers used for the surgical treatment of BPH, the holmium and thulium lasers are currently the most widely used in clinical practice.

INTRODUCTION

In the last few decades, there has been intensive development in the field of laser construction, such that the knowledge gained by modern physics finds wide applications in various fields of science and technology. All the advantages of the phenomenon of laser light emission are compatible with the needs of practical medicine, especially surgical branches. A large number of scientific and professional papers in this field testify to the expansion of this relatively young discipline of scientific knowledge and how it is applied in the field of biomedicine. Scientific progress in the field of laser medicine is accompanied by the production of adequate laser medical equipment. Modern lasers represent a synthesis of the most modern achievements of quantum electronics, precision mechanics, optics, and electrical engineering. Laboratory and clinical research have resulted in the understanding of a number of advantages of laser techniques over classical methods. These advantages include: ease of handling, low risk of intervention, non-contact of tissue manipulators, precise tissue destruction, access to inaccessible organs and tissues, minimal damage to surrounding healthy tissue, absence of side effects (bleeding, pain, infection), rapid wound healing, possibility repetitions of the procedure, etc.

The word laser comes from the initial letters of the English name: Light Amplification by Stimulated Emission of Radiation (1). This name contains two important physical characteristics that explain this special way of obtaining light, and which, both in the recent past and today, are in the

center of interest of almost all modern scientific branches, especially medicine and biology. Laser light emission is a quantum phenomenon and its origin is based on the laws of quantum physics, i.e. the interaction of photons with matter. The absorption coefficient can be considered negative, which means that the light intensity increases. More precisely, the laser is not a light amplifier, but a kind of oscillator that generates light, i.e. represents a light source.

Another phenomenon we encounter here is stimulated radiation emission. It is interesting that in 1917, Albert Einstein predicted the possibility of such a show, and that the laser was realized only in 1960. The principle of stimulated radiation was first used in the generation of microwaves (masseurs) (2).

Laser-tissue interaction

The application of laser as a method and means in medicine for the treatment of many diseases, will be much clearer if the reaction of tissues to laser radiation is explained. In fact, there is a laser-tissue interaction, which results in functional, structural and biochemical changes that occur in the body as a result of irradiation. At the very beginning of the development of laser technology, the essential importance of laser-tissue interaction was noticed, and then tests in developed countries showed that laser radiation is, in fact, an artificial physical agent, and its effects on living organisms depend on laser emission parameters and physiological properties, irradiated tissue. To explain the reaction of laser radiation on biological tissues, a multidisciplinary approach is necessary in the further study of this phenomenon, i.e. the application of methods that are primarily based on the principles of biology and physics. There is no doubt that there are positive therapeutic effects based on these mechanisms, but further scientific clarification of the interaction of laser radiation with bio tissues is necessary, along with monitoring the input values in the interaction, i.e. laser output parameters, on the one hand, and the results of the total effect of laser radiation on health, on the other (3).

Factors of laser energy action on biological tissues can be classified thus: thermal, photochemical, electrical, mechanical, quantum and multiphonic effects, which result in local increase of pressure, temperature and photobiological reactions in irradiated tissues. Not only the parameters of

laser radiation (laser type, wavelength, radiation power density, pulse frequency, etc.), but also the physiological characteristics of irradiated tissues and organs (blood flow intensity, thermal conductivity, absorption and reflection coefficient, heterogeneity, microstructure, etc.) determine the final the result of the biological effect of laser radiation on biological objects.

Biogenic effects resulting from the laser-tissue interaction can be conditionally divided into three basic categories:

- Primary effects (change of global energy of electronic levels of mercury and the matter of the molecules, restructuring of living matter molecules, coagulation of protein structures);
- Secondary effects (photodynamic effect, photoreactivation effect, stimulation of bioprocesses);
- Tertiary effects, so-called subsequent effects (elastic oscillations of molecules protein, formation of toxic products in tissues, etc.).

When analyzing secondary effects, it is necessary to take into account the corrections of the state of biological systems related to nonlinear optical effects, electrostriction, acoustic and ultrasonic oscillations and standing waves. Little is known about these significant factors and there is little data in the literature. Due to the generation II harmonics, which represent radiation with new parameters, nonlinear optical effects are related to tissue excitation. Tissues that are irradiated with infrared light begin to emit green light. Addition, subtraction and frequency eruptions may also occur (3). The effect of electrostriction results in deformations and probably, destruction of macromolecules of living matter. When the state of protein structures (colloidal changes) of irradiated tissues changes, acoustic, ultrasonic oscillations, and standing waves appear, which are all resonant processes. Secondary effects are actually a complex of reactions in the organism and are correlated with the primary effects that are responsible for the immediate changes in the tissues.

Having in mind the existence of many different factors that are at play in the creation of appropriate biogenic effects, researchers encounter complex systems, diverse in nature, with a consequent series of events that take place in the irradiated part of the organism. All this is part of an integral picture of the final effects of the interaction of laser radiation within living systems. The integrity of such a picture, however, cannot exclude the

dominance of one of the above components of interaction over another, which depend on the character of the biological object and laser parameters.

Low and moderate power densities of laser radiation (10-103 Wcm-2) lead to an increase in tissue temperature (photothermal action), or initiate chemical reactions (photochemical action). A moderate increase in temperature (up to 10 ° C) causes coagulation of molecules in the tissue and therefore a visible thermal reaction (photocoagulation) occurs. A further increase in temperature causes evaporation of intracellular and extracellular fluid (water) and makes an incision in the tissue (photovaporization). By injecting certain substances, it is possible to make the tissue sensitive to a certain wavelength, and when such tissue is irradiated with laser light of the same wavelength, destructive free radicals (photoradiation) are created. The excimer laser emits in the UV (ultraviolet) region of the spectrum, so that the initiated photochemical reaction breaks the molecular bond in the tissue, which can create precise defects in the tissue, without heat damage, which is removed by molecule fragments (ablative photodecomposition).

Short-term pulses (30ps-15ns), high power density (1010 Wcm-2) generated by a Nd: YAG laser, cause the breakdown of electrons in tissue molecules and thus lead to ionization of molecules and tissue disintegration. Additional destruction of the target tissue is achieved by the rapid expansion of the resulting plasma, which creates a mechanical wave. By absorbing or scattering each subsequent pulse of the laser beam, the plasma protects the tissue behind the target. This process is called photodisruption. A laser is a powerful beam of radiation that produces predominant thermal damage in the target tissue. Each type of laser produces a beam of one wavelength or at most two similar wavelengths. The effect of the laser depends on the wavelength, as well as on the ability of the target tissue to absorb radiation energy. Thus, to choose the best laser for a given type of surgery, one should keep in mind the differences between lasers, the variability of their use and the factors that determine how the laser affects the tissue. In general, only two types of lasers are important for medical and dental use. These are "soft" (low-energy) lasers, so thermal effects are excluded and their main purpose is to stimulate cellular activity; the second type are "hard" lasers, which are dominated by thermal effects and are used in surgery for precise cutting, evaporation (evaporation, evaporation) and tissue coagulation (4,5).

Physical principles of laser operations

The laser beam is unique in existence, composed of photons of the same wavelength, which travel in phase and parallel to each other. The radiation is emitted from an active medium-substance composed of atoms, which, when excited, emit radiation of a usable wavelength. In order to achieve light amplification, the active medium must first be supplied with some form of energy (light, heat, electricity), so that a large number of atoms absorb enough energy to pass into the excited state. At this point, spontaneous photon emission begins to take place, as some of the excited atoms return to their previous state. When each type of active medium is excited to a certain energy state and spontaneously emits excess energy, photons with a wavelength characteristic of that medium are released. The stimulated emission originates from the moment when one emitted photon collides with one excited atom, which has absorbed an amount of energy equal to that of the photon, which, colliding with that atom, causes the emission of a photon from it. When both photons have the same wavelength, they are in phase, and they leave the atom going in the same direction. Amplification occurs by striking a certain number of photons back and forth through an active medium between two mirrors placed both below and above the medium. The mirror at the output end is partially reflective, so that it transmits a certain proportion of photons, directing them like a laser beam, while the others are reflected back into the active medium to continue stimulating the release of other photons from the excited atoms. The mirror at the other end reflects 100% of the photons, directing them back to the partially reflecting mirror at the output end. In this way, a certain percentage of the created photons strike back and pass between the two mirrors at the speed of light, stimulating the emission of photons from the excited atoms. The laser beam is monochromatic, coherent and very intense. It is created as a result of the stimulated emission of the amplification process. The coherent nature of the beam allows it to be focused by the lens at points, whose radius can theoretically be equal to half the wavelength of the beam. All photons from a ray can be compressed into a very tiny point, resulting in the flux or number of photons passing through that point at the same time. This means that the laser beam can be used to concentrate energy and deliver it in extremely high doses to the target area, even to a very small area (6,7).

Air power. Power density

The power of a laser beam is the property of the beam to transmit energy to a specific target point in a unit of time and thus increase the vibrational motion of the target atoms and molecules. Power density (PD) is the amount of power per area and is calculated by dividing the power (W) emitted by the laser, by the area of the target point. PD = W / πr2 (r-radius of the circle), which shows that PD varies inversely with the square of the radius of the point. It is important that the surgeon knows and intuitively understands how strength density affects the tissue. First, the level of ablation varies with power density. Higher power density means faster ablation. Second, the zone of thermal damage below the tissue surface varies depending on the strength density. At low power density values, the tissue heats up more slowly and has time for the heat to be conducted to the layers that lie below the actual layer. When the PD value is high, the surface tissues heat up rapidly to the boiling point of intracellular water, when a large part of the air energy is dissipated in the form of water vapor, which partially carbonizes the organic cellular components. The rest of the heat is conducted to the lower layers, but because this process is relatively slow to evaporate, the heat does not penetrate long before the evaporation field reaches the zone of thermal damage. Thus, at lower power density values, the zone of thermal damage below the tissue surface increases. Conversely, at high strength density values, the zone of thermal damage below the tissue surface decreases (4,8).

CW and pulsed lasers

There are two basic ways of delivering energy for any type of laser, and they are pulsed and continuous wave. The continuous wave (CW) is the same as with a flashlight; the intensity of light, in a unit of time, is constant. Pulsed lasers are similar to stroboscopic light: energy is delivered through a series of intense flashes with a very short duration (ms, ns). As with stroboscopic light, if the pulses (oscillations) are delivered in high doses, they can make the air look continuous, but the key difference is that the rate of energy transfer during the pulse is much higher than the rate at which the CW laser transmits energy during the same time period. Maximum power indicates the maximum value of energy transfer during the pulse. The

pulsation of the laser beam can be performed in two different ways: either by transmitting activation energy to the active medium in pulses, or by a process called Q-switching. Q-switching is performed by introducing a fast shutter between the active medium and the output mirror. By simultaneously holding the shutter closed and pumping the activation energy into the active medium, the activation energy is "captured" as a photon within the active medium. Thus, there is an extremely large increase in energy in the active medium. When the shutter opens, that energy is released almost instantly, and as a result, the peak of the pulse energy is high. Q-interruption is common for industrial lasers, but is not widely used for medical lasers, except in the fragmentation of ureteral calculi. The main limiting factor is the conduction of laser energy through the optical fibers. The energy flux at the input to the optical fiber exceeds the damage threshold of the fibrous material even at wavelengths that are easily transmitted by the CW mode. Storz introduced the Q-switching Nd: YAG (neodymium: yttrium-aluminum-garnet) laser, which is capable of fiberoptic transmission and is good for endoscopic lithotripsy (stone breaking) in medicine.

Advances in laser technology applied in the treatment of BPH have been inspired primarily by the need to find a comparable alternative therapeutic option to transurethral resection of the prostate (TURP). Aspirations were aimed at creating innovative procedures, which would not have limitations in terms of prostate volume and whose clinical results, related to perioperative morbidity, hemorrhage, and hospitalization period, would be at least identical to the clinical results achieved by the TURP procedure. In the early 1990s, Nd: YAG visual prostate ablation (VLAP) and Nd: Yag interstitial laser coagulation (ILC) of the prostate were enthusiastically used. The results of these procedures were shown to be significantly weaker in relation to TURP, both in relation to urination parameters and in relation to the rate of reinterventions and reoperations. As a result, these procedures were abandoned. Later, through the framework of the stated needs, the following types of lasers were reviewed for their use: Neodymium: yttrium aluminum garnet (Nd: YAG), Holmium (Ho): YAG and Nd: Yag (Potassium titanyl phosphate) (KTP) laser. Each of these lasers acts at a different wavelength, and the effects in the tissue are achieved by heating, the degree and speed of which depend on the immediate surgical effects. Modern lasers have far greater energy power compared to older generations of lasers (9,10).

Th Nd: YAG (Neodymium: Yttrium Aluminum Garnet) laser

The beam wavelength of Nd: YAG lasers is 1064 nm, close to the infrared spectrum. The air is invisible and achieves a tissue penetration depth of about 10 mm. Due to the action of this laser, an increase in temperature occurs in the tissue, which causes coagulation necrosis. Therefore, the resection and hemostasis characteristics of this laser are good and are useful for soft tissue incision (urethral stenosis, ureteral stenosis, bladder neck sclerosis), tissue ablation (BPH) and skin changes (condyloma, penile cancer). Alternatively, the contact technique achieves the effects of tissue vaporization (11,12).

Holmium: YAG (Holmium: yttrium aluminum garnet) laser

This type of laser emits a wavelength of 2100nm, with a high coefficient of absorption in water. It is used primarily in pulse mode, rarely in continuous. Its absorption length in prostate tissue is short (0.4 mm), which provides excellent characteristics for vaporization. In addition, damage to the surrounding racetrack is minimal (0.5-1mm). It is used for prostate enucleation in BPH and lithotripsy. There are numerous lasers of this type in use, and each type operates at a different wavelength (12).

KTP (Potassium Titanyl Phosphate) laser

The potassium-titanyl phosphate (KTP) laser beam is in the region of the visible green spectrum, with a wavelength of 532 nm. It occurs as a consequence of the passage of Nd: YAG-waves (1064 nm) through the KTP crystal, which doubles the frequency and halves the wavelength. The actions of this laser are similar to the actions of an argon laser, with a maximum power of about 60W. The absorption characteristics of KTP lasers are good for the effects of vaporization, coagulation and hemostasis (10).

PVP (Photoselective vaporization of the prostate)

The potassium-titanyl phosphate (KTP) laser beam is in the region of the visible green spectrum, with a wavelength of 532 nm. The absorption characteristics of KTP lasers are good for the effects of vaporization,

coagulation, and hemostasis. When using high power, the action of this laser in the prostate tissue causes photothermal vaporization of intracellular tissue water, which is used in a procedure known as photoselective vaporization of the prostate (PVP). Because KTP energy is strongly absorbed by hemoglobin, tissue penetration is less pronounced. The hemostatic characteristics of this laser are extraordinary, while the zone of coagulation necrosis is only 2 mm, which achieves a vaporization coefficient of 0.3-0.5 g / min. As such, it is possible to achieve a reduction in prostate volume of up to 43%, which has been shown to be sufficiently effective for acute deconstruction, even in patients using oral anticoagulant therapy. A good feature of this laser is the possibility of changing the air pressure during work. This is useful considering that the lateral lobes of the prostate are more adenomatous, while the medial lobe has a pronounced muscular-collagen structure. The disadvantage of this method is that it does not provide a sample of prostatic tissue for PH analysis. The 80W KTP laser and the 120W KTP laser are the two current KTP laser models on the market.

Although long-term results are not yet available, published results of one-year follow-ups of patients undergoing PVP with KTP laser 80W show that compared to TURP, PVP is associated with uniform parameters of micturition and IPSS score, lower blood loss and shorter catheterization and hospitalization periods. The rate of early postoperative complications is low and comparable with HoLEP and TURP results. Some authors report a slightly higher rate of urethral stenosis (7%).

The new 120W KTP HPS (high performance system) laser was developed with the aim of increasing the vaporization coefficient and shortening the operation time. It includes a lithium borate laser. Despite the acceleration of the vaporization effect, the high power of this laser poses a potential danger that, due to increased depth of penetration and reduced vision (more bubbles in the field of view), perforation of the prostate capsule, bladder perforation and ureteral orifice injuries can occur, especially when the medial lobe is pronounced. The results of a five-year follow-up of patients who underwent PVP KTP with a 120W laser show a significant improvement in key parameters (AUA-symptom score, maximum urine flow, post-micturition residual urine, prostate volume), without statistically significant differences in relation to TURP (11-15).

Holmium laser enucleation of the prostate (HoLEP)

Except for prostate volume, all remaining indications for surgical treatment of BPH with this procedure are identical to indications for transurethral resection (TURP). In relation to prostate volume, indications for HoLEP include larger prostates, even up to 200ml. Exclusion criteria are urination disorders that are not caused by benign prostatic hyperplasia, previous surgical interventions on the prostate, bladder neck or urethra, as well as the existence of prostate cancer. According to the published results of randomized studies, the rate of perioperative morbidity is very low, with slight blood transfusions, the absence of TUR syndrome and the need for reintervention. The rate of postoperative urinary retention ranges up to 8%. The occurrence of possible postoperative dysuria or urgent incontinence (1%) is usually resolved with drug therapy in a shorter period of time. In large series, the incidence of urethral stenosis after the procedure is stated to be 1.9%, while the incidence of bladder neck sclerosis is 1.5% (17-20).

The published results of this procedure indicate its growing popularity. Compared to open prostatectomy, HoLEP is associated with significantly lower rates of hemorrhage and blood transfusions, with a shorter period of hospitalization and catheterization. Regarding symptomatology, as well as the occurrence and frequency of late complications, there is no difference between these two procedures. So far, the results of many randomized controlled studies comparing the results of TURP and HoLEP in the treatment of BPH have been published. In most studies, results related to the resection coefficient, symptom score, postmictive residual urine, and urine flow were better in the HoLEP group. A meta-analysis of these results shows that the HoLEP procedure is superior to TURP, in terms of perioperative hemorrhage, as well as periods of catheterization and hospitalization. In addition, the reduction in PSA values after the HoLEP procedure is up to 90%, while after TURP it is 71%. The main disadvantage compared to TURP is the longer learning curve for HoLEP (> 50 cases). Distant HoLEP results after 7 years of follow-up show durability in relation to the IPSS score, QoL score, as the maximum urine flow, resulting in the expressed satisfaction of the applied procedure in 92% of patients. Compared with TURP, there is no difference in the outcome of surgical treatment. Although initially described as an alternative to TURP, the HoLEP

procedure has great potential to become a "volume-independent," new gold standard in the surgical treatment of BPH (21-24).

The first holmium-laser enucleation of the prostate (HoLEP) in Serbia was performed in 2011, at the Urology Clinic of the Clinical Center of Niš. The first results of a comparative study in relation to TURP were published, with a follow-up period of one year. The results of this study show that, in relation to the period of catheterization and hospitalization, a decrease in hemoglobin levels, as well as a rate of early and late postoperative complications, the results are statistically significant and better in the HoLEP group. And, in relation to the results of postoperative monitoring related to QoL, IPSS and PVR, after 6 months and after 12 months from the operation, there is a statistically significant difference in favor of the HoLEP procedure. The operative time was statistically significant in duration during the HoLEP procedure, thus confirming the suggestions from the literature that a broader learning curve is inherent for complete mastery of this operative technique (27).

Holmium laser ablation (vaporization) of the prostate (HoLAP)

This method uses side-firing fibers (60° and 90°) and a 60W device. The results published in previous years show that it is a safe procedure that achieves effective deconstruction and improvement of urination quality (including IPSS and AUA symptom score), without significant perioperative morbidity and with a very low rate of re-operations after 7 years of follow-up. This procedure can also be used to treat larger volumes of prostate, with a lower rate and extent of hemorrhage and a shorter period of catheterization and hospitalization. The speed and efficiency of the procedure was limited by the low power of the device (60W), and the procedure itself was later overshadowed by the HoLRP and HoLEP procedures. Thanks to the appearance of a more powerful 100W device, as well as the growing popularity of the PVP KTP laser, interest in HoLAP has been renewed in recent years, and this procedure is recommended for small and medium-volume prostates (28-29).

Holmium laser resection of the prostate (HoLRP)

Holmium laser resection of the prostate is a procedure similar to transurethral resection, because the laser beam performs segmental resection

of the adenoma all the way to the capsule. In relation to the volume of the prostate, the indication area is limited to 100 ml. Resection removes the tissue in small pieces, which are later exteriorized through the resectoscope sheath. The mass of the removed adenoma tissue is smaller in relation to the mass of the tissue removed by transurethral resection, due to the additional effect of HoLRP which vaporizes almost 50%. At the end of the operation, the appearance of the prostatic lodge is almost identical to the appearance it has after transurethral resection. Comparative studies in relation to TURP, after a follow-up period of 4 years, show lower perioperative morbidity, with similar results in relation to urodynamic parameters, IPSS, potency and continence (30,31).

CW2 Thulium laser

The CW2 thulium laser represents a new generation of lasers. With a wavelength of 2000nm, it provides better effects in continuous mode than in pulse mode. It can incise extraordinarily and has great hemostatic characteristics. Compared to the holmium laser, the thulium laser enables finer and more precise incisions It is also used for incisions of stenoses, tumors or renal parenchyma. The disadvantage in relation to the holmium laser is that the thulium laser is not used for lithotripsy. Surgical techniques in prostate surgery are similar to those of holmium lasers (HoLEP, HoLRP). Current studies show that the results of the holmium laser, when compared with the thulium laser, prostate enucleation are almost equal. Long term studies have not yet been conducted to confirm these preliminary results (31,32).

Conclusion

The evolution of lasers from coagulation to evaporation and enucleation of the prostate has provided urologists with a quality alternative for minimally invasive (operative) treatment of BPH, both in relation to transurethral resection of the prostate and in relation to open prostatectomy.

REFERENCES

1. Maiman TH. Stimulated optical radiation in ruby. Nature. 1960; 187: 493-4.
2. Einstein A. Zur Quantentheorie der Strahlung. Phys Z. 1917; 18: 121-8.

3. Teichmann HO, Herrmann TR, Bach T. Technical aspects of lasers in urology. World J Urol. 2007; 25: 221–5.
4. Bašić D. Fizičke osnove primene snažnih lasera u medicini. Magistarski rad; Medicinski fakultet u Nišu. Niš; 1997: 2-8.
5. Bašić D. Minimalno invazivno i hirurško lečenje benigne hiperplazije prostate. U: Prostata (Tomislav Pejčić, Jovan Hadži Đokić, Dragoslav Bašić, autori); Elit Medica, Beograd, 2014: 77-125 (ISBN 978-86-7222-104-8).
6. Bach T, Muschter R, Sroka R, Gravas S, Skolarikos A, Herrmann TR, Bayer T, Knoll T, Abbou CC, Janetschek G, Bachmann A, Rassweiler JJ. Laser treatment of benign prostatic obstruction: basics and physical differences. Eur Urol. 2012 Feb; 61(2): 317-25.
7. Grande M, Facchini F, Moretti M, Larosa M, Leone M, Ziglioli F, Pozzoli GL, Frattini A. Storia del Laser nella terapia dell'IPB [History of laser in BPH therapy]. Urologia. 2014 Jan-Mar; 81 Suppl 23: S38-42. Italian. doi: 10.5301/RU.2014.11985. Epub 2014 Mar 6. PMID: 24665033.
8. Rassweiler J, Teber D, Kuntz R, Hofmann R. Complications of transurethral resection of the prostate (TURP)—incidence, management, and prevention. Eur Urol. 2006; 50: 969–80.
9. Gratzke C, Schlenker B, Seitz M, et al. Complications and early postoperative outcome after open prostatectomy in patients with benign prostatic enlargement: results of a prospective multicenter study. J Urol. 2007; 177: 1419–22.
10. de la Rosette J, Collins E, Bachmann A, Choi B, Muir G, Reich O, Gomez-Sancha F , Tabatabaei S , Woo H. Historical Aspects of Laser Therapy for Benign Prostatic Hyperplasia. Eur Urol. 2012; 61: 317–325.
11. Dołowy Ł, Krajewski W, Dembowski J, Zdrojowy R, Kołodziej A. The role of lasers in modern urology. Cent European J Urol. 2015;68(2):175-82. doi: 10.5173/ceju.2015.537.
12. Kuntz RM. Current role of lasers in the treatment of benign prostatic hyperplasia (BPH). Eur Urol. 2006; 49: 961-969.
13. Te AE, Malloy TR, Stein BS, et al. Photoselective vaporization of the prostate for the treatment of benign prostatic hyperplasia: 12-month results from the first United States multicenter prospective trial. J Urol. 2004; 172: 1404–8.
14. Malek RS, Kuntzman RS, Barrett DM. Photoselective potassium-titanyl-phosphate laser vaporization of the benign obstructive prostate: observations on long-term outcomes. J Urol. 2005; 174: 1344–8.
15. Bachmann A, Schu¨ rch L, Ruszat R, et al. Photoselective vaporization (PVP) versus transurethral resection of the prostate (TURP): a prospective bi-centre study of perioperative morbidity and early functional outcome. Eur Urol. 2005; 48: 965–72.

16. Bouchier-Hayes DM, Anderson P, Van Appledorn S, Bugeja P, Costello AJ. KTP laser versus transurethral resection: early results of a randomized trial. J Endourol. 2006; 20: 580–5
17. Boxall NE, Georgiades F, Miah S, Dragos L, Armitage J, Aho TF. A call for HoLEP: AEEP for mega-prostates (≥ 200 cc). World J Urol. 2021 May 12. doi: 10.1007/s00345-021-03708-3. Epub ahead of print. PMID: 33978810.
18. Becker B, Gross AJ, Netsch C. Safety and efficacy using a low-powered holmium laser for enucleation of the prostate (HoLEP): 12-month results from a prospective low-power HoLEP series. World J Urol. 2018 Mar; 36(3): 441-447. doi: 10.1007/s00345-017-2159-5. Epub 2017 Dec 23. PMID: 29275506.
19. Netsch C, Becker B, Tiburtius C, Moritz C, Becci AV, Herrmann TRW, Gross AJ. A prospective, randomized trial comparing thulium vapoenucleation with holmium laser enucleation of the prostate for the treatment of symptomatic benign prostatic obstruction: perioperative safety and efficacy. World J Urol. 2017 Dec; 35(12): 1913-1921. doi: 10.1007/s00345-017-2071-z. Epub 2017 Jul 11. PMID: 28698991.
20. Becker B, Herrmann TRW, Gross AJ, Netsch C. Thulium vapoenucleation of the prostate versus holmium laser enucleation of the prostate for the treatment of large volume prostates: preliminary 6-month safety and efficacy results of a prospective randomized trial. World J Urol. 2018 Oct; 36(10): 1663-1671. doi: 10.1007/s00345-018-2321-8. Epub 2018 May 5. PMID: 29730838.
21. Enikeev D, Morozov A, Taratkin M, Misrai V, Rijo E, Podoinitsin A, Gabdullina S, Herrmann TRW. Systematic review of the endoscopic enucleation of the prostate learning curve. World J Urol. 2020 Sep 17. doi: 10.1007/s00345-020-03451-1. Epub ahead of print. PMID: 32940737.
22. Enikeev D, Glybochko P, Rapoport L, Gahan J, Gazimiev M, Spivak L, Enikeev M, Taratkin M. A Randomized Trial Comparing The Learning Curve of 3 Endoscopic Enucleation Techniques (HoLEP, ThuFLEP, and MEP) for BPH Using Mentoring Approach-Initial Results. Urology. 2018 Nov; 121: 51-57. doi: 10.1016/j.urology.2018.06.045. Epub 2018 Jul 24. PMID: 30053397.
23. Shigemura K, Fujisawa M. Current status of holmium laser enucleation of the prostate. Int J Urol. 2018 Mar; 25(3): 206-211. doi: 10.1111/iju.13507. Epub 2017 Dec 3. PMID: 29205507.
24. Kim A, Hak AJ, Choi WS, Paick SH, Kim HG, Park H. Comparison of long-term effect and complications between holmium laser enucleation and transurethral resection of prostate: Nations-wide health insurance study. Urology. 2021 Apr 29:S0090-4295(21)00349-6. doi: 10.1016/j.urology.2021.04.019. Epub ahead of print. PMID: 33933503.

25. Westenberg A, Gilling P, Kennett K, Frampton C, Fraundorfer M. Holmium laser resection of the prostate versus transurethral resection of the prostate: results of a randomized trial with 4-year minimum long-term followup. J Urol. 2004; 172: 616-619.
26. Tan A, Liao C, Mo Z, Cao Y. Meta-analysis of holmium laser enucleation versus transurethral resection of the prostate for symptomatic prostatic obstruction. Br J Surg. 2007; 94: 1201-1208.
27. Basić D, Stanković J, Potić M, Ignjatović I, Stojković I. Holmium laser enucleation versus transurethral resection of the prostate: a comparison of clinical results. Acta Chir Iugosl. 2013; 60(1): 15-20.
28. Thurmond P, Bose S, Lerner LB. Holmium laser for the surgical treatment of benign prostatic hyperplasia. Can J Urol. 2016 Aug;23(4): 8356-62. PMID: 27544558.
29. Barski D, Richter M, Winter C, Arsov C, de Geeter P, Rabenalt R, Albers P. Holmium laser ablation of the prostate (HoLAP): intermediate-term results of 144 patients. World J Urol. 2013 Oct; 31(5): 1253-9. doi: 10.1007/s00345-012-0901-6. Epub 2012 Jul 11. PMID: 22782618.
30. Whiles BB, Martin AJ, Brevik A, Carrera RV, Thompson JA, Molina WR, Thurmon KL. Utilization of MOSES Modulated Pulse Mode Results in Improved Efficiency in Holmium:YAG Laser Ablation of the Prostate. Urology. 2021 Mar; 149: 187-192. doi: 10.1016/j.urology.2020.12.024. Epub 2021 Jan 5. PMID: 33412223.
31. Tan A, Liao C, Mo Z, Cao Y. Meta-analysis of holmium laser enucleation versus transurethral resection of the prostate for symptomatic prostatic obstruction. Br J Surg. 2007; 94: 1201-1208.
32. Shao Q, Zhang FB, Shang DH, Tian Y. [Comparison of holmium and thulium laser in transurethral enucleation of the prostate]. Zhonghua Nan Ke Xue. 2009; 15: 346-349.

doi: 10.20903/zpopov-2022-0020 UDK: 616.69-008.14:616.13-004.6

Ognen IVANOVSKI MD, PhD[1]

PATHOPHYSIOLOGY OF ERECTILE DYSFUNCTION: FROM BENCH TO BEDSIDE

Abstract

Background. Erectile dysfunction (ED) and cardiovascular diseases share the same risk factors. Atherosclerosis not only affects the coronary arteries but also the penile arteries in men, thus contributing to organic causes of ED. Recently, the hypercholesterolemic atherosclerotic apolipoprotein $E^{-/-}$ deficient ($ApoE^{-/-}$) mouse model was introduced as a powerful experimental tool in ED research. The aim of this review is to characterize the development and distribution of atherosclerosis development in $ApoE^{-/-}$ mice and to try to test different treatment strategies in order to prevent or cure ED.

Experimental model. Serum cholesterol and triglycerides were significantly increased in $ApoE^{-/-}$ mice as compared to wild type mice. $ApoE^{-/-}$ mice displayed not only fatty streaks, but also widespread fibrous plaques at vascular sites that are typically affected in human atherosclerosis. The atherosclerotic lesions covered 15 ± 1.1 % of the aortic root section, 0.9% ± 0.2 of the aortic luminal surface, 21 ± 4.2 % of the aortic arch region and 26 ± 2.2% of the renal artery. In contrast, no atherosclerotic lesion formation was observed in wild type C57BL6/J mice. Interestingly, no atherosclerosis was observed in penile arteries. However, we have found increased staining of vasculature calcification, nitrotyrosin, macrophage/monocyte content as well as total collagen content at the vascular sites typically affected by atherosclerosis. Uremia significantly increases the degree of atherosclerosis as compared to the controls. Of note, even mild renal dysfunction, for example after uninephrectomy, increases the calcification score and aggravates endothelial function of cavernosal bodies in $apoE^{-/-}$ mice,

[1] University Clinic of Urology, Medical Faculty, University SS Cyril and Methodius, Skopje, North Macedonia; e-mail: ognen.ivanovski@medf.ukim.edu.mk

and this effect might be linked to increased oxidative stress in penile endothelium. Simvastatin significantly improved endothelial function of aortic and cavernosal tissues in uremic ApoE$^{-/-}$ mice via reduction of oxidative stress, and this effect does not seem to be associated with the lipid lowering effect of this drug.

Conclusions: The ApoE$^{-/-}$ mouse is a well-characterized model to study disorders associated with hypercholesterolemia and atherosclerosis in cardiovascular research. We anticipate that this mouse model will be useful to test treatment strategies aiming to target both atherosclerosis and erectile dysfunction.

Erectile dysfunction and cardiovascular disease

Erectile dysfunction (ED) is the persistent inability to achieve or maintain an erection sufficient to permit satisfactory sexual performance, and the resulting stress often impacts interaction with others (1). In the Western industrialized countries, prevalence of ED in the general population is approximately 20 to 30%, with an economical and significant psychological impact (2). In cardiovascular high-risk patients, prevalence of ED rises up to 75%, indicating the strong association of ED with the known cardiovascular risk factors and cardiovascular diseases (CVD) (3).

Atherosclerosis, which can be defined as a chronic and progressive disease characterized by an inflammatory response of the arterial wall, is still a leading cause of death, mainly in the Western world [4,5]. Atherosclerosis is an inflammatory process of the arterial walls and is initiated by endothelial dysfunction accompanied by an imbalance in the production of reactive oxygen species (ROS) and nitric oxide (NO). Erectile dysfunction (ED) and cardiovascular diseases share the same risk factors. Atherosclerosis not only affects the coronary arteries but also the penile arteries in men, thus contributing to organic causes of ED.

Explanation of the model

Recently, the hypercholesterolemic atherosclerotic apolipoprotein ApoE deficient (ApoE$^{-/-}$) mouse model was introduced as a powerful expe-

rimental tool in ED research. We and others have addressed important questions more directly in a mouse model of accelerated atherosclerosis (6), vascular calcification (7) and ED (8). Since wild type rats or mice do not easily develop atheromatous lesions, we used the most common mouse models to study atherogenic mechanisms, namely the apolipoprotein E gene knock-out ($ApoE^{-/-}$) model. $ApoE^{-/-}$ mice have delayed clearance of lipoproteins, and when placed on low-cholesterol, low-fat diets, their total serum cholesterol levels reach 11 to 13 mM as a result of the accumulation of chylomicrons and cholesterol-rich VLDL remnants, as compared with 2 to 3 mM in wild-type mice. Importantly, these genetically engineered mice develop not only fatty streaks but also widespread fibrous plaques at vascular sites that are typically affected in human atherosclerosis (9). Atherosclerotic lesion formation was evaluated with oil-red O staining on the following vascular sites: aortic root, aortic arch with its main branches: brachiocephalic artery, right common carotid artery and left subclavian artery, longitudinal aorta and renal arteries. Serum total cholesterol, triglycerides and urea levels were determined. The results were compared with a group of wild type C57BL6/J male mice. Interestingly, we have discovered that atherosclerotic lesions covered 15 ± 1.1 % of the aortic root section, 0.9% ± 0.2 of the aortic luminal surface, 21 ± 4.2 % of the aortic arch region and 26 ± 2.2% of the renal artery. In contrast, no atherosclerotic lesion formation was observed in wild type C57BL6/J mice. In addition, no atherosclerosis was observed in penile arteries in both types of mice. However, microscopic structural changes of the arterial wall were observed (see below).

Erectile dysfunction in uremia

Chronic kidney disease (CKD) in males causes sexual dysfunction with a prevalence ranging between 71% and 98% [10]. ED's physiopathology in uremia is complex and multifactorial, involving a combination of classical risk factors (obesity, glucose intolerance, hypercholesterolemia, smoking, and hypertension) and specific uremia-related risk factors (increased oxidative stress, endothelial dysfunction, inflammation). Endothelial dysfunction is associated with loss of nitric oxide (NO) bioavailability due to either reduced formation or accelerated degradation of NO. In turn, degradation of NO by increased reactive oxidant species including supero-

xide anion and oxidized lipoproteins might be a major mechanism underlying ED in these patients [11]. In addition to the uremic milieu, peripheral neuropathy, autonomic insufficiency, peripheral vascular disease, and pharmacologic therapy all play an important role in the genesis of this problem.

Endothelial dysfunction and accelerated atherosclerosis are some of the primary causes of morbidity and mortality in patients with CKD [12]. ED and CVD share the same risk factors. Accelerated atherosclerosis not only affects the coronary arteries but also may affect penile arteries in men, thus contributing to arteriogenic causes of ED in CKD patients. Thus atherosclerosis as a general health problem may be a link between these two entities [13]. We have recently published the effect of CKD on vascular calcification and endothelial function of cavernosal bodies in apoE$^{-/-}$ mice as a new model of ED research (14). We have created 2 uremic groups with different degree of uremia by using serum urea levels to assess renal function. At 16 weeks after uremia-inducing surgery, serum urea concentrations in uremic mice were 200% increased, as compared with those in the sham-op group. Likewise, uninephrectomized mice showed significant increase in serum urea level compared to the controls. Serum total cholesterol and triglyceride levels also were significantly higher in uremic and uninephrectomized mice. Both groups had higher serum calcium concentrations), whereas the serum phosphate level did not differ between groups (P = NS, Table 1). Mean arterial blood pressure did not differ between the groups, either. Atherosclerotic lesions in thoracic aorta were significantly larger both uremic groups compared to the non-uremic controls. There was no atheromatous lesions in cavernosal bodies or penile arteries observed in any group. However, uremic animals showed a significant increase in calcification score in both aorta and cavernosal bodies when compared with controls (Figure 1A, 1B). In addition, calcification score in the cavernosal bodies was significantly higher in uninephrectomized mice, as compared with the controls (Figure 1B). No such difference was observed in the aorta between uninephrectomized mice and the controls (Figure 1A). To investigate whether uremia had an impact on the cavernosal body's morphology, detailed histological studies analyzing nitrotyrosine expression, collagen content, and macrophage infiltration, were performed. Collagen content was higher in cavernosal bodies of uremic mice than in those of the controls. (Table 2, Figure 2A). Furthermore, uremic mice showed increased nitrotyrosine expression in the cavernosal bodies

compared with sham-operated controls (Table 2, Figure 2B). The percentage of the lesion cross-section area occupied by macrophages, as revealed by MOMA-2 staining, was comparable between the 3 groups (P=NS, Table 2).

Partial nephrectomy and atherosclerosis

Radical nephrectomy (RN) as compared to partial nephrectomy (PN) increases the risk of CKD in patients with kidney tumors. CKD is a significant risk factor for cardiovascular events and death. Given equivalent oncological efficacy of both surgical approaches in patients with small (15) and large renal tumors (16), several recent studies report that patients treated with PN have better cardiovascular health and global survival rates than those undergoing RN (17, 18) This survival advantage was also observed after surgery for histologically benign renal tumors, comparing PN with RN (19) Thus, the widespread use of RN may result in an unnecessary removal of functional nephron mass in many patients with kidney tumors and increase the risk of cardiovascular and all-cause mortality, particularly in individuals with small kidney tumors who are unlikely to die of renal cancer.

There is an increasing body of literature on the deleterious effects of decreased renal function on overall survival in the general population (20). After stratification for age, gender, race, and the presence or absence of diabetes, cardiovascular mortality in patients with CKD is 10 to 20 times higher than in the general population (21) due to a variety of CKD-linked complications including accelerated atherosclerosis and arterial stiffening.

The association between poor renal functional outcomes and cardiovascular outcomes is relevant for kidney cancer management as well. Several studies have demonstrated poor kidney function outcomes in patients treated with RN rather than PN (22, 23). Recently, Huang et al reported that a 3-year probability of freedom from new onset CKD was only 35% in patients who underwent RN compared with 80% in those who underwent PN. The average excess loss of renal function observed with RN was associated with a 25% increase in the risk of cardiac death and a 17% increase in the risk of death from any cause (19)

The clinical significance of iatrogenic CKD and in particular the impact of kidney surgery on long-term cardiovascular outcomes remain

research topics of great importance. Even mild CKD is associated with numerous metabolic and endocrine disturbances, including arterial hypertension, abnormalities of calcium and phosphate metabolism, and a state of chronic inflammation and oxidative stress. They contribute to the development and progression of arteriosclerosis, atherosclerosis, vascular and valvular calcification, and cardiac disease.

It is important to further investigate the pathophysiological mechanisms involved in the increased cardiovascular risk of patients with mild CKD. We have shown, for the first time, that PNX, in contrast to UNX, does not stimulate CKD-enhanced atherosclerosis progression in the experimental model of the apoE$^{-/-}$ mouse. Our study lends further support to the still controversial view that even mild renal dysfunction, such as that occurring after unilateral nephrectomy, may cause an increase in atherosclerotic plaque size in presence of pre-existing atheromatous disease. Our finding is in agreement with two other recent reports on the deleterious effects of UNX on the apoE$^{-/-}$ mouse model (14-26) and extends these results by showing that unlike UNX, PNX prevents accelerated atherosclerosis and plaque composition changes in this mouse model.

Partial nephrectomy preserves functional nephron mass and offers cancer specific survival, equivalent to that of radical nephrectomy. The results obtained in our experimental study, if they can be extrapolated to the human condition, represent further support for an expanded use of nephron sparing techniques among patients with kidney cancer. In addition, we have shown that even uninephrectomy has a deterious effect on erectile function by making structural changes to the cavernosal bodies.

Treatment measures

Statin is an inhibitor of the enzyme 3-hydroxy-methylglutaryl-CoA reductase. It suppresses the conversion of 3-hydroxy-methylglutaryl-CoA to mevalonate, which is the rate-limiting step in *de novo* synthesis of cholesterol. 10 Recent evidence suggests that even in the absence of cholesterol lowering, statins may be beneficial in the treatment of ED and endothelial dysfunction, due to direct anti-inflammatory and anti-oxidative actions at the arterial site. These local actions may occur via an upregulation of endothelial nitric oxide (NO) synthase with increased bioavailability of NO, a

decrease in cellular proliferation, an increase in apoptosis and/or an interference with local oxidative injury [6]. Many of these pleiotropic statin effects are mediated by their ability to block the production of isoprenoid intermediates such as farnesyl or geranylgeranyl pyrophosphates. These isoprenoid derivatives play an important role in the activation of small GTP-binding proteins, including Rho, Ras, and Rac, through an isoprenylation process [7]. Moreover, a mevalonate pathway–independent effect of statins has been reported previously, although the mechanism has not yet been elucidated [8].

Several studies found that statins could rapidly improve endothelial function, even before changing the lipid profile.12, 13. However, the precise involvement of such pleiotropic effects in the efficacy of statins in patients remains unclear because of the difficulty of separating lipid-related versus non-lipid related responses in clinical trials. In addition, some studies suggested that statin therapy was associated even with reduced levels of testosterone and even symptoms of hypogonadism. 15, 16

One of the differences with the clinical setting, however, is that, in apolipoprotein E deficient models, statins do not decrease serum cholesterol levels. Therefore, any potential statin benefit in these animals can be interpreted in the absence of a change of this confounding variable [9].

Simvastatin treatment led to a significant decrease in both nitrotyrosine expression and collagen content in the corpus cavernosum and in the atheromatous plaques of CKD mice (Fisher's exact test, p 0.02, table 2). As revealed by monocyte-macrophage-2 staining, the percent of lesion cross-sectional area occupied by macrophages was comparable in the 4 groups.

We evaluated the effects of the HMG-CoA reductase inhibitor on uremia enhanced atherosclerosis and vascular calcification in apoE$^{-/-}$ mice with superimposed CKD. We found that CKD enhanced aortic plaque development in apoE$^{-/-}$ mice compared with the controls, in agreement with previous reports by us and others.10, 12 Simvastatin treatment did not reduce the atherosclerotic lesions in the aortic root or thoracic aorta in this experimental model, in line with its failure to decrease serum total cholesterol. Nevertheless, simvastatin therapy led to a significant decrease in intima calcium content, that is, the calcium content of atheromatous plaques. It did not, however, change medial calcium deposition. Vascular calcification is a prominent feature of CKD13, and it is predictive of increased

cardiovascular morbidity and mortality.13 Coronary artery plaques in patients with end stage renal failure are characterized by increased medial thickness and marked intimal and medial calcification.3 Treatment with statins has been shown to be associated with a decrease in vascular calcification in two retrospective clinical studies 4,14 and in animal models such as the apoE$^{-/-}$ Leiden mouse15 but not in another prospective trial.5 This effect is generally associated with a decrease in serum lipid levels, which was not the case in the current study. The beneficial effect of simvastatin on CKD mice had severe atherosclerotic lesions in the thoracic aorta that were significantly increased compared to those in the non-CKD controls (fig. 2). Long-term Simvastatin treatment did not decrease uremia associated atherosclerosis in the aortic root or in the thoracic aorta. The same was true in the control non-CKD mice (fig. 2). Analyses of corpus spongiosum did not show any Red O Oli positive lesions in all groups. As expected, the apoE$^{-/-}$ mice showed higher plasma cholesterol than the WT animals [17-20], exhibiting approximately a 14-fold increase in total cholesterol, as recently reviewed.

Figure 1

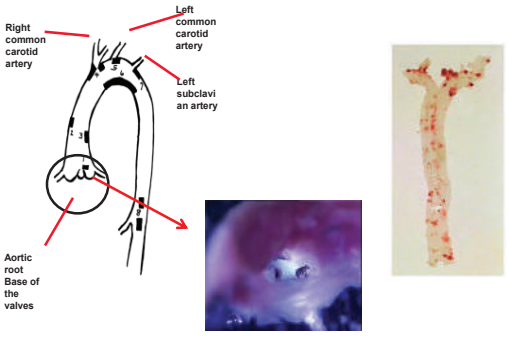


Figure 2

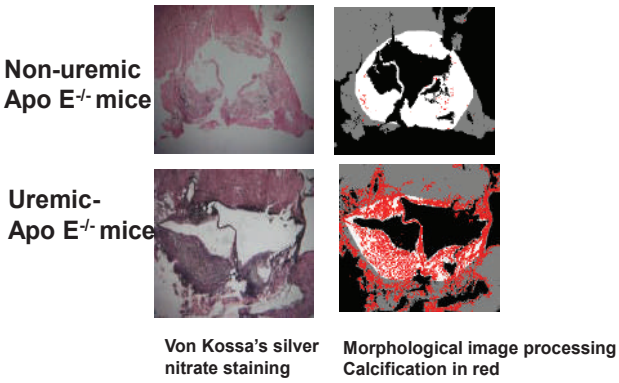


Figure 3A

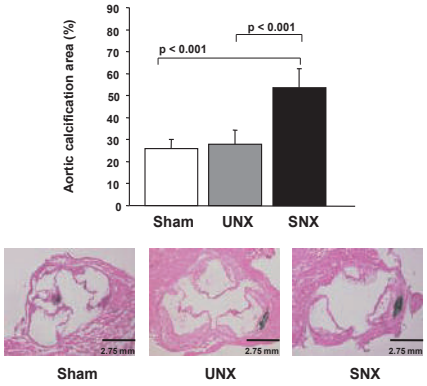


Figure 3A

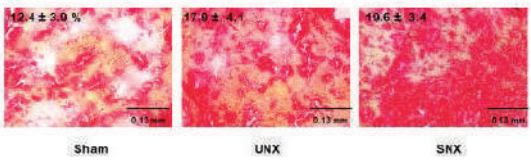
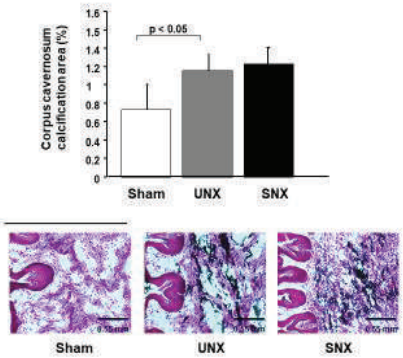


Figure 3B



REFERENCES

1. Lue T.F. Erectile dysfunction. N Engl J Med. 2000; 342: 1802-1813
2. Braun M, Wassmer G, Klotz T, Reifenrath B, Mathers M, Engelmann U. Epidemiology of erectile dysfunction: results of the 'Cologne Male Survey'. Int J Impot Res. 2000; 12: 305-11.
3. Montorsi P, Ravagnani PM, Galli S, Rotatori F, Briganti A, Salonia A, Rigatti P, Montorsi F (2005) The artery size hypothesis: a macrovascular link between erectile dysfunction and coronary artery disease. Am J Cardiol 96 (12B):19M-23M. doi:10.1016/j.amjcard.2005.07.006
4. Ross R. Atherosclerosis - an inflammatory disease. N Engl J Med. 1999; 340: 115–126.
   doi: 10.1056/NEJM199901143400207. [PubMed] [CrossRef] [Google Scholar]

5. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011; 473: 317–325.
doi: 10.1038/nature10146. [PubMed] [CrossRef] [Google Scholar]
6. Buzello M, Tornig J, Faulhaber J, Ehmke H, Ritz E, Amann K (2003) The apolipoprotein e knockout mouse: a model documenting accelerated atherogenesis in uremia. Journal of the American Society of Nephrology : JASN 14 (2): 311-316
7. Massy ZA, Ivanovski O, Nguyen-Khoa T, Angulo J, Szumilak D, Mothu N, Phan O, Daudon M, Lacour B, Drueke TB, Muntzel MS (2005) Uremia accelerates both atherosclerosis and arterial calcification in apolipoprotein E knockout mice. Journal of the American Society of Nephrology: JASN 16 (1):109-116. doi:10.1681/ASN.2004060495
8. Xie D, Odronic SI, Wu F, Pippen AM, Donatucci CF, Annex BH (2007) A mouse model of hypercholesterolemia-induced erectile dysfunction. The journal of sexual medicine 4 (4 Pt 1):898-907.
doi:10.1111/j.1743-6109.2007.00518.x
9. 8. Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL (1992) Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 71 (2): 343-353
10. Navaneethan SD, Vecchio M, Johnson DW, Saglimbene V, Graziano G, Pellegrini F, Lucisano G, Craig JC, Ruospo M, Gentile G, Manfreda VM, Querques M, Stroumza P, Torok M, Celia E, Gelfman R, Ferrari JN, Bednarek-Skublewska A, Dulawa J, Bonifati C, Hegbrant J, Wollheim C, Jannini EA, Strippoli GF Prevalence and correlates of self-reported sexual dysfunction in CKD: a meta-analysis of observational studies. Am J Kidney Dis 56 (4): 670-685
11. Beckman JS, Koppenol WH (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 271 (5 Pt 1):C1424-1437
12. Foley RN, Parfrey PS, Sarnak MJ (1998) Epidemiology of cardiovascular disease in chronic renal disease. Journal of the American Society of Nephrology : JASN 9 (12 Suppl): S16-23
13. Montorsi P, Ravagnani PM, Galli S, Rotatori F, Briganti A, Salonia A, Rigatti P, Montorsi F (2005) The artery size hypothesis: a macrovascular link between erectile dysfunction and coronary artery disease. Am J Cardiol 96 (12B):19M-23M. doi:10.1016/j.amjcard.2005.07.006
14. Ivanovski O, I, Davceva O, Petrushevska G. Calcification of the cavernosal bodies may be responsible for development of erectile dysfunction in uremic apolipoprotein E deficient (apoE-/-) mice. Rev Int Androl Jan-Mar 2021; 19(1): 25-3

15. Patard JJ, Pantuck AJ, Crepel M, et al. Morbidity and clinical outcome of nephron-sparing surgery in relation to tumour size and indication. Eur Urol. 2007; 52: 148-154.
16. Becker F, Roos FC, Janssen M, et al. Short-term functional and oncologic outcomes of nephron-sparing surgery for renal tumours >/= 7 cm. Eur Urol. 2011; 59: 931-937.
17. Thompson RH, Siddiqui S, Lohse CM, et al. Partial versus radical nephrectomy for 4 to 7 cm renal cortical tumors. J Urol. 2009; 182: 2601-2606.
18. Weight CJ, Larson BT, Fergany AF, et al. Nephrectomy induced chronic renal insufficiency is associated with increased risk of cardiovascular death and death from any cause in patients with localized cT1b renal masses. J Urol. 2010; 183: 1317-1323.
19. Weight CJ, Lieser G, Larson BT, et al. Partial nephrectomy is associated with improved overall survival compared to radical nephrectomy in patients with unanticipated benign renal tumours. Eur Urol. 2010; 58: 293-298.
20. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004; 351: 1296-1305.
21. Foley RN, Parfrey PS, Sarnak MJ. Epidemiology of cardiovascular disease in chronic renal disease. J Am Soc Nephrol. 1998; 9: S16-23.
22. McKiernan J, Simmons R, Katz J, Russo P: Natural history of chronic renal insufficiency after partial and radical nephrectomy. Urology. 2002; 59: 816-820.
23. Lau WK, Blute ML, Weaver AL, Torres VE, Zincke H. Matched comparison of radical nephrectomy vs nephron-sparing surgery in patients with unilateral renal cell carcinoma and a normal contralateral kidney. Mayo Clin Proc. 2000; 75: 1236-1242.
24. Ivanovski O, Nikolov IG, Davceva O, Gjorgjievska K, Janevska V, Petrushevska G. Compared With Radical Nephrectomy, Nephron-sparing Partial Nephrectomy Protects Apolipoprotein E-deficient Mice From Atherosclerosis Progression. Urology. 2015 May; 85(5)
25. Buzello M, Tornig J, Faulhaber J, et al. The apolipoprotein E knockout mouse: a model documenting accelerated atherogenesis in uremia. J Am Soc Nephrol. 2003; 14: 311-316.
26. Piecha G, Koleganova N, Gross ML, Geldyyev A, Ritz E. Oxidative stress after uninephrectomy alters heart morphology in the apolipoprotein E-/- mouse. J Hypertens. 2008; 26: 2220-2229.

doi: 10.20903/zpopov-2022-0021 UDK: 616.348/.35-006.6-089.819(497.7)"2015/2019"

Nikola JANKULOVSKI[1] Svetozar ANTOVIC[1],
Vladimir JOKSIMOVIC[1], Biljana KUZMANOVSKA[2]

PERIOPERATIVE OUTCOMES OF THE LAPAROSCOPIC TREATMENT FOR COLORECTAL CANCER AT THE CLINIC FOR DIGESTIVE SURGERY, SKOPJE IN A 5-YEAR INTERVAL

Abstract

Colorectal cancer (CRC) is the third leading cause of cancer related death in the world, and its incidence is rising in developing nations. Taking into account the increase in the incidence of this disease, the purpose of this review was to evaluate perioperative outcomes (in the first 30 days after surgery) for **laparoscopic treatment** of this malignancy, a treatment that is steadily becoming standard of patient care in the world.

Our review showed that at the Clinic for Digestive Surgery, from 2015 to 2019, 115 patients with colorectal cancer were treated laparoscopically. The figures show a growing trend during this period. 10% of all colorectal cancers in 2019 were completed laparoscopically. In most cases (88.7%) tumor staging was pT2 and pT3. Perioperative outcomes showed wound infection in 2 patients, pulmonary complications in 1, anastomotic leakage in 1, bleeding in 1 patient, no readmission, and no mortality. The rate of conversion to open access is 5.7%, the operating time was 198 minutes on average, the average number of hospital stays was 9 days, and the average number of extirpated lymph nodes 13. There was no need for blood transfusion.

[1] University Clinic for Digestive Surgery, Medical Faculty, University of Ss. Cyrill and Methodius, Skopje, R. North Macedonia

[2] University Clinic for Anesthesiology, Reanimatology and Intensive Care, Medical Faculty, University of Ss. Cyril and Methodius, Skopje, R. North Macedonia

Perioperative results for laparoscopic treatment of colorectal cancer at our institution show a low morbidity and mortality rate in these patients, with a clearly rising number in laparoscopically operated patients each year. Long term results are yet to be seen. Follow up with these patients will provide results later.

Key words: colorectal cancer, laparoscopy, outcomes

Introduction

CRC is the third most commonly diagnosed cancer in males and the second most in females. The global incidence of CRC in 2018 was 1.8 million new cases and nearly 860,000 deaths (World Health Organization GLOBOCAN database). Age-standardized (world) incidence rates per 100,000 of CRC in both sexes is 19.7: 23.7 in males, and 16.2 in females. Rates of colorectal cancer in younger patients have been increasing over the past few years, making this disease even more important to observe. Recent advances in screening for early detection and treatment have reduced CRC mortality in developed nations, despite the circumstances of growing incidence.

The emergence of laparoscopy has brought forth a revolution in the surgical approach to colonic resections for cancers. Laparoscopic colectomy was first established in 1991. Initially, it was not widely accepted as a cancer treatment because there were some technical difficulties (working in multiple intra-abdominal quadrants, ligation of vessels and re-establishment of intestinal continuity, as well as oncological concerns which included the retrieval of lymph nodes, surgical resection margin and survival results). These controversies died down as surgeons gained more experience as well as the technological progress of instrumentation.[1] Large prospective randomized trials comparing these two approaches of treatment (Lap vs Open) have found no significant differences between open and laparoscopic colectomy, with regards to the intraoperative or postoperative complications (perioperative mortality rates, readmission or reoperation rates, or rate of surgical wound recurrence). Also the results of oncologic outcomes (cause-specific survival, disease recurrence, number of gathered lymph nodes), are likewise comparable.[2] Although laparoscopic surgery continues to be considered the foremost common approach for the treatment of colorectal cancer, new surgical technologies are emerging including transanal total mesorectal

excision, laparoscopic lateral pelvic lymphatic node tissue dissection and robotic surgery.

Implementing laparoscopic treatment for CRC is by no means an easy task for even experienced surgeons and hospitals. It involves a steep learning curve and requires a dedicated team that, with persistent endurance, will accumulate enough skills for a safe and quality laparoscopy. This is why perioperative outcomes are very important in assessing the quality of laparoscopy itself as a treatment method for this disease.

The aim of this study is not to compare laparoscopic with the open treatment of colorectal cancer, nor to establish which is better, since many studies have already addressed this issue. It is simply to show our ability to perform laparoscopy for colorectal cancer in our clinic and to show the perioperative results from it.

Materials and methods

Our paper represents a retrospective analysis of laparoscopic surgical treatment in patients with colorectal cancer who were operated on at the Clinic for Digestive Surgery, Skopje in the period from 2015 to 2019. The analysis covers the trend of the number of operated patients in the given period, the proportion of different surgical procedures, the relationship between laparoscopic and open access, the rates of perioperative morbidity and perioperative outcomes.

The incidence of patients with colorectal cancer in R.N. Macedonia is 25.7/100,000 inhabitants. The number of annual newly diagnosed cases is around 600, of which about 300-350 are surgically treated at the Clinic for Digestive Surgery in Skopje. Three-quarters of patients have advanced stages of the disease, at least stage III.

As a developing country, the first case of laparoscopic surgery at our Clinic coincides with the year when the world's first laparoscopic colectomy was performed, 1991. The first laparoscopic resection of the rectum in our country was performed in 2003.

The review of our data shows that in the period of 2015-2019, a total of 115 laparoscopic operations for colon and rectal cancer were performed at our Clinic. The numbers of laparoscopic treatment of the colon and rectum show a yearly upward trend. This trend has increased thus: 16 (2015),

18 (2016), 22 (2017), 30 (2018), 29 (2019). In 2019, 10% of all surgically treated patients with colorectal cancer at our Clinic were treated laparoscopically (29 out of 290 operated patients). Out of a total of 115 cases for the 5-year time interval, 28 (24.3%) patients underwent right hemicolectomy, 37 (32.1%) left hemicolectomy, anterior resection of the rectum was observed in 32 (27.8%) patients, while 18 (15.6%) patients underwent rectal amputation. As for the tumor staging, among 115 patients, the distribution of cases is 5.2% (pT1), 34.7% (pT2), 53% (pT3), 6.95% (pT4).

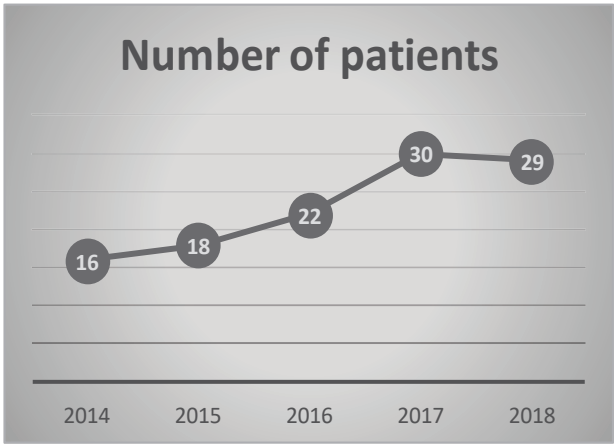


Figure 1 – Number of patients

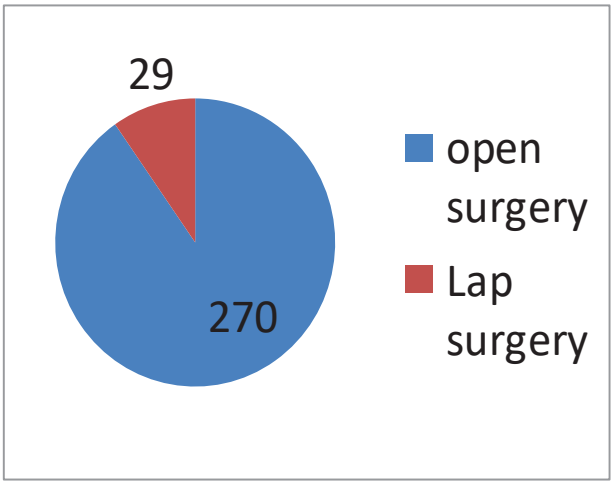


Figure 2 – Laparoscopy for colon/rectum 2019

Table 1

Laparoscopy for colon/rectum - 5 year period

Operation performed	Number of cases
Right hemicolectomy	28
Left hemicolectomy	37
Anterior resection	32
Amputation of rectum	18
Total	115

Table 2

Laparoscopy for colon/rectum- Tumor stage

Tumor stage	Number of cases
pT1	6 5,2%
pT2	40 34.7%
pT3	61 53%
pT4	8 6,95%
Total	115 100%

The analysis of the perioperative outcomes gave the following results: conversion to open surgery 5.7%, average duration of the operation 198 min (125-285 min), the need for blood transfusions - 0%, hospital stay an average of 9 days (7-16), and the average number of dissected lymph nodes 13 (8–25).

Perioperative morbidity analysis, on the other hand, covered the following variables: wound infection 1.7% (2 patients), pulmonary complications in only 1 patient, bleeding, stepler port bleeding in only 1 patient, anastomotic leakage in 1 patient (conservative care), readmission to hospital 0, mortality 0.

Table 3

Perioperative outcomes

Variable	**Number (%)**
Conversion to open	7/122 (5.7%)
Operative time (min)	198 min (125-285)
Blood transfusion	0
Length of stay (day	9 (7-16)
Lymph node yield	13 (8-25)

Table 4

Perioperative morbidity

Variable	**Number (%)**
Wound infection	2 (1.7%)
Pulmonary	1
Bleeding	1Stapler port bleeding (operated)
Anastomotic leakage	1 Conservative treatment
Readmission to hospital	0
Mortality	0

Discussion

Although this paper reflects our perioperative experience with the laparoscopic treatment of colorectal cancer within the 5-year interval, without direct comparison with open surgical access, the results show that patients who receive laparoscopic treatment have a short hospital stay, operating time equivalent to the open surgery approach, very low rates of wound infections, bleeding and anastomotic leakage, low pulmonary complications, no admission to the ICU unit or need for mechanical ventilation, no need for blood transfusions, no repeated hospitalizations and no mortality. Although our clinical long-term oncological outcomes of laparoscopic vs. open surgery remain to be seen, these perioperative results show that recovery, physiological function, and other short-term outcome measures are improved with the laparoscopic approach.[3-6]

It has been reported that hospital volume, surgeon volume, and, therefore, the rate of laparoscopic surgery may affect the results of colorectal surgery. Higher hospital and surgeon volume are generally related to better outcomes after laparoscopic surgery for colorectal cancer.[7] However, what is evident is that although our Clinic does not have a high rate of laparoscopic colorectal surgery, the rates of complications are still very low. This could be result of the high rate of laparoscopic treatment for other digestive pathologies at our clinic and the right selection of cases for laparoscopy.

In our study, laparoscopic surgery for colorectal cancer was associated with comparable operation time to the open access surgery, which was slightly lower but generally compatible with most previous reports. [4,15] Although prolonged operation time was a suggested possible risk factor for the development of postoperative pulmonary complications, our results show that laparoscopic surgery was associated with no ICU admission and postoperative mechanical ventilation. This was outweighed by other factors like incision size and pain. Conversion from laparoscopic to open surgery has been related to worse outcome, but this has probably been associated with issues stemming from the learning curve. Risk factors for conversion for various populations are widely reported within the literature. Clancy et al. recently performed a meta-analysis of 15 studies and reported a mean conversion rate of 17.9% (± 10.1%) with males, rectal tumor, T3/T4 stage and node-positive disease as factors that negatively influence the completion

of laparoscopic surgery.[16] Our analysis, however, shows that although most of our cases - 53% - were T3, the conversion rate remained low - only 5.7%. Conversion rates are expected to scale back over time. The CLASICC trial, for example, had a conversion rate of 34% [17] for rectal cancer, while this was 16% [18] in additional recently published trials from Western population regions. Surgical experience is one of the most crucial elements for quality patient care in laparoscopic procedures. Recent studies show that with increasing laparoscopic hospital volume, conversion decreases below 10% with only a minimal impact of conversion on short-term postoperative outcome. To perform an early conversion may be an appropriate decision, and this kind of conversion should not be considered a failure.[19]

Some oncological parameters like tumor size, number of lymph nodes retrieved, and surgical margin, are important to assess the oncological adequacy of the operation. Of those, lymph node status is probably the strongest pathologic predictor of patient outcome, and it represents a high quality indicator for cancer care. Sufficient node staging (TNM) is absolutely essential to establish definitive diagnosis and prognosis of the patient and is essential for planning further oncologic treatment. Several studies support that number of lymph nodes harvested during an operation (minimum 12 lymph nodes) is one of the strongest predictor for cancer treatment because it is associated with a better survival rate. In our study, the average number of harvested lymph nodes was 13, which is a very important result, showing adequate oncological resection. In terms of tumor size, our data shows that almost all of the operated patients had PT2, PT3 lesions (87.7% together), which is a reflection of the low screening rate within our population. Laparoscopic surgery for advanced colorectal cancer has become widespread, with demonstrated short-term benefits and better long-term oncological outcomes than open surgery.[4,16,21-23]However, for locally advanced pathological T4 (pT4) carcinoma based on the American Joint Committee on Cancer (AJCC) TNM staging system,[24] the safety and feasibility of laparoscopic procedures remain controversial. In pT4 carcinoma, technically demanding surgical procedures, including en bloc resection of adjacent infiltrated organs or structures, are generally required. It is well-known that open multivisceral resection for pT4 colon cancer includes a high postoperative morbidity and a high risk of microscopically positive surgical margins.[25] For these reasons, some authors consider pT4 colon cancer to be a relative contraindication to laparoscopic surgery which could lead to prolon-

ged operative time, an increased conversion rate, higher postoperative morbidity, and, most significantly, suboptimal oncological results.[26] In our case, the selective approach provided a comparatively low rate of operated patients in pT4 colorectal cancer of only 6.5%.

It is essential to perform strict oncologic resections and for pathologists to conduct a meticulous evaluation of specimens.[27]

Conclusion

Laparoscopic surgery, which is becoming a standard treatment for colon and rectal cancer in the USA and Europe, has several benefits over open surgery in terms of short-term outcomes such as decreased pain, improved pulmonary function in the postoperative period, lower rates of postoperative ileus, lower incidence of wound infection, faster recovery, and shorter hospital stay. Further, as shown by the results of several randomized controlled trials, the long-term outcomes after laparoscopic surgery for colorectal cancer are comparable to those after open surgery. An overview of our data has shown excellent short-term perioperative outcomes as well as solid oncological surgical parameters. The long-term outcome of these cases remains to be considered.

REFERENCES

1. Clinical Outcomes of Surgical Therapy Study Group. *A comparison of laparoscopically assisted and open colectomy for colon cancer. N Engl J Med. 2004 May 13; 350(20): 2050–9*
2. Veldkamp R, Kuhry E, Hop WC, et al. *Laparoscopic surgery versus open surgery for colon cancer: short-term outcomes of a randomised trial. Lancet Oncol*. 2005 Jul. 6(7): 477-84.
3. Clinical Outcomes of Surgical Therapy Study, G. *et al*. *A comparison of laparoscopically assisted and open colectomy for colon cancer*. *N. Engl. J. Med.*, 2004; 350, 2050–2059,
4. Lacy, A. M. *et al*. *Laparoscopy-assisted colectomy versus open colectomy for treatment of non-metastatic colon cancer: a randomised trial*. *Lancet*, 2002; 359, 2224–2229,

5. Veldkamp, R. *et al*. Laparoscopic surgery versus open surgery for colon cancer: short-term outcomes of a randomised trial. *Lancet Oncol.*, 2005; 6 477–484,
6. Jayne, D. G. *et al*. *Randomized trial of laparoscopic-assisted resection of colorectal carcinoma: 3-year results of the UK MRC CLASICC Trial Group*. *J. Clin. Oncol.*, 2007; 253061–3068,
7. Huo, Y. R., Phan, K., Morris, D. L. & Liauw, W. *Systematic review and a meta-analysis of hospital and surgeon volume/outcome relationships in colorectal cancer surgery*. *J. Gastrointest. Oncol.* 2017; 8 534–546,
8. Doyle, R. L. *Assessing and modifying the risk of postoperative pulmonary complications*. *Chest* 1999; 115.77S–81S
9. Fougeres, E. *et al*. *Hemodynamic impact of a positive end-expiratory pressure setting in acute respiratory distress syndrome: importance of the volume status. Crit. Care Med.* 2010; 38 802–807,
10. Schweickert, W. D. *et al*. *Early physical and occupational therapy in mechanically ventilated, critically ill patients: a randomised controlled trial*. *Lancet*, 2010; 3731874–1882,
11. Southall J C, Lee S W, Allendorf J D, Bessler M, Whelan R L. *Colon adenocarcinoma and B-16 melanoma grow larger following laparotomy vs. pneumoperitoneum in a murine model. Dis Colon Rectum.* 1998; 41(5): 564–569
12. Allendorf J DF, Bessler M, Kayton M L, et al. *Increased tumor establishment and growth after laparotomy vs laparoscopy in a murine model. Arch Surg.* 1995; 130(6): 649–653
13. Lee S W, Feingold D L, Carter J J, et al. *Peritoneal macrophage and blood monocyte functions after open and laparoscopic-assisted cecectomy in rats. Surg Endosc*. 2003; 17(12): 1996–2002.
14. Fleshman J, Sargent DJ, Green E, Anvari M, Stryker SJ, Beart RW, Jr, et al. *Laparoscopic colectomy for cancer is not inferior to open surgery based on 5-year data from the COST Study Group trial. Ann Surg.* 2007; 246: 655–662.
15. Shantha Kumara H M, Feingold D, Kalady M, et al. *Colorectal resection is associated with persistent proangiogenic plasma protein changes: postoperative plasma stimulates in vitro endothelial cell growth, migration, and invasion. Ann Surg.* 2009; 249(6): 973–977.
16. Jayne, D. G. *et al. Randomized trial of laparoscopic-assisted resection of colorectal carcinoma: 3-year results of the UK MRC CLASICC Trial Group. J. Clin. Oncol.* 2007; 25: 3061–3068,

17. Clancy C, O'Leary DP, Burke JP, Redmond HP, Coffey JC, Kerin MJ, et al. *A meta-analysis to determine the oncological implications of conversion in laparoscopic colorectal cancer surgery. Colorectal Dis.* 2015; 17: 482–490.
18. Guillou PJ, Quirke P, Thorpe H, Walker J, Jayne DG, Smith AM, et al. *Short-term endpoints of conventional versus laparoscopic-assisted surgery in patients with colorectal cancer (MRC CLASICC trial): multicentre, randomised controlled trial. Lancet.* 2005; 365: 1718–1726.
19. van der Pas MHGM, Deijen CL, Abis GSA, de Lange-de Klerk ESM, Haglind E, Fürst A, Lacy AM, Cuesta MA, Bonjer HJ *Conversions in laparoscopic surgery for rectal cancer. Surg Endosc* 2017; 31: 2263–2270
20. Michael P. M. de Neree tot Babberich, Julia T. van Groningen, Evelien Dekker, Theo Wiggers, Michel W. J. M. Wouters, Willem A. Bemelman, Pieter J. Tanis, Laparoscopic *Surg Endosc.* 2018; 32(7): 3234–3246Surg Endosc. 2018; 32(7): 3234–3246
21. Baxter NN, Virgin D, et al. Lymph Node Evaluation in Colorectal Cancer Patients: *A Population-Based Study. Journal of the National Cancer Institute.* 2005; 97(3): 219–225
22. Yamamoto S, Inomata M, Katayama H, Mizusawa J, Etoh T, Konishi F, et al. *Short-term surgical outcomes from a randomized controlled trial to evaluate laparoscopic and open D3 dissection for stage II/III colon cancer: Japan Clinical Oncology Group Study JCOG 0404.* Ann Surg. 2014; 260: 23–30.
23. Kitano S, Inomata M, Mizusawa J, Katayama H, Watanabe M, Yamamoto S, et al. *Survival outcomes following laparoscopic versus open D3 dissection for stage II or III colon cancer (JCOG0404): a phase 3, randomised controlled trial. Lancet Gastroenterol Hepatol.* 2017; 2: 261–268.
24. Edge SB, Compton CC. *The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol*. 2010; 17: 1471–1474.
25. Mohan HM, Evans MD, Larkin JO, Beynon J, Winter DC. *Multivisceral resection in colorectal cancer: a systematic review. Ann Surg Oncol.* 2013; 20: 2929–2936.
26. Buunen M, Veldkamp R, Hop WC, Kuhry E, Jeekel J, Haglind E, et al. *Survival after laparoscopic surgery versus open surgery for colon cancer: long-term outcome of a randomised clinical trial. Lancet Oncol.* 2009; 10: 44–52.
27. Wong S. *Lymph Node Counts and Survival Rates After Resection for Colon and Rectal Cancer. Gastrointestinal Cancer Research*. 2009; 2(3) Supplement 1: S33–35.

doi: 10.20903/zpopov-2022-0022 UDK: 616.6-089.844-055.2

Васил Н. ИЛИЕВ[1], Весна И. КОТЕВСКА[2]

СИНТЕТСКИ ХЕТЕРОГРАФТИ (МРЕЖИЧКИ) ВО РЕКОНСТРУКТИВНАТА ГИНЕКОЛОШКА ХИРУРГИЈА

Нарушување на статиката на пелвичните органи кај жената, т. е. пролапсот на пелвичните органи (ППО) кај жената, е честа состојба и се среќава кај повеќе од 40 % од жените постари од 50 години. Околу 11 % од жените се подложени на корективна операција за ППО и/или на уринарна инконтиненција до возраст на 80 години, и приближно 30 % од нив имаат потреба за ревизија поради рецидив. Покрај ППО, друг клинички ентитет од урогинекологијата, кој има значајна контрибуција во пелвичната реконструктивна хирургија, е стрес-инконтиненција на урината (СИУ). Стрес-инконтиненцијата на урината е нарушување кое ги погодува жените, најчесто во пери- и постменопаузалниот период од животот. Преваленцата на СИУ се движи од 19 % до 55 % за различни возрасни групи и заедници, со преваленца од 27,8 % во Р Македонија во општата женска популација. Се смета дека процентот на жени со СИУ е поголем од официјалната статистика, поради тоа што не секогаш се пријавува од страна на жената и поретко се дијагностицира од лекарите (1). СИУ има големо негативно влијание на квалитетот на животот и претставува здравствен, хигиенски и социјален проблем кај афектираната популација. Постојат два примарни хируршки патишта во реконструктивната пелвична хирургија: абдоминален пристап (лапаротомија или лапароскопија) и вагинален пристап. Првиот рефериран запис за користењето на синтетски хетерологен материјал (мерсилен, марлекс-мрежата) во третманот на рецидивантен пролапс на предниот вагинален sид датира од 1995-тата година, и во 2000 година,

[1] Клиничка болница „Жан Митрев“, ул. „Бледски договор“ бр. 8, Скопје; Медицински факултет, Универзитет „Св. Кирил и Методиј“, Skopje, РС Македонија

[2] Институт за микробиологија и паразитологија, Медицински факултет, УКИМ, Скопје, РС Македонија

Хардиман и сор. (2) први ја опишале употребата на полипропиленската мрежа за корекција на цистоцелата. Во следните години, многу гинеколози/урогинеколози за корекција на позицијата на пелвичните органи и на карличното дно користеле синтетички мрежички во обид да се подобрат стапките на успех и да се намалат рецидивите.

Постојат четири типа синтетички материјал кој се употребува во корективната урогинеколошка хирургија:

ТИП 1 се макропорен, монофиламентен.

ТИП 2 се микропорни.

ТИП 3 се макропорни, мултифиламентни.

ТИП 4 се субмикронски биоматеријали чии пори се помали од 1 микрон.

Мрежичките од тип 1 (макропорни, монофиламентни) покажуваат највисок степен на биокомпатибилност со најмала тенденција кон отфрлање од организмот и инфекција. Бидејќи се покажа дека овој тип мрежичка има поповолни својства во однос на другите достапни синтетички мрежи, брзо стана синтетички материјал (мрежа) на избор за повеќето урогинеколози. Со употребата на овој тип мрежичка, во литературата беше реферирано намалување на објективните и субјективните стапки на рецидиви, кога тие ќе се споредат со корекциите без хетерологен материјал, односно корективни интервенции при кои е користено локалното ткиво (3). Студиите реферираат дека кај приближно една третина од операциите за пролапс на карличните органи се користела синтетичка мрежа (90 % од сакро/спино/колпоплексии, 25 % од трансвагиналните корекции). Повеќе од 75 % од интервенциите за корекција на стрес-инконтиненција вклучуваат синтетичка мрежичка (4). Но, зголемената употреба на синтетички хетерографти резултираше со зголемување на бројот на компликациите поврзани со мрежата кои се добро документирани во литературата (5). Иако разни синтетички материјали (апсорбирачки и трајни) биле користени за корекција на пролапс на карличните органи многу години пред неговото објавување, во 2001 година, Администрацијата за храна и лекови на САД (FDA) ја одобри првата хируршка мрежа на производи специјално

дизајнирани за хируршки корекции на пролапс на карличните органи кај жената. Следува период на широка примена на разни мрежички во корективната гинеколошка хирургија со различен степен на успех. Така, ретропубичната бестензиска трансвагинална корекција на стрес-инконтиненцијата на урина (ТВТ), воведена во 1996 година, беше поврзана со ризик од повреди на мочниот меур, уретрата и крвните садови, па таа беше заменета со трансобтураторен пат на пласирање на полипропиленската мрежичка на ниво на мидуретра (ТВТ-О). За корекција на гениталниот пролапс беа имплементирани голем број мрежички од различен материјал и со различен облик, со или без посебни апликатори за нив, но за жал тоа резултираше со зголемен број несакани ефекти, компликации и рецидиви, поради што во неколку наврати регулаторните тела во САД и во ЕУ ги забрануваа или беа повлекувани од пазарот токму од производителите. До денес опстанаа разни форми на ТВТ-О полипропиленската макропорна мрежичка како златен стандард во минимално инвазивниот асортиман за хируршки третман на стрес-инконтиненција на уринатa. Употребата на мрежички за корекција на гениталниот пролапс, денес е прилично рестриктивно.

Техника за поставување мрежа

Точките на фиксација за трансвагинално пласирање на мрежичката се аркус тендинеус, фасциите на карлицата, мускулот илеококцигеус или сакроспинален лигамент. Мрежата може да биде поставена со употреба на систем за наведување, со троакар како при трансбутратор и/или трансглутеален пристап за прецизно пласирање на мрежичката, како и за нејзина фиксација. Кога мрежата е поставена, се препорачува стандардизирана техника и систематски пристап во пласирањето со цел да се минимизираат постоперативните компликации. Предоперативно, кај пери- и постменопаузални пациентки, вагинален естроген крем треба да се користи 2-3-пати неделно, за да се обезбеди сигурност дека вагината е добро естрогенизирана пред поставувањето на мрежата. Дисекцијата за поставување мрежа е малку поинаква од дисекцијата за предната колпографија, и оваа суптилна разлика е клуч за минимизирање на постоперативната ерозија на мрежата. Додека кај

предната колпографија е неопходна дисекција на вагиналниот епител од мускулниот слој, дисекција, за да се пласира мрежичка, треба да се направи под мускулатурата, така што мрежичката треба да биде под целата ткивна структура на вагиналниот флеп. Вака обезбедениот простор овозможува пласирање на мрежичката прецизно и без тензија. И на крајот, треба да се направи цистоскопија и/или дигитален ректален преглед за да се провери дали има интраоперативна повреда на мочниот меур, уретрата или ректумот.

Чести компликации поврзани со мрежичките

Истиснувањето на мрежичката од околното ткиво е најчеста компликација поврзана со мрежата и реферирано е дека може да се појави до 10,4 % во случаите каде што полипропиленската мрежа се користи за предни корекции на вагиналниот ѕид (6). Други компликации поврзани со користењето синтетички материјал по трансвагинално поставување мрежичка за корекција на пролапс на пелвичен орган и мидуретрална трансобтураторна мрежичка за корекција на стрес-уринарна инконтиненција вклучуваат интраоперативни перфорации на мочниот меур, ерозија или перфорација во некој соседен орган, вклучувајќи ги мочниот меур, уретрата и ректумот, како и контракција на мрежичката, што доведува до хронична пелвична болка и диспареунија, инфекција и формирање фистула. Во ретроспективната анализа на 347 жени со компликации, поврзани со апликација на мрежичката при примарната хируршка интервенција за пролапс на гениталните органи и за стрес-инконтиненција на урина, Абот и сор. (7) откриле дека 30 % од пациентите имале диспареунија, 33 % од пациентите имале ерозија предизвикана од мрежичката, а 35 % од пациентите имале карлична болка. За време на оперативната ревизија, мрежата е најдена во мочниот меур (1 %) и во цревата (1%) во наведениот процент на случаи. Податоците за повторна операција за рецидив на стрес-инконтиненцијата на уринатa по мидуретрална апликација на мрежичка (TBT-O) се движат од 1 % до 5 %, а проценетата 9-годишна кумулативна стапка на ревизија на проблемот со мрежичката е 1,3 % за ретенција на урина и 2,5 % за мрежична ерозија (8). Пациентите со

микциона дисфункција и/или со ретенција по апликација на TBT-O честопати се ослободуваат со пресекување на мрежичката, така шо се губи субуретралниот слинг-ефект, додека ерозијата, екструзијата и постоењето на болка, чест0о бараат ексцизиона хирургија. На постоперативна болка поврзана со слинговидната положба на мрежичката се реферира во околу 2 % од случаите (9).

Третман на компликациите од поставена мрежичка во корективната хирургија

Опциите за третман вклучуваат употреба на вагинален естроген, локално пресекување на мрежичката или делумна или целосна ексцизија на мрежичката во операционата сала. Кај пациентите кои не се сексуално активни и асимптоматски за постоење на мала ерозија (<3мм), се препорачува апликација на вагинален естроген во облик на крем. За поголеми мрежни ерозии (>3мм), кога вагиналниот естроген е контраиндициран или не третира успешно симптоматски пациенти, индицирана е ексцизија на вагиналната мрежа. Исто така, важно е да се има предвид дека таа мрежа може да се сретне на неочекувани места во вагината и/или во околните структури за време на операцијата. Во нивниот ретроспективен преглед, Крозби и сор. (14) откриле дислокација на мрежата кај 5 % од нивните случаи. Понекогаш отстранувањето на краците на мрежичката, кои минуваат низ обтураторната фоса и иширектални јами, не е можно и треба да се избегнува, бидејќи неоваскуларизацијата на краците на мрежичката во овие области може да доведе до крвавење, кое понекогаш е многу тешко да се контролира. Затоа се препорачува пресекување на краците на мрежичката непосредно пред тие да влезат во овие простори. Ако дојде до тешко крвавење, тоа е често венско по природа и најчесто сутура во облик на осумка помага во воспоставувањето хемостаза. Хемостатските агенси (на пр., сургицел или др.) треба да се пласираат најдлабоко во дисекцијата и да се затвори вагината со поединечни ресорптивни сутури.

Кога да употребиме хетерографт во реконструктивната урогинеколошка корективна хирургија?

Како резултат од пријавените компликации поврзани со мрежичката и од објавените предупредувања од разни регулаторни тела, може да се заклучи дека постојат малку податоци кои потврдуваат кои се најдобрите пациенти за пласирање мрежичка при трансвагинална корекција на урогенитален пролапс. Анализата на достапните податоци може да ја сугерира препораката за користењето мрежичка при трансвагиналната урогенитална корекција, тоа да биде резервирано за лица со висок ризик каде што придобивката од поставување мрежа може да го оправда ризикот како што е тоа кај одделни пациентки со рецидивен урогенитален пролапс (особено кај рецидивен пролапс на предниот компартман). Полемиката поврзана со поставувањето синтетички материјал не се однесува на употребата на полипропиленската мрежичка за третман на стрес-уринарната инконтиненција (TBT, TBT-O) или при абдоминалниот пристап за корекција на пролапот (сакро/спино/колпопексија), бидејќи ризиците поврзани со овие процедури не се чести и тешки, за што зборуваат контролирани, рандомизирано направени студии со кои се потврдуваат нивната безбедност и ефикасноста (1). Американската асоцијација за урогинекологија (AUGS) препорачува поставувањето мрежа за пролапс на карличните органи да се извршува од гинеколози/урогинеколози со помината обука и со стекнат сертификат, кои имаат соодветно знаење, хируршки вештини и искуство во реконструктивната хирургија на пелвичните органи.

Наше искуство

Материјал – Евалуирани се 124 пациентки оперирани од стрес-инконтиненција на урината, со или без придружен пролапс на гениталните органи, со бестензична трансвагинална трансобтураторна апликација (TBT-O) како метода на избор за третман на стрес-инконтиненцијата на урината, во период од три години.

Резултати – Анализиравме 124 TBT-O апликации кај пациентки со следните општи податоци (табела 1):

Табела 1

Демографски податоци од анализираните 124 TBT-O апликации кај пациентки

Демографски податоци	
Возраст (години)	60,5 +/- 12,7
БМИ	25,2 +/- 3,9
Менопауза	85
Оп. за пролапс	4
Предоп. ургентна инконтиненција	25
Хистеректомија	10
TBT-O	124

Иако бројот на анализираните случаи е релативно мал, добиените резултати за компликации од поставувањето на хетерологниот материјал се компарабилни со податоците референрани во стручната литература (табела 2).

Табела 2

Компликации од мрежичката

Компликации	Вкупно TBT-O (124)
Ерозија на траката	2 (1,6 %)
Болки во препоните	1 (0,8 %)

Дискусија: Добиените резултати го потврдуваат податокот дека тип еден (монофиламентен макропорен) вид на хетерологен материјал е безбеден за корекција во урогинеколошката хирургија поради што тој и остана надвор од рестрикциите на регулаторните тела за контрола на импланти. Ефикасноста на TBT-O-процедурата за третман на стрес-инконтиненција на урината која изнесува околу 92 % по пет години, ја потврдува оправданоста на оваа метода на избор за хируршки третман на стрес-инконтиненција на урината кај жената.

Заклучок: Трансвагиналната апликација на хетерологен материјал од тип еден има одредена анатомска предност во однос на корекциите со употреба на локалното ткиво за третман на пролапс на карличните органи и уринарната стрес-инконтиненција, претежно за пред-

ниот компартман. Сепак, секогаш треба да се има предвид ризикот од компликации што е добро документирано во литературата. Употребата на бестензионата трансвагинална лента за третман на стрес-уринарната инконтиненција (ТВТ-О) останува хируршки третман на избор или златен стандард во третманот на оваа болест.

ЛИТЕРАТУРА

1. Илиев, В., Лазаревски, М., Чакамаков, Д. *Радна способност након корективних операција за генитални пролапс и уринарну стрес инконтиненцију*. XI-ти Конгрес гинеколога опстетричара Југославије, Зборник радова, Нови Сад, 1988, 248.
2. Hardiman, P., Oyawoye, S., Browning, J. *Cystocele repair using polypropylene mesh*. Br J Obstet Gynaecol. 2000; 107: 825–826.
3. Nguyen, J. N., Burchette, R. J. *Outcome after anterior vaginal prolapse repair: a randomized controlled trial*. Obstet Gynecol. 2008; 111: 891–898.
4. Jonsson Funk, M., Levin, P. J., Wu, J. M. *Trends in the surgical management of stress urinary incontinence*. Obstet Gynecol. 2012; 119: 845–851.
5. US Food and Drug Administration. *FDA safety communication: update on serious complications associated with transvaginal placement of surgical mesh in repair of pelvic organ prolapse*. 2011. Accessed February 10, 2015.
6. Maher, C., Feiner, B., Baessler, K. et al. *Surgical management of pelvic organ prolapse in women*. Cochrane Database Syst Rev. 2013; 4: CD004014.
7. Abbott, S., Unger, C. A., Evans, J. M. et al. *Evaluation and management of complications from synthetic mesh after pelvic reconstructive surgery: a multicenter study*. AmJObstetGynecol. 2014; 210: e1–e8.
8. Jonsson Funk, M., Levin, P. J., Wu, J. M. *Trends in the surgical management of stress urinary incontinence*. Obstet Gynecol. 2012; 119: 845–851.
9. Brubaker, L., Norton, P. A., Albo, M. E. et al. *Adverse events over two years after retropubic or transobturator midurethral sling*

surgery: findings from the Trial of Midurethral Slings (TOMUS) study. Am J Obstet Gynecol. 2011; 205: 498.e1–498.e6.

10. Siddiqui, N. Y., Grimes, C. L., Casiano, E. R. et al. *Mesh sacrocolpopexy compared with native tissue vaginal repair: a systematic review and metaanalysis*. Obstet Gynecol. 2015; 125: 44–55.
11. Maher, C., Feiner, B., Baessler, K. et al. *Surgical management of pelvic organ prolapse in women*. Cochrane Database Syst Rev. 2013; 4: CD004014.
12. Muffly, T. M., Barber, M. D. *Insertion and removal of vaginal mesh for pelvic organ prolapse*. Clin Obstet Gynecol. 2010; 53: 99–114.
13. Brubaker, L., Norton, P. A., Albo, M. E. et al. *Adverse events over two years after retropubic or transobturator midurethral sling surgery: findings from the Trial of Midurethral Slings (TOMUS) study*. Am J Obstet Gynecol. 2011; 205: 498.e1–498.e6.
14. Crosby, E. C., Abernathy, M., Berger, M. B. et al. *Symptom resolution after operative management of complications from transvaginal mesh*. Obstet Gynecol. 2014; 123: 134–139.

Vasil N. ILIEV, Vesna I. KOTEVSKA

SYNTHETIC HETEROGRAPHS (MESH) IN RECONSTRUCTIVE GYNECOLOGICAL SURGERY

Abstract

The intensions to obtain good results in pelvic reconstructive surgery and reduce rate of recidivism urge surgeons to adopt different surgical procedures using heterology synthetic materials. The rise in transvaginal mesh use was accompanied by a surge of adverse events and mesh-related complications. As a result, the Food and Drug Administration (USA) and EU regulatory commission put several efforts to regulate the manufacturing of vaginal mesh products. From the other side, an International Urogynecological Association (IUGA) and International Continence Society (ICS) joint report on the terminology and classification of complications arising from the insertion of

prostheses and grafts in female pelvic floor surgery and recommendations for evaluation and management of mesh-related complications.

Aim- Evaluate mesh complications in reconstructive pelvic surgery

Material- We evaluated patients operated for urinary stress incontinence with or without some prolapse of genital organs during period of three years.

Results- We report our experience with transvaginal tension free miduretra obturator tape applicated for treatment of urinary stress incontinence on 124 patients. During follow-up period of three years we had erosion in 2 patients (1,6%) and permanent groin pain in 0,8% (one patient). We did not have bladder injuries and reoperations within 12 months.

Conclusion-Our results on mesh complications and efficacy of TVT-O procedure for surgical treatment of urinary stress incontinence are comparable with reported in literature.

Keywords: mesh, genital prolapse, urinary stress incontinence, tension-free tape

ПОГЛАВЈЕ V

РОБОТСКА ХИРУРГИЈА ВО УРОЛОГИЈАТА

doi: 10.20903/zpopov-2022-0023 UDK: 616.6-089:007.52

Academician Zeljko KASTELAN[1,2] MD PhD,
Assistant Professor Tvrtko HUDOLIN[1] MD PhD
and Tomislav KULIS[1] MD PhD.

ROBOTICS IN UROLOGY – UNIVERSITY HOSPITAL CENTER ZAGREB, OUR EXPERIENCE

Abstract

Robotic surgery as a minimally invasive procedure has now been considered, for some time, the best operative method in many surgical fields, including urology. We present here the advantages of robotic surgery with an emphasis on the Senhance® robotic platform, which we have been using for more than 2 years, mainly for radical prostatectomy, but also for other upper urinary tract surgeries at our institution. This system has an open cockpit and four robotic arms, eye-tracking technology and 3D vision, articulated instruments and feedback function, a comfortable and adjustable seat for optimal position and, most important, reusable instruments, signify-cantly reducing costs, therefore bringing this new and exciting technology with all its benefits for patients and surgeons at a lower cost.

Key words: robotic surgery, radical prostatectomy, Senhance®, minimally invasive surgery

Introduction

Since 2000, when the DaVinci® robotic surgical system was first introduced in urology, there has been a significant shift from open to robotic

[1] Department of urology University Hospital Center Zagreb and School of Medicine Zagreb, Croatia

[2] Croatian Academy of Sciences and Arts, Zagreb, Croatia

surgery, especially in radical prostatectomy (RP). Although it was initially planned as a robot for cardiothoracic surgery, its application has expanded to many areas of surgery over these past two decades.[1] Open surgery will not go away, but it has been significantly replaced by a robotic approach, especially in the United States (US), where the vast majority of RPs are now performed using the DaVinci® platform which has been considered the gold standard for minimally invasive treatment of prostate cancer for some time now.[2]

Robotic surgery offers several important advantages over open surgery, to both the patient and the surgeon.[3] Patients operated on by the robot have less blood loss, spend fewer days in the hospital, have smaller incisions and scars, and thus less pain, and also mobilize shortly after surgery. They return to their usual daily activities earlier, but they also return to work earlier. There are benefits for the surgeon as well. For example, it is certainly more comfortable to sit and work, while tremor and movements are corrected or amplified by the robotic arms, compared to standing and applying the physical force needed to create space to access or to manipulate the target organ or tissue. Furthermore, robotic platforms allow for augmentation and thus better visualization of anatomical structures located deep in the pelvis, which is important for the oncological and functional outcomes of RP. But those benefits come with a price that, while significant, can be justified even for lower-income countries compared to Western countries, especially the US, when all benefits are taken into account.

The cost of DaVinci®'s robotic platform is approximately US$2 million, with maintenance costs around US$200,000 per year, plus the additional cost of disposable instruments for each operation. These disposable instruments can vary significantly, from several hundreds to several thousand of US dollars, therefore it is not a cheap procedure.[4]

There is a logical question as to what we receive for this price? In order to answer this question many variables must be taken into account, but still, we will not receive a universal answer. Why? Because many of these variables are different for different countries. For example, in the Western world, especially in the US, health care providers encourage hospitals to reduce the length of hospital stay (in days), which is considered one of the most important variables which reduces the cost of the procedure, thus increasing hospital earnings. That's why there is such a strong desire for hospitals to increase their use of robotic RP, after which, patients can be

discharged on the first day or even the same day. This is becoming an increasingly common practice in the US.[5] In many other countries, especially former Socialist ones, including Croatia, hospitals are still not under any strong pressure to discharge patients as quickly as possible. In a recent analysis of the 25 European Union member states, for which data are available for 2016, the two countries with the highest average length of hospital stays were the Czech Republic and Croatia.[6] There are several reasons for this. One of the most important is traditional (in the former Socialist countries, patients are used to stay longer in the hospital). It is also important to state that we still do not have large private hospitals or large private insurance companies that will support shorter hospitalization in order to reduce costs and increase profits. But that is changing because the benefits of one or two days in the hospital made possible by robotic surgery are becoming more and more obvious, and we will surely go towards this in the near future.

Another important advantage of robotic surgery is minimal blood loss, especially compared to open RP, where intra-operative blood loss can be more than 500 mL.[7] This is also something that must be considered when comparing the cost of open and robotic prostatectomy. The use of blood has its price, moreover, there is always, albeit a very small, nevertheless permanent risk of getting blood-borne diseases if a patient needs a transfusion. Less pain after robotic RP is also something that may not be crucial, but it is certainly important for the patient as well as early mobilization, which can all reduce costs but also reduce the likelihood of some complications associated with prolonged bed rest.

Furthermore, patients operated on with robotic platforms return earlier to their usual activities and work compared to open surgery and therefore significantly reduce sick leave costs.

There is also one very important question we need to ask ourselves when thinking about robotic surgery, its need, cost, and benefits. What kind of urologists we would like to be? Those whose time is slowly passing, or those whose time is coming or rather has come? This is especially important for young urologists: if they do not learn minimally invasive urology, they will not be competitive, and it will be much harder for them to find a job. But this should not be limited to them, and older urologists could and should learn new methods. Their experience from open surgery can be used as an advantage when switching to minimally invasive procedures. Of course, this

is something we are only partially responsible for because hospital management has to buy a robot and has to send the surgeon for training. But if one is not aware of all these benefits and if one does nothing to present them to hospital management, one will certainly not receive a robot.

It is also important to say that a good and motivated assistant is very important for robotic prostatectomy, but also that the support of the entire surgical team is needed: from nurses and technicians in the operating room to anesthesiologists, especially at the introduction of the robotic method.

Our experience

At the end of 2018, our hospital purchased the Senhance® robotic platform. This platform was originally designed and manufactured by an Italian company, which was later bought by the American company Trans Enterix, Morrisville, NC, US, and was approved by the Food and Drug Administration in 2017. It is an open cockpit platform with four robotic arms (Fig 1a and b). This was our first real contact with a robotic platform. After initial education at our institution, members of the surgical team went to Italy to an animal farm for additional training on pigs and we visited one hospital in the Netherlands, where we watched RRP. Upon our return to Zagreb, we started surgery, mainly on the adrenal gland, kidneys, and radical prostatectomy. Our surgical technique and our experience have already been published in detail.[8,9,10] Here we will present few important aspects related to robotic RP.

We decided to use an extraperitoneal approach for two reasons. First, we had experience with it because we were using it for laparoscopic RP, and the same approach was used in the Netherlands. We think that the extraperitoneal approach is a good approach for RP. The only problem we had was when we accidentally opened the peritoneum and had to increase the opening in the peritoneum to reduce the pneumoperitoneum. Using an extraperitoneal approach, the surgeon avoids the abdominal cavity and potential associated morbidity.

We still do lymph node dissection, when indicated by a laparoscopic approach, for the same two reasons. We hope to improve our technique, step by step, and as a next step we plan to perform a robotic lymph node dissection. This will likely require more planning and consultations as well as learning by doing.

What we noticed in our series is a relatively higher rate of positive surgical margins for the disease stage. It has already been shown that the risk of positive margins decreases with surgeon experience.[11] We also hope to decrease that rate by increasing the number of procedures we complete. Another fact that is important to note is that since we used a laparoscopic instrument to hold and manipulate the prostate, there is also the possibility of iatrogenic prostatic disruption, as already shown. Minor surface abrasions or lacerations of the prostate are seen in a significant proportion of laparoscopic RP. The act of grasping and manipulating the prostate *in situ* commonly traumatizes the surface of the gland, resulting in iatrogenic positive margins.[12]

We also showed that with increased experience, we reduced the number of days in the hospital from 6 to 5, as well as a lower estimated blood loss, from 300 to 200 ml. Our average operative time was the same, but we started to see an increase in the number of patients who had lymphadenectomy, further increasing the time of the procedure. This explains the same operative time for the first 40 and 75 cases.[8,9] We expect to improve our results further as we gain more experience. What is also important to note is that, in the beginning, it took us a lot of time to prepare everything for the surgery. Furthermore, we spent extra time removing and setting up the robotic instruments, but with increasing experience we became faster, and now we can change the instruments almost as fast as in laparoscopy. Our docking time for a robot is less than 5 minutes.

We think the Senhance® system has several important advantages over laparoscopy and some other robotic platforms, such as eye-tracking (this is significantly improved with the newer, updated software) and 3D vision, for faster and better visualization by enlarging important anatomical structures. Articulated instruments add additional benefits, and the force feedback function works very well, enabling the surgeon to sense whether he has grabbed the needle or to feel stitch tension when puling, for example. A comfortable and adjustable seat for optimal position, especially for longer surgical procedures, is also important. What is also very important is the lower cost of maintenance and operation compared to the DaVinci® machine. Although it is not easy to directly compare the two platforms, as prices can be different (especially for the instruments), depending on the supplier and the country, but this could probably only be done if both systems were in the same institution, i.e. the same country. We can thus say that Senhance®

instruments are very good and robust and can be used for many surgical procedures (as in laparoscopy). This allows for a significant reduction in costs, as compared to single use instruments (Fig. 2).

An additional advantage is that since the system is based on laparoscopy, the conversion to laparoscopy can be done very simply, one must only remove the robotic arms and replace the robotic instruments with laparoscopic ones. It may be something that is very rarely seen in robotic RP, but we must also consider possible conversions to laparoscopy for other urological, gynecological, or surgical procedures that are being done, or can be done, with this robotic platform. Since this is a new robotic system, there will probably be cases that will have to be converted into laparoscopy, at least until surgeons gain enough experience. We can therefore say that this is also a possible advantage of the platform.

Based on our experience, but also based on personal communication with other urologists using this platform, as well as based on studies published on PubMed, we can say that Senhance® is a safe robotic platform that can perform numerous urological surgical procedures while reducing costs. Studies of a large number of patients, as well as comparisons with other robotic platforms and other types of radical prostatectomy, are needed to actually assess its oncological and functional outcomes.

In addition to Senhance®, there are a number of new robotic platforms that are in the final stages of development, some are already under testing, and some are in the approval phase or have been approved for clinical use. The increased number of robotic platforms will certainly make this technology more accessible and hopefully cheaper than it is now. As previously mentioned, when we consider the system costs, maintenance, and instruments, there are platforms which have reusable instruments, and this can bring significant cost reductions, making them attractive to hospitals in lower-income countries. Hence, the costs are not really that high if we have carefully selected and if we consider the advantages and disadvantages of each platform, as well as the savings achieved by reducing the number of hospital days, less blood loss, and a faster return to work, just to name a few. In any case, robotic surgery is not the future, it is the present, and it is imperative that this must be recognized by every hospital that wants to follow world trends and provide its patients with the best possible care.

Figure 1a. and b. – Open cockpit platform and two (out of four) robotic arms (which can be easily moved around the operating room).

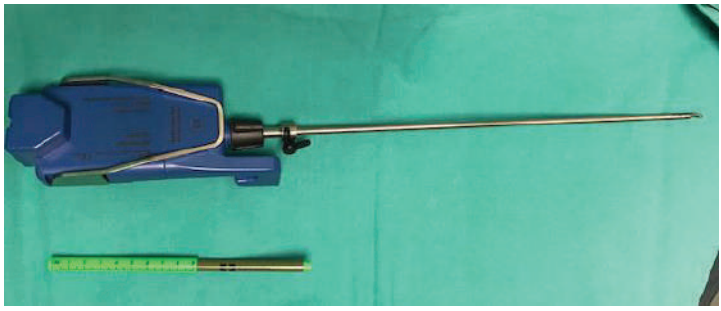


Figure 2 – Senhance® robotic instruments are sturdy and robust and can be reused for many procedures.

REFERENCES

1. McGuinness LA, Prasad Rai B. Robotics in urology. Ann R Coll Surg Engl 2018; 100(6_sup): 38-44.
2. Descazeaud A, Peyromaure M and Zerbib M. Will robotic surgery become the gold standard for radical prostatectomy? Eur Urol. 2007; 51: 9-11. PMID: 17070985
3. Hudolin T, Kuliš T, Knežević, N, Penezić L, Zakulić T, Hauptman D et al. A short review and a single-centre experience with Senhance®TM robotics system. RAD HAZU. 2021; 547(54-44): 64-68.
4. Schroeck FR, Jacobs BL, Bhayani SB, Nguyen PL, Penson D, Hu J. Cost of New Technologies in Prostate Cancer Treatment: Systematic Review of Costs and Cost Effectiveness of Robotic-assisted Laparoscopic Prostatectomy, Intensity-modulated Radiotherapy, and Proton Beam Therapy. Eur Urol. 2017; 72(5): 712-35.
5. Abaza R, Martinez O, Ferroni MC, Bsatee A and Gerhard RS Same Day Discharge after Robotic Radical Prostatectomy. J Urol. 2019; 202(5): 959-963.
6. https://gateway.euro.who.int/en/indicators/hfa_540-6100-average-length-of-stay-all-hospitals/visualizations/#id=19635
7. Pereira R, Joshi A, Roberts M, Yaxley J, Vela I. Open retropubic radical prostatectomy. Transl Androl Urol. 2020 Dec; 9(6): 3025-3035.
8. Kaštelan Ž, Knežević N, Hudolin T, Kuliš T, Penezić L, Goluža E et al. Extraperitoneal radical prostatectomy with the Senhance® Surgical System robotic platform. Croat Med J 2019; 60(6): 556-559.
9. Kastelan Z, Hudolin T, Kulis T, Penezic L, Gidaro S, Bakula M et al. Extraperitoneal Radical Prostatectomy with the Senhance® Robotic Platform: First 40 Cases. Eur Urol 2020; 78(6): 932-934.
10. Kastelan Z, Hudolin T, Kulis T, Knezevic N, Penezic L, Maric M et al. Upper urinary tract surgery and radical prostatectomy with Senhance® ® robotic system: Single center experience-First 100 cases. Int J Med Robot 2021; 17(4): e2269.
11. Bravi CA, Tin A, Vertosick E, Mazzone E, Martini A, Dell'Oglio P et al. The Impact of Experience on the Risk of Surgical Margins and Biochemical Recurrence after Robot-Assisted Radical Prostatectomy: A Learning Curve Study. J Urol. 2019; 202: 108-113.
12. Kumar M, Mansour P, Vesey GS. Reducing Laparoscopic Radical Prostatectomy False-Positive Margin Rates Using Cyanoacrylate Tissue Glue. Eur Urol 2009; 56(4): 651-7.

doi: 10.20903/zpopov-2022-0024 UDK: 616.65-089.87:007.52

Clement Claude ABBOU[1], Ahmed ABDELNABY[2]

ANATOMICAL CONSIDERATIONS FOR THE IMPROVEMENT OF SIDE EFFECTS OF RADICAL PROSTATECTOMY: THE INFLUENCE OF ROBOTIC SURGERY

Introduction

Despite good results regarding oncologic outcomes, well-known adverse events follow radical prostatectomy; including incontinence and erectile dysfunction. The first revolution came in the 1980's from the work of Walsh and Donker; aided by laboratory dissections, a systematic operation was described in order to provide adequate cancer control, in addition to preserving continence and erectile function. The 2000's have seen another revolution in the form of the introduction of robotic radical prostatectomy[1, 2]. Urinary incontinence is, by far, the most feared side effect by the patient being a major cause of distress, social withdrawal, increased psychological and the financial burden of pads, this also includes secondary procedures such as urethral slings, urethral bulking procedures and artificial sphincter implants [21]. These side effects often impact the choice of treatment. Immediate and short term results of urinary control at catheter removal, first, second, and third months after surgery range from (0 – 80%), (22 – 80%), and (40 - 90%) respectively, these results leave a lot to be desired [22, 23, 24, 25, 26, 27, 28]. With improved oncologic outcomes, urologists and their patients are becoming increasingly ambitious regarding functional outcomes and quality of life, leading to the development of the

[1] Full Professor of Urology, Former Head of Urology Department, University Hospital Centre Henri Mondor, XII University of Paris Val de Marne, France; Foreign Member of Macedonian Academy of Sciences and Arts, Skopje, North Macedonia

[2] University of Paris, France

concept of a “Trifecta” of oncologic control, recovery of urinary control, and potency [29, 30].

Principles of surgical anatomy

Understanding the principles of surgical anatomy is the first key step to any successful surgery. More than a century has passed since the first radical prostatectomy by Hugh Young, and, yet, several uncertainties still exist as to which surgical technique offers the best immediate functional outcome, while also respecting the oncologic principles. As mentioned in the seminal article by Koraitem, the structural plan of the human body is characterized by a “*duplication of safety mechanisms to maintain function*” [3]. The etiology of post prostatectomy incontinence (PPI) is multifactorial; consisting of non-modifiable variables, including advanced age, obesity, pre-operative incontinence, short urethral length, detrusor instability, prostate volume, and disruption of normal anatomy [4, 5]. Modifiable variables include surgeon/institution volume [6], preservation of urethral sphincter [7], and presservation of suspensory anatomical support [8].

Urethral sphincter complex

The external urethral sphincter complex is considered by the majority of authors to be the most important structure contributing to the maintenance of post-operative continence. According to Dorschner and Stolzenburg, the distal urethral sphincter is composed of an outer striated and inner smooth muscle component. The smooth muscular part of the external sphincter, “lissosphincter”, is the most likely structure to ensure continence at rest after resection of the internal vesical sphincter following radical prostatectomy or transurethral resection (TURP) [9]. Under normal conditions, urine is stopped at the level of the vesical orifice, after TURP it arrests at the distal limit of the prostatic cavity where the lissosphincter is intact. Following prostatectomy, a variable portion of lissosphincter is resected. This might contribute to post-prostatectomy incontinence. The striated component exerts its function from the prostate apex to the penile bulb, whereas the inner smooth muscle component extends at least to the verumontanum [10]. In some studies, the striated portion was shown to contain both “fast twitch”

and “slow twitch” fibers; the fatigue-resistant slow twitch fibers being partially responsible for sustained continence at rest, and fast twitch fibers contributing to continence during periods of sudden increase in intra-abdominal pressure [11]. As mentioned previously, **apical configuration** is another challenge which confronts the surgeon attempting to preserve as much urethral length while also avoiding a positive urethral margin. Lee and colleagues have shown that the prostatic apex overlaps with the striated sphincter anteriorly, posteriorly, bilaterally or unilaterally in 85% of their patients [12]. These facts mandate precise dissection of the apex in order to avoid inadvertent resection of valuable functional urethral tissue. In addition to urethral length, urethral integrity is also an important determinant of recovery of continence. The striated sphincter is related anteriorly to the dorsal venous complex which may invaginate the sphincter’s anterior portion [13]. Theoretically, this fact makes the muscle fibers of the striated sphincter vulnerable to entrapment by the standard DVC stitch, which was illustrated in an anatomic study by Ganzer and colleagues [14]. According to many authors, apical dissection is arguably the most important technical predictor of recovery of continence after radical prostatectomy [15], unfortunately it is surgeon dependent and is difficult to measure objectively.

Neurovascular bundle (NVB) sparing

The pelvic plexus is the central neural plexus that provides autonomic innervation to male urogenital organs. Dissection and preservation, whenever possible, of the cavernous nerves that run from the NVB to the cavernosal bodies during radical prostatectomy are essential to preserve erectile function. This is an especially challenging task considering that the arrangement of these nerves is characterized by marked anatomic variabilities [16]. Contemporary studies on adult specimens describe the neural structures as widely dispersed along the anterolateral, lateral, and posterolateral aspect of the prostate [16, 17], as opposed to the classic “localized posterolateral bundle” description in fetal dissections [18]. Nerve-sparing surgery allows for a better chance at preserving potency [5]. Also, of note is that some anatomic studies have shown that the neurovascular bundle provides at least some neural contribution to the membranous urethra [19, 20]. Cadaveric dissections demonstrated that the sympathetic nerves from sacral segments S2 to S4 provide autonomic supply to the smooth muscle

sphincter of the membranous urethra, and the somatic nervous branches from the pudendal nerve innervate the striated urethral sphincter [21]. This anatomic hypothesis was associated with a faster return of continence in a number of clinical studies [22, 23, 24].

Supporting anatomy

The prostate is attached anteriorly and antero-laterally to the pubic bone with the puboprostatic/pubovesical ligaments. These ligaments are paired fibrous bands; they appear in the sagittal plane as a triangular fascial structure that attaches the pubic bone to the fascia of the striated sphincter, anterior surface of the prostate and the urinary bladder [25]. The ligaments then blend laterally with a fibrous thickening of the endopelvic fascia "arcus tendinous fascia pelvis"; representing a tough structure that is used in urethral suspension procedures for stress urinary incontinence [26]. Together with the puboperinealis portion of the levator ani muscle the ligaments and the arcus tendinous fascia form the "puboprostatic collar" which is believed to provide important structural support to the urethra and maintain urinary control.

The previously mentioned anatomic facts have prompted many surgeons to experiment with a variety of reconstruction techniques including; anterior suspension stitch, pubourethral ligament sparing, puboprostatic collar sparing, posterior reconstruction of the musculo-fascial plate [27, 28, 29], and Retzius-sparing prostatectomy [30, 31].

Influence of robotic surgery

One can argue against the superiority of robotic prostatectomy, compared to open and laparoscopic approaches, since level I evidence is still lacking. However, it is difficult to disagree that since the introduction of the robot, many new research avenues have been opened. A PubMed search (keywords; prostatectomy, robotic prostatectomy) indicates that the number of publications has substantially increased (**Figure 1, Figure 2**). Since the introduction of robotic prostatectomy in the year 2000, a surge of new

techniques regarding radical prostatectomy have been introduced to the literature, possibly due to the fact that a robot provides the surgeon with the ergonomics to dissect exactly how and where he wants to.

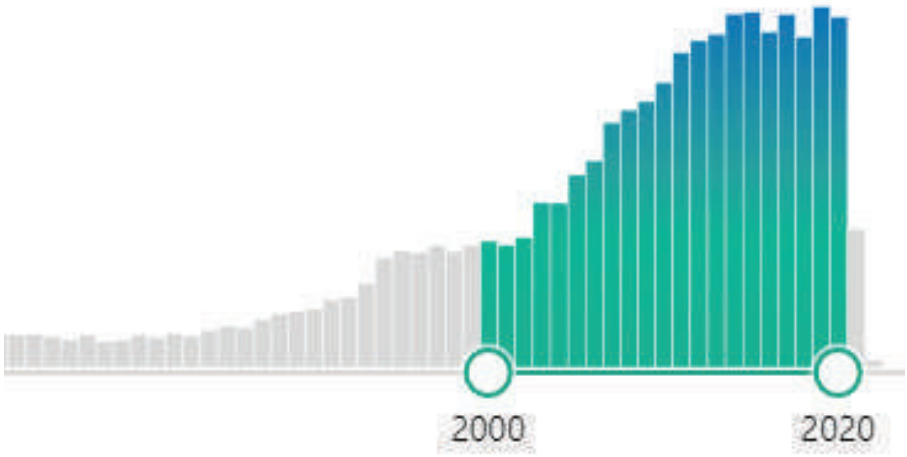


Figure 1 – Pubmed search results for "Prostatectomy"

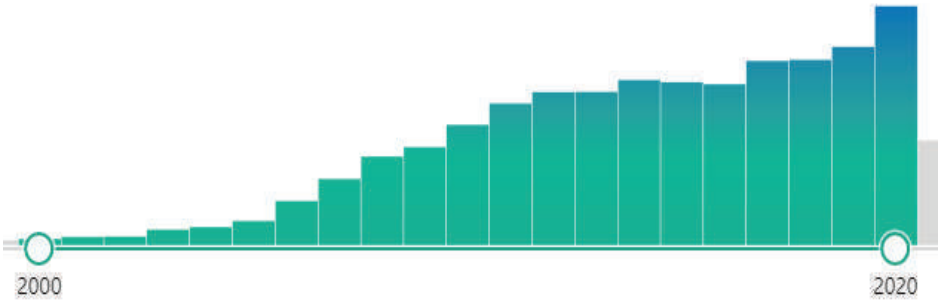


Figure 2 – Pubmed search results for "Robotic Prostatectomy"

Robotic surgery has offered the minimally invasive advantages of laparoscopy, with the added benefits of stereoscopic vision, 10-15 times

magnification, wristed instruments enabling precise dissection and complex reconstruction. In other words, robotic surgery was designed to overcome the shortcomings of conventional laparoscopy and potentially reduce the learning curve of complex minimally invasive surgery [32].

Some of the techniques described (i.e. puboprostatic collar sparing approach, total anatomical reconstruction, Retzius-sparing) are difficult to perform using traditional laparoscopy, or even open surgery. Pneumoperitoneum allows for better vision due to the tamponade of small venous bleeding. Enhanced optical magnification has provided potentially better identification of anatomic details. Video recording in robotic surgery facilitates with the teaching of early career surgeons.

Due to its prohibitive cost, the robot must clearly demonstrate superiority when compared to open retropubic, and laparoscopic assisted radical prostatectomy.

Table 1, summarizes the data of the most relevant studies. And despite the fact that many studies were characterized by heterogeneous data, small numbers and non-randomized nature/low methodological level, higher quality studies are being observed in more recent publications. A trend towards earlier continence recovery, better potency, lower blood loss and rates of transfusion is being consistently observed with the robot. The results of this data suggest that robotic assisted radical prostatectomy is at least equivalent to other approaches.

The only randomized study which has compared robotic to open prostatectomy has shown equivalence of functional outcomes, and superior outcomes of the robot in terms of intra-operative complications, blood loss and hospital stay. The robotic arm was operated by a fellowship-trained surgeon, having performed 200 robotic cases, compared to an experienced open surgeon with more than 1500 completed cases. If the robot allows a young surgeon to achieve equivalent outcomes to an experienced open surgeon in a short period of time, then this reduction in the learning curve should be considered an advantage [43].

Table 1

Robot compared to open retropubic and laparoscopic assisted radical prostatectomy: The most relevant studies (Modified from Abbou & Abdelbary **Error! Bookmark not defined.**)

Author	Study design	Continence	Potency	Other
Huang et al., 2017 [33]	**Meta-analysis** 24 studies (2 RCT) (9178 patients) (LRP vs. RARP)	Better with RARP	Better with RARP	-Less transfusion with RARP -Similar BCR rate -Similar complication rate
Tang et al., 2017 [34]	**Meta-analysis** No RCTs (Open vs. RARP)	No difference	Better with RARP	-Less transfusion with RARP -Less complications with RARP -Less +ve margins with RARP
Seo et al., 2016 [35]	**Meta-analysis** No RCTs (Open vs. RARP)	Better with RARP	Better with RARP	-Less transfusion with RARP -Similar BCR rate -Less complications with RARP
Moran et al., 2013 [36]	**Meta-analysis** 51 studies (1 RCT) (Open vs. LRP vs. RARP)	Better with RARP (in comparison to open)	Better with RARP (in comparison to open)	-Less complications, LOS with RARP -Less +ve margins with RARP
Ficarra et al., 2012 [8]	**Meta-analysis** (Open vs. LRP vs. RARP)	Better with RARP	Better with RARP	-Less blood transfusion with RARP -Less surgery for incontinence with RARP
Robertson et al., 2013 [37]	**Meta-analysis** (LRP vs. RARP)	No difference	NR	-Less +ve margin with RARP -Less organ injury with RARP

Yaxley et al., 2016 [38]	**RCT** (163 vs. 163 patients) (Open vs. RARP)	No difference	No difference	-Less hospital stay with RARP -Lower blood loss -Similar +ve margin rate
Porpiglia et al., 2013 [39]	**RCT** (60 vs. 60 patients) (LRP vs. RARP)	Better with RARP	Better with RARP	-Similar margin rates -Similar blood loss
Ong et al., 2016 [40]	**Prospective, comparative** (1117 vs. 885 patients) (Open vs. RARP)	Better at 1 year with RARP Similar at 2 years	No difference	-Less +ve margins with RARP -Less BCR with RARP
Haglind et al., 2015 [1]	**Prospective, comparative** (778 vs. 1847 patients) (Open vs. RARP)	No difference	Marginally better with RARP	-Similar margin rates
Beauval et al., 2015 [42]	**Prospective, comparative** (129 vs. 175 patients) (Open vs. RARP)	No difference	Better with RARP	-------
Jackson et al., 2016 [43]	**Prospective, Retrospective comparison** (Early RARP experience vs. Experienced open)	No difference	No difference	-Shorter LOS with RARP -Longer OR time with RARP -Similar +ve margin rate
Jeong et al., 2014 [44]	**Retrospective** (Open vs. RARP)	Better with RARP	NR	
O'Neil et al., 2016 [45]	**Retrospective** (Open vs. RARP)	Better at 6 months with RARP	Better with RARP	
Du et al., 2018	**Meta-analysis** (Open vs. LRP vs. RARP)	Better with RARP	Better with RARP	-Less blood loss -Less +ve margins

- **“Veil” technique**

The “Veil” technique was described by Menon and colleagues [46]. Their first publication reported a 96% potency rate at one year, which was higher than most contemporary series. In this technique, the prostatic fascia is lifted off the prostate without incising the endopelvic fascia or ligating the Santorini plexus. This helps achieve two goals: minimizing traction on the neurovascular bundle, as well as preserving the integrity of the distal sphincter complex by avoiding mass ligation stitch. While this technique has been described later in open surgery [47], it was not used in robotic assisted radical prostatectomy until it was innovated, and repeatedly tried and tested robotically.

- **Total anatomical reconstruction after robotic prostatectomy**

Radical prostatectomy is an operation of resection and reconstruction. Wu and colleagues have hypothesized that posterior reconstruction of the musculofascial plate by suturing the DF to the median dorsal raphe, followed by the vesico-urethral anastomosis and reconstruction of the detrusor apron and the puboprostatic ligaments, could contribute to urethral stabilization and accelerated recovery of continence [48]. This complex reconstruction requires superior ergonomics which is facilitated by the endowrist technology provided by the robotic system.

- **Lateral approach**

Following the concept of zealous preservation of peri-prostatic tissues, Gaston’s group described a technique which allows the prostate to be dissected from the surrounding fascial envelope. Their robotic exposure involves the incision of the fascia lateral to the prostate, thus leaving the rest of the endopelvic fascia and detrusor apron undisturbed**Error! Bookmark not defined.**, effectively following the same principles of the popular “Retzius sparing approach” which is described later. Although technically demanding, they reported a remarkable 80% pad-free rate at catheter removal in their cohort of 30 patients. Despite the small sample size of the study, and the cohort being comprised of relatively young patients, the results were encouraging.

- **Retzius sparing prostatectomy**

Described by Galfano and colleagues [29], the concept of Retzius-sparing approach aims to minimize dissection around the prostate. Recent reports have elucidated the importance of the suspensory structures of the pelvis surrounding the prostate in maintaining post-prostatectomy urinary control. In addition, the approach theoretically maximizes sparing the peri-prostatic fascia including the neural structures contributing to potency. Dissection involves working in the field of exposure which is very limited and is extremely difficult to implement in open surgery. The inherent advantages of the robot include; 7 degrees of freedom, high fidelity magnification as well as camera access to the depth of the pelvis, thus allowing the surgeon to execute these demanding surgical maneuvers efficiently.

- **Perineal prostatectomy**

The first attempt at robotic perineal prostatectomy on a cadaver was performed by the Cleveland Clinic group [49]. The procedure was performed on patients in 2019 [50]. While the number of cases performed is still too small to draw any comparative conclusions with the traditional approach, robotic perineal prostatectomy was found to be feasible and safe. In addition, it has the potential advantages of avoiding an abdominal incision and intraperitoneal adhesions, and better access to the prostate in obese patients [51]. Limiting factors to this approach can be dissection of large prostates, and the requirement of an Xi or Single port robot in order to perform with less difficulty.

- **Single port prostatectomy**

The Da Vinci single port (SP) preclinical model was purposefully designed to accommodate the robotic camera and instruments, to tackle confined spaces through a single incision. The authors demonstrated the feasibility of robotic SP transabdominal prostatectomy, perineal prostatectomy as well as robotic pelvic lymphadenectomy from the perineal approach [52, 53]. The introduction of the new machine opens new frontiers for robotic surgery, but also raises some controversy regarding the limited wor-

king space, instrument clashing and longer operative time. It also requires a highly experienced operator and a skilled assistant [54]. It is argued that the single port robot opposes the intuitive concept of robotic surgery which is designed to reduce complexity rather than increase it.

- **Robotic surgery for benign disease of the prostate**

The standard treatment for benign prostatic hypertrophy for huge (>80 gm) prostates has been open transvesical or retropubic prostatectomy. Robotic simple prostatectomy offers the advantages of being less invasive than its open counterpart, is relatively easy to perform for an operator with experience in robotic urologic surgery, lower risk of urethral injury, and is associated with a less step learning curve when compared to new effective modalities such as HoLeP (Holmium laser enucleation of the prostate) [55]. A reported advantage for robotic simple prostatectomy over HoLeP is the lower risk of urine incontinence (4.6 – 22%) [56].

- **Application of new technologies in robotic prostatectomy**

Improving surgical outcomes is another dynamic goal for which urologists continuously strive to achieve. One component of improving surgical outcomes is enhancing the level of training which the novice surgeon receives. The "Agency for healthcare research and quality", estimated the annual cost to US healthcare from medical errors is €17.1 billion, a large proportion of which were believed to be avoidable [57]. Consistent video recording in robotic surgery has provided opportunities to assess performance and competence with an unprecedented zeal, so much so, that some authors have ambitiously envisioned computer-based assessment using machine learning [58, 59, 60, 61].

Another technology which has seen integration in robotic surgery is the combined use of near-infrared fluorescence (NIRF) and Indocyanine green (ICG); which enables the enhancement of anatomy and the mapping of relevant vasculature. This technology has seen some promising applications in robot-assisted partial nephrectomy; especially in identifying suitable tissue for resection included within the ischemic area of the kidney, and allowing super-elective vascular identification and clamping. It also aids in guiding tumor resection with an appropriate safety margin. NIRF + ICG has

also been investigated as a way to enhance the delineation of the neurovascular bundle during nerve-sparing surgery.[62] In robotic radical prostatectomy, the technology has been tested to guide lymph node dissection, and has demonstrated a potential to identify sentinel lymph nodes after coupling ICG to nanocolloid [63]. However, that feasibility was not replicated by Chennamsetty and colleagues, and they concluded that ICG guided lymph node dissection cannot replace established lymph node dissection [64].

Machine learning and artificial neural networking can also be applied in other domains; one study evaluated the use of artificial intelligence to predict recovery of urinary control using automated performance metrics, and reported 85.9% accuracy in this prediction [65]. Other investigators have explored the application of augmented reality 3D imaging during robotic surgery; one group has devised a technique to superimpose a real-time image of the prostate neoplasm based on pre-operative MRI findings and intra-operative transrectal ultrasound during robotic prostatectomy [66]. In their study, Porpiglia and colleagues have reported that using a 3D model to guide resection during robotic prostatectomy has led to a reduction of positive surgical margin rates in pT3 disease (5.7% in the 3D group versus 26.7% in the control group, $p < 0.05$) [67]. Another study used a tablet computer to provide real time mapping to aid in identification and preservation of important anatomical structures during RARP in four patients [68].

While this effort is still in its infancy, it can be a source of limitless applications. One can only wonder if these applications can lead to a fully automated prostate cancer operation in the not too distant future.

Conclusion

It is evident from the trend of publications during the last two decades that robotic surgery will continue to expand and further evolve. Regarding prostatectomy, the robotic interface has popularized prostate cancer surgery across the globe. It did not compromise oncologic control, but brought forward important advantages as reduced blood loss, early recovery and a trend towards better urinary and erectile function. The technology allowed the urologists and their patients to become more ambitious regarding functional outcome; the procedure now focuses on preserving quality of life as much as ablating the cancer. Moreover, there has been an

evident increase in academic competition in devising new techniques to achieve these goals. From a broader point of view, robotic surgery has the potential to become integrated with various artificial intelligence tools, allowing for unprecedented efficiency and autonomous decision making.

REFERENCES

1. Abbou CC, Hoznek A, Salomon L, Olsson LE, Lobontiu A, Saint F, Cicco A, Antiphon P, Chopin D (2001) Laparoscopic radical prostatectomy with a remote controlled robot. J Urol 165(6 Pt 1): 1964–1966
2. J. Binder, W. Kramer. Robotically-assisted laparoscopic radical prostatectomy. BJU Int, 87 (2001), pp. 408-410
3. Koraitem M (2008). The Male Urethral Sphincter Complex Revisited: An Anatomical Concept and its Physiological Correlate. The Journal of Urology, Vol. 179, 1683-1689, May 2008.
4. von Bodman C, Matsushita K, Savage C, Matikainen MP, Eastham JA, Scardino PT, Rabbani F, Akin O, Sandhu JS (2012). Recovery of urinary function after radical prostatectomy: predictors of urinary function on preoperative prostate magnetic resonance imaging. J Urol. 2012 Mar; 187(3): 945-50.
5. Montorsi F, Wilson TG, Rosen RC, Ahlering TE, Artibani W, Carroll PR, Costello A, Eastham JA, Ficarra V, Guazzoni G, Menon M, Novara G, Patel VR, Stolzenburg JU, Van der Poel H, Van Poppel H, Mottrie A (2012). Pasadena Consensus Panel. Best practices in robot-assisted radical prostatectomy: recommendations of the Pasadena Consensus Panel. Eur Urol. 2012 Sep; 62(3): 368-81.
6. Trinh QD, Bjartell A, Freedland SJ, Hollenbeck BK, Hu JC, Shariat SF, Sun M, Vickers AJ (2013). A Systematic Review of the Volume–Outcome Relationship for Radical Prostatectomy. European Urology, 64(5), 786–798.
7. Asimakopoulos AD, Annino F, D'Orazio A, Pereira CF, Mugnier C, Hoepffner JL, Piechaud T, Gaston R (2010). Complete periprostatic anatomy preservation during robot-assisted laparoscopic radical prostatectomy (RALP): the new pubovesical complex-sparing technique. European Urology 2010 Sep; 58(3): 407-17.
8. Dalela D, Jeong W, Prasad MA, Sood A, Abdollah F, Diaz M, Karabon P, Sammona J, Jamil M , Baize B, Simone A, Menon M (2017). A Pragmatic Randomized Controlled Trial Examining the Impact of the Retzius sparing

Approach on Early Urinary Continence Recovery After Robot-assisted Radical Prostatectomy. Eur Urol. 2017 Nov; 72(5): 677-685.

9. Dorschner W, Stolzenburg JU (1994). A new theory of micturition and urinary continence based on histomorphological studies. 3. The two parts of the Musculus sphincter urethrae: physiological importance for continence in rest and stress. Urol Int 52: 185–188.
10. Hakimi AA, Faleck DM, Agalliu I, Rozenblit AM, Chernyak V, Ghavamian R (2011). Preoperative and intraoperative measurements of urethral length as predictors of continence after robot-assisted radical prostatectomy. J Endourol. 2011 Jun; 25(6): 1025-30
11. Burnett AL and Mostwin JL (1998). In situ anatomical study of the male urethral sphincteric complex: relevance to continence preservation following major pelvic surgery. J. Urol. 1301 – 1306.
12. Lee H, Kim K, Hwang SI, Lee HJ, Byun SS, Lee SE, Hong SK (2014). Impact of prostatic apical shape and protrusion on early recovery of continence after robot-assisted radical prostatectomy. Urology. 2014; 84(4): 844–9.
13. Steiner MS (1994). The puboprostatic ligament and the male urethral suspensory mechanism: An anatomic study. Urology 1994; 44: 530-534.
14. Ganzer R, Stolzenburg J, Neuhaus J, Weber F, Burger M, Bründl J (2014). Is the Striated Urethral Sphincter at Risk by Standard Suture Ligation of the Dorsal Vascular Complex in Radical Prostatectomy? An Anatomic Study. UROLOGY 84: 1453e1460, 2014.
15. Hamilton ZA, Kane CJ (2016). Nerve-sparing Technique During Radical Prostatectomy and its Effect on Urinary Continence. EUROPEAN UROLOGY 69 (2016) 590–591
16. Abbou CC, Abdelbary A (2019). Neuro-anatomic basis of potency recovery after radical prostatectomy: an expert's point of view. Minerva Chirurgica. 2019 Feb; 74(1):28-36. DOI: 10.23736/s0026-4733.18.07848-3.
17. Lunacek A, Schwentner C, Fritsch H, Bartsch G, Strasser H (2005). Anatomical radical retropubic prostatectomy: ‘curtain dissection’ of the neurovascular bundle. 2005 BJU INTERNATIONAL | 95, 1226–1231
18. Walsh PC, Donker PJ (1982). Impotence following radical prostatectomy. Insight into etiology and prevention. J Urol 1982; 128: 492–7
19. Costello AJ, Brooks M, Cole OJ (2004). Anatomical studies of the neurovascular bundle and cavernosal nerves. BJU Int. 2004 Nov; 94(7): 1071-6.
20. Takenaka A, Leung RA, Fujisawa M, Tewari AK (2006). Anatomy of autonomic nerve component in the male pelvis: the new concept from a

perspective for robotic nerve sparing radical prostatectomy. World J Urol. 2006 Jun; 24(2): 136-43. Epub 2006 May 24.

21. Song LJ, Lu HK, Wang JP, Xu YM (2010). Cadaveric study of nerves supplying the membranous urethra. Neurourol Urodyn. 2010; 29(4): 592–595.
22. Reeves F, Preece P, Kapoor J, Everaerts W, Murphy DG, Corcoran NM, Costello AJ (2015). Preservation of the neurovascular bundles is associated with improved time to continence after radical prostatectomy but not long-term continence rates: results of a systematic review and meta-analysis. Eur Urol. 2015 Oct; 68(4): 692-704. Epub 2014 Oct 29.
23. Steineck G, Bjartell A, Hugosson J, Axén E, Carlsson S, Stranne J, Wallerstedt A, Persson J, Wilderäng U, Thorsteinsdottir T, Gustafsson O, Lagerkvist M, Jiborn T, Haglind E, Wiklund P; LAPPRO steering committee (2015). Degree of preservation of the neurovascular bundles during radical prostatectomy and urinary continence 1 year after surgery. Eur Urol. 2015 March; 67(3): 559-68.
24. Ko YH, Coelho RF, Chauhan S, Sivaraman A, Schatloff O, Cheon J, Patel VR (2012). Factors affecting return of continence 3 months after robot-assisted radical prostatectomy: analysis from a large, prospective data by a single surgeon. J Urol. 2012; 187: 190-194
25. Wimpissinger TF, Tschabitscher M, Feichtinger H, Stackl W (2003). Surgical anatomy of the puboprostatic complex with special reference to radical perineal prostatectomy. BJU Int. 2003 Nov; 92(7): 681-4.
26. Pit MJ, De Ruiter MC, Lycklama A, Nijeholt AA, Marani E, Zwartendijk J (2003). Anatomy of the arcus tendinous fasciae pelvis in females. Clinical Anatomy 2003 Mar; 16(2): 131-7.
27. Patel VR, Coelho RF, Palmer KJ, Rocco B (2009). Periurethral suspension stitch during robot-assisted laparoscopic radical prostatectomy: description of the technique and continence outcomes. Eur. Urol. 2009; 56: 472–8
28. Hurtes X, Rouprêt M, Vaessen C, Pereira H, Faivre d'Arcier B, Cormier L, Bruyère F (2012). Anterior suspension combined with posterior reconstruction during robot-assisted laparoscopic prostatectomy improves early return of urinary continence: a prospective randomized multicentre trial. BJU Int. 2012; 110: 875–83.
29. Hurtes X, Rouprêt M, Vaessen C, Pereira H, Faivre d'Arcier B, Cormier L, Bruyère F (2012). Anterior suspension combined with posterior reconstruction during robot-assisted laparoscopic prostatectomy improves early return of urinary continence: a prospective randomized multicentre trial. *BJU Int.* 2012; 110: 875–83.

30. Galfano A, Ascione A, Grimaldi S, Petralia G, Strada E, A. Bocciardi M. A new anatomic approach for robot-assisted laparoscopic prostatectomy: a feasibility study for completely intrafascial surgery. Eur Urol, 58 (2010), pp. 457-461
31. Galfano A, Di Trapani D, Sozzi F, Strada E, Petralia G, Bramerio M, Ascione A, Gambacorta M, Bocciardi AM (2013). Beyond the learning curve of the Retzius-sparing approach for robot-assisted laparoscopic radical prostatectomy: oncologic and functional results of the first 200 patients with ≥ 1 year of follow-up. Eur Urol. 2013 Dec; 64(6): 974-80.
32. Merseburger AS, Herrmann TRW, Shariat SF, Kyriazis J, Nagele U, Traxer O, Evangelos N (2013). EAU Guidelines on Robotic and Single-site Surgery in Urology, August 2013 Volume 64, Issue 2, Pages 277–291
33. Huang X, Wang L, Zheng X, Wang X (2017). Comparison of perioperative, functional, and oncologic outcomes between standard laparoscopic and robotic-assisted radical prostatectomy: a systemic review and meta-analysis. Surgical Endoscopy, March 2017, Volume 31, Issue 3, pp 1045–1060.
34. Tang K, Jiang K, Chen H, Chen Z, Xu H, Ye Z (2017). Robotic vs. Retropubic radical prostatectomy in prostate cancer: A systematic review and a meta-analysis update. Oncotarget. 2017; 8(19): 32237-32257.
35. Seo H-J, Lee NR, Son SK, Kim DK, Rha KH, Lee SH (2016). Comparison of Robot-Assisted Radical Prostatectomy and Open Radical Prostatectomy Outcomes: A Systematic Review and Meta-Analysis. Yonsei Medical Journal. 2016; 57(5): 1165-1177.
36. Moran PS, O'Neill M, Teljeur C, Flattery M, Murphy LA, Smyth G, and Ryan M (2013). Robot-assisted radical prostatectomy compared with open and laparoscopic approaches: a systematic review and meta-analysis. International Journal of Urology 2013; 20(3): 312-321.
37. Robertson C, Close A, Fraser C, Gurung T, Jia X, Sharma P, Vale L, Ramsay C, Pickard R (2013). Relative effectiveness of robot-assisted and standard laparoscopic prostatectomy as alternatives to open radical prostatectomy for treatment of localised prostate cancer: a systematic review and mixed treatment comparison meta-analysis. BJU Int. 2013 Oct; 112(6): 798-812. doi: 10.1111/bju.12247. Epub 2013 Jul 26.
38. Yaxley JW, Coughlin GD, Chambers SK, Occhipinti S, Samaratunga H, Zajdlewicz L, Dunglison N, Carter R, Williams S, Payton DJ, Perry-Keene J, Lavin MF, Gardiner RA (2016). Robot-assisted laparoscopic prostatectomy versus open radical retropubic prostatectomy: early outcomes from a randomised controlled phase 3 study. The Lancet, Volume 388, No. 10049, p1057–1066, 10 September 2016.

39. Porpiglia F, Morra I, Lucci M, Matteo C, Fabrizio M, Grande S, Ragni F, Poggio M, Fiori C (2013). Randomised Controlled Trial Comparing Laparoscopic and Robot-assisted Radical Prostatectomy. European Urology, Volume 63, Issue 4, April 2013, Pages 606-614.
40. Ong WL, Evans SM, Spelman T, Kearns PA, Murphy DG, Millar JL (2016). Comparison of oncological and health-related quality of life outcomes between open and robot-assisted radical prostatectomy for localised prostate cancer - findings from the population-based Victorian Prostate Cancer Registry. BJU Int. 2016 Oct; 118(4): 563-9. Epub 2015 Dec 19.
41. Haglind E, Carlsson S, Stranne J, Wallerstedt A, Wilderäng U, Thorsteinsdottir T, Lagerkvist M, Damber JE, Bjartell A, Hugosson J, Wiklund P, Steineck G; LAPPRO steering committee (2015). Urinary Incontinence and Erectile Dysfunction After Robotic Versus Open Radical Prostatectomy: A Prospective, Controlled, Nonrandomised Trial. Eur Urol. 2015 Aug; 68(2): 216-25. Epub 2015 Mar 12.
42. Beauval JB, Roumiguié M, Ouali M, Doumerc N, Thoulouzan M, Mazerolles C, Rischmann P, Malavaud B, Soulié M (2015). [A prospective trial comparing consecutive series of open retropubic and robot-assisted laparoscopic radical prostatectomy in a centre: Oncologic and functional outcomes]. Prog Urol. 2015 Jun; 25(7): 370-8. Epub 2015 May 1.
43. Jackson MA, Bellas N, Siegrist T, Haddock P, Staff A, Laudone V, Wagner JR (2016). Experienced Open vs Early Robotic-assisted Laparoscopic Radical Prostatectomy: A 10-year Prospective and Retrospective Comparison. Urology, Volume 91, May 2016, Pages 111-118.
44. Jeong SJ, Yeon JS, Lee JK, Cha WH, Jeong JW, Lee BK, Lee SC, Jeong CW, Kim JH, Hong SK, Byun S, Lee SE (2014). Development and validation of nomograms to predict the recovery of urinary continence after radical prostatectomy: comparisons between immediate, early, and late continence. World J Urol. 2014 Apr; 32(2): 437-44. Epub 2013 Jul 6.
45. O'Neil B, Koyama T, Alvarez J, Conwill RM, Albertsen PC, Cooperberg MR, Goodman M, Greenfield S, Hamilton AS, Hoffman KE, Hoffman RM, Kaplan SH, Stanford JL, Stroup AM, Paddock LE, Wu XC, Stephenson RA, Resnick MJ, Barocas DA, Penson DF (2016). The Comparative Harms of Open and Robotic Prostatectomy in Population Based Samples. The Journal of urology. 2016; 195(2): 321-329.
46. Kaul S, Savera A, Badani K, Fumo M, Bhandari A, Menon M. Functional outcomes and oncological efficacy of Vattikuti Institute prostatectomy with Veil of Aphrodite nerve-sparing: an analysis of 154 consecutive patients. BJU Int. 2006 Mar; 97(3): 467-72. doi: 10.1111/j.1464-410X.2006.05990.x.

47. Shigemura K, Yamanaka N, Yamashita M: Veil Nerve-Sparing Technique and Postoperative Urinary Continence in Open Antegrade Radical Prostatectomy. Urol Int 2012; 89: 283-289. doi: 10.1159/000339921
48. Wu YP, Xu N, Wang ST, et al. The efficacy and feasibility of total reconstruction versus nontotal reconstruction of the pelvic floor on short-term and long-term urinary continence rates after radical prostatectomy: a meta-analysis. World J Surg Oncol 2017; 15: 228.
49. Laydner H, Akça O, Autorino R, Eyraud R, Zargar H, Brandao LF, Khalifeh A, Panumatrassamee K, Long JA, Isac W, Stein RJ, Kaouk JH. Perineal robot-assisted laparoscopic radical prostatectomy: feasibility study in the cadaver model. J Endourol. 2014 Dec; 28(12): 1479-86. doi: 10.1089/end.2014.0244.
50. Bertolo R, Garisto J, Eltemamy M, Kaouk J (2019). Pure single-site transperineal robotic radical prostatectomy: first clinical report using the SP surgical system. Eur Urol Suppl 18: e2282
51. Tuğcu V, Akça O, Şimşek A, Yiğitbaşı İ, Şahin S, Yenice MG, Taşçı Aİ. Robotic-assisted perineal versus transperitoneal radical prostatectomy: A matched-pair analysis. Turk J Urol. 2019 Apr 3; 45(4): 265-272. doi: 10.5152/tud.2019.98254. PMID: 30978167; PMCID: PMC6619843.
52. Ramirez, D., Maurice, M.J. and Kaouk, J.H. (2016). Robotic perineal radical prostatectomy and pelvic lymph node dissection using a purpose-built single-port robotic platform. BJU Int, 118: 829-833. https://doi.org/10.1111/bju.13581
53. Jihad Kaouk, Alireza Aminsharifi, Clark A. Wilson, Guilherme Sawczyn, Juan Garisto, Simone Francavilla, Michael Abern, and Simone Crivellaro. Extraperitoneal versus Transperitoneal Single Port Robotic Radical Prostatectomy: A Comparative Analysis of Perioperative Outcomes. https://doi.org/10.1097/JU.0000000000000700
54. Chang Y, Lu X, Zhu Q, Xu C, Sun Y, Ren S. Single-port transperitoneal robotic-assisted laparoscopic radical prostatectomy (spRALP): Initial experience. Asian J Urol. 2019 Jul; 6(3): 294-297. doi: 10.1016/j.ajur.2018.08.002.
55. Cho JM, Moon KT, Yoo TK. Robotic Simple Prostatectomy: Why and How? Int Neurourol J. 2020 Mar; 24(1):12-20. doi: 10.5213/inj.2040018.009. Epub 2020 Mar 31.
56. Cho KJ, Koh JS, Choi JB, Kim JC. Factors associated with early recovery of stress urinary incontinence following holmium laser enucleation of the prostate in patients with benign prostatic enlargement. Int Neurourol J. 2018; 22: 200–5.

57. Chen, IH.A., Ghazi, A., Sridhar, A. et al. Evolving robotic surgery training and improving patient safety, with the integration of novel technologies. World J Urol (2020). https://doi.org/10.1007/s00345-020-03467-7
58. Aier-Hein, L., Vedula, S.S., Speidel, S. et al. Surgical data science for next-generation interventions. Nat Biomed Eng 1, 691–696 (2017). https://doi.org/10.1038/s41551-017-0132-7
59. Stoyanov, D. Surgical Vision. Ann Biomed Eng 40, 332–345 (2012). https://doi.org/10.1007/s10439-011-0441-z
60. Xuan Anh Nguyen, Damir Ljuhar, Maurizio Pacilli, Ramesh Mark Nataraja, Sunita Chauhan. Surgical skill levels: Classification and analysis using deep neural network model and motion signals, Computer Methods and Programs in Biomedicine, Volume 177, 2019, Pages 1-8, ISSN 0169-2607, https://doi.org/10.1016/j.cmpb.2019.05.008.
61. Hung AJ, Chen J, Che Z, Nilanon T, Jarc A, Titus M et al (2018). Utilizing machine learning and automated performance metrics to evaluate robot-assisted radical prostatectomy performance and predict outcomes. J Endourol 32(5): 438–444
62. Mangano MS, De Gobbi A, Beniamin F, Lamon C, Ciaccia M, Maccatrozzo L (2018). Robot-assisted nerve-sparing radical prostatectomy using near-infrared fluorescence technology and indocyanine green: initial experience. Urologia 85(1): 29–31
63. KleinJan GH, van den Berg NS, de Jong J, Wit EM, Thygessen H, Vegt E et al (2016). Multimodal hybrid imaging agents for sentinel node mapping as a means to (re)connect nuclear medicine to advances made in robot-assisted surgery. Eur J Nucl Med Mol Imaging 43(7): 1278–1287
64. Chennamsetty A, Zhumkhawala A, Tobis SB, Ruel N, Lau CS, Yamzon J et al (2017). Lymph node fluorescence during robot-assisted radical prostatectomy with indocyanine green: prospective dosing analysis. Clin Genitourin Cancer 15(4): e529–e534
65. Hung, AJ, Chen, J, Ghodoussipour, S, et al. A deep-learning model using automated performance metrics and clinical features to predict urinary continence recovery after robot-assisted radical prostatectomy. BJU Int 2019; 124: 487–495.
66. Goldenberg, S., Nir, G. & Salcudean, S.E. A new era: artificial intelligence and machine learning in prostate cancer. Nat Rev Urol 16, 391–403 (2019). https://doi.org/10.1038/s41585-019-0193-3
67. Porpiglia F, Manfredi M, Checcucci E, Mele F, Bertolo R, De Luca S, Garrou D, Cattaneo G, Amparore D, Fiori C (2017). 66–3D prostate MRI

reconstruction for cognitive robot assisted radical prostatectomy: Is it able to reduce the positive surgical margin rate? Eur Urol Suppl 16(3): e110

68. Kratiras Z, Gavazzi A, Belba A, Willis B, Chew S, Allen C, Amoroso P, Dasgupta P. Phase I study of a new tablet-based image guided surgical system in robot-assisted radical prostatectomy. Minerva Urol Nefrol. 2019 Feb; 71(1): 92-95. doi: 10.23736/S0393-2249.18.03250-2.

doi: 10.20903/zpopov-2022-0025 UDK: 616.65-089.87:007.52

Deyan ANAKIEVSKI[1]

ROBOT-ASSISTED RADICAL PROSTATECTOMY WITH DA VINCI XI- OUR INITIAL EXPERIENCE

Introduction: The last two decades have seen a dramatic change in the surgical treatment of most urological diseases with the advent of robotic surgical platforms. Technical improvements over the years have led to improved results in terms of oncological and functional outcomes. In fact, surgical treatment of prostate cancer has undergone the most dramatic change, with most cases now performed with a robot. Robotic surgery is also used for the surgical treatment of bladder cancer, kidney cancer, ureteral reconstruction, and other benign conditions. With the accumulation of additional experience and the realization of longer-term results, robotic surgery plays a growing role in the surgical treatment of many urological conditions.

In this abstract, we report our initial experience with the use of the da Vinci Xi system in prostate cancer.

Material and Methods: For the period from 01.01.2020 to 01.05.2021, 137 robot-assisted radical prostatectomies were performed in our clinic on patients with prostate cancer.

Results: All patients were operated on with the da Vinci Xi robotic system. The operations were performed transperitoneally using 6 ports, 4 for the robot and 2 for the assistant. The average operative time for the performed operations was 160 minutes, with minimal blood loss. In two patients we performed transfusion, no conversion of operations was required in other patients. About half of the patients underwent extended pelvic lymph node dissection due to the high risk of the patients.

[1] Clinic of Urology, University Hospital "St. Marina" – Varna Medical University "Prof. Paraskev Stoyanov" – Varna

Conclusion: The Da Vinci robotic system achieves a minimally invasive technique with the lowest levels of trauma and postoperative pain. Recovery is significantly faster than with open surgery, and patients can return to their normal routine as soon as possible.

Keywords: Robotic surgery, oncological, functional, robotic surgical platform

Introduction

Prostate cancer is the most common cancer in men and second leading cause of cancer-related deaths (C. J. Magnani *et al*., 2021). It represents the most frequent cancer diagnosed in men in Germany and Europe (N. Westhoff *et al*., 2021).

According to the International Agency for Research on Cancer GLOBOCAN, which provides cancer statistics for the year 2020 from 185 countries or territories worldwide and is based on the best available data of cancer incidence from population-based cancer registries, prostate cancer, with an incidence rate of 1,41 comes in second after lung cancer with an incidence rate of 2,21 (J. Ferlay *et al.*, 2021).

According to an update on the global cancer burden using the GLOBOCAN 2020, estimates of cancer incidence produced by the International Agency for Research on Cancer, there are 1,414,259 newly-diagnosed prostate cancer cases or 7.3% of all estimated 19,292,789 new cancer cases in 185 countries worldwide (H. Sung *et al.*, 2021). Efforts to build a sustainable infrastructure for the dissemination of cancer prevention measures and provision of cancer care in transitioning countries is critical for global cancer control.

The most recent annual dynamics of age-adjusted (world standard) incidence rates per 100,000 by year of prostate cancer diagnosis in Bulgaria is illustrated in Fig. 1 (Cancer Incidence in Bulgaria, 2017). After a stable increase until 2012, there is a decrease in the subsequent years, based on available information.

Figure 1 – Annual dynamics of age-adjusted (world standard) incidence rates per 100,000 by year of prostate cancer diagnosis in Bulgaria

According to an update on the global cancer burden using the GLOBOCAN 2020, estimates of cancer mortality rates, produced by the World Health Organization mortality database with information from 185 countries or territories worldwide, there are a total of 375,304 deaths of prostate cancer and 3.8% of all estimated 9,958,133 cancer deaths in 185 countries worldwide (H. Sung *et al.*, 2021).

The most recent annual dynamics of age-adjusted (world standard) mortality rates per 100,000 by year of prostate cancer diagnosis in Bulgaria is illustrated in Fig. 2 (Cancer Incidence in Bulgaria, 2017). It remains relatively high during the last five years of registration available.

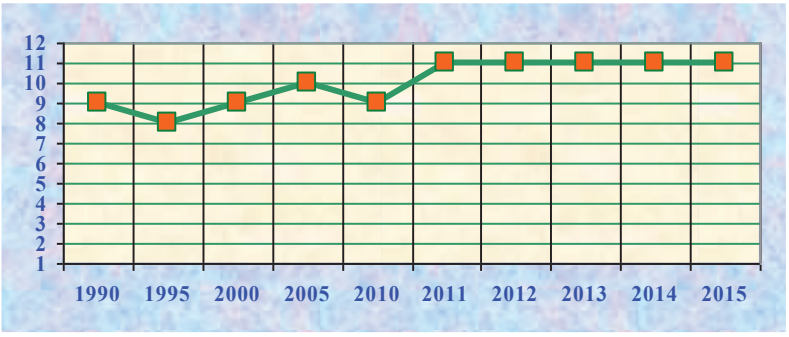


Figure 2 –. Annual dynamics of age-adjusted (world standard) mortality rates per 100,000 by year of prostate cancer diagnosis in Bulgaria

In the past, the European and American societies have not recommended prostate cancer screening with a prostate specific antigen, allowing physicians to make this decision themselves (C. Juliá-Romero *et al.*, 2021). In 2012, the American United States Preventive Task Force recommended to abandon the use of the prostate-specific antigen. This resulted in an increased incidence rate of the metastatic prostate cancer and its mortality rate as well. For the first time in 2018, the European Association of Urology released new recommendations in favour of screening, based on the prostate-specific antigen. In 2019, guidelines were updated with no changes in their recommendations.

Results of the largest screening trials reveal that prostate-specific antigen testing reduces the incidence rate of locally advanced and metastatic prostate cancer and shows an effect on cancer-specific mortality as well (N. Westhoff *et al*., 2021). Early diagnosis results in overdiagnosis and overtreatment of insignificant cancer cases with comorbidity, and thus a more individualized and risk-tailored modern strategy is needed. The German Federal Joint Committee declines the financial coverage of this testing by health insurance firms. Available validated instruments should accompany the baseline prostate-specific antigen to optimize detection of clinically significant prostate cancer.

Changes in screening guidelines, adoption of active surveillance, and implementation of high-cost technologies have changed treatment costs of prostate cancer.

The real-world costs of first-line prostate cancer management over 24 or 60 months following diagnosis using clinical electronic health records for 2008-2018 linked with the California Cancer Registry and the Medicare Fee Schedule are assessed in C. J. Magnani *et al*. (2021). In 3433 patients, surgery (54.6% of cases) is more common than radiation (22.3% of cases) or active surveillance (in 23% of cases). Two years following diagnosis, active surveillance ($2.97 per day) is cheaper than surgery ($5.67 per day) or radiation ($0.34 per day) in a favourable disease, while surgery ($7.17 per day) is less expensive than radiation ($16.34 per day) for unfavourable prostate cancer.

The health care costs and use one year after open radical prostatectomy (in 9853 patients) and robotic-assisted radical prostatectomy (in 1604 patients) are comparatively assessed within a retrospective cohort study of a US commercial claims database from January 1, 2013, to December 31, 2018 (K. E. Okhawere *et al*., 2021). The patients with robotic-assisted radical prostatectomy have a statistically significantly higher cost at the index hospitalization ($p<0.001$) but similar total cumulative costs observed within 180 days and one year after discharge. In these patients, one-year post discharge health care use is statistically significantly lower for mean numbers of emergency department visits ($p<0.001$) and hospital outpatient visits as well visits ($p<0.001$). The reduced use of health care services among these patients translates into additional savings of $2,929 ($p<0.001$) and approximately 1.69 fewer days ($p<0.001$) missed from work for health care visits.

Robot-assisted radical prostatectomy (RARP) is indicated for men with prostate cancer with an acceptable lifetime expectancy. Historically, the primary indication for RARP has been localized disease, but there has been recent evidence that men with non-localized disease will likely experience significant improvement in survival and, as such, are an indication as long as a complete discussion of risks, benefits, and complications has been completed. Contraindications that may impact the decision for RARP include a history of extensive abdominal or pelvic surgery, morbid obesity, or extremely large prostates.

Materials and methods

For the period from 01.01.2020 to 01.05.2021, 137 robot-assisted radical prostatectomies were performed in our clinic on patients with prostate cancer.

Clinicodemographic information of our patient cohort is presented in Table 1.

n = 137	
Median age, yrs.(range)	62(50-78)
PSA ng/ml, n% <5 5-10 >10	 17(12.4) 97(71) 23(16.5)
Median BMI kg/m^2 (range)	22.65 (18.14- 30.08)
ASA score, n (%) 1 2 3 4	 37 (27%) 70 (51%) 15 (11%) 15 (11%)
Previous abdominal or pelvic surgery, n. Repair of bowel perforation Herniorrhaphy Laparoscopic cholecystectomy	 12 14 9
IIEF-5, n (%) 22-25 15-21	 92 (67%) 24 (17.5%)
pGScore, n (%) 3+3 3+4 4+3 4+4 3+5 >4+5	 48(35%) 31(23%) 30 (22%) 18 (13%) 2 (0.8%) 8 (21.2%)
P- stage, n (%) pT2 pT3/T4	 98 (71.5%) 49 (28.5%)

All patients were operated with the robotic da Vinci Xi system. The operations were performed transperitoneally using 6 ports, 4 for the robot and 2 for the assistant. (Fig.1).

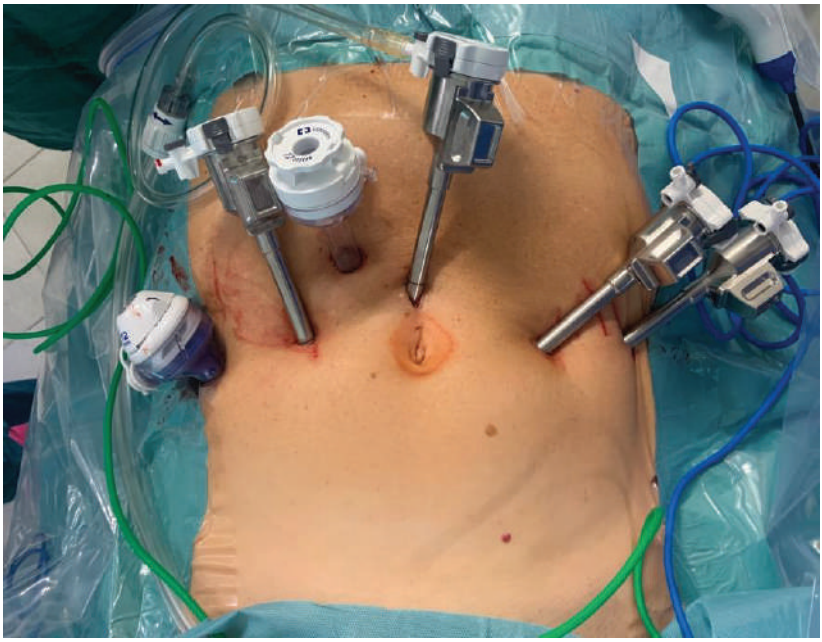


Fig. 1 – Patient position and port placement

All procedures are performed under general anaesthesia. After the patient is prepped and draped and a standard "time-out" completed, a Foley catheter is gently placed. Next, a Veress needle is placed in Palmer's point in the left upper quadrant. Once the needle reaches the peritoneal cavity, carbon dioxide is pumped into the abdomen via tubing from an insufflator, creating a pneumoperitoneum at 20 mm Hg. As the pressure slowly rises to 20, the port sites are prepared. Once at 20 mm Hg, the first port, the camera port is placed through a transverse incision just above the navel followed by the remaining five ports all under direct vision. Once all ports are positioned, AirSeal is installed and activated and the pneumoperitoneum is reduced to 10 mm Hg for the procedure, and in some cases to 8 mm Hg. Patients are then positioned in the Trendelenburg position, allowing gravity to gently pull the abdominal contents out of the pelvis, facilitating access to the bladder and prostate, and reducing the risk of injury to abdominal organs.

The legs are separated to facilitate docking of the da Vinci robot. Once the patient is positioned, the robot is docked.

The main Surgical Steps are:

1. Releasing the bladder
2. Endopelvic fascia
3. Anterior and posterior bladder neck
4. Seminal vesicles and rectum
5. "Clipless" transection of the prostatic pedicles and the NVBs
6. DVC and urethral transection
7. Rocco and Van Velthoven anastomosis

Results

The average operative time for the performed operations was 160 minutes, with minimal blood loss. In two patients we performed transfusion, no conversion of operations was required in other patients. About half of the patients underwent extended pelvic lymph node dissection due to the high risk of the patients.

Discussion

A. Nathan *et al*. (2021) state that robot-assisted radical prostatectomy is associated with fewer intraoperative adverse events, reduced blood loss and lower complication rates in localized prostate cancer patients than open and laparoscopic surgery but delivers comparable oncological and functional outcomes. The use of enhanced recovery after surgery pathways improves patient's recovery and experience, reduces costs and maintains patient's safety. New recommendations to reduce unnecessary postoperative blood tests are suggested.

According to E.F. Faria *et al*. (2021), robot-assisted radical prostatectomy for localized/locally advanced prostate cancer is comparable to open radical prostatectomy in terms of cancer control and complication rates while new evidence suggests that the robot-assisted radical prostatectomy

may have better functional outcomes, especially with respect to patient's urinary incontinence and erectile dysfunction.

The differences in perioperative characteristics, surgical complications as well as in oncological and functional control between the extraperitoneal and transperitoneal robot-assisted radical prostatectomy are evaluated using contemporary systematic review and meta-analysis of a total of 16 studies including 3,897 prostate cancer patients (M. Uy *et al*., 2021). The extraperitoneal robot-assisted radical prostatectomy offers faster operative time (mean difference of 14.4 min.); shorter postoperative stay length (mean difference of 0.9 days) as well as decreased postoperative ileus rates (relative risk of 0.2; between 0.1 and 0.7 at a confidence interval of 95%) and inguinal hernia formation (relative risk of 0.2; between 0.1 and 0.5 at a confidence interval of 95%).

J. U. Stolzenburg *et al*., (2021) compare a multicentre, randomized, patient-blinded controlled trial in Germany, focusing on the functional and oncological outcomes between robot-assisted and laparoscopic radical prostatectomy at a follow-up of 3 months of 718 prostate cancer patients. The difference in continence rates is 8.7% in favour of robot-assisted radical prostatectomy (54% versus 46%; p=0.027). Robot-assisted radical prostatectomy remains superior to the laparoscopic radical prostatectomy even after adjustment for the randomization stratum nerve sparing and age >65 years (hazard ratio of 1.40; between 1.09 and 1.81; p=0.008). A significant benefit in early potency recovery is also identified.

Within a prospective, single-center, single-surgeon cohort of 70 consecutive prostate cancer patients undergoing robot-assisted radical prostatectomy between January and December 2019, 35 patients operated on with the urethral fixation technique in which the urethral stump is fixed to the dorsal median raphe posteriorly and to the medial portion of the *m. levator ani* posterolaterally are compared with a control group of 35 patients receiving standard vesicourethral anastomosis only (V. Ficarra *et al*., 2021). There is urinary continence recovery at three months after catheter removal in 34 patients (in 97.14%) in group one and in 28 patients (in 80% of the cases) in the control group (p=0.02). The patients in group one report statistically significantly higher urinary continence rates even at one week

and one month after catheter removal than those in the control group (68.6% versus 45.7%; p=0.04 and 80% versus 54.3%; p=0.04, respectively). Ninety-day postoperative complications are observed in one patient in group one (in 2.86%) and in four patients (in 11.43% of the cases) in the control group.

The anatomy and concepts of the nerve-sparing radical prostatectomy are first conceptualized by P. C. Walsh & P. J. Donker in 1982. These authors conclude that impotence after radical prostatectomy results from injury to the pelvic nerve plexus that provides autonomic innervation to the corpora cavernosa (P. C. Walsh & P. J. Donker, 2017). The various mechanisms of injury to the neurovascular bundle lying in proximity to the prostate have further compounded the concept of nerve-sparing in radical prostatectomy.

The nerve-sparing techniques can be intrafascial or interfascial based on fascial dissections as well as antegrade or retrograde based on the surgical approach (A. Kumar *et al*., 2021).

A. Kumar *et al*. (2021) list the following nerve-sparing techniques: veil of Aphrodite technique (high anterior release), super veil technique, early retrograde release, hypothermic nerve-sparing robot-assisted laparoscopic prostatectomy, modified clipless antegrade nerve-sparing robot-assisted laparoscopic prostatectomy, flexible carbon dioxide laser fibre guided nerve-sparing robot-assisted laparoscopic prostatectomy, potassium titanyl phosphate laser nerve-sparing radical prostatectomy, laparoscopic Doppler ultrasound probe in nerve-sparing robot-assisted laparoscopic prostatectomy, and transrectal ultrasound-guided energy-free nerve-sparing laparoscopic radical prostatectomy.

A. Kumar *et al*. (2021) review the introduction of nerve-sparing to standard robot-assisted radical prostatectomy, which has positive results in terms of functional outcomes in addition to the oncological outcomes. A. Kumar *et al*. (2021) also review the current perspectives of nerve-sparing robot-assisted radical prostatectomy in terms of applied anatomy of the prostatic fascial planes, the neurovascular bundle, various nerve-sparing techniques and postoperative functional outcomes. Variables such as preoperative risk assessments, baseline potency, surgical anatomy of individual patients and surgeons' expertise play a major role in the outcomes. A tailored approach for each patient is required for applying the nerve-sparing approach during robot-assisted radical prostatectomy.

E. A. Sokolov *et al*. (2021) compare the efficacy and safety of unilateral or bilateral nerve-saving technique of radical prostatectomy between 117 prostate cancer patients aged ≥65 years and with 333 control patients from January 2012 to December 2019. There are minimal differences between both groups in erectile function 24 months after radical prostatectomy with bilateral nerve-saving technique (84.2% towards 87.9%), and more relevant differences with unilateral nerve-saving technique (53.8% towards 66.7%) (p=0.033). The performance of radical prostatectomy with nerve-saving technique in elderly patients is not associated with additional oncological risks. The bilateral nerve-saving technique provides high potency recovery results regardless of age.

Conclusion

RARP has shown to be an easily acquired laparoscopic technique, with shorter learning curves that rival the open procedure as best practice. We have witnessed a paradigm shift from open to robotic radical prostatectomy as the procedure of choice worldwide. When compared with the open approach, early studies indicate that robotic prostatectomy has equal outcomes in short-term oncologic control, continence, and potency with potentially favourable perioperative outcomes such as blood loss and transfusion rates, minor complications, narcotic use, convalescence, and length of hospital stay. Initial long-term oncologic and quality-of-life outcomes have also demonstrated similar outcomes to open radical prostatectomy.

REFERENCES

1. Al-Monajjed R, Arsov C, Albers P. Risikoadaptierte Früherkennung des Prostatakarzinoms - Update 2021. Urologe A. 2021 Apr 1. doi: 10.1007/s00120-021-01505-9.
2. Alves MR, Galvão ND, Souza RAG, Andrade ACS, Oliveira JCS, Souza BDSN, Azevedo EFS. Spatial and temporal distribution of cancer mortality in a Brazilian Legal Amazon State between 2000 and 2015. Rev Bras Epidemiol. 2021; 24(suppl 1): e210009.

3. Atere-Roberts J, Gray SC, Hall IJ, Smith JL. Racial and ethnic disparities in health status, chronic conditions, and behavioral risk factors among prostate cancer survivors, United States, 2015. Prev Chronic Dis. 2021 Apr 22; 18: E39. doi: 10.5888/pcd18.200523.
4. Bosland MC, Nettey OS, Phillips AA, Anunobi CC, Akinloye O, Ekanem IA, Bassey IE, Mehta V, Macias V, van der Kwast TH, Murphy AB. Prevalence of prostate cancer at autopsy in Nigeria - A preliminary report. Prostate. 2021 Apr 27. doi: 10.1002/pros.24133.
5. Bygrave A, Whittaker K, Paul C, Fradgley EA, Varlow M, Aranda S. Australian experiences of out-of-pocket costs and financial burden following a cancer diagnosis: a systematic review. Int J Environ Res Public Health. 2021 Mar 2; 18(5): 2422. doi: 10.3390/ijerph18052422.
6. Callender T, Emberton M, Morris S, Pharoah PDP, Pashayan N. Benefit, harm, and cost-effectiveness associated with magnetic resonance imaging before biopsy in age-based and risk-stratified screening for prostate cancer. JAMA Netw Open. 2021 Mar 1; 4(3): e2037657.
doi: 10.1001/jamanetworkopen.2020.37657.
7. Cancer Incidence in Bulgaria, 2014 & 2015. Sofia, National Oncological Hospital and Bulgarian National Cancer Registry, 2017. Vol. 25.
8. Choi HG, Bang WJ, Jo JK, Oh CY, Shim M, Cho JS. The association between family history of prostate cancer and development of prostate cancer among Korean population: A prospective cohort study using KoGES data. Medicine (Baltimore). 2021 Feb 19; 100(7): e24757.
doi: 10.1097/MD.0000000000024757.
9. Coughlin SS, Ayyala DN Luque JS, Moore JX. Predictors of prostate cancer screening among African American men treated at an Academic Medical Center in the Southern United States. Curr Cancer Rep. 2021; 3(1): 81-94.
10. de Oliveira RAR, Guimarães GC, Mourão TC, Favaretto RL, Santana TBM, Lopes A, Zequi SC. Prostate cancer screening in Brazil: a single center experience in the public health system. Int Braz J Urol. 2021; 47(3): 558-565.
11. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F. Cancer statistics for the year 2020: An overview. Int J Cancer. 2021 Apr 5. doi: 10.1002/ijc.33588.
12. Georgantopoulos P, Eberth JM, Cai B, Rao G, Bennett CL, Emrich CT, Haddock KS, Hébert JR. A spatial assessment of prostate cancer mortality-to-incidence ratios among South Carolina veterans: 1999-2015. Ann Epidemiol. 2021 Apr 6:S1047-2797(21)00054-5.
doi: 10.1016/j.annepidem.2021.03.010.

13. Gutiérrez-Pérez MI, González-Sagrado M, Conde-Vicente R, Amón-Sesmero JH. Influence of the diagnostic protocol and age on the prostate cancer incidence rate in Castilla y León according to the national registry 2010. Actas Urol Esp. 2021 Feb 17:S0210-4806(21)00005-X.
doi: 10.1016/j.acuro.2020.11.007.
14. Hamano T, Li X, Sundquist J, Sundquist K. Neighborhood social capital and incidence and mortality of prostate cancer: a Swedish cohort study. Aging Clin Exp Res. 2021 Apr 22. doi: 10.1007/s40520-021-01852-9.
15. Hippisley-Cox J, Coupland C. Predicting the risk of prostate cancer in asymptomatic men: a cohort study to develop and validate. Br J Gen Pract. 2021; 71(706): e364-e371.
16. Huynh-Le MP, Karunamuni R, Fan CC, Thompson WK, Muir K, Lophatananon A, Tye K, Wolk A, Håkansson N, Mills IG, Andreassen OA, Dale AM, et al.; PRACTICAL Consortium. Common genetic and clinical risk factors: association with fatal prostate cancer in the cohort of Swedish men. Prostate Cancer Prostatic Dis. 2021 Mar 15.
doi: 10.1038/s41391-021-00341-4.
17. Ibarrondo O, Lizeaga G, Martínez-Llorente JM, Larrañaga I, Soto-Gordoa M, Álvarez-López I. Health care costs of breast, prostate, colorectal and lung cancer care by clinical stage and cost component. Gac Sanit. 2021 Feb 18:S0213-9111(21)00009-1. doi: 10.1016/j.gaceta.2020.12.035.
18. Ikeda S, Sobue T, Kitamura T, Ishihara J, Kotemori A, Zha L, Liu R, Sawada N, Iwasaki M, Tsugane S, On behalf of the JPHC Study Group. Dietary acrylamide intake and the risks of renal cell, prostate, and bladder cancers: a Japan Public Health Center-Based prospective study. Nutrients. 2021 Feb 27;13(3):780. doi: 10.3390/nu13030780.
19. Iyer HS, Gomez SL, Chen JT, Trinh QD, Rebbeck TR. Trends in mortality among Black and White men with prostate cancer in Massachusetts and Pennsylvania: race and neighborhood socioeconomic position. Cancer. 2021 Apr 2. doi: 10.1002/cncr.33506.
20. Jochems SHJ, Wood AM, Häggström C, Orho-Melander M, Stattin P, Stocks T. Waist circumference and a body shape index and prostate cancer risk and mortality. Cancer Med. 2021; 10(8): 2885-2896.
21. Juliá-Romero C, Rechi-Sierra K, Juan-Escudero JU. Nuevas recomendaciones en el screening de cáncer de próstata mediante PSA. Semergen. 2021 Apr 7:S1138-3593(21)00062-9. doi: 10.1016/j.semerg.2020.12.009.
22. Karlsson AA, Hao S, Jauhiainen A, Elfström KM, Egevad L, Nordström T, Heintz E, Clements MS. The cost-effectiveness of prostate cancer scre-

ening using the Stockholm3 test. PLoS One. 2021 Feb 25; 16(2): e0246674. doi: 10.1371/journal.pone.0246674.

23. Lagacé F, Ghazawi FM, Le M, Savin E, Zubarev A, Powell M, Moreau L, Sasseville D, Popa I, Litvinov IV. Incidence and mortality of prostate cancer in Canada during 1992-2010. Curr Oncol. 2021; 28(1): 978-990.
24. Laroche E, L'Espérance S. Cancer incidence and mortality among firefighters: an overview of epidemiologic systematic reviews. Int J Environ Res Public Health. 2021 Mar 3; 18(5): 2519. doi: 10.3390/ijerph18052519.
25. Lécuyer L, Victor Bala A, Demidem A, Rossary A, Bouchemal N, Triba MN, Galan P, Hercberg S, Partula V, Srour B, Latino-Martel P, Kesse-Guyot E, et al. NMR metabolomic profiles associated with long-term risk of prostate cancer. Metabolomics. 2021 Mar 11; 17(3): 32.
doi: 10.1007/s11306-021-01780-9.
26. Lee EJ, Suh JD, Cho JH. The incidence of prostate cancer is increased in patients with obstructive sleep apnea: Results from the national insurance claim data 2007-2014. Medicine (Baltimore). 2021 Feb 12; 100(6): e24659. doi: 10.1097/MD.0000000000024659.
27. Lima CA, da Silva BEB, Hora EC, Lima MS, Brito EAC, Santos MO, da Silva AM, Nunes MAP, Brito HLF, Lima MMM. Trends in prostate cancer incidence and mortality to monitor control policies in a northeastern Brazilian state. PLoS One. 2021 Mar 25;16(3):e0249009.
doi: 10.1371/journal.pone.0249009.
28. Magnani CJ, Bievre N, Baker LC, Brooks JD, Blayney DW, Hernandez-Boussard T. Real-world evidence to estimate prostate cancer costs for first-line treatment or active surveillance. Eur Urol Open Sci. 2021; 23: 20-29.
29. Meissner VH, Jahnen M, Herkommer K. Familiäres Prostatakarzinom und genetische Prädisposition. Urologe A. 2021 Mar 15. doi: 10.1007/s00120-021-01491-y.
30. Michaeli T, Michaeli D. Prostate cancer follow-up costs in Germany from 2000 to 2015. J Cancer Surviv. 2021 Mar 1. doi: 10.1007/s11764-021-01006-w.
31. Ngcamphalala C, Ostensson E, Hlongwa M, Ginindza TG. Mapping evidence on the distribution of the costs associated with cancer of prostate, cervix, and female breast in the sub-Saharan Africa: protocol for a scoping review. Syst Rev. 2021 Apr 17; 10(1): 113.
doi: 10.1186/s13643-021-01672-y.
32. Ni Raghallaigh H, Eeles R. Genetic predisposition to prostate cancer: an update. Fam Cancer. 2021 Jan 24. doi: 10.1007/s10689-021-00227-3.

33. Okhawere KE, Shih IF, Lee SH, Li Y, Wong JA, Badani KK. Comparison of 1-year health care costs and use associated with open vs robotic-assisted radical prostatectomy. JAMA Netw Open. 2021 Mar 1; 4(3): e212265. doi: 10.1001/jamanetworkopen.2021.2265.
34. Plym A, Penney KL, Kalia S, Kraft P, Conti DV, Haiman C, Mucci LA, Kibel AS. Evaluation of a multi-ethnic polygenic risk score model for prostate cancer. J Natl Cancer Inst. 2021 Apr 1:djab058. doi: 10.1093/jnci/djab058.
35. Prizment AE, McSweeney S, Pankratz N, Joshu CE, Hwang JH, Platz EA, Ryan CJ. Prostate cancer mortality associated with aggregate polymerphisms in androgen-regulating genes: The Atherosclerosis Risk in the Communities (ARIC) Study. Cancers (Basel). 2021 Apr 19; 13(8): 1958.
36. Roshandel G, Ferlay J, Ghanbari-Motlagh A, Partovipour E, Salavati F, Aryan K, Mohammadi G, Khoshaabi M, Sadjadi A, Davanlou M, Asgari F, Abadi H, et al. Cancer in Iran 2008 to 2025: Recent incidence trends and short-term predictions of the future burden. Int J Cancer. 2021 Apr 21. doi: 10.1002/ijc.33574.
37. Sattayapiwat O, Wan P, Hernandez BY, Le Marchand L, Wilkens L, Haiman CA. Anthropometric measures and risk of prostate cancer in the multi-ethnic cohort. Am J Epidemiol. 2021 Mar 6:kwab054. doi: 10.1093/aje/kwab054.
38. Novara G, Ficarra V, Mocellin S, et al. Systematic review and meta-analysis of studies reporting oncologic outcome after robot-assisted radical prostatectomy. Eur Urol 2012; 62: 382–404.
39. Box GN, Ahlering TE. Robotic radical prostatectomy: Long- term outcomes. Curr Opin Urol 2008; 18: 173–179.
40. Skarecky D, Morales CA, Ahlering TE. Simple method to predict return of continence after robot-assisted radical prostatectomy. J Endourol 2011; 9: 1451–1455.

ПОГЛАВЈЕ VI

МОЛЕКУЛАРНА БИОЛОГИЈА И ГЕНЕТИКА ВО УРО-ОНКОЛОГИЈАТА, НЕФРОЛОГИЈАТА И МЕДИЦИНАТА

doi: 10.20903/zpopov-2022-0026 UDK: 616.62-006.6

François RADVANYI[1]

BLADDER CANCER, WHEN BASIC AND CLINICAL RESEARCH MEET

It is both an honor and a pleasure for me to write about bladder cancer research on the occasion of the 70th birthday of Professor Popov, my friend Zivko.

I have always enjoyed meeting up with Zivko, sharing his passion and enthusiasm, and watching the expression on his face switch between facetious and extremely serious. It has never ceased to amaze me how such a skilled surgeon also manages to display such dedication to his research projects. I have been lucky enough to have the opportunity to work with him, both while he was working at Henri Mondor Hospital in Créteil, and in Skopje, in the framework of a European project tackling a difficult question: why some non-muscle-invasive bladders recur, whereas others do not.

It is no overestimate to say that Zivko's research work with Dominique Chopin in France (Chopin *et al*., 1993; Mazerolles *et al*., 1994; Popov *et al*. 1997, 2000, 2004; Saint *et al*., 2004) shaped my career. Zivko and Dominique, whose names and contributions are often associated in my mind, were among the first to understand the importance of annotated tumor banks and the key role of surgeons in research studies based on this invaluable resource. They were also well ahead of the curve in understandding the value of multidisciplinary collaborations in the field of cancer research. I learnt a lot from may interactions with Zivko, Dominique and the young urologists training with them at the time at Henri Mondor Hospital, who were attracted by the possibility of performing research in an environment in which laboratory and clinic were closely linked: Marc Colombel,

[1] CNRS research director, Institut Curie – UMR144 CNRS Cell Biology and Cancer, Paris, France

Jean-Jacques Patard, Vincent Ravery, and Fabien Saint, to name but a few. I also remember Sixtina Gil Diez de Medina, an MD who trained in Chile before joining the team at Henri Mondor, as an essential element of this collaborative atmosphere. Through these interactions, I got to know many influential MDs from France and elsewhere including, in particular, two pathologists: Yves Allory, a long-standing collaborator of mine who is now at Institut Curie, and Theo van der Kwast, who was working in Rotterdam at the time and is now in Toronto. Unfortunately, Dominique is no longer with us, but he will always remain in our memories.

Zivko, the time I spent in Skopje, at your invitation, will remain with me forever as an unforgettable moment of science, culture and friendship. I wish we could have worked more together, and had more opportunities to meet.

We have witnessed the progress in both basic and clinical aspects of bladder cancer research, and have, on occasions, contributed to it. This progress was made possible by the persistence of several teams, including several teams in Europe, and by technical advances, particularly in molecular biology and data analyses. The success of recent clinical trials of immune-therapies and targeted therapies has added a further impetus to this progress.

Many technical advances have been made in the last few years, driving considerable progress in our molecular understanding of normal and pathological processes, including cancer. This technical revolution began with the sequencing of the human genome. The advent of microarray technology was the first major step forward in this process, making it possible to study the expression of thousands of genes simultaneously. This technology was then applied to studies of the genomic alterations occurring in cancer. A few years later, high-throughput sequencing was developed. This technology revolutionized sequencing, by greatly decreasing its cost, and making it possible to identify polymorphisms (the differences between two individuals at the DNA level), somatic mutations (mutations occurring during tumorigenesis), copy number alterations, and translocations. Not only was high-throughput sequencing used to study the genetics of cancer, but it was also used to investigate changes at the epigenetic level (the epigenome is all the modifications making two cells with identical genomes different). High-throughput sequencing can be used to study the DNA methylome, but also the proteins bound to DNA and their chemical modifications, DNA accessi-

bility and the three-dimensional structure of the DNA (two regions of DNA, even if separated by large distances, can interact, through the creation of loops). The revolution continues, and it is now possible to perform single-cell studies of many parameters that just a few years ago could only be studied on thousands of cells at a time. Transcriptomes, genomic alterations, methylomes, histone modifications, and DNA accessibility can be now studied in single cells, and such studies have revealed an unsuspected level of heterogeneity in tumors. Single-cell analyses are generally performed on cells obtained by tissue digestion, resulting in a loss of spatial information. Such information is crucial to place the cell in its context, and to identify its neighbors and possible mechanisms of cell-to-cell communication. Fortunately, *in situ* techniques have progressed at the same pace as techniques for precisely localizing proteins (by multiplex immunofluorescence) and RNA (spatial transcriptomics, named technology of the year in 2020 by the journal Nature). With the resulting data avalanche, data analysis has become crucial, and bioinformaticians and statisticians have become essential actors in biology generally, and in cancer research in particular, resulting in an ever-growing need for multidisciplinarity in research.

I would like to take the opportunity here to introduce our work on FGFR3, an Ariadne's thread of a research adventure building on the foundations laid at Henri Mondor Hospital. FGFR3 is now recognized as a major actor in bladder cancer. It is activated by point mutations in about 50% of non-muscle-invasive bladder cancers (NMIBC) and 15% of muscle-invasive bladder cancers (MIBC) (Cappellen *et al*., 1999; Billerey *et al*., 2001; Neuzillet *et al*., 2012). In rarer cases (about 4% of MIBC), it may also be activated by fusion (Williams *et al*., 2013). FGFR3 is a tyrosine kinase receptor, and is therefore a possible therapeutic target. Indeed, the favorable results obtained in recent clinical trials (Loriot *et al*., 2019) have led to FDA approval for anti-FGFR therapy in patients with advanced bladder cancer with tumors presenting *FGFR3* mutation or fusions of either FGFR2 or FGFR3 (*FGFR3* mutations are the commonest of these three events, occurring in 75% of cases).

The first identification of *FGFR3* mutations (Cappellen *et al*., 1999) by my team (then part of Jean Paul Thiery's laboratory), in collaboration with Henri Mondor Hospital, was only possible because of the bank of frozen tumors set up by Dominique and Zivko. This bank was highly

diverse, containing bladder tumors of various stages and grades, and it was entirely unique at the time. We are still using it today, more than thirty years after its establishment. The protocols we used to obtain not only DNA, but also RNA and proteins, from the tumors in this tumor bank were robust. We took our time at the start, carefully checking the quality of the protocol initially described by the group of M. Knowles (Coombs *et al.,* 1990), but it paid off. The RNA and DNA samples we obtained remain undegraded and can still be used for all the techniques our research demands. We initially performed targeted studies on a few genes/proteins, such as FGFs and FGFRs, then large-scale DNA and RNA analyses (Stransky *et al*., 2006; Biton *et al*., 2014; Rebouissou *et al*., 2014), and we are now performing large-scale analyses on proteins (Sanchez-Quiles *et al*., 2021). The techniques are evolving and the tumor bank is still going strong.

Before FGFR3, FGFR2

Why did we start to work on FGFR3, one of the four members of the FGFR tyrosine kinase receptor family? When we started this work, large-scale mutations analyses had not yet been developed, so we had to focus on a small number of genes. The team at Henri Mondor Hospital was interested in FGFs, the ligands of FGFRs, because these factors could be found in the urine. For this reason, we focused on the FGF receptors in bladder tumors, although, with hindsight, we have still not found the link between the important role of FGFR3 in bladder cancer and the presence of FGFs in urine. The first FGFR we studied was FGFR2. The main isoform of this receptor expressed in the bladder urothelium (the tissue of origin of most bladder cancers) is FGFR2b. We unexpectedly found that FGFR2b was lost during tumor progression and that it inhibited the growth of bladder tumor cells. So, FGFR2b, which, as a tyrosine kinase receptor, we had expected to play a protumorigenic role, actually had the opposite effect in bladder tumors (Gil Diez de Medina *et al*., 1997, 1999; RIcol *et al*., 1999). Many examples of proteins that play opposite, inhibitory or protumorigenic roles in different tissues and differentiation states are now known. FGFR3 is another striking example. It inhibits bone growth (activating mutations of *FGFR3* in the germline are responsible for several forms of mild to severe dwarfism), and has a protumorigenic role in various tumors.

Activating *FGFR3* mutations in bladder tumors

Our results for FGFR2 were not what we had expected. We were searching for tyrosine kinase receptors with a tumorigenic role to serve as potential therapeutic targets. We therefore moved on to another FGFR expressed in the urothelium: FGFR3. We initially feared that history would repeat itself and, indeed, we observed a downregulation of FGFR3 in a significant proportion of bladder tumors and several other indications of a possible tumor suppressor role for FGFR3. We therefore sequenced *FGFR3*, looking for inactivating mutations. To our surprise, we found a large number of recurrent mutations (Cappellen *et al*., 1999), all of which had already been reported as germline mutations in a severe form of dwarfism, thanatophoric dysplasia (Tavormina *et al*., 1995; Rousseau *et al*., 1995). These mutations had already been shown to be activating mutations (Naski *et al*., 1996), providing genetic evidence that FGFR3 can act as an oncogene in bladder cancers. We then joined forces with the team of Ellen Zwarthoff and Theo van der Kwast. Theo was already collaborating with the team at Henri Mondor Hospital, and we performed a systematic search for *FGFR3* mutations in bladder cancer. Remarkably, most of the mutations we found were mutations already implicated in thanatophoric dysplasia, but other rarer mutations were picked up that had also already been reported as germline mutations in other forms of dwarfism or bone diseases (achondroplasia, hypochondroplasia, Crouzon syndrome with acanthosis nigricans) (van Rhijn *et al*. 2002).

Funnily enough, David Cappellen — the student in the team working on FGFR2 and FGFR3, who brought the techniques he had learnt at the Gustave Roussy Institute that were essential for the identification of *FGFR3* mutations to the laboratory — described only the possible tumor suppressor role of FGFR3 in his PhD thesis. We discovered the activating mutations just after he submitted his thesis. We had also already submitted a manuscript in which we described the inhibitory role of FGFR3. I called the editor to explain that we now had genetic evidence of an oncogenic role for this receptor. To my surprise, the editor was immediately convinced, willing to wait for the modifications, and he then sent the manuscript for review. It turns out that FGFR3 in the bladder urothelium, may have either an oncogenic role or a tumor suppressor role, and the story of its dual role does not end here. We filed a patent for the identification of *FGFR3* activating

mutations, as this discovery revealed that FGFR3 could be used as a therapeutic target and diagnostic marker for certain types of bladder cancer. Institut Curie helped us with the patent application, but we also had help from another David, David Ricol, a former student from the team who had worked on FGFR2 and FGFR3 during his PhD before studying to become a patent attorney. This patent was contested by Genentech and Roche, which annoyed me considerably, but David Ricol told me not to worry, it was a good sign, indicating that they were interested. Genentech and Roche got hold David Cappellen's PhD thesis, in an attempt to show that we had disclosed the information before the patent application was filed, which would have invalidated the patent. However, as the activating mutations were not discovered until after the submission of this thesis manuscript, the oncogenic role of FGFR3 was not in it and only the tumor suppressor role of the receptor was described!

Inverse correlations of FGFR3 mutations with stage and grade

Thanks to the annotations of the Henri Mondor tumor bank (pathological and clinical data were available for all the tumor samples) and the involvement of a young pathologist, Marie-Hélène Aubriot-Lorton, whose Masters project focused on FGFR3, we were able to detect an inverse correlation between the *FGFR3* mutation frequency on the one hand, and grade and stage on the other. The percentage of *FGFR3* mutations was high in G1, lower in G2, and very low in G3 tumors (including carcinoma *in situ*, which is always of high grade). A similar pattern was observed for stage, with the percentage of *FGFR3* mutations high in Ta tumors (papillary tumors not invading the basement membrane), lower in T1 tumors (invading the basement membrane but sparing the smooth muscle) and even lower in T2-4 (tumors invading the smooth muscle). Discussions with two pathologists specializing in bladder cancer, Claude Billerey and Dominique Vieillefond, suggested that the inverse relationship with stage might be even stronger, due to the possible misclassification of some Ta as T1. Dominique Chopin and the Head of the Pathology Department at Henri Mondor Hospital, Serge Zafrani, agreed that Claude Billerey and a pathologist from the Department, Marie-Pierre Bralet, should re-examine the slides. This review

of the slides, blind to *FGFR3* mutation results, indeed revealed that several of the mutated T1 tumors were actually Ta tumors.

This inverse correlation between *FGFR3* mutations and stage or grade (Billerey *et al*., 2001) was initially puzzling. However, it turned out to be easily explained by the existence of two pathways of tumor progression in bladder cancer: the low-grade Ta pathway and the CIS pathway, consistent with the initial observations of clinicians (progression is rarely observed for low-grade Ta tumors, but frequently observed for carcinoma *in situ* (CIS), high-grade tumors that do not invade the basement membrane. *FGFR3* is a driver gene for the Ta pathway, but not for the CIS pathway. The percentages of *FGFR3* mutations found were completely consistent with this hypothesis: 70% in TaG1 and TaG2 tumors, none in CIS, and 15% in T2-4 tumors (about 80% of MIBC arise from CIS). An association had previously been found between *TP53* mutation and the CIS pathway. *TP53* mutations are absent from Ta low-grade tumors, but are frequent in CIS and MIBC (Spruck *et al*., 1994). It is essential to think in terms of the existence of different pathways. Not doing so would results in the conclusion that FGFR3 is initially important for tumorigenesis but not after progression. This conclusion is entirely incorrect. Tumors with *FGFR3* mutations continue to express FGFR3 when they progress and continue to be dependent on this receptor. This situation is reminiscent of that in chronic myeloid leukemia, in which the progression of the tumor is built around the activation of an oncogene, BCR-ABL.

Take care when sampling*:* *FGFR3* may also be mutated in cervical cancer, albeit rarely.

After discovering *FGFR3* mutations in bladder cancer, we decided to check for the presence of these mutations in other carcinomas. We were already working on cervical cancers in collaboration with Xavier Sastre-Garau, Head of the Pathology Department at Institut Curie. This seemed like a good place to start, so we measured FGFR3 expression in cervical cancers. Expression levels ranged from absent to very high. We selected 12 tumors with different levels of FGFR3 expression and sequenced them to check for

FGFR3 mutations. We found activating mutations in three of these tumors, which led us to conclude that *FGFR3* mutations were also frequent in cervical cancer. We subsequently realized that *FGFR3* mutations were less frequent in cervical than in bladder cancers (Rosty *et al*., 2005). Our initial observation was biased due to the small number of cervical tumors studied and the non-random nature of their selection (based on FGFR3 expression). All the mutations were found in cervical tumors with high levels of FGFR3 expression (as for bladder cancers).

Functional evidence for an oncogenic role of FGFR3 *in vitro*

Activating mutations provided genetic evidence for a protumorigenic role of FGFR3 in bladder cancer. We then looked for functional evidence of this oncogenic role. Whilst visiting Yves Fradet's laboratory in Canada (we were introduced by Dominique Chopin), I was informed that they had a a cell line derived from NMIBC — the MGHU3 cell line. This cell line was therefore possibly mutated for FGFR3. They sent it to us. we checked for and found activating mutations of *FGFR3* in this cell line, which we were then able to use to investigate the functional role of FGFR3 in bladder cancer. Fortunately, the arrival of this cell line coincided with the arrival in the laboratory of a young researcher, Isabelle Bernard-Pierrot, who was highly motivated by this project and had expertise in functional studies. We showed that FGFR3 inactivation by siRNA approaches or with an FGFR inhibitor in this cell line led to a loss of its tumor properties. We also showed that mutated *FGFR3* could transform an immortalized cell line (Bernard-Pierrot *et al*., 2006), thus demonstrating a tumorigenic role for mutated FGFR3 *in vitro*.

Functional evidence for an oncogenic role of FGFR3 *in vivo*: from bladder tumors to benign skin tumors

We investigated the possible tumorigenic role of FGFR3 *in vivo*, by generating transgenic mice expressing mutated *FGFR3*. We studied the most common mutation of *FGFR3*, FGFR3 S249C. As our goal was to demonstrate the possible oncogenic role of FGFR3 in the bladder urothelium in

particular, but possibly more generally in epithelia, we used different promoters to target the expression of the mutated *FGFR3* to the urothelium (the promoter of the uroplakin 2 gene, which I obtained after my visit to Tung-Tien Sun's laboratory in New York), and to other epithelia (the promoter of the keratin 5 gene, obtained from Jose Jocarno in Madrid). We derived several lines for each construct. Our first striking observation concerned the keratin 5 promoter – FGFR3 S249C-transgenic mice. They had verrucous lesions on the snout and eyelids, and older mice also had skin lesions on the throat and upper chest. Histologically, these lesions resembled benign skin tumors. In parallel, we investigated *FGFR3* mutations in a panel of various non-bladder carcinomas, including skin carcinomas (squamous cell carcinoma and basal cell carcinoma); we found no *FGFR3* mutations in these lesions (Karoui *et al*., 2001). In addition to observing these skin lesions in keratin 5 promoter – FGFR3 S249C mice, we also performed laser microdissection on various epithelia, and we found that FGFR3 was strongly expressed in the urothelium and epidermis. We therefore expected to observe frequent *FGFR3* mutations in skin cancers, as in bladder cancer. During a discussion of these results at one of our laboratory meetings, a pathologist, Christophe Rosty, said that the skin lesions in the transgenic mice resembled benign skin tumors observed in humans and suggested that we look at *FGFR3* mutations in the most common benign tumor in humans, seborrheic keratosis. The tumor bank at Institut Curie included several such lesions, so we rapidly investigated *FGFR3* mutations in these benign tumors. We identified the very same *FGFR3* mutations that we had found in bladder cancer and had previously been identified in patients with thanatophoric dysplasia at a high frequency in these lesions (Logié *et al*., 2005). Thus, activating mutations of *FGFR3* inhibit growth in bone, they induce proliferation but never transformation in the epidermis, and they cause proliferation and transformation leading to low-grade tumors in the urothelium that may, in rare cases, progress to MIBC. The reasons for these differences remain unclear. We found that even though the *FGFR3* mutations observed in bladder cancer and seborrheic keratoses were the same, their distributions were different, with FGFR3 S249C the main mutation in bladder cancer, whereas this mutation was no more frequent than other *FGFR3* mutations in seborrheic keratoses. At the time, we interpreted this observation incorrectly as indicating that FGFR3 S249C was more transforming than other *FGFR3*

mutations. We performed a number of observations to test this hypothesis, but were eventually forced to conclude that the transforming ability of FGFR3 S249C was no higher than that of another mutation we tested, Y375C.

We also generated transgenic mice expressing mutated *FGFR3* under the control of the uroplakin 2 promoter. Initial observations revealed hyperplasia of the urothelium in these mice (this model is more difficult to study than keratin 5 promoter – FGFR3 S249C mice, in which the tumors are on the skin and their appearance is easy to follow). Isabelle Bernard-Pierrot had the patience to follow a large enough number of mice for sufficiently long periods of time to observe lesions, which Yves Allory and Jacqueline Fontugne, a pathologist who had joined his team, identified as low-grade Ta tumors. The expression of mutated *FGFR3* in mouse urothelium therefore reproduced the very frequent association of *FGFR3* mutation with low-grade Ta tumors observed in humans. A bias toward higher tumor penetrance in males was observed in the mouse model. We checked the human data and found that the same was true for humans: the male-to-female ratio was higher in patients with *FGFR3*-mutated tumors than in patients with non-mutated tumors, suggesting a role for the androgen receptor in the process of carcinogenesis in tumors with *FGFR3* mutations.

An explanation for the higher frequency of FGFR3 S249C in certain cancers, including bladder cancer

There are observations for which the explanations remain elusive for long periods, suddenly crystalizing years later. In response to an invitation from David McConkey, I once gave a seminar at Johns Hopkins University focusing on FGFR3, in which I suggested that the high frequency of the FGFR3 S249C mutation was due to a greater transforming activity of this mutation. At the time, I was 100% convinced by this theory. The following day, I went to Bethesda/Rockville to visit several other scientists, including, in particular, Mila Prokunina-Olsson at the NIH, who was working on polymorphisms associated with bladder cancer, which was a subject of great interest to our laboratory, as it can help to identify genes of importance in bladder carcinogenesis. Whilst preparing for my meeting with her, I was in my hotel room reading one of her papers on a polymorphism associated

with a gene coding encoding one of the APOBEC enzymes (a large proportion of the mutations in bladder cancer are due to APOBECs). Looking at the sequence of the motif recognized by APOBEC, it suddenly hit me that the higher frequency of FGFR3 S249C could not be due to an advantage of this mutation over other *FGFR3* mutations, but to the mutagenesis process, which preferentially targeted the S249 site. I checked and found that S249 was the only *FGFR3* mutation with an APOBEC motif. At this point, all this was just an idea, but I discussed this idea with Mila and then with the members of my laboratory on my return. Having an idea is just the first step. Many ideas remain just that if they are not realized, choosing the right idea and demonstrating is validity, is probably the most important step. Two very motivated students in the laboratory, Mingjun Shi and XIangYu Meng, were immediately convinced by this idea, as was Isabelle Bernard-Pierrot. They interrupted the studies they were working on, and investigated it. If FGFR3 S249C was due to APOBEC, then APOBEC signatures should be observed in tumors carrying the FGFR3 S249C mutation than in tumors carrying other *FGFR3* mutations. A modest but significant correlation was observed in the TCGA data, but these data include only MIBC tumors, relatively few of which have *FGFR3* mutations. We wrote to a Danish researcher, Lars Dyrskjøt (I was a member of his PhD panel, and he now heads a very active group working on bladder cancer). Lars had molecular data for a large series of NMIBC tumors, thanks once again to the existence of a large tumor bank set up over a number of years and initiated by his former boss, Torben Orntoft. The frequency of *FGFR3*-mutated tumors was high in this tumor bank, and he found a highly significant correlation between FGFR3 S249C mutation and the APOBEC mutational signature. This and other observations in other tumor types with and without a higher frequency of FGFR3 S249C mutation than of other *FGFR3* mutations led us to conclude that the carcinogenesis process may be biased toward particular recurrent mutations. These recurrent mutations could be driver mutations, like FGFR3 S249C (Shi *et al*., 2019) or even passenger mutations (Shi *et al*., 2020).

FGFR3 mutation and molecular subtypes of MIBC

Bladder cancer is a very heterogeneous disease, both clinically and histologically. Many attempts have been made to identify molecularly

homogeneous classes of tumors that could be used to predict the outcome for a given patient and the response to treatment. One of the first cancers for which such a strategy, based on transcriptome data, was applied was breast cancer (Perou *et al*., 2000; Sorlie *et al*., 2001). Several subtypes, including the basal and luminal subtypes, were identified. Basal tumors express genes that are also expressed by the cells of the basal layer of stratified epithelia, and have low levels of differentiation markers. Luminal tumors express the estrogen receptor and differentiation markers. Luminal breast tumors are usually hormone-dependent, whereas basal tumors are not. We and other teams have also used transcriptomic data to identify homogeneous subtypes of bladder cancer (for example, Cancer Genome Atlas Research Network 2014; Hurst *et al*., 2017; Robertson *et al*., 2017; Marzouka *et al*., 2019; Kamoun *et al*., 2020; Lindskrog *et al*., 2021). In these studies, it was important to consider NMIBC and MIBC separately, because these two groups of tumors constitute different diseases in terms of their clinical features. Separating them improves the granularity of each category. Strikingly, in all these studies, attempts to identify molecularly homogeneous classes identified a main division separating the tumors into two groups: basal (also called basal/squamous) and luminal tumors. As for breast cancer, basal bladder tumors express markers of the basal layer of stratified epithelia and have low levels of differentiation markers, whereas luminal bladder tumors express high level of differentiation markers. The similarities between bladder and breast cancers extend even further: both basal bladder cancers and basal breast cancers are particularly aggressive, with deaths occurring earlier than for luminal cancers; furthermore, luminal bladder cancers are also dependent on a nuclear receptor, not the estrogen receptor as for luminal breast cancers, but PPARγ (Biton *et al*., 2014; Rochel *et al*., 2019). The luminal subtypes of MIBC include one particular subtype identified by several teams: the luminal papillary subtype (described as “papillary” on the basis of the frequent papillary morphology of these tumors). Most of the *FGFR3* mutations are found in tumors of this subtype (2/3 of tumors with *FGFR3* mutations belong to this subtype, and 40% of the tumors of this subtype present mutations of this receptor gene). In addition, all the tumors of this subtype (not just those with *FGFR3* mutations) present a transcriptomic signature of activation for this receptor. Several questions have been posed: do the luminal papillary subtype tumors without *FGFR3* muta-

tions respond to anti-FGFR therapy? Does the response to anti-FGFR3 therapy differ between tumors with *FGFR3* mutations of the luminal papillary subtype and other subtypes? Given that the stroma of the luminal papillary tumors is particularly poor (small numbers of normal cells in the tumor microenvironment), it also remains unclear whether the response to immunotherapy or combination therapy (anti-FGFR and hormonotherapy) is likely to differ between patients with luminal papillary tumors with *FGFR3* mutations and patients with mutated tumors of other subtypes.

Dividing tumors into molecular categories (not necessarily based on the transcriptome) may be useful not only for predicting treatment response, but also for identifying prognostic markers. For example, in studies considering only NMIBC tumors with *FGFR3* mutations, we identified a marker of progression: *CDKN2A* loss. This finding was based on the observation that mutated MIBC tumors often display CDKN2A loss, whereas mutated NMIBC tumors do not, suggesting that *CDKN2A* loss is an important event in *FGFR3*-mutated tumors (Rebouissou *et al*., 2012).

The mutated FGFR3 signaling pathway: the adventure continues

Not all patients with *FGFR3*-mutated MIBC respond to anti-FGFR3 therapy, and initial responders eventually relapse. It is, therefore, important to identify the patients likely to present an initial response to treatment, and to develop additional treatments. Studies of the signaling pathway of mutated FGFR3 could potentially lead to the identification of additional therapeutic targets. Isabelle Bernard-Pierrot has tackled this problem, successfully identifying important actors in this signaling pathway. In particular, she identified a positive feedback loop between mutated FGFR3 or fusion proteins involving FGFR3 and the transcription factor MYC. Mutated FGFR3 and FGFR3 fusion proteins activate MYC, in turn activating FGFR3 expression. She has also begun to identify intermediate proteins involved in this loop that could potentially serve as therapeutic targets (Mahé *et al*., 2018). One key aspect of resistance to treatment is heterogeneity (the existence of several types of tumor cell, differing genetically and/or epigenetically, within a tumor), and another is plasticity (tumor cells can change their phenotype in response to the tumor microenvironment and treatment). Heterogeneity and

plasticity are mediated by changes in signaling pathways and the activation of transcription factors. Host polymorphisms and, probably, the history of patients (the environmental factors to which patients have been exposed) may also be important for predicting the response to treatment. Many exciting discoveries of benefit to patients are undoubtedly yet to come.

I must thank Zivko and Dominique for their enthusiasm for bladder cancer research, for attracting me into this field, for introducing me to talented researchers and MDs, for having understood the importance of annotated tumor banks before it became widely apparent, for teaching me about the clinical aspects of this disease and for demonstrating the importance of multidisciplinarity.

REFERENCES

1. Bernard-Pierrot I, Brams A, Dunois-Lardé C, Caillault A, Diez de Medina SG, Cappellen D, Graff G, Thiery JP, Chopin D, Ricol D, Radvanyi F. Oncogenic properties of the mutated forms of fibroblast growth factor receptor 3b. Carcinogenesis. 2006; 27: 740-7.
2. Billerey C, Chopin D, Aubriot-Lorton MH, Ricol D, Gil Diez de Medina S, Van Rhijn B, Bralet MP, Lefrere-Belda MA, Lahaye JB, Abbou CC, Bonaventure J, Zafrani ES, van der Kwast T, Thiery JP, Radvanyi F. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. Am J Pathol. 2001; 158: 1955-9.
3. Biton A, Bernard-Pierrot I, Lou Y, Krucker C, Chapeaublanc E, Rubio-Pérez C, López-Bigas N, Kamoun A, Neuzillet Y, Gestraud P, Grieco L, Rebouissou S, de Reyniès A, Benhamou S, Lebret T, Southgate J, Barillot E, Allory Y, Zinovyev A, Radvanyi F. Independent component analysis uncovers the landscape of the bladder tumor transcriptome and reveals insights into luminal and basal subtypes. Cell Rep. 2014; 9: 1235-45.
4. Cappellen D, De Oliveira C, Ricol D, de Medina S, Bourdin J, Sastre-Garau X, Chopin D, Thiery JP, Radvanyi F. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat Genet. 1999; 23: 18-20.
5. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature. 2014; 507: 315-22.

6. Chopin DK, Popov Z, Ravery V, Bellot J, Hoznek A, Patard JJ, Abbou CC, Colombel M. Prognostic factors in superficial bladder cancer. World J Urol. 1993; 11: 148-52.
7. Coombs LM, Pigott D, Proctor A, Eydmann M, Denner J, Knowles MA. Simultaneous isolation of DNA, RNA, and antigenic protein exhibiting kinase activity from small tumor samples using guanidine isothiocyanate. Anal Biochem. 1990; 188: 338-43.
8. Diez de Medina SG, Chopin D, El Marjou A, Delouvée A, LaRochelle WJ, Hoznek A, Abbou C, Aaronson SA, Thiery JP, Radvanyi F. Decreased expression of keratinocyte growth factor receptor in a subset of human transitional cell bladder carcinomas. Oncogene. 1997; 14: 323-30.
9. Diez de Medina SG, Popov Z, Chopin DK, Southgate J, Tucker GC, Delouvée A, Thiery JP, Radvanyi F. Relationship between E-cadherin and fibroblast growth factor receptor 2b expression in bladder carcinomas. Oncogene. 1999; 18: 5722-6.
10. Hurst CD, Alder O, Platt FM, Droop A, Stead LF, Burns JE, Burghel GJ, Jain S, Klimczak LJ, Lindsay H, Roulson JA, Taylor CF, Thygesen H, Cameron AJ, Ridley AJ, Mott HR, Gordenin DA, Knowles MA. Genomic subtypes of non-invasive bladder cancer with distinct metabolic profile and female gender bias in KDM6A mutation frequency. Cancer Cell. 2017; 32: 701-715.e7.
11. Kamoun A, de Reyniès A, Allory Y, Sjödahl G, Robertson AG, Seiler R, Hoadley KA, Groeneveld CS, Al-Ahmadie H, Choi W, Castro MAA, Fontugne J, Eriksson P, Mo Q, Kardos J, Zlotta A, Hartmann A, Dinney CP, Bellmunt J, Powles T, Malats N, Chan KS, Kim WY, McConkey DJ, Black PC, Dyrskjøt L, Höglund M, Lerner SP, Real FX, Radvanyi F; Bladder Cancer Molecular Taxonomy Group. A consensus molecular classification of muscle-invasive bladder cancer. Eur Urol. 2020; 77: 420-433.
12. Karoui M, Hofmann-Radvanyi H, Zimmermann U, Couvelard A, Degott C, Faridoni-Laurens L, Ahomadegbe JC, Gazzeri S, Brambilla E, Clerici T, Charbonnier P, Tresallet C, Mitry E, Penna C, Rougier P, Boileau C, Thiery JP, Nordlinger B, Franc B, Radvanyi F. No evidence of somatic *FGFR3* mutation in various types of carcinoma. Oncogene. 2001; 20:5059-61.
13. Lindskrog SV, Prip F, Lamy P, Taber A, Groeneveld CS, Birkenkamp-Demtröder K, Jensen JB, Strandgaard T, Nordentoft I, Christensen E, Sokac M, Birkbak NJ, Maretty L, Hermann GG, Petersen AC, Weyerer V, Grimm MO, Horstmann M, Sjödahl G, Höglund M, Steiniche T, Mogensen K, de Reyniès A, Nawroth R, Jordan B, Lin X, Dragicevic D, Ward DG, Goel A, Hurst CD, Raman JD, Warrick JI, Segersten U, Sikic D, van

Kessel KEM, Maurer T, Meeks JJ, DeGraff DJ, Bryan RT, Knowles MA, Simic T, Hartmann A, Zwarthoff EC, Malmström PU, Malats N, Real FX, Dyrskjøt L. An integrated multi-omics analysis identifies prognostic molecular subtypes of non-muscle-invasive bladder cancer. Nat Commun. 2021; 12: 2301.

14. Logié A, Dunois-Lardé C, Rosty C, Levrel O, Blanche M, Ribeiro A, Gasc JM, Jorcano J, Werner S, Sastre-Garau X, Thiery JP, Radvanyi F. Activating mutations of the tyrosine kinase receptor FGFR3 are associated with benign skin tumors in mice and humans. Hum Mol Genet. 2005; 14: 1153-60.
15. Loriot Y, Necchi A, Park SH, Garcia-Donas J, Huddart R, Burgess E, Fleming M, Rezazadeh A, Mellado B, Varlamov S, Joshi M, Duran I, Tagawa ST, Zakharia Y, Zhong B, Stuyckens K, Santiago-Walker A, De Porre P, O'Hagan A, Avadhani A, Siefker-Radtke AO; BLC2001 Study Group. Erdafitinib in locally advanced or metastatic urothelial carcinoma. N Engl J Med. 2019; 381: 338-348.
16. Mahé M, Dufour F, Neyret-Kahn H, Moreno-Vega A, Beraud C, Shi M, Hamaidi I, Sanchez-Quiles V, Krucker C, Dorland-Galliot M, Chapeaublanc E, Nicolle R, Lang H, Pouponnot C, Massfelder T, Radvanyi F, Bernard-Pierrot I. An FGFR3/MYC positive feedback loop provides new opportunities for targeted therapies in bladder cancers. EMBO Mol Med. 2018; 10: e8163.
17. Marzouka NA, Eriksson P, Rovira C, Liedberg F, Sjödahl G, Höglund M. A validation and extended description of the Lund taxonomy for urothelial carcinoma using the TCGA cohort. Sci Rep. 2018; 8: 3737.
18. Mazerolles C, Rishmann P, Chopin D, Popov Z, Malavaud B, Selves J, Neulat-Duga I, Bellot J, Delsol G. Usefulness of MIB1 monoclonal antibody in assessing the proliferative index in human bladder carcinoma: comparison with Ki-67 antibody. Histopathology. 1994; 25: 563-8.
19. Naski MC, Wang Q, Xu J, Ornitz DM. Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. Nat Genet. 1996; 13: 233-7.
20. Neuzillet Y, Paoletti X, Ouerhani S, Mongiat-Artus P, Soliman H, de The H, Sibony M, Denoux Y, Molinie V, Herault A, Lepage ML, Maille P, Renou A, Vordos D, Abbou CC, Bakkar A, Asselain B, Kourda N, El Gaaied A, Leroy K, Laplanche A, Benhamou S, Lebret T, Allory Y, Radvanyi F. A meta-analysis of the relationship between FGFR3 and TP53 mutations in bladder cancer. PLoS One. 2012; 7: e48993.
21. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov

A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature. 2000; 406: 747-52.

22. Popov Z, Hoznek A, Colombel M, Bastuji-Garin S, Lefrere-Belda MA, Bellot J, Abboh CC, Mazerolles C, Chopin DK. The prognostic value of p53 nuclear overexpression and MIB-1 as a proliferative marker in transitional cell carcinoma of the bladder. Cancer. 1997; 80: 1472-81.
23. Popov Z, Gil-Diez de Medina S, Lefrere-Belda MA, Hoznek A, Bastuji-Garin S, Abbou CC, Thiery JP, Radvanyi F, Chopin DK. Low E-cadherin expression in bladder cancer at the transcriptional and protein level provides prognostic information. Br J Cancer. 2000; 83: 209-14.
24. Popov Z, Gil-Diez-De-Medina S, Ravery V, Hoznek A, Bastuji-Garin S, Lefrere-Belda MA, Abbou CC, Chopin DK. Prognostic value of EGF receptor and tumor cell proliferation in bladder cancer: therapeutic implycations. Urol Oncol. 2004; 22: 93-101.
25. Rebouissou S, Hérault A, Letouzé E, Neuzillet Y, Laplanche A, Ofualuka K, Maillé P, Leroy K, Riou A, Lepage ML, Vordos D, de la Taille A, Denoux Y, Sibony M, Guyon F, Lebret T, Benhamou S, Allory Y, Radvanyi F. CDKN2A homozygous deletion is associated with muscle invasion in FGFR3-mutated urothelial bladder carcinoma. J Pathol. 2012; 227: 315-24.
26. Rebouissou S, Bernard-Pierrot I, de Reyniès A, Lepage ML, Krucker C, Chapeaublanc E, Hérault A, Kamoun A, Caillault A, Letouzé E, Elarouci N, Neuzillet Y, Denoux Y, Molinié V, Vordos D, Laplanche A, Maillé P, Soyeux P, Ofualuka K, Reyal F, Biton A, Sibony M, Paoletti X, Southgate J, Benhamou S, Lebret T, Allory Y, Radvanyi F. EGFR as a potential therapeutic target for a subset of muscle-invasive bladder cancers presenting a basal-like phenotype. Sci Transl Med. 2014; 6: 244ra91.
27. Ricol D, Cappellen D, El Marjou A, Gil-Diez-de-Medina S, Girault JM, Yoshida T, Ferry G, Tucker G, Poupon MF, Chopin D, Thiery JP, Radvanyi F. Tumour suppressive properties of fibroblast growth factor receptor 2-IIIb in human bladder cancer. Oncogene. 1999; 18: 7234-43.
28. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, Hinoue T, Laird PW, Hoadley KA, Akbani R, Castro MAA, Gibb EA, Kanchi RS, Gordenin DA, Shukla SA, Sanchez-Vega F, Hansel DE, Czerniak BA, Reuter VE, Su X, de Sa Carvalho B, Chagas VS, Mungall KL, Sadeghi S, Pedamallu CS, Lu Y, Klimczak LJ, Zhang J, Choo C, Ojesina AI, Bullman S, Leraas KM, Lichtenberg TM, Wu CJ, Schultz N, Getz G, Meyerson M, Mills GB, McConkey DJ; TCGA Research Network, Weinstein JN, Kwi-

atkowski DJ, Lerner SP. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell. 2017; 171: 540-556.e25.

29. Rochel N, Krucker C, Coutos-Thévenot L, Osz J, Zhang R, Guyon E, Zita W, Vanthong S, Hernandez OA, Bourguet M, Badawy KA, Dufour F, Peluso-Iltis C, Heckler-Beji S, Dejaegere A, Kamoun A, de Reyniès A, Neuzillet Y, Rebouissou S, Béraud C, Lang H, Massfelder T, Allory Y, Cianférani S, Stote RH, Radvanyi F, Bernard-Pierrot I. Recurrent activeting mutations of PPARγ associated with luminal bladder tumors. Nat Commun. 2019; 10: 253.
30. Rosty C, Aubriot MH, Cappellen D, Bourdin J, Cartier I, Thiery JP, Sastre-Garau X, Radvanyi F. Clinical and biological characteristics of cervical neoplasias with FGFR3 mutation. Mol Cancer. 2005; 4: 15.
31. Rousseau F, Saugier P, Le Merrer M, Munnich A, Delezoide AL, Maroteaux P, Bonaventure J, Narcy F, Sanak M. Stop codon FGFR3 mutations in thanatophoric dwarfism type 1. Nat Genet. 1995; 10: 11-2.
32. Saint F, Le Frere Belda MA, Quintela R, Hoznek A, Patard JJ, Bellot J, Popov Z, Zafrani ES, Abbou CC, Chopin DK, de Medina SG. Pretreatment p53 nuclear overexpression as a prognostic marker in superficial bladder cancer treated with Bacillus Calmette-Guérin (BCG). Eur Urol. 2004; 45: 475-82.
33. Sanchez-Quiles V, Shi MJ, Dingli F, Krucker C, Loew D, Bernard-Pierrot I, Radvanyi F. Triple extraction method enables high quality mass spectrometry-based proteomics and phospho-proteomics for eventual multi-omics integration studies. Proteomics. 2021; 21: e2000303.
34. Shi MJ, Meng XY, Lamy P, Banday AR, Yang J, Moreno-Vega A, Chen CL, Dyrskjøt L, Bernard-Pierrot I, Prokunina-Olsson L, Radvanyi F. APOBEC-mediated Mutagenesis as a Likely Cause of FGFR3 S249C Mutation Over-representation in Bladder Cancer. Eur Urol. 2019; 76: 9-13.
35. Shi MJ, Meng XY, Fontugne J, Chen CL, Radvanyi F, Bernard-Pierrot I. Identification of new driver and passenger mutations within APOBEC-induced hotspot mutations in bladder cancer. Genome Med. 2020; 12: 85.
36. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001; 98: 10869-74.
37. Spruck CH 3rd, Ohneseit PF, Gonzalez-Zulueta M, Esrig D, Miyao N, Tsai YC, Lerner SP, Schmütte C, Yang AS, Cote R, et al. Two molecular path-

ways to transitional cell carcinoma of the bladder. Cancer Res. 1994; 54: 784-8.

38. Stransky N, Vallot C, Reyal F, Bernard-Pierrot I, de Medina SG, Segraves R, de Rycke Y, Elvin P, Cassidy A, Spraggon C, Graham A, Southgate J, Asselain B, Allory Y, Abbou CC, Albertson DG, Thiery JP, Chopin DK, Pinkel D, Radvanyi F. Regional copy number-independent deregulation of transcription in cancer. Nat Genet. 2006; 38: 1386-96.
39. Tavormina PL, Shiang R, Thompson LM, Zhu YZ, Wilkin DJ, Lachman RS, Wilcox WR, Rimoin DL, Cohn DH, Wasmuth JJ. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. Nat Genet. 1995; 9: 321-8.
40. van Rhijn BW, van Tilborg AA, Lurkin I, Bonaventure J, de Vries A, Thiery JP, van der Kwast TH, Zwarthoff EC, Radvanyi F. Novel fibroblast growth factor receptor 3 (FGFR3) mutations in bladder cancer previously identified in non-lethal skeletal disorders. Eur J Hum Genet. 2002; 10: 819-24.
41. Williams SV, Hurst CD, Knowles MA. Oncogenic FGFR3 gene fusions in bladder cancer. Hum Mol Genet. 2013; 22: 795-803.

doi: 10.20903/zpopov-2022-0027 UDK: 616.379-008.64-06:616.61]-097

Gorazd ROSOKLIJA[1,2], MD, Ph.D.

DIABETIC NEUROPATHY-IMMUNOPATHOGENESIS

Abstract

Quantitative immunocytochemical analysis of complement proteins (CP) was performed on sural nerve biopsies from 15 patients with diabetic neuropathy (DN) and 18 nondiabetic patients with other forms of chronic neuropathy (ON). The mean age of the patients and the pathological severity of the neuropathy were similar in both groups. The percentage of patients that expressed strongly immunoreactive CP in the walls of endoneurial microvessels was significantly greater in DN than in ON for all proteins tested. C3d neoantigen was expressed in 100% of DN cases compared with 17% of ON; and membrane attack complex (MAC), C5b-9 neoantigen, in 93% of DN and 17% of ON. In the cases with DN, 81% of endoneurial microvessels, as identified by the endothelial marker, *Ulex europaeus*, contained C5b-9 neoantigen deposits, compared with 22% in those of ON, and the staining in DN was significantly more intense. Expression of the neoantigens of C3d and C5b-9 in nerve implies local activation of the complement system. In DN, activation of the complement pathway and formation of the MAC could injure blood vessels and adversely affect the circulation in the endoneurium.

Key words: Diabetes mellitus, Diabetic neuropathy, Complement components, Membrane attack complex, Microvessels

---

[1] Associate Professor, College of Physicians and Surgeons, Columbia University-New York
[2] Foreign member of Macedonian Academy of Sciences and Arts, Skopje, North Macedonia

Introduction

Neuropathy is common in patients with diabetes mellitus. The cause of diabetic neuropathy (DN) is not well understood, although metabolic and vascular abnormalities are suspected. Recent studies suggest that immunological mechanisms contribute to the pathogenesis of the disorder.

The metabolic defects are thought to be caused by hyperglycemia and altered metabolism of glucose, resulting in increased sorbitol and fructose, depletion of myoinositol, impaired turnover of phosphoinositides and reduced activation of protein kinase C. These metabolic alterations in nerve are thought to result in diminished (Na, K)-ATPase activity, osmotic perturbations, disturbance of cellular oxidation and reduction mechanisms, and abnormal metabolism of fatty acids and prostaglandins. These, in turn, could be responsible for the nerve conduction abnormalities [9, 11,14, 31, 35]. Accelerated nonenzymatic glycation of proteins by glucose or its metabolites in diabetes could also alter protein structure and interfere with normal cellular function [6, 7, 23, 30, 32, 36, 38]. The metabolic disorder may directly injure the axons and Schwann cells or it may act indirectly by altering interstitial tissue or the microenvironment of nerve fibers.

There is increasing evidence that DN may be caused by abnormal function of the small blood vessels in the endoneurium [10]. Early pathological changes of microangiopathy are found in the walls of the capillaries, arterioles and venules of nerves. The structural abnormalities are postulated to cause decreased perfusion and eventual ischemic injury of nerve fibers. Microangiopathy appears to precede the onset of neuropathy in diabetic patients [12] and the quantified abnormalities in vessels increase in parallel with the severity of the neuropathy [12, 39]. Accordingly, both reduced blood flow and reduced oxygen have been documented in the peripheral nerve, and similar abnormalities have been found in animal models of the disease [22]. Microangiopathic changes are also strongly implicated in the pathogenesis of retinopathy and nephropathy, which are important complications of diabetes mellitus.

The contribution of autoimmune mechanisms to the pathogenesis of DN was first suggested by Duchen et al. [8], who found inflammatory infiltrates in autonomic ganglia from affected patients. Other investigators observed similar mononuclear cell infiltrates in peripheral nerves [3, 18, 21,

28, 29, 37, 40]. Deposits of immunoglobulins and complement components, C3 and C4, in diabetic nerves were first described by Graham and Johnson [13]. In our own studies of diabetic nerves, epineurial microvasculitis was associated with deposits of activated complement proteins located chiefly within the walls of the endoneurial microvessels [40]. Diabetes itself is an autoimmune disease that targets the pancreatic islets [1, 17] and is associated with other autoimmune phenomena, such as the anti-glutamate dehydrogenase antibodies of stiff man syndrome [15] and antibodies to sympathetic ganglia [5].

In this study, we used quantitative immunohistochemistry to evaluate complement deposition in the endoneurial microvessels of patients with DN, and compared the abnormalities with those found in nerves from patients with other types of neuropathy (ON). We used the Endoneurial cell marker, *Ulex europaeus* agglutinin (UEA-I) to identify blood vessels, and measured the activated forms of C4, C3 and C5b-9, and the complement regulatory proteins, S-protein and SP40,40. The findings indicate local activation of the complement pathway in microvessels of DN, leading to formation of membrane attack complex (MAC).

Materials and methods

Subjects

After obtaining informed consent, biopsies of the whole sural nerve were performed just above the lateral malleolus in 15 patients with progressive DN and in 18 with other forms of chronic neuropathy. The 15 patients with DN were included in a previous immunohistochemical study of lymphocytes, immunoglobulins, and the complement proteins, C3 and C5b-9 [40]. The sural nerves were submitted for routine histology (paraffin sections of formalin-fixed tissue), resin histology (semithin sections of epoxy resin-embedded tissue), and immunohistochemical analysis (cryosections of unfixed, frozen tissue). The patients who served as disease controls were chosen so as to have a similar range of age and pathological severity of neuropathy as the diabetic group. Of the 15 patients with DN, 9 had non-insulin-dependent diabetes mellitus (NIDDM), and 6 had an insulin-dependent form (IDDM). Seven of the patients had proximal DN (PDN), 6 had

distal symmetrical polyneuropathy (DSPN), and 2 had mononeuropathy multiplex (MNM) (see Table 2). The controls included 13 patients with a chronic axonopathy, 3 with chronic inflammatory demyelinating polyneuropathy and 2 with amyotrophic lateral sclerosis (ALS) (see Table 3). The sural nerves of the 2 ALS patients had minor "neuropathic" features, a common finding in the disease.

Histology and immunohistochemistry

Serial cryosections of sural nerves were cut 6 μm in thickness and stained with UEA-I and with antibodies to C4d, C4d neoantigen, C3d, C3d neoantigen, C5b-9 neoantigen, S-protein/vitronectin and SP40,40/clusterin. The antigens, antibodies, dilutions and commercial sources are indicated in Table 1. The primary antibodies that recognize C4d and C3d bind to the whole complement protein as well as the proteolytic fragments that are formed upon activation of the complement system and subsequent degradation to inactive peptides. Hence, these antibodies do not distinguish the whole protein from the activated state or the inactive split peptides. However, three of the monoclonal antibodies recognize neoantigens of C4d, C3d and C5b-9, and these epitopes are expressed only upon activation of the complement system. Two monoclonal antibodies have specificity for two regulatory proteins that inhibit activity of the terminal components of the complement system. One of the monoclonal antibodies binds to the S-protein (Quidel) either as a single protein or as a component of the SC5b-9 complex, an inactive form of the MAC. The second monoclonal antibody reacts with human SP40,40 (Quidel), which is another protein that also becomes a component of the SC5b-9 complex. UEA-I, a lectin that binds to a-linked fucose residues of glycoproteins and glycolipids, is a marker for human endothelial cells and was used to determine the location of blood vessels in the nerves. The avidin-biotinylated peroxidase (ABC) method employed the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, Calif.) and diaminobenzidine (DAB) as chromogen and detected the binding of these antibodies or the lectin to tissue sections.

On the UEA-I-stained sections, endoneurial vascular profiles (microvessels) were counted manually. (To test whether UEA-I staining was quantitatively reproducible, we localized and counted the lectin-stained blood vessels on nine serial 6-μm transverse and longitudinal sections of normal nerve. The number and localization were identical on all nine sections.) The total endoneurial area and UEA-I -stained area were determined by computerized image analysis. The immunostaining intensity of complement com-

ponents and regulatory proteins was measured semiquantitatively on a scale of 0 (none) to 3 (intense). The number of vascular profiles that express neoantigens of C4d, C3d and C5b-9 on each section was counted by hand. The area and optical density of the same three immunoreactive neoantigens in blood vessels were determined by computerized image analysis (see below). In 22 cases (10 DN, 12 ON) Epon-embedded tissue was available to quantify the histological severity of the neuropathies. Transverse semithin sections of these specimens were stained with *p*-phenylenediamine, and the number of myelinated fibers was counted in each nerve fascicle and expressed as the number per mm of cross-sectional area of endoneurium (myelinated fiber density or MFD).

Table 1
List of antibodies (*ABC* avidin biotin complex, *IF* immunofluorescence, *FITC* fluorescein isothiocyanate-conjugated)

Antibody description	Dilution	Catalog no	Source	Method
Murine monoclonal anti-human C4d	1:300	A 213	Quidel	ABC
Murine monoclonal anti-human C3d	1:300	CLSIF 33001	Quidel	ABC
Murine monoclonal anti-human C4d neo	1:400	CLSIF 25701	Quidel	ABC
Murine monoclonal anti-human C3d neo	1:300	CLSIF 35501	Quidel	ABC
Murine monoclonal anti-human C5b-9	1:100	M 777	Dako	ABC
Murine monoclonal anti-human S-protein	1:200	A237 F29101	Quidel	ABC
Murine monoclonal anti-human SP40,40	1:200	A241 F35201	Quidel	ABC
Ulex europaeus agglutinin I	1:50	L-1060	Vector	ABC
Rabbit anti-human albumin-FITC	1:50	F 117	Dako	IF
Rabbit anti-human fibrinogen-FITC	1:50	F 111	Dako	IF
Goat anti-human IgG-FITC	1:100	F 1641	Sigma	IF
Goat anti-human IgM-FITC	1:20	F 5384	Sigma	IF
Goat anti-human IgA-FITC	1:20	F 5259	Sigma	IF

Computerized image analysis

To quantify immunoreactivity, digitized images were acquired by a video camera mounted on a microscope and attached to a Macintosh IIfx computer using the public domain NIH Image program. Using standardized illumination, optical density (OD) values were calibrated from a photographic gray scale. A series of images acquired with a ×4 objective comprised the endoneurium of the entire nerve in each section. A threshold set by the operator distinguished “stained” from “unstained” objects. Once a threshold

was chosen for a given component of complement, the same threshold was used for all cases.

Analysis of results

The following measurements were performed on the 33 nerve biopsies (15 patients with DN and 18 with ON): (1) the intensity of immunoreactivity of each complement neoantigen and regulatory proteins measured semiquantitatively in endoneurial microvessel walls; (2) the proportion of immunoreactive proteins in vascular profiles to total number of UEA I-labeled endoneurial microvessels; (3) the mean OD of suprathreshold immunoreactivity for each neoantigens in the microvessels; (4) the integrated complement neoantigens immunoreactivity normalized for UEA I-stained area (neoantigens OD x neoantigen area of immunoreactivity/UEA I-labeled area); (5) the number of UEA I-labeled vascular profiles per mm; and (6) the fraction of endoneurial area occupied by UEAI-labeling. The effects of diabetes on semiquantitative intensity of immunoreactivity were evaluated by Mantel-Haenzel test. Other comparisons between DN and ON were performed by ANOVA. These were repeated with MFD as an additional covariate for the 22 cases on which Epon-embedded tissue was available with age as covariate. For evaluation of the number of UEA I-labeled blood vessels per mm, orientation of the section was included as a categorical covariate. Linear regression was used to evaluate the relationship of MFD with complement deposits and vascular density. The above analyses revealed deposits of activated complement in the thickened blood vessels of DN biopsy samples. To rule out the possibility that these were related to thickening of blood vessels alone, we subsequently stained a series of nerve biopsy specimens from four patients with Charcot-Marie-Tooth disease, type 1 (younger than the DN cases but matched to them by semiquantitative assessment of myelinated fiber loss) and five patients with neuropathy and an IgM paraprotein reactive to myelin-associated glycoprotein (anti-MAG neuropathy) (matched to the DN cases by age and semiquantitative assessment of myelinated fiber loss). Complement deposits in these cases were assessed visually.

Results

Severity of neuropathy

Epon-embedded material was available for 10 cases of DN and 12 cases of ON (Tables 2, 3). The spatial density of myelinated fibers in DN (2427 ± 1489 fibers/mm^2) and ON (1840 ± 718 fibers/mm^2) did not differ significantly (t = 1.21, df = 20, P = 0.24). In addition, microscopic semiquantitative assessment of cryosections from all 33 subjects demonstrated a similar loss of myelinated fibers in both groups.

Immunohistochemistry

Immunoreactivity of complement proteins

Semiquantitative assessment revealed more C4d, C3d, and C5b-9 immunoreactivity in the walls of endoneurial blood vessels in the 15 patients with DN than 18 with ON using monoclonal antibodies that recognize neoantigens (see Fig. 1 C–1F). The differences between the diabetic patients and the disease controls were statistically significant (C4d neoantigen, Mantel-Haenzel χ^2 = 7.1, df = 1, P = 0.008; C3d neoantigen, Mantel-Haenzel χ^2 = 22.4, df = 1, $P < 10^{-5}$; C5b-9 neoantigen, Mantel-Haenzel χ^2 = 20, df = 1, $P = 10^{-5}$) (Tables 2, 3). Semiquantitative ratings of 2 or 3 were obtained for C4d neoantigen in 67% of DN and 28% of ON, for C3d neoantigen in 100% of DN and 17% of ON; and for C5b-9 neoantigen in 93% of DN and 17% of ON. These neoantigens are only expressed upon activation of the complement components. Expression of C4 and C3 was also tested using two other monoclonal antibodies, which each recognize as a corresponding epitope of the whole protein (C4 or C3) and split fragment (C4d or C3d). Semiquantitative assessment of these components revealed more C3/C3d immunoreactivity in the endoneurial microvessels of DN than ON (Mantel-Haenzel χ^2 = 13.6, df = 1, P = 0.0002) but similar levels of C4/C4d (Mantel-Haenzel χ^2 = 0.98, df = 1, P = 0.32).

Table 2

Summary of diabetic neuropathy cases. Immunostaining intensity of complement components and regulatory proteins is measured semiquantitatively on a scale of 0 (none) to 3 (intense) [*MFD* myelinated fiber density (number of myelinated fibers/mm), *DSPN* distal symmetrical polyneuropathy, *PDN* proximal diabetic neuropathy, *MNM* mononeuropathy multiplex, *IDDM* insulin-dependent diabetes mellitus, *NIDDM* non-insulin-dependent diabetes mellitus, *N/A* not available]

Case no	C3d neo	C4d neo	C5b-9 neo	S-protein	SP40,40	MFD	Age	Sex	Diabetic type	Clinical diagnosis
1	3	2	3	3	2	548	65	F	IDDM	PDN
2	3	3	3	3	2	N/A	59	F	IDDM	DSPN
3	3	3	3	2	3	N/A	62	M	NIDDM	DSPN
4	2	1	3	2	2	203	89	M	IDDM	PDN
5	2	0	1	2	2	2550	43	M	NIDDM	PDN
6	3	1	2	2	2	N/A	52	M	IDDM	PDN
7	3	3	3	3	3	3869	49	M	IDDM	PDN
8	2	1	3	2	2	1580	67	M	NIDDM	DSPN
9	3	2	3	2	2	N/A	58	M	IDDM	MNM
10	3	3	3	3	2	N/A	64	M	NIDDM	MNM
11	3	2	3	2	2	3141	74	M	NIDDM	DSPN
12	3	3	3	2	2	4279	63	F	IDDM	DSPN
13	3	1	3	2	2	2648	83	F	NIDDM	PDN
14	3	3	3	3	3	1229	59	F	IDDM	DSPN
15	3	3	3	3	2	4223	84	F	NIDDM	PDN

Table 3

Other neuropathies. Immunostaining intensity of complement components and regulatory proteins is measured on a scale of 0 (none) to 3 (intense) (*PN* peripheral neuropathy, *CIDP* chronic inflammatory demyelinating polyneuropathy, *ALS* amyotrophic lateral sclerosis, *N/A* not available)

Case no.	C3d neo	C4d neo	C5b-9 neo	S-protein	SP40,40	MFD	Age	Sex	Clinical diagnosis
16	0	1	0	1	1	3198	51	F	PN
17	0	1	1	1	1	1298	63	M	CIDP
18	3	3	3	2	2	1237	75	M	CIDP
19	1	2	1	1	1	1343	72	F	PN
20	0	0	0	N/A	N/A	2525	74	F	PN
21	0	0	0	1	1	N/A	48	F	PN
22	0	0	0	1	1	1519	76	F	PN
23	0	0	0	1	1	N/A	64	F	ALS
24	0	0	0	1	1	1283	51	F	PN
25	2	3	2	2	2	N/A	62	M	CIDP
26	0	1	0	1	1	N/A	45	F	PN
27	1	2	1	1	1	2183	75	M	PN
28	0	1	1	1	1	N/A	78	M	PN
29	2	2	2	1	1	1339	65	M	PN
30	1	0	0	1	0	N/A	59	M	ALS
31	0	0	0	1	1	1668	66	M	PN
32	1	0	1	1	1	3061	39	M	PN
33	0	0	0	N/A	N/A	1436	67	M	PN

$P = 0.008$; C3d neoantigen, Mantel-Haenzel $\chi^2 = 22.4$, $df = 1$, $P < 10^{-5}$; C5b-9 neoantigen, Mantel-Haenzel $\chi^2 = 20$, $df = 1$, $P = 10$) (Tables 2, 3). Semiquantitative ratings of 2 or 3 were obtained for C4d neoantigen in 67% of DN and 28% of ON, for C3d neoantigen in 100% of DN and 17% of ON; and for C5b-9 neoantigen in 93% of DN and 17% of ON. These neoantigens are only expressed upon activation of the complement components. Expression of C4 and C3 was also tested using two other monoclonal antibodies, which each recognize as a corresponding epitope of the whole protein (C4 or C3) and split fragment (C4d or C3d). Semiquantitative assessment of these components revealed more C3/C3d immunoreactivity in the endoneurial microvessels of DN than ON (Mantel-Haenzel $\chi^2 = 13.6$, $df = 1$, $P = 0.0002$) but similar levels of C4/C4d (Mantel-Haenzel $\chi^2 = 0.98$, $df = 1$, $P = 0.32$).

Multivariate ANOVA of the densitometric measurements revealed significant effects of diabetes on neoantigens deposits, whether expressed as a fractional number of UEA-I-stained vessels, OD of staining, or integrated immunoreactivity (see Methods). The proportion of Endoneurial microvessels that displayed each complement protein was greater in the DN group than in the ON group. By univariate ANOVA on these 33 subjects, with age as a covariate, the differences were statistically significant for all three neoantigens (Fig. 2). The OD of the immunostained vessels was also greater in DN than in ON. The differences were significant for C3d and C5b-9 neoantigens, but not for C4d (Fig. 3). Likewise, integrated immunoreactivity, normalized for the area of UEA-I labeling, provided significant differences for C3d and C5b-9 neoantigens, but not for C4d (Fig. 4). Similar results were obtained with fiber density as an additional covariate, for the 22 subjects with Epon-embedded specimens.

All complement proteins including the neoantigens, but not UEA-I, were expressed in the perineurium of all nerves of the DN and ON patients (Fig. 1). We have routinely immunostained C3d and C5b-9 of over 1000 nerve biopsies as part of the diagnostic evaluation, and these complement proteins are expressed in the perineurium of most normal and diseased nerves. Hence, we attach no significance to the apparent immunoreactivity of the perineurium in DN.

All 15 cases of DN but one exhibited prominent hyaline thickening of the blood vessel walls within the endoneurium. Sural nerves with similar vessel thickening were present in 4 patients with Charcot-Marie-Tooth disease, type 1 (CMT), and 5 patients with anti-MAG neuropathy. No detectable C3d neoantigen was found in the blood vessel walls of any of the 9 patients. C5b-9 was absent in the vessels of the CMT cases, but expressed weakly immunoreactive MAC in the vessel walls of anti-MAG neuropathy. All of the anti-MAG nerves displayed C3d on the surface of myelin sheaths, but no C5b-9.

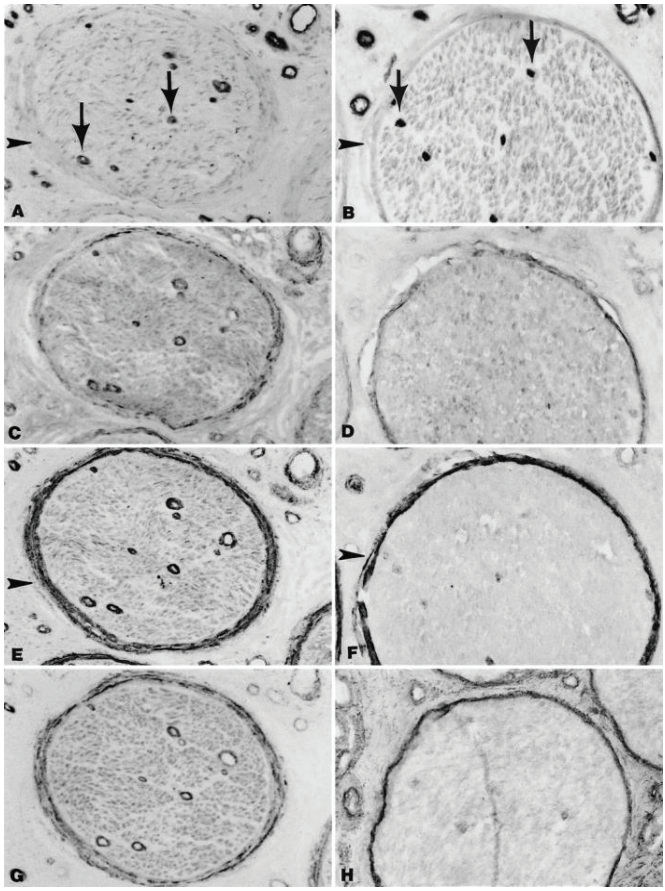


Fig. 1 – **A–H** Immunoperoxidase stains of the endoneurial microvessels using the lectin, UEA-I and monoclonal antibodies to complement proteins in sural nerve biopsies of DN and a disease control (ON). The nerve in DN is depicted in the same field of four semiserial cryosections (**A**, **C**, **E**, **G**). The endoneurium contains nine microvessels, as indicated by the endothelial marker, UEA-I (**A**, *arrows*). The C3d neoantigen (**C**), C5b-9 (**E**) and S-protein (**G**) are expressed in the same nine blood vessels. The nerve in ON shows similar semiserial sections (**B**, **D**, **F**, **H**) except that **H** is a different field. The nerve fascicle encloses six blood vessels marked by UEA-I (**B**, *arrows*), but there is little or no immunoreactive C3d neoantigen (**D**), C5b-9 (**F**) or S-protein (**H**). Note that the same complement proteins (**C–H**), but not UEA-I (**A**, **B**), are also located in the perineurium (*arrowheads*), a common finding in many different types of neuropathies (*UEA-I Ulex europaeus*-I, *DN* diabetic neuropathy, *ON* other neuropathy). **A–H** × 120

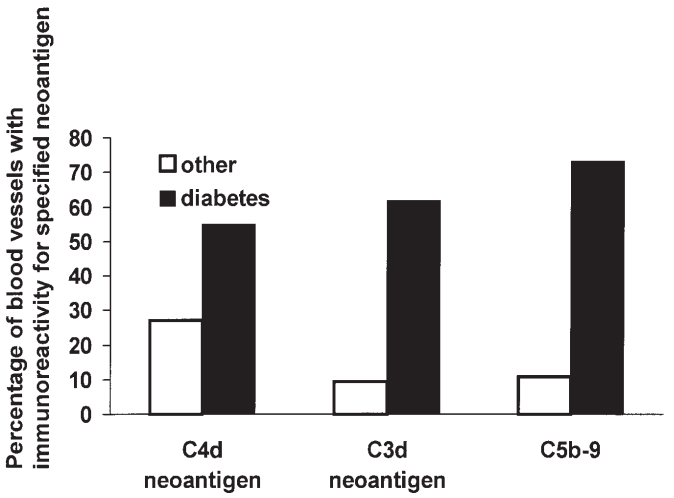


Fig. 2 – Age-adjusted mean percentage of blood vessels displaying immunoreactivity for each neoantigen. ANOVA (*df* = 30,1,1); C4d: $F = 5.49$, $P = 0.026$; C3d: $F = 32.79$, $P = < 0.0005$; C5b-9: $F = 47.11$, $P = < 0.0005$

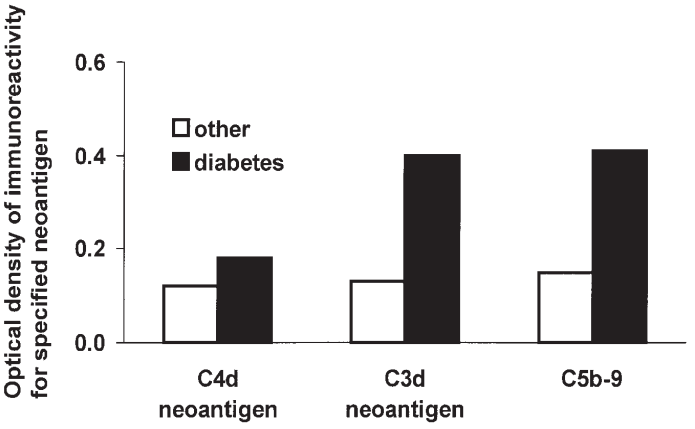


Fig. 3 – Age-adjusted optical density of immunoreactive vessel walls for each neoantigen. ANOVA (*df* = 30,1,1); C4d: $F = 2.71$, $P = 0.11$; C3d: $F = 29.48$, $P = < 0.0005$; C5b-9: $F = 31.08$, $P = < 0.0005$

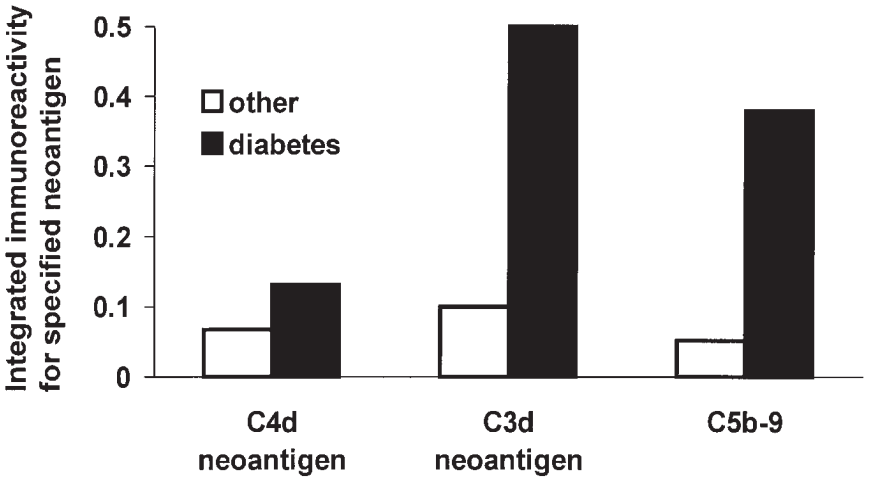


Fig. 4 – Age-adjusted integrated immunoreactivity for each neoantigen. ANOVA (df = 30,1,1); C4d: $F = 1.87$, $P = 0.182$; C3d: $F = 13.42$, $P = < 0.0005$; C5b-9: $F = 28.48$, $P = < 0.0005$

Immunoreactivity of S protein (vitronectin) and SP40,40 (clusterin)

The expression of S-protein and SP40,40 (Fig. 1 G, H; Tables 2, 3) in the walls of endoneurial microvessels of DN exceeded that of ON based on semiquantitative analysis (S-protein, Mantel-Haenzel $\chi^2 = 22.0$, $df = 1$, $P < 10^{-5}$; SP40,40, Mantel-Haenzel $\chi^2 = 20.0$, $df = 1$, $P = 10^{-5}$). S protein and SP40,40 were generally colocalized with all three neoantigens within the walls of the endoneurial microvessels in DN (Fig. 1).

Immunoreactivity of immunoglobulins, fibrinogen and albumin

Immunoreactivity for IgG, IgM, and IgA was previously demonstrated in DN and normal nerves [40], and in this study, these immunoglobulins were also present in ON. Albumin was expressed diffusely in the endoneurium in both DN and ON. No deposits of fibrinogen were observed in the tissue of any of the biopsy specimens.

Correlation between severity of neuropathy, the age of the patient and complement deposits

The extent of complement neoantigen immunoreactivity, measured by any of the above parameters, was not correlated with severity of neuropathy or age, neither among the whole group of subjects, nor within either group separately ($r^2 < 0.1$ for all correlations).

Correlation between severity of neuropathy, the age of the patient and complement deposits

The extent of complement neoantigen immunoreactivity, measured by any of the above parameters, was not correlated with severity of neuropathy or age, neither among the whole group of subjects, nor within either group separately ($r^2 < 0.1$ for all correlations).

Density of endoneurial blood vessels

The number of UEA-I-labeled vascular profiles was expressed per unit of endoneurial area. The density of these microvessels was significantly greater in the DN group than in the ON group, (means adjusted for age, MFD, and orientation of section: DN 34.3/mm ; ON 14.1/mm^2 ; F =12.13, df = 17, 3, 1, P = 0.003; all cases, covarying for age and orientation only: DN 29.2, ON 15.9, F = 10.61, df = 29, 2, 1, P = 0.003). Likewise, when the fraction of the endoneurial area occupied by UEA-I staining was considered, the difference was statistically significant (means adjusted for age and MFD: DN 1.67%, ON 0.61%, F = 21.13, df = 18,2,1, $P < 0.0005$; all cases, covarying for age only: DN 1.45, ON 0.8, F = 8.41, df = 30, 1, 1, P = 0.007). There was no statistically significant correlation between the age and the number or area of UEA-I-labeled vascular profiles.

Discussion

The findings described here indicate an activation of the complement system in DN, as was found by expression of the neoantigens C3d and C5b-9 in sural nerve biopsy specimens. These complement deposits were located in the thickened walls of endoneurial microvessels, and they were more

intensely immunoreactive and more extensive than in other types of neuropathy. The quantitative differences were significant and imply that the abnormalities are not simply a by-product of an injury of nerve fibers. The results are also not a general response to vascular thickening, as similar abnormalities were present in other chronic neuropathies in this study.

Widespread pathological alterations occur in microvessels of diabetes mellitus, and they have been postulated to cause major complications of the disease, namely, neuropathy, retinopathy, and nephropathy. In a diabetic nerve, the endoneurial microvessels exhibit thickening and proliferation or reduplication of the basal lamina, injury with loss of pericytes and hyperplasia of endothelial cells [10, 12, 39]. This microangiopathy has been linked to increased permeability of the vessels, edema, and hypoxemia of the nerve [22, 26] and the development of neuropathy. The vascular abnormalities have previously been attributed to metabolic abnormalities and advanced glycated end products, but the pathogenesis of the microangiopathy is not understood.

MAC has the potential to induce cell injury or death, and it could be responsible, in part, for the microangiopathy seen in diabetic nerves. MAC acts by becoming inserted into the surface membrane of host cells, where it creates abnormal pores. The alteration disrupts the integrity of the membrane and results in leakage of the cell membrane with dysfunction and death of cells [19, 24, 25]. The interaction of the MAC with endothelial cell (EC) membrane could also result in the release of soluble macromolecules including fibroblast and platelet-derived growth factors, which have been shown to be potent mitogens for mesangial cells or pericytes in the kidney [2]. In addition, activation of the complement system releases C3a and C5a into the extracellular fluid, where they act to increase permeability of blood vessels and serve as chemokines to promote influx of polymorphonuclear leukocytes and recruitment of monocytes. Diabetic nerves exhibit chronic inflammation [3, 18, 21, 28, 29, 37, 40], numerous histiocytes and macrophages in the endoneurium [40] and endoneurial edema [26], and these findings are consistent with a chronic low-level state of complement activation.

The mechanisms responsible for activation of the complement system in DN are not clear. In general, the classical pathway is activated by antibody-antigen interactions, whereas carbohydrates initiate the alternative pathway [25]. In addition, the mannose binding protein (MBP) activates the

classical pathway by binding to carbohydrate groups, a process described recently as the MBP pathway [34]. These three pathways converge to generate C3b as a result of sequential proteolytic cleavage of the complement components [25]. The active fragment, C3b, binds covalently to macromolecules on the surface membrane of cells, where it amplifies the action of the alternative pathway to yield abundant C3b. It acts, in turn, to split C5 yielding C5b, and this fragment assembles the terminal components, C6, C7, C8 and C9 in sequence to form the C5b-9 or MAC. In this study, analysis of the early complement pathways was limited to the C4d neoantigen, an activation marker of the classical and MBP pathways. Expression of this complement component was inconstant in different diabetic nerves and OD of the protein was not significantly greater than disease controls. Hence, further investigation of the activation of the complement system is necessary.

Whether C3b proceeds to activate the terminal components with assembly of C5b-9 depends critically on regulatory proteins. These molecules are located in extracellular fluid and cell membranes where they tightly control complement function. Some of these proteins effectively restrict the action of C3b to the immediate vicinity of the bound fragment, and others degrade the peptide to inactive fragments. Still other proteins inhibit the terminal pathway and limit formation of C5b-9. In this study, the diabetic nerves expressed two strongly immunoreactive complement regulatory proteins, S-protein and SP40,40, in the walls of the endoneurial microvessels of DN. The intensity and extent of immunostaining exceeded that of ON, and these regulatory proteins were generally colocalized with the neoantigens of C4d, C3d and C5b-9. S-protein, also known as vitronectin, associates with the terminal components of complement as they are assembled to form the inactive complex, SC5b-9 [27, 33]. This complex is soluble and cannot be inserted into target cell membranes [19]. SP40,40 (clusterin) inhibits the function of MAC and is also an integral component of the inactive soluble complex, SC5b-9 [25]. The increased immunoexpression of the neoantigen, C5b-9, in diabetic nerves implies that S-protein, clusterin, and other regulatory proteins, such as CD 59, have failed to suppress the terminal pathway, thereby, permitting formation of MAC. Possible mechanisms of this pathological state include an overwhelming acceleration of complement activation or impaired activity of regulatory proteins, possibly rendered by advanced glycation, as has previously been shown for S-protein (vitronectin) in the diabetic state [16].

The expression of active complement components in microvessel walls of DN was found in all 15 cases, but the abnormality is not unique as 3 of the 18 controls expressed a similar pattern of complement proteins. Two of the three control patients had chronic inflammatory demyelinating polyneuropathy, a disorder that is thought to be autoimmune [4]. The trigger for complement local activation in vessel walls in diabetic nerves is unknown. Possible triggers include anti-endothelial cell or pericyte antibodies, interaction of the complement pathways with abnormally glycated proteins, or exposure of normal cellular molecules, such as sulfatide [20]. Loss or impaired function of regulatory proteins may also promote deposition of lytic C5b-9. Identification of the mechanisms responsible for activation and dysregulation of the complement system could lead to a better understanding of the disease.

REFERENCES

1. Atkinson MA, Maclaren NK (1994) The pathogenesis of insulin dependent diabetes mellitus. N Engl J Med 331: 1428–1436
2. Benzaquen LR, Nicholson-Weller A, Helperin AJ (1994) Terminal complement proteins C5b-9 release basic fibroblast growth factor and platelet-derived growth factor from endothelial cells. J Exp Med 179: 985–992
3. Bradley WG, Chad D, Verghese JP, Liu HC, Good P, Gabbai AA, Adelman LS (1984) Painful lumbosacral plexopathy with elevated erythrocyte sedimentation rate: a treatable inflammatory syndrome. Ann Neurol 15: 457–464
4. Briani C, Brannagan TH III, Trojaborg W, Latov N (1996) Chronic inflammatory demyelinating polyneuropathy. Neuromuscl Disord 6: 311–325
5. Brown FM, Stuart JB, Roy F, Steven LB (1989) Anti-sympathetic nervous system autoantibodies. Diminished catecholamines with orthostasis. Diabetes 38: 938–941
6. Brownlee M, Cerami A, Vlassara H (1988) Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 318 : 1315–1321
7. Bucala R, Tracey KJ, Cerami A (1991) Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest 87: 432–4388. Duchen LW, Anjorin MB, Watkins MD, Mackay MB (1980) Pathology of autonomic neuropathy in diabetes mellitus. Ann Intern Med 92: 301–303

9. Dvornik D (1987) Hyperglycemia in the pathogenesis of diabetic complications. In: Porte D (ed) Aldose reductase inhibition. An approach to the prevention of diabetic complications. Biomedical Information Corporation, New York, pp 69–151
10. Dyck PJ, Giannini C (1996) Pathologic alterations in the diabetic neuropathies of humans: a review. J Neuropathol Exp Neurol 55: 1181–1193
11. Gabbay KH, Merola LO, Field RA (1966) Sorbitol pathway: presence in nerve and cord with substrate accumulation in diabetes. Science 151: 209–210
12. Giannini C, Dyck PJ (1995) Basement membrane reduplication and pericyte degeneration precede development of diabetic polyneuropathy and are associated with its severity. Ann Neurol 37: 498–504
13. Graham AR, Johnson PC (1985) Direct immunofluorescence findings in peripheral nerve from patients with diabetic neuropathy. Ann Neurol 17: 450–454
14. Greene DA, Winegrad AI (1979) In vitro studies of the substrates for energy production and the effects of insulin on glucose utilization in the neural components of the peripheral nerve. Diabetes 28: 878–887
15. Grimaldi LM, Martino G, Braghi S, Quattrini A, Furlan R, Bosi E, Comi G (1993) Heterogeneity of autoantibodies in stiff-man syndrome. Ann Neurol 34: 57–64
16. Hammes HP, Weiss A, Hess S, Araki N, Horiuchi S, Brownlee M, Preissner KT (1996) Modification of vitronectin by advanced glycation alters functional properties in vitro and in the diabetic retina. Lab Invest 75: 325–338
17. Hanninen A, Jalkanen S, Salmi M, Toikanen S, Nikolakaros G, Simell O (1992) Macrophages, T cell receptor usage, and endothelial cell activation in the pancreas at the onset of insulin dependent diabetes mellitus. J Clin Invest 90: 1901–1910
18. Johnson PC, Doll SC, Cromey DW (1986) Pathogenesis of diabetic neuropathy. Ann Neurol 19: 450–457
19. Kolb WP, Muller-Eberhard HJ (1975) The membrane attack mechanism of complement. Isolation and subunit composition of the C5b-9 complex. J Exp Med 141: 724–735
20. Koski CL, Vanguri JP (1987) C1 fixation by lipids of human peripheral nerve myelin in absence of specific antibody. Complement 4: 181
21. Llewelyn JG, Thomas PK, King RH (1998) Epineurial microvasculitis in proximal diabetic neuropathy. J Neurol 245: 159–165
22. Low PA, Lagerlund TD, McManis PG (1989) Nerve blood flow and oxygen delivery in normal, diabetic and ischemic neuropathy. Int Rev Neurobiol 31: 355–438

23. Monnier VM, Kohn RR, Cerami A (1984) Accelerated age related browning of human in diabetes mellitus. Proc Natl Acad Sci USA 81: 583–587
24. Muller-Eberhard HJ (1986) The membrane attack complex of complement. Annu Rev Immunol 4 : 503–528 25. Muller-Eberhard HJ (1988) Molecular organization and function of the complement system. Annu Rev Biochem 57: 321–347
26. Poduslo JF, Curran GL, Dyck PJ (1988) Increase in albumin, IgG, and IgM blood-nerve barrier indices in human diabetic neuropathy. Proc Natl Acad Sci USA 85: 4879–4883
27. Preissner KT (1991) Structure and biological role of vitronectin. Annu Rev Cell Biol 7: 275–310
28. Raff MC, Asbury AK (1968) Ischemic mononeuropathy and mononeuropathy multiplex in diabetes mellitus. N Engl J Med 279: 17–21
29. Said G, Goulon-Goeau C, Lacroix C, Moulonguet A (1994) Nerve biopsy findings in different patterns of proximal diabetic neuropathy. Ann Neurol 35: 559–569
30. Schmidt AM, Hasu M, Popov D, Zhang JH, Chen J, Yan SD, Brett J, Cao R, Kuwabara K, Costache G (1994) Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE proteins. Proc Natl Acad Sci USA 91: 8807–8811
31. Stevens MJ, Feldman EL, Greene DA (1995) The aetiology of diabetic neuropathy: the combined roles of metabolic and vascular defects. Diabet Med 12: 566–579
32. Stitt AW, Li YM, Gardiner TA, Bucala R, Archer D, Vlassara H (1997) Advanced glycation end products (AGEs) localize with AGE receptors in the retinal vasculature of diabetic and of AGE-infused rats. Am J Pathol 150: 523–531 33. Tomasini BR, Mosher DF (1991) Vitronectin. Prog Hemost Thromb 10: 269–305
34. Turner MW (1995) The lectin pathway of complement activation. Res Immunol 147: 110–115
35. Van Heyningen R (1965) The metabolism of glucose by the rabbit lens in the presence and absence of oxygen. Biochem J 96: 419–431
36. Vlassara H, Fuh H, Makita Z, Krungkrai S, Cerami A, Bucala R (1992) Exogenous advanced glycosylation end products induce complex vascular dysfunction in normal animals: a model for diabetic and aging complications. Proc Natl Acad Sci USA 89: 12 043–12 047
37. Woltman HW, Wilder RM (1929) Diabetes mellitus: pathologic changes in spinal cord and peripheral nerves. Arch Intern Med 44: 576–603

38. Yamagishi S, Hsu CC, Taniguchi M, Harada S, Yamamoto Y, Ohsawa K, Kobayashi K, Yamamoto H (1995) Receptor mediated toxicity to pericytes of advanced glycosylation end products: a possible mechanism of pericyte loss in diabetic microangiopathy. Biochem Biophys Res Commun 213: 681–687
39. Yasuda H, Dyck PJ (1987) Abnormalities of endoneurial microvessels and sural nerve pathology in diabetic neuropathy. Neurology 37: 20–28
40. Younger DS, Rosoklija G, Hays AP, Trojaborg W, Latov N (1996) Diabetic peripheral neuropathy: a clinicopathologic and immunohistochemical analysis of sural nerve biopsies. Muscle Nerve 19: 722–727

doi:10.20903/zpopov-2022-0028 UDK: 616.155.392.2-092

Dimitar G. EFREMOV[1],[2]

THE PATHOGENESIS OF RICHTER TRANSFORMATION IN CHRONIC LYMPHOCYTIC LEUKEMIA

Abstract

Chronic lymphocytic leukemia (CLL) is the paradigm of intrinsic and extrinsic tumor heterogeneity with respect to both cellular and interpatient variance and response to treatment. The multiple genetic, epigenetic, functional and microenvironmental alterations underlying the pathophysiology of CLL limit the efficacy of currently applied treatments. Moreover, single-agent treatments present a high risk of tumor resistance, with a substantial proportion of patients relapsing during treatment or undergoing Richter transformation. The latter condition represents the transformation of CLL into a secondary high-grade and aggressive lymphoma and is considered the most important unmet clinical need in CLL because of the lack of any effective treatment option. The development of more effective treatments for Richter transformation requires a more complete understanding of the molecular mechanisms that drive this condition. This paper will describe some novel insights in the pathogenesis of Richter Transformation that may potentially provide the basis for the development of personalized therapeutic approaches for this condition in the future.

Keywords: chronic lymphocytic leukemia, diffuse large B cell lymphoma, Richter transformation, B-cell receptor, cell cycle, targeted therapy.

[1] Dimitar G. Efremov, MD, PhD, Head, Molecular Haematology Unit, International Centre for Genetic Engineering and Biotechnology, Padriciano 99, 34149 Trieste, Italy, Tel: +39-040-3757212, E-mail: efremov@icgeb.org; https://www.icgeb.org/dimitar-efremov/

[2] Member of Macedonian Academy of Sciences and Arts, Skopje, North Macedonia

Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in Europe and America, accounting for more than 40% of all adult leukemia diagnoses. The disease is approximately 10 times less frequent in Asian countries, which is believed to reflect different prevalence of inherited risk factors. The risk of developing CLL increases with age, with a median age at diagnosis ranging from 70 to 72 years (1).

CLL is characterized by a highly variable clinical course and outcome. Approximately 60% of the patients have a relatively indolent disease and many of them remain symptom-free and fully active for decades, whereas the remaining patients experience a rapidly progressive disease that requires treatment soon after diagnosis, and this might result in early death due to disease-related complications (2).

The diagnosis of CLL is established based on the presence of ≥5,000 per μl clonal B cells with a distinct immunophenotype consisting of co-expression of CD5, CD19 and CD23 and a weak expression of CD79B, CD22 and surface immunoglobulin (2). This finding in most patients is the only sign of the disease at diagnosis. However, patients that progress will subsequently develop lymphadenopathy, splenomegaly and hepatomegaly, whereas anemia and thrombocytopenia occur in the most advanced stages of the disease. In addition, CLL patients have progressive defects in both cell-mediated and antibody-mediated immunity, including hypogammaglobulinemia and B cell and T cell quantitative and functional defects. These defects result in an increased risk of infections, which are the main cause of death. Another serious complication of CLL is the transformation into an aggressive lymphoma, which is a syndrome known as Richter transformation (RT) or Richter syndrome (RS). This complication occurs in 5-10% of the patients, with an annual incidence of 0.5-1.0%, and currently represents the most important unmet clinical need of CLL (3).

This review will focus on the pathogenetic mechanisms that drive Richter Transformation and on recently developed *in vivo* models that have been used to study novel combinatorial treatments for this condition. Considering the close relationship between CLL and Richter Transformation, the first part of the paper will cover the genetic lesions and microenvironmental signals that drive the pathogenesis of CLL, whereas the second part will describe the genetic lesions most frequently associated with Richter

transformations and their capacity to induce transformation in *in vivo* murine models of the disease.

Pathogenesis of CLL: Genetic lesions

Table 1 lists the most frequent genetic lesions in CLL. By far the most frequent abnormality is the 13q14 deletion, which can be detected by fluorescence in situ hybridization (FISH) in approximately 50-60% of patients at diagnosis. This deletion represents an early event in the malignant transformation, considering that it is typically present in all or most cells of the malignant clone. The deletion invariably involves the microRNAs mir-15a and mir-16-1, which are two small noncoding RNAs that negatively regulate the expression of several important apoptosis and cell-cycle regulatory proteins (4). In particular, mir-15a and mir-16-1 have been shown to negatively regulate BCL-2. BCL-2 is an antiapoptotic protein that is strongly overexpressed by the malignant B cells, and it is believed to be the main reason for the greater apoptosis resistance of CLL compared to normal B cells (5, 6). In addition, these microRNAs downmodulate the expression of several genes that control cell-cycle progression, including the cyclins CCND2 and CCND3 and the cyclin-dependent kinases CDK4 and CDK6 (7). The increased expression of these genes as a consequence of the 13q14 deletion provides the leukemic cells with a greater capacity to respond to proliferative signals.

Table 1. Major genetic lesions and affected pathways in chronic lymphocytic leukemia

Genetic lesion	Frequency	Cellular pathway
13q14 deletion / miR-15/16 deletion	50%-60%	Apoptosis (BCL2) and cell cycle (CCND2, CCND3, CDK4, CDK6)
trisomy 12	15%-25%	Unknown
11q22 deletion and ATM mutations	15%-25%	DNA damage response
17p13 deletion and TP53 mutations	10%-15%	DNA damage response
NOTCH1 mutations	10%-15%	NOTCH1 signaling
SF3B1 mutations	10%-15%	RNA processing
BIRC3 mutations	4%-5%	NF-kB signaling
NFKBIE mutations	3%-6%	NF-kB signaling
MyD88 mutations	3%-5%	TLR / NF-kB signaling

Another frequent genetic lesion is trisomy 12, which can be detected by FISH in approximately 15-25% of patients at diagnosis. This lesion is also frequently clonal and is typically mutually exclusive with the 13q14 deletion. The mechanism how this abnormality contributes to the pathoge-

nesis of CLL is still unknown, although recent data indicate that this may be related to increased expression and activity of the receptor EDRNB and the kinase IRAK4, which transduce signals through the endothelin- and Toll-like receptor pathways, respectively (8).

Mutations or deletions of the ATM and TP53 genes, which are located on chromosome 11q22 and 17p13, respectively, are present in 10-25% of cases at diagnosis and are typically subclonal, suggesting that they are acquired at later stages of the disease (9). Importantly, both ATM and TP53 are tumor suppressors that are involved in DNA damage response. ATM and TP53 preserve the genomic integrity and stability of the cells by activating DNA repair proteins upon DNA damage recognition and arresting cellular growth by holding the cell cycle at the G1/S regulation point, or initiating apoptosis if the DNA damage proves to be irreparable. Consequently, deficiency of ATM or TP53 results in increased genomic instability and greater risk for acquisition of additional genetic lesions. In addition, deficiency of ATM and particularly TP53 is associated with resistance to cytotoxic agents that function by inducing DNA damage. For these reasons, the frequency of TP53 and ATM genetic lesions increases in more advanced stages of the disease and in patients that have become refractory to chemotherapy.

Mutations in NOTCH1 are present in 10-15% of patients at diagnosis, but are more frequent in patients at advanced stages of the disease and, as will be discussed later, in patients with Richter transformation. NOTCH1 acts as a transcription factor that induces the expression of various proteins involved in cell survival, proliferation, chemotaxis and homing (10). These include the antiapoptotic proteins MCL-1, c-IAP2, BCL-2 and XIAP, the cyclin CCND3, the proto-oncogene MYC, the B cell receptor pathway components LYN, SYK, BLK and BLNK, the chemokine receptor CXCR4 and the integrin CD49d (11-13). In addition, NOTCH1 signaling has also been reported to downregulate surface expression of CD20, which might explain why such patients derive limited benefits from anti-CD20-based immunotherapies. Interestingly, NOTCH1 mutations in CLL do not result in constitutive activation of this pathway, but rather cause prolonged signaling once NOTCH1 has been activated by binding to one of its ligands, which are various members of the SERRATE/JAGGED or DELTA-like families that are expressed on adjacent cells. Activated NOTCH1 is normally rapidly

degraded because of the presence of signal sequences for ubiquitination and proteasomal degradation in its C-terminal PEST domain. This domain is either truncated or entirely removed by the NOTCH1 mutations, resulting in reduced degradation and prolonged biological activity of NOTCH1 (11).

SF3B1 is another frequently mutated gene, with a mutational frequency of 10-15% in different studies (14, 15). The SF3B1 gene encodes a protein that acts as a component of the spliceosome machinery and is involved in the binding of the spliceosomal U2 small nuclear ribonucleoprotein (snRNP) to the branch point of 3' intronic splicing sites. SF3B1 mutations promote the use of an alternative branch point, leading to the inclusion of 3' intronic sequences in the mature RNA (16) and, therefore, to splicing changes affecting the structure and coding potential of gene transcripts across multiple pathways, including the DNA damage response, NOTCH1 signaling, apoptosis, and cell proliferation (17). SF3B1 mutations are also typically subclonal at diagnosis and have been associated with more aggressive disease and unfavorable overall survival.

Genetic abnormalities that affect members of the NF-kB pathway are also common in CLL. The two most frequent abnormalities are loss-of-function mutations in NFKBIE and BIRC3, which are negative regulators of the canonical and non-canonical NF-kB pathway, respectively. Mutations in NFKBIE and BIRC3 are seen in approximately 3-6% of cases at diagnosis (18,19). However, BIRC3 is also frequently affected by the 11q22 deletion, which, in 80% of the cases, involves both ATM and BIRC3. Deficiency of NFKBIE or BIRC3 has been associated with increased activity of the various members of the NF-kB family of transcription factors, which play an essential role in regulating several cardinal cellular processes, including cell survival and proliferation. NFKBIE and BIRC3 mutations are enriched in cases with advanced disease and are associated with poor-prognostic markers, suggesting their likely involvement in disease progression.

Another genetic abnormality that results in activation of the NF-kB pathway are mutations in MyD88, which is an adaptor protein that is involved in signaling downstream of various Toll-like receptors (TLRs). Mutations in MyD88 have been observed at a relatively low frequency in CLL (3-5% of cases), but are considerably more frequent in some other B cell malignancies, such as diffuse large B cell lymphoma (DLBCL, 30% of cases) and Waldenstrom's macroglobulinemia (90% of cases) (20,21). The

most common recurrent MYD88 mutation (p.L265P) is a missense mutation, which leads to increased binding of MYD88 to the downstream kinase IRAK1 (22). MyD88 mutations are typically clonal and have been associated with a favorable prognosis.

In addition to these genetic lesions, at least 50 other genetic lesions have been identified in putative CLL driver genes at frequencies ranging from 1 to 5 percent. Although the exact mechanisms as to how these lesions contribute to the pathogenesis of CLL are still unknown, most of them would be expected to affect one of the cellular pathways that were previously mentioned, including cell cycle regulation (e.g., CCND2, CDKN1B, CDKN2A/CDKN2B, MGA), DNA damage response (e.g., POT1), NOTCH1 signaling (e.g., FBXW7, SPEN), RNA processing (e.g., DDX3X, XPO1, U1 snRNA, RPS15), chromatin remodeling (e.g., SETD1A, SETD2, CHD2) and signaling through the NF-kB, TLR- and B cell receptor (BCR) pathways (e.g., TRAF3, IRF4, TLR2, IRAK1, EGR2)(23).

Pathogenesis of CLL: Microenvironmental signals

In addition to genetic lesions, signals from the microenvironment have been shown to play a major role in the pathogenesis of CLL. Evidence for this stems from the fact that CLL cells die rapidly by spontaneous apoptosis *in vitro*, whereas their survival in the blood and lymph nodes of patients has been estimated by *in vivo* labelling studies to be in the order of several months (24-27). Moreover, CLL cells do not proliferate when placed in culture, although a substantial proportion of them proliferates in the lymph nodes within specialized structures called proliferation centers (28). At these sites, CLL cells have been shown to interact directly with various other cell types, including T helper cells, macrophages, stromal cells, and follicular dendritic cells. Interaction with these cells has been shown to increase the survival of the leukemic cells *in vitro* and, in some cases, induce their proliferation. These interactions are mediated by various cell surface and soluble ligands, such as CD40L, IL-4 and IL-21 (expressed by T helper cells), BAFF, APRIL and IL-15 (expressed by macrophages) or the NOTCH1-ligand Jagged and the integrin-ligand VCAM-1 (expressed by stromal cells).

CLL cells receive additional growth-promoting signals from the BCR, which is a signaling complex composed of a surface immunoglobulin and a heterodimer of the proteins CD79A and CD79B. The BCR is expressed on all B cells, both normal and malignant. However, in contrast to normal B cells, where the BCR is only transiently activated following stimulation with a microbial antigen, the BCR in CLL and other malignant B cells is frequently chronically activated (29).

Initial evidence for a role of the BCR in the pathogenesis of CLL was provided by immunogenetic studies showing that CLL BCRs have a restricted usage of immunoglobulin heavy chain variable (IGHV) and immunoglobulin light chain variable (IGLV) genes, indicating selection for particular antigen-binding properties. BCRs encoded by these IGHV/IGLV combinations and having particular HCDR3 structures have been named "stereotyped BCRs" and have been identified in approximately one-third of the CLL cases, whereas they are rarely seen in normal B lymphocytes (30-32). Subsequent studies have shown that freshly isolated CLL cells express high levels of BCR target genes and constitutively activated BCR signaling molecules, such as the kinases LYN, SYK, PI3K and BTK, suggesting that the BCR pathway is chronically activated in these cells (33-36). Definite evidence for a major role of the BCR pathway in the pathogenesis of CLL came from clinical studies showing that drugs that inhibit signaling through this receptor induce clinical responses in the majority of CLL patients. Most of these drugs target the kinases BTK or PI3K, although inhibitors of SYK, SRC family kinases, and mTOR have also demonstrated activity in clinical trials (29).

Mechanisms of BCR pathway activation in CLL B cells

Studies conducted during the previous decade have identified two main mechanisms of BCR activation in CLL cells (Figure 1). The first mechanism involves binding to low-affinity external autoantigens that are typically generated during apoptosis or oxidation (37, 38). This mechanism is primarily seen with CLL cells belonging to the more aggressive, IGHV unmutated (U-CLL) subset, and would be predicted to result in intermittent activation of the BCR pathway in CLL-infiltrated lymphoid tissues, where apoptotic cells are commonly present (39, 40). The second mechanism is a peculiar mechanism that involves binding of CLL BCRs to internal motifs

located in neighboring immunoglobulin molecules, resulting in BCR-BCR interactions that occur in the absence of any external ligand (41). These cell-autonomous BCR-BCR interactions have been detected with both IGHV-mutated (M-CLL) and U-CLL BCRs and would be expected to generate a continuous low-intensity BCR signal in all tissue compartments (39).

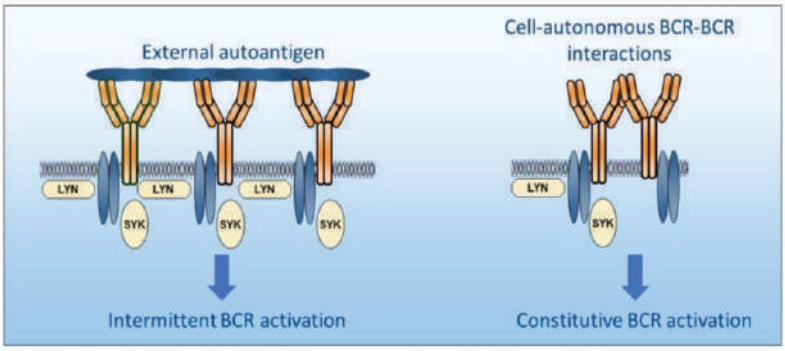


Figure 1. Mechanisms of B cell receptor activation in chronic lymphocytic leukemia

The pathogenetic role of these two mechanisms of BCR activation was explored in a study conducted by our group, in which we investigated the capacity of different antigen-BCR and BCR-BCR interactions to induce leukemia in the well-established Eμ-TCL1 transgenic mouse model of CLL [42]. These mice, which are predisposed to develop CLL because of targeted overexpression of the TCL1 oncogene in the B cell compartment, were bred with mice that expressed transgenic BCRs with different antigen specificity. Prolonged follow-up of these mice showed that only B cells that expressed BCRs with cell-autonomous activity or BCRs reactive with low-affinity self-antigens enter into the leukemogenic process and become CLL cells. By contrast, B cells that expressed BCRs that did not react with any antigen, or that reacted with high-affinity antigens, did not undergo malignant transformation, regardless of antigen form (soluble or membrane-tethered) or presentation (foreign or self). These findings provided direct *in vivo* evidence that self-reactivity is a major driving force in CLL pathogenesis and suggested that only BCR signals of certain quality can promote the growth of the malignant cells.

The exact nature of the cellular processes regulated by the two different mechanisms of BCR pathway activation has still not been fully

elucidated, because most of the studies so far have evaluated only responses induced by external ligands. However, these studies identified several BCR-driven processes that could contribute to the expansion and accumulation of the malignant clones, including increased apoptosis resistance (mediated by induction of the antiapoptotic protein MCL-1 and downregulation of the proapoptotic proteins BIM and HRK), increased adhesion to stromal cells and the extracellular matrix (mediated by activation of integrins and induction of adhesion molecules) and recruitment of T cells and macrophages (mediated by induction of the chemokines CCL3, CCL4 and CCL22) (35, 43-48). In addition, BCR engagement with an external ligand has been shown to induce CLL cells to enter into the G1 phase of the cell cycle, although, unlike normal B cells, this did not result in subsequent progression into the S phase of the cell cycle (49-51). A possible explanation for this inability of BCR signals to drive CLL cell proliferation was provided by a recent study from our group, showing that BCR stimulation of human and murine CLL cells simultaneously induces the expression of both the positive regulators MYC, CCND1, CCND2 and CDK4, which induce cell cycle entry, and the negative regulators CDKN1A, CDKN2A and CDKN2B, which inhibit CDK4 and CDK6 and block G1/S phase progression (52). This could explain why CLL cells require co-stimulatory signals from T cells (i.e., CD40L + IL-4 + IL-21) or TLR ligands (i.e., unmethylated DNA) for their proliferation (53-55).

Richter Syndrome: Clinical and diagnostic features

Richter syndrome is defined in the World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues as the development of a secondary and aggressive lymphoma arising on the background of CLL or small lymphocytic lymphoma (SLL) (57). The first case was described in 1928 by Dr Maurice Richter as a “reticular cell sarcoma of lymph nodes” arising in the context of “lymphatic leukemia” (58), and was nominated in his honor in 1964 by Lortholary et al. (59).

Richter Syndrome occurs in approximately 5–10% of CLL patients during their lifetime, with an annual incidence of 0.5-1.0% (60). It may present as two different pathologic entities: the diffuse large B cell lymphoma (DLBCL) variant, which accounts for approximately 90% of the cases, and the Hodgkin lymphoma (HL) variant, which accounts for the remaining cases (61).

The main clinical signs of Richter syndrome are new onset B symptoms, rapidly-growing and/or asymmetrical lymphadenopathy or extranodal masses, and rapidly rising LDH levels or new onset hypercalcemia. These signs and symptoms should raise suspicion of Richter syndrome and prompt an excisional biopsy, which is best guided by the results of an 18-fluorodeoxyglucose (18FDG) positron emission tomography/computed tomography (PET/CT) scan. The lesions that display the most avid 18FDG uptake with the highest standardized uptake value (SUV) should be selected for bioptic sampling (62). In contrast, lesions that have a SUV<5 are unlikely to be transformed and should not be biopsied.

The diagnosis is established based on the results of the histological analysis, which, in the case of the HL variant, requires the presence of classical Reed-Sternberg cells harboring a CD30+/CD15+/CD20- phenotype in a polymorphous background of T cells, epithelioid histiocytes, eosinophils and plasma cells. In most cases the Reed-Sternberg cells are EBV positive and have a different IG gene rearrangement from the CLL clone, suggesting that they represent *de novo*, EBV-driven lymphomas (61).

The diagnosis of the DLBCL variant is more complicated and requires an experienced pathologist, because it may mimic aggressive/accelerated CLL, which is characterized by expanded and confluent proliferation centers (57,62). The diagnosis is based on the finding of a diffuse infiltration of large B cells with a nuclear size equal to or larger than macrophage nuclei or more than twice the size of a normal lymphocyte. The tumor cells invariably express the CD20 antigen, whereas CD5 expression is maintained in only a fraction (~30%) of cases, and CD23 is expressed even less frequently (~15% of cases) (63). Most tumor cells express the Ki67 antigen, suggesting that they are actively proliferating.

Immunogenetic studies have shown that in more than 80% of the cases the tumor cells are clonally related to the CLL cells, as evidenced by the presence of identical IG gene rearrangements (63). The remaining cases carry IG gene rearrangements that are different from those of the CLL cells and therefore represent *de novo* lymphomas. The determination of the clonal relationship between the lymphoma and CLL cells is critical for the proper management of Richter syndrome, as patients with clonally-related and clonally-unrelated RT respond differently to treatment and have a different prognosis. In particular, patients with clonally-related RT are resistant to

chemoimmunotherapy and have an average survival time of less than 12 months, whereas patients with clonally-unrelated DLBCL respond well to standard DLBCL treatment and have a similar survival as *de novo* DLBCL, which is approximately 65 months.

Richter Syndrome: Genetic lesions

Another difference between clonally-related and clonally-unrelated Richter syndrome is the genetic profile of the malignant cells. The clonally-unrelated tumors carry the same genetic lesions as *de novo* DLBCL, such as inactivating mutations in the acetyltransferase genes CREBBP and EP300 and the histone methyltransferase gene KMT2D, or translocations of the BCL2 and BCL6 oncogenes. These genetic lesions do not occur in the clonally-related tumors, further suggesting that DLBCL transformed from CLL and *de novo* DLBCL represent distinct disease entities.

In clonally related RT-DLBCL, the most frequent genetic abnormalities are mutations or deletions of TP53, which occur in 60% to 80% of the cases and can be either present or acquired at the time of transformation (Table 2)(63). The second most frequent abnormality is the 9p21 deletion, which disrupts the previously mentioned cell cycle inhibitors CDKN2A and CDKN2B. Deletion of CDKN2A and CDKN2B has been reported in over 30% of Richter syndrome tumors and typically occurs at the time of transformation, whereas it has been detected in only 1.7% of unselected CLL cases (64-66). Aberrant MYC expression or activation is also frequently acquired at the time of transformation and is usually due to somatic structural alterations, such as the t (8;14) translocation or the 8q24 amplification, which are detected in approximately 30% of cases and result in MYC overexpression, or by truncating mutations and deletions of the MYC-antagonist MGA, which occur in approximately 10% of the cases and result in increased MYC activity (64,65, 67, 68). In addition, MYC is overexpressed in an additional 30% of cases because of gain-of-function NOTCH1 mutations.

Table 2. Major genetic lesions and affected pathways in Richter Syndrome

Genetic lesion	Frequency in CLL	Frequency in RT	Cellular Pathway
TP53 mutation and/or deletion (del17p13)	10%-15%	**60%**	DNA damage response and cell cycle
CDKN2A/CDKN2B deletion (del9p21)	1.5%	**35%**	Cell cycle
MYC abnormalities: t(8;14); 8q24 amplification	5-7%	**30%**	Cell cycle (increased MYC expression)
MGA deletion (del15q)	4%	**10%**	Cell cycle (increased MYC activity)
NOTCH1 mutations	10%-15%	**30%**	NOTCH1 signaling (increased MYC expression)

Based on their genetic profiles, two major RT-DLBCL subsets have been identified, each accounting for approximately one third of the cases: 1) a subset characterized by CDKN2A/CDKN2B deletions that are always associated with TP53 mutations/deletions and are frequently associated with MYC abnormalities, and 2) a subset characterized by NOTCH1 mutations that, in most cases, are associated with trisomy 12. Cases belonging to the latter subset frequently express BCRs belonging to the stereotyped subset 8, which is characterized by broad polyreactivity to multiple autoantigens. In one study, usage of a subset 8 stereotyped BCR increased the risk for development of Richter Syndrome by 24-fold, suggesting that BCR signaling may represent an important driving force during Richter transformation (69).

Richter Syndrome: *In vivo* murine models

The capacity of the above-mentioned genetic lesions to induce Richter transformation was investigated in several recent studies using the previously described Eμ-TCL1 transgenic mouse model of CLL. Crossing these mice with TP53 mice to mimic the effects of TP53 deficiency resulted in the development of aggressive lymphomas with features of Richter syndrome in 15% of the cases. This suggests that TP53 abnormalities increase the risk for Richter transformation but are unable to drive this complication on their own (70). In another study, Eμ-TCL1 transgenic mice were crossed with mice overexpressing MYC in the B cell compartment (71). These mice developed aggressive lymphomas in all cases, but the tumors were derived from B cells at an earlier maturation stage and had distinct immunogenetic features compared to the CLL cells, suggesting that they represent clonally-unrelated lymphomas. More recently, Eμ-TCL1 transgenic mice were cros-

sed with mice with B cell-restricted overexpression of the NOTCH1 intracellular domain to investigate the effects of constitutive NOTCH1 activation (72). Although these mice developed aggressive lymphomas with a high penetrance, this only occurred in mice at an advanced age, suggesting that NOTCH1-activating mutations are not sufficient to drive Richter transformation on their own and that additional genetic lesions need to be acquired for this to occur.

My own group recently investigated whether combined genetic lesions in CDKN2A, CDKN2B and TP53 can drive Richter transformation in the Eμ-TCL1 transgenic model (52). This combination piqued our interest because of the previously mentioned observations that CDKN2A/CDKN2B deletions are almost always acquired at the time of Richter Transformation and always associated with TP53 mutations or deletions. Further rationale to investigate this combination was provided by our recent finding that stimulation of CLL cells through the BCR simultaneously induces the cell cycle inhibitors CDKN1A, CDKN2A and CDKN2B. Because CDKN1A is a transcriptional target of TP53, we reasoned that genetic lesions in TP53, CDKN2A and CDKN2B may prevent the upregulation of these cell cycle inhibitors in BCR-stimulated CLL cells and, consequently, allow them to proliferate in the absence of co-stimulatory signals (Figure 2).

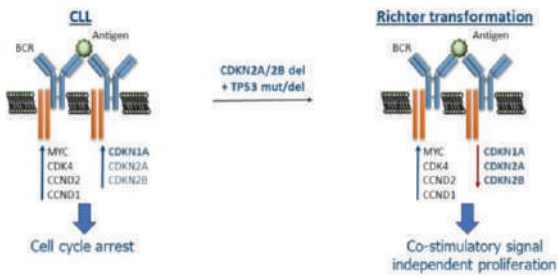


Figure 2. Mechanism of Richter Transformation in cases with combined TP53, CDKN2A and CDKN2B abnormalities.

To test this hypothesis, we simultaneously targeted the TP53, CDKN2A and CDKN2B genes in primary Eμ-TCL1 leukemia cells by CRISPR/Cas9 genome editing. The targeted and control leukemia cells were then transplanted in syngeneic mouse recipients and the impact of combined TP53, CDKN2A

and CDKN2B genetic lesions was assessed by comparing the *in vivo* growth of the leukemic cells. Mice that received TP53/CDKN2A/CDKN2B-targeted cells showed accelerated leukemia growth and developed splenic tumors with morphological features of Richter transformation, including more diffuse infiltration, larger and more pleomorphic cells, and a higher proliferative rate compared to the control leukemia cells (Figure 3). Importantly, *in vitro* culture experiments with the targeted cells showed that they had acquired the capacity to proliferate in the absence of any co-stimulatory signals, which was not the case for cells in which only TP53 or CDKN2A and CDKN2B had been disrupted. Moreover, the spontaneously proliferating cells displayed homozygous defects in all three genes, suggesting that biallelic inactivation of TP53, CDKN2A and CDKN2B is required for co-stimulatory signal-independent proliferation. These cells, however, remained dependent on BCR signals, as evidenced by their reduced growth and gradual disappearance upon CRISPR/Cas9-mediated knockdown of the BCR or treatment with the BCR inhibitors ibrutinib, idelalisib, entospletinib or fostamatinib. The latter finding was further exploited to investigate the therapeutic activity of combinations of BCR inhibitors with other drugs, which resulted in the identification of a highly potent combination of ibrutinib with the CDK4/CDK6 inhibitor palbociclib. Collectively, these data provide evidence that BCR signals are directly involved in driving Richter transformation and suggest that frequently co-occurring genetic lesions in TP53 and CDKN2A/2B contribute to Richter transformation by allowing for BCR-dependent/costimulatory signal-independent proliferation. In addition, they suggest that the combination of a BCR and a CDK4/6 inhibitor could represent an effective treatment for Richter syndrome patients with combined TP53 and CDKN2A/2B abnormalities and provide the preclinical rationale to evaluate this possibility in the clinic.

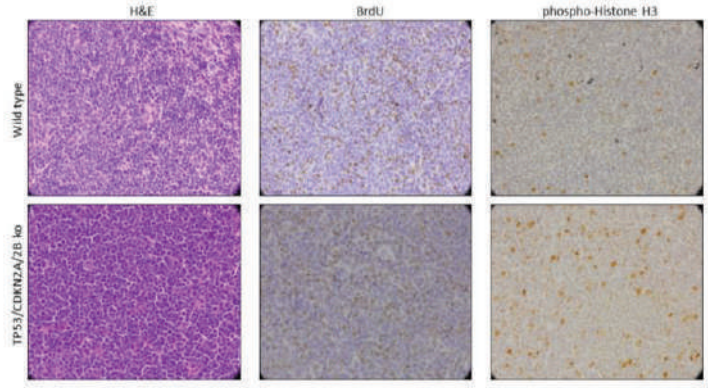


Figure 3. Histology of tumors with wild type or mutated TP53, CDKN2A and CDKN2B. Splenic sections were stained with Haematoxylin and Eosin (H&E), anti-BrdU or anti-phospho-Histone H3 antibodies. Scale bars 20 μm; 60x objective.

In summary, data from the murine models confirm the pathogenic role of TP53, CDKN2A/CDKN2B, MYC and NOTCH1 abnormalities in Richter transformation. These models, therefore, represent invaluable tools for preclinical testing of novel drugs and drug combinations that target the molecular programs that are altered in patients with Richter transformation, and these findings should also facilitate the development of personalized therapeutic approaches for this condition.

Acknowledgements and Dedication

I would like to thank the numerous members of my research group for their most valuable contributions to the studies that I described in this paper. The paper was written to celebrate the 70th birthday of my dear friend and colleague, Academician Prof. Dr. Zivko Popov, to whom I wish that he continues with the same pace, enthusiasm and productivity for many years to come.

REFERENCES

1. Kipps TJ, Stevenson FK, Wu CJ, Croce CM, Packham G, Wierda WG, O'Brien S, Gribben J, Rai K. Chronic lymphocytic leukemia. Nat Rev Dis Primers. 2017; 3: 17008.
2. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, Hillmen P, Keating M, Montserrat E, Chiorazzi N, Stilgenbauer

S, Rai KR, Byrd JC, Eichhorst[0- B, O'Brien S, Robak T, Seymour JF, Kipps TJ. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018; 131(25): 2745-2760.

3. Rossi D, Spina V, Gaidano G. Biology and treatment of Richter syndrome. Blood. 2018; 131(25): 2761-2772.
4. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc. Natl Acad. Sci. USA. 2002; 99: 15524–15529.
5. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc. Natl Acad. Sci. USA. 2005; 102: 13944–13949.
6. Valentin R, Grabow S, Davids MS. The rise of apoptosis: targeting apoptosis in hematologic malignancies. Blood. 2018; 132: 1248-1264.
7. Klein U, Lia M, Crespo M, Siegel R, Shen Q, Mo T, Ambesi-Impiombato A, Califano A, Migliazza A, Bhagat G, Dalla-Favera R. The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. Cancer Cell. 2010; 17: 28-40.
8. Reid JC, Golubeva D, Boyd AL, Hollands CG, Henly C, Orlando L, Leber A, Hébert J, Morabito F, Cutrona G, Agnelli L, Gentile M, Ferrarini M, Neri A, Leber B, Bhatia M. Human pluripotent stem cells identify molecular targets of trisomy 12 in chronic lymphocytic leukemia patients. Cell Rep. 2021; 34(11): 108845.
9. Bosch F, Dalla-Favera R. Chronic lymphocytic leukemia: from genetics to treatment. Nat Rev Clin Oncol. 2019; 16(11):684-701.
10. Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. Development. 2011; 138(17): 3593-612.
11. Rosati E, Baldoni S, De Falco F, Del Papa B, Dorillo E, Rompietti C, Albi E, Falzetti F, Di Ianni M, Sportoletti P. NOTCH1 Aberrations in Chronic Lymphocytic Leukemia. Front Oncol. 2018; 8: 229.
12. Arruga F, Gizdic B, Bologna C, Cignetto S, Buonincontri R, Serra S, Vaisitti T, Gizzi K, Vitale N, Garaffo G, Mereu E, Diop F, Neri F, Incarnato D, Coscia M, Allan J, Piva R, Oliviero S, Furman RR, Rossi D, Gaidano G, Deaglio S. Mutations in NOTCH1 PEST domain orchestrate

CCL19-driven homing of chronic lymphocytic leukemia cells by modulating the tumor suppressor gene DUSP22. Leukemia. 2017;31(9): 1882-1893.

13. Benedetti D, Tissino E, Pozzo F, Bittolo T, Caldana C, Perini C, Martorelli D, Bravin V, D'Agaro T, Rossi FM, Bomben R, Santinelli E, Zaja F, Pozzato G, Chiarenza A, Di Raimondo F, Del Poeta G, Rossi D, Gaidano G, Dal Bo M, Gattei V, Zucchetto A. NOTCH1 mutations are associated with high CD49d expression in chronic lymphocytic leukemia: link between the NOTCH1 and the NF-κB pathways. Leukemia. 2018; 32(3): 654-662.
14. Quesada V, Conde L, Villamor N, Ordóñez GR, Jares P, Bassaganyas L, Ramsay AJ, Beà S, Pinyol M, Martínez-Trillos A, López-Guerra M, Colomer D, Navarro A, Baumann T, Aymerich M, Rozman M, Delgado J, Giné E, Hernández JM, González-Díaz M, Puente DA, Velasco G, Freije JM, Tubío JM, Royo R, Gelpí JL, Orozco M, Pisano DG, Zamora J, Vázquez M, Valencia A, Himmelbauer H, Bayés M, Heath S, Gut M, Gut I, Estivill X, López-Guillermo A, Puente XS, Campo E, López-Otín C. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. Nat Genet. 2011; 44(1): 47-52.
15. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, Kluth S, Bozic I, Lawrence M, Böttcher S, Carter SL, Cibulskis K, Mertens D, Sougnez CL, Rosenberg M, Hess JM, Edelmann J, Kless S, Kneba M, Ritgen M, Fink A, Fischer K, Gabriel S, Lander ES, Nowak MA, Döhner H, Hallek M, Neuberg D, Getz G, Stilgenbauer S, Wu CJ. Mutations driving CLL and their evolution in progression and relapse. Nature. 2015; 526(7574): 525-30.
16. Kesarwani AK, Ramirez O, Gupta AK, Yang X, Murthy T, Minella AC, Pillai MM. Cancer-associated SF3B1 mutants recognize otherwise inaccessible cryptic 3' splice sites within RNA secondary structures. Oncogene. 2017; 36(8): 1123-1133.
17. Yin S, Gambe RG, Sun J, Martinez AZ, Cartun ZJ, Regis FFD, Wan Y, Fan J, Brooks AN, Herman SEM, Ten Hacken E, Taylor-Weiner A, Rassenti LZ, Ghia EM, Kipps TJ, Obeng EA, Cibulskis CL, Neuberg D, Campagna DR, Fleming MD, Ebert BL, Wiestner A, Leshchiner I, DeCaprio JA, Getz G, Reed R, Carrasco RD, Wu CJ, Wang L. A Murine Model of Chronic Lymphocytic Leukemia Based on B Cell-Restricted Expression of Sf3b1 Mutation and Atm Deletion. Cancer Cell. 2019; 35(2): 283-296.e5.
18. Diop F, Moia R, Favini C, Spaccarotella E, De Paoli L, Bruscaggin A, Spina V, Terzi-di-Bergamo L, Arruga F, Tarantelli C, Deambrogi C, Rasi S, Adhinaveni R, Patriarca A, Favini S, Sagiraju S, Jabangwe C, Kodipad AA, Peroni D, Mauro FR, Giudice ID, Forconi F, Cortelezzi A, Zaja F,

Bomben R, Rossi FM, Visco C, Chiarenza A, Rigolin GM, Marasca R, Coscia M, Perbellini O, Tedeschi A, Laurenti L, Motta M, Donaldson D, Weir P, Mills K, Thornton P, Lawless S, Bertoni F, Poeta GD, Cuneo A, Follenzi A, Gattei V, Boldorini RL, Catherwood M, Deaglio S, Foà R, Gaidano G, Rossi D. Biological and clinical implications of BIRC3 mutations in chronic lymphocytic leukemia. Haematologica. 2020 Jan 31; 105(2): 448-456.

19. Mansouri L, Sutton LA, Ljungström V, Bondza S, Arngården L, Bhoi S, Larsson J, Cortese D, Kalushkova A, Plevova K, Young E, Gunnarsson R, Falk-Sörqvist E, Lönn P, Muggen AF, Yan XJ, Sander B, Enblad G, Smedby KE, Juliusson G, Belessi C, Rung J, Chiorazzi N, Strefford JC, Langerak AW, Pospisilova S, Davi F, Hellström M, Jernberg-Wiklund H, Ghia P, Söderberg O, Stamatopoulos K, Nilsson M, Rosenquist R. Functional loss of IκBε leads to NF-κB deregulation in aggressive chronic lymphocytic leukemia. J Exp Med. 2015 Jun 1;212(6):833-43.
20. Vermaat JS, Somers SF, de Wreede LC, Kraan W, de Groen RAL, Schrader AMR, Kerver ED, Scheepstra CG, Berenschot H, Deenik W, Wegman J, Broers R, de Boer JD, Nijland M, van Wezel T, Veelken H, Spaargaren M, Cleven AH, Kersten MJ, Pals ST. MYD88 mutations identify a molecular subgroup of diffuse large B-cell lymphoma with an unfavorable prognosis. Haematologica. 2020; 105(2): 424-434.
21. Treon SP, Xu L, Guerrera ML, Jimenez C, Hunter ZR, Liu X, Demos M, Gustine J, Chan G, Munshi M, Tsakmaklis N, Chen JG, Kofides A, Sklavenitis-Pistofidis R, Bustoros M, Keezer A, Meid K, Patterson CJ, Sacco A, Roccaro A, Branagan AR, Yang G, Ghobrial IM, Castillo JJ. Genomic Landscape of Waldenström Macroglobulinemia and Its Impact on Treatment Strategies. J Clin Oncol. 2020; 38(11): 1198-1208.
22. Puente XS, Pinyol M, Quesada V, Conde L, Ordóñez GR, Villamor N, Escaramis G, Jares P, Beà S, González-Díaz M, Bassaganyas L, Baumann T, Juan M, López-Guerra M, Colomer D, Tubío JM, López C, Navarro A, Tornador C, Aymerich M, Rozman M, Hernández JM, Puente DA, Freije JM, Velasco G, Gutiérrez-Fernández A, Costa D, Carrió A, Guijarro S, Enjuanes A, Hernández L, Yagüe J, Nicolás P, Romeo-Casabona CM, Himmelbauer H, Castillo E, Dohm JC, de Sanjosé S, Piris MA, de Alava E, San Miguel J, Royo R, Gelpí JL, Torrents D, Orozco M, Pisano DG, Valencia A, Guigó R, Bayés M, Heath S, Gut M, Klatt P, Marshall J, Raine K, Stebbings LA, Futreal PA, Stratton MR, Campbell PJ, Gut I, López-Guillermo A, Estivill X, Montserrat E, López-Otín C, Campo E. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukemia. Nature. 2011; 475(7354): 101-5.

23. Fabbri G, Dalla-Favera R. The molecular pathogenesis of chronic lymphocytic leukemia. Nat Rev Cancer. 2016; 16(3): 145-62.
24. Collins RJ, Verschuer LA, Harmon BV, Prentice RL, Pope JH, Kerr JF. Spontaneous programmed death (apoptosis) of B-chronic lymphocytic leukemia cells following their culture in vitro. Br J Haematol. 1989 Mar; 71(3): 343-50.
25. Messmer BT, Messmer D, Allen SL, Kolitz JE, Kudalkar P, Cesar D, Murphy EJ, Koduru P, Ferrarini M, Zupo S, Cutrona G, Damle RN, Wasil T, Rai KR, Hellerstein MK, Chiorazzi N. In vivo measurements document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. J Clin Invest. 2005; 115(3): 755-64.
26. van Gent R, Kater AP, Otto SA, Jaspers A, Borghans JA, Vrisekoop N, Ackermans MA, Ruiter AF, Wittebol S, Eldering E, van Oers MH, Tesselaar K, Kersten MJ, Miedema F. In vivo dynamics of stable chronic lymphocytic leukemia inversely correlate with somatic hypermutation levels and suggest no major leukemic turnover in bone marrow. Cancer Res. 2008; 68(24): 10137-44.
27. Burger JA, Li KW, Keating MJ, Sivina M, Amer AM, Garg N, Ferrajoli A, Huang X, Kantarjian H, Wierda WG, O'Brien S, Hellerstein MK, Turner SM, Emson CL, Chen SS, Yan XJ, Wodarz D, Chiorazzi N. Leukemia cell proliferation and death in chronic lymphocytic leukemia patients on therapy with the BTK inhibitor ibrutinib. JCI Insight. 2017; 2(2): e89904.
28. Herndon TM, Chen SS, Saba NS, Valdez J, Emson C, Gatmaitan M, Tian X, Hughes TE, Sun C, Arthur DC, Stetler-Stevenson M, Yuan CM, Niemann CU, Marti GE, Aue G, Soto S, Farooqui MZH, Herman SEM, Chiorazzi N, Wiestner A. Direct in vivo evidence for increased proliferation of CLL cells in lymph nodes compared to bone marrow and peripheral blood. Leukemia. 2017; 31(6): 1340-1347.
29. Efremov DG, Turkalj S, Laurenti L. Mechanisms of B Cell Receptor Activation and Responses to B Cell Receptor Inhibitors in B Cell Malignancies. Cancers (Basel). 2020; 12(6): 1396.
30. Efremov DG, Ivanovski M, Siljanovski N, Pozzato G, Cevreska L, Fais F, Chiorazzi N, Batista FD, Burrone OR. Restricted immunoglobulin VH region repertoire in chronic lymphocytic leukemia patients with autoimmune hemolytic anemia. Blood. 1996; 87(9): 3869-76.
31. Messmer BT, Albesiano E, Efremov DG, Ghiotto F, Allen SL, Kolitz J, Foa R, Damle RN, Fais F, Messmer D, Rai KR, Ferrarini M, Chiorazzi N. Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. J Exp Med. 2004; 200(4): 519-25.

32. Agathangelidis A, Darzentas N, Hadzidimitriou A, Brochet X, Murray F, Yan XJ, Davis Z, van Gastel-Mol EJ, Tresoldi C, Chu CC, Cahill N, Giudicelli V, Tichy B, Pedersen LB, Foroni L, Bonello L, Janus A, Smedby K, Anagnostopoulos A, Merle-Beral H, Laoutaris N, Juliusson G, di Celle PF, Pospisilova S, Jurlander J, Geisler C, Tsaftaris A, Lefranc MP, Langerak AW, Oscier DG, Chiorazzi N, Belessi C, Davi F, Rosenquist R, Ghia P, Stamatopoulos K. Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. Blood. 2012; 119(19): 4467-75.
33. Herishanu Y, Pérez-Galán P, Liu D, Biancotto A, Pittaluga S, Vire B, Gibellini F, Njuguna N, Lee E, Stennett L, Raghavachari N, Liu P, McCoy JP, Raffeld M, Stetler-Stevenson M, Yuan C, Sherry R, Arthur DC, Maric I, White T, Marti GE, Munson P, Wilson WH, Wiestner A. The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. Blood. 2011; 117(2): 563-74.
34. Contri A, Brunati AM, Trentin L, Cabrelle A, Miorin M, Cesaro L, Pinna LA, Zambello R, Semenzato G, Donella-Deana A. Chronic lymphocytic leukemia B cells contain anomalous Lyn tyrosine kinase, a putative contribution to defective apoptosis. J Clin Invest. 2005; 115(2): 369-78.
35. Gobessi S, Laurenti L, Longo PG, Carsetti L, Berno V, Sica S, Leone G, Efremov DG. Inhibition of constitutive and BCR-induced Syk activation downregulates Mcl-1 and induces apoptosis in chronic lymphocytic leukemia B cells. Leukemia. 2009;23(4): 686-97.
36. Ringshausen I, Schneller F, Bogner C, Hipp S, Duyster J, Peschel C, Decker T. Constitutively activated phosphatidylinositol-3 kinase (PI-3K) is involved in the defect of apoptosis in B-CLL: association with protein kinase Cdelta. Blood. 2002; 100(10): 3741-8.
37. Lanemo Myhrinder A, Hellqvist E, Sidorova E, Söderberg A, Baxendale H, Dahle C, Willander K, Tobin G, Bäckman E, Söderberg O, Rosenquist R, Hörkkö S, Rosén A. A new perspective: molecular motifs on oxidized LDL, apoptotic cells, and bacteria are targets for chronic lymphocytic leukemia antibodies. Blood. 2008; 111(7): 3838-48.
38. Catera R, Silverman GJ, Hatzi K, Seiler T, Didier S, Zhang L, Hervé M, Meffre E, Oscier DG, Vlassara H, Scofield RH, Chen Y, Allen SL, Kolitz J, Rai KR, Chu CC, Chiorazzi N. Chronic lymphocytic leukemia cells recognize conserved epitopes associated with apoptosis and oxidation. Mol Med. 2008; 14(11-12): 665-74.
39. Chiorazzi N, Efremov DG. Chronic lymphocytic leukemia: a tale of one or two signals? Cell Res. 2013; 23(2): 182-5.

40. Mongini PK, Gupta R, Boyle E, Nieto J, Lee H, Stein J, Bandovic J, Stankovic T, Barrientos J, Kolitz JE, Allen SL, Rai K, Chu CC, Chiorazzi N. TLR-9 and IL-15 Synergy Promotes the In Vitro Clonal Expansion of Chronic Lymphocytic Leukemia B Cells. J Immunol. 2015; 195(3):901-23.
41. Dühren-von Minden M, Übelhart R, Schneider D, Wossning T, Bach MP, Buchner M, Hofmann D, Surova E, Follo M, Köhler F, Wardemann H, Zirlik K, Veelken H, Jumaa H. Chronic lymphocytic leukemia is driven by antigen-independent cell-autonomous signaling. Nature. 2012; 489(7415): 309-12.
42. Iacovelli S, Hug E, Bennardo S, Duehren-von Minden M, Gobessi S, Rinaldi A, Suljagic M, Bilbao D, Bolasco G, Eckl-Dorna J, Niederberger V, Autore F, Sica S, Laurenti L, Wang H, Cornall RJ, Clarke SH, Croce CM, Bertoni F, Jumaa H, Efremov DG. Two types of BCR interactions are positively selected during leukemia development in the Eμ-TCL1 transgenic mouse model of CLL. Blood. 2015; 125(10):1578-88.
43. Petlickovski A, Laurenti L, Li X, Marietti S, Chiusolo P, Sica S, Leone G, Efremov DG. Sustained signaling through the B-cell receptor induces Mcl-1 and promotes survival of chronic lymphocytic leukemia B cells. Blood. 2005; 105(12): 4820-7.
44. Longo PG, Laurenti L, Gobessi S, Sica S, Leone G, Efremov DG. The Akt/Mcl-1 pathway plays a prominent role in mediating antiapoptotic signals downstream of the B-cell receptor in chronic lymphocytic leukemia B cells. Blood. 2008; 111(2): 846-55.
45. Bojarczuk K, Sasi BK, Gobessi S, Innocenti I, Pozzato G, Laurenti L, Efremov DG. BCR signaling inhibitors differ in their ability to overcome Mcl-1-mediated resistance of CLL B cells to ABT-199. Blood. 2016; 127(25): 3192-201.
46. Burger JA, Quiroga MP, Hartmann E, Bürkle A, Wierda WG, Keating MJ, Rosenwald A. High-level expression of the T-cell chemokines CCL3 and CCL4 by chronic lymphocytic leukemia B cells in nurselike cell cocultures and after BCR stimulation. Blood. 2009; 113(13): 3050-8.
47. de Rooij MF, Kuil A, Geest CR, Eldering E, Chang BY, Buggy JJ, Pals ST, Spaargaren M. The clinically active BTK inhibitor PCI-32765 targets B-cell receptor- and chemokine-controlled adhesion and migration in chronic lymphocytic leukemia. Blood. 2012; 119(11): 2590-4.
48. Tissino E, Benedetti D, Herman SEM, Ten Hacken E, Ahn IE, Chaffee KG, Rossi FM, Dal Bo M, Bulian P, Bomben R, Bayer E, Härzschel A, Gutjahr JC, Postorino M, Santinelli E, Ayed A, Zaja F, Chiarenza A, Pozzato G, Chigaev A, Sklar LA, Burger JA, Ferrajoli A, Shanafelt TD, Wiestner A, Del Poeta G, Hartmann TN, Gattei V, Zucchetto A. Functional

and clinical relevance of VLA-4 (CD49d/CD29) in ibrutinib-treated chronic lymphocytic leukemia. J Exp Med. 2018; 215(2): 681-697.

49. Deglesne PA, Chevallier N, Letestu R, Baran-Marszak F, Beitar T, Salanoubat C, Sanhes L, Nataf J, Roger C, Varin-Blank N, Ajchenbaum-Cymbalista F. Survival response to B-cell receptor ligation is restricted to progressive chronic lymphocytic leukemia cells irrespective of Zap70 expression. Cancer Res. 2006; 66(14): 7158-66.
50. Guarini A, Chiaretti S, Tavolaro S, Maggio R, Peragine N, Citarella F, Ricciardi MR, Santangelo S, Marinelli M, De Propris MS, Messina M, Mauro FR, Del Giudice I, Foà R. BCR ligation induced by IgM stimulation results in gene expression and functional changes only in IgV H unmutated chronic lymphocytic leukemia (CLL) cells. Blood. 2008; 112(3): 782-92.
51. Krysov S, Dias S, Paterson A, Mockridge CI, Potter KN, Smith KA, Ashton-Key M, Stevenson FK, Packham G. Surface IgM stimulation induces MEK1/2-dependent MYC expression in chronic lymphocytic leukemia cells. Blood. 2012; 119(1): 170-9.
52. Chakraborty S, Martines C, Porro F, Fortunati I, Bonato A, Dimishkovska M, Piazza S, Yadav BS, Innocenti I, Fazio R, Vaisitti T, Deaglio S, Zamò A, Dimovski AJ, Laurenti L, Efremov DG. B Cell Receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter Transformation. Blood. 2021 Apr 30:blood.2020008276.

    doi: 10.1182/blood.2020008276. Online ahead of print.
53. Modulation of malignant B cell activation and apoptosis by bcl-2 antisense ODN and immunostimulatory CpG ODN.
54. Jahrsdörfer B, Jox R, Mühlenhoff L, Tschoep K, Krug A, Rothenfusser S, Meinhardt G, Emmerich B, Endres S, Hartmann G. Modulation of malignant B cell activation and apoptosis by bcl-2 antisense ODN and immunostimulatory CpG ODN. J Leukoc Biol. 2002; 72(1): 83-92.
55. Pascutti MF, Jak M, Tromp JM, Derks IA, Remmerswaal EB, Thijssen R, van Attekum MH, van Bochove GG, Luijks DM, Pals ST, van Lier RA, Kater AP, van Oers MH, Eldering E. IL-21 and CD40L signals from autologous T cells can induce antigen-independent proliferation of CLL cells. Blood. 2013; 122(17): 3010-9.
56. Schleiss C, Ilias W, Tahar O, Güler Y, Miguet L, Mayeur-Rousse C, Mauvieux L, Fornecker LM, Toussaint E, Herbrecht R, Bertrand F, Maumy-Bertrand M, Martin T, Fournel S, Georgel P, Bahram S, Vallat L. BCR-associated factors driving chronic lymphocytic leukemia cells proliferation ex vivo. Sci Rep. 2019; 9(1): 701.

57. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J. WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. In WHO Classification of Tumors, Revised 4th Edition, Volume 2. 2017.
58. Richter MN. Generalized Reticular Cell Sarcoma of Lymph Nodes Associated with Lymphatic Leukemia. Am J Pathol. 1928; 4(4): 285-292.7.
59. LORTHOLARY P, RIPAULT M, BOIRON M. [RICHTER'S SYNDROME]. Nouv Rev Fr Hematol. 1964; 4: 456-7.
60. Parikh SA, Rabe KG, Call TG, Zent CS, Habermann TM, Ding W, Leis JF, Schwager SM, Hanson CA, Macon WR, Kay NE, Slager SL, Shanafelt TD. Diffuse large B-cell lymphoma (Richter syndrome) in patients with chronic lymphocytic leukemia (CLL): a cohort study of newly diagnosed patients. Br J Haematol. 2013; 162: 774–82.
61. Condoluci A, Rossi D. Richter Syndrome. Curr Oncol Rep. 2021; 23(3):26.
62. Soilleux EJ, Wotherspoon A, Eyre TA, Clifford R, Cabes M, Schuh AH. Diagnostic dilemmas of high-grade transformation (Richter's syndrome) of chronic lymphocytic leukemia: results of the phase II National Cancer Research Institute CHOP-OR clinical trial specialist haemato-pathology central review. Histopathology. 2016; 69(6): 1066-1076.
63. Rossi D, Spina V, Gaidano G. Biology and treatment of Richter syndrome. Blood. 2018; 131(25): 2761-2772.
64. Fabbri G, Khiabanian H, Holmes AB, Wang J, Messina M, Mullighan CG, Pasqualucci L, Rabadan R, Dalla-Favera R. Genetic lesions associated with chronic lymphocytic leukemia transformation to Richter syndrome. J Exp Med. 2013; 210(11): 2273-88.
65. Chigrinova E, Rinaldi A, Kwee I, Rossi D, Rancoita PM, Strefford JC, Oscier D, Stamatopoulos K, Papadaki T, Berger F, Young KH, Murray F, Rosenquist R, Greiner TC, Chan WC, Orlandi EM, Lucioni M, Marasca R, Inghirami G, Ladetto M, Forconi F, Cogliatti S, Votavova H, Swerdlow SH, Stilgenbauer S, Piris MA, Matolcsy A, Spagnolo D, Nikitin E, Zamò A, Gattei V, Bhagat G, Ott G, Zucca E, Gaidano G, Bertoni F.. Two main genetic pathways lead to the transformation of chronic lymphocytic leukemia to Richter syndrome. Blood. 2013; 122(15): 2673-82.
66. Leeksma AC, Baliakas P, Moysiadis T, Puiggros A, Plevova K, Van der Kevie-Kersemaekers AM, Posthuma H, Rodriguez-Vicente AE, Tran AN, Barbany G, Mansouri L, Gunnarsson R, Parker H, Van den Berg E, Bellido M, Davis Z, Wall M, Scarpelli I, Österborg A, Hansson L, Jarosova M, Ghia P, Poddighe P, Espinet B, Pospisilova S, Tam C, Ysebaert L, Nguyen-Khac F, Oscier D, Haferlach C, Schoumans J, Stevens-Kroef M, Eldering E, Stamatopoulos K, Rosenquist R, Strefford JC, Mellink C, Kater AP. Genomic

arrays identify high-risk chronic lymphocytic leukemia with genomic complexity: a multi-center study. Haematologica. 2020; 106(1): 87-97.

67. De Paoli L, Cerri M, Monti S, Rasi S, Spina V, Bruscaggin A, Greco M, Ciardullo C, Famà R, Cresta S, Maffei R, Ladetto M, Martini M, Laurenti L, Forconi F, Marasca R, Larocca LM, Bertoni F, Gaidano G, Rossi D. MGA, a suppressor of MYC, is recurrently inactivated in high risk chronic lymphocytic leukemia. Leuk Lymphoma. 2013; 54(5): 1087-90
68. Edelmann J, Holzmann K, Tausch E, Saunderson EA, Jebaraj BMC, Steinbrecher D, Dolnik A, Blätte TJ, Landau DA, Saub J, Estenfelder S, Ibach S, Cymbalista F, Leblond V, Delmer A, Bahlo J, Robrecht S, Fischer K, Goede V, Bullinger L, Wu CJ, Mertens D, Ficz G, Gribben JG, Hallek M, Döhner H, Stilgenbauer S. Genomic alterations in high-risk chronic lymphocytic leukemia frequently affect cell cycle key regulators and NOTCH1-regulated transcription. Haematologica. 2020; 105(5): 1379-1390.
69. Rossi D, Spina V, Cerri M, Rasi S, Deambrogi C, De Paoli L, Laurenti L, Maffei R, Forconi F, Bertoni F, Zucca E, Agostinelli C, Cabras A, Lucioni M, Martini M, Magni M, Deaglio S, Ladetto M, Nomdedeu JF, Besson C, Ramponi A, Canzonieri V, Paulli M, Marasca R, Larocca LM, Carbone A, Pileri SA, Gattei V, Gaidano G. Stereotyped B-cell receptor is an independent risk factor of chronic lymphocytic leukemia transformation to Richter syndrome. Clin Cancer Res. 2009; 15(13): 4415-22.
70. Knittel G, Rehkämper T, Korovkina D, Liedgens P, Fritz C, Torgovnick A, Al-Baldawi Y, Al-Maarri M, Cun Y, Fedorchenko O, Riabinska A, Beleggia F, Nguyen PH, Wunderlich FT, Ortmann M, Montesinos-Rongen M, Tausch E, Stilgenbauer S, P Frenzel L, Herling M, Herling C, Bahlo J, Hallek M, Peifer M, Buettner R, Persigehl T, Reinhardt HC. Two mouse models reveal an actionable PARP1 dependence in aggressive chronic lymphocytic leukemia. Nat Commun. 2017; 8(1): 153.
71. Lucas F, Rogers KA, Harrington BK, Pan A, Yu L, Breitbach J, Bundschuh R, Goettl VM, Hing ZA, Kanga P, Mantel R, Sampath D, Smith LL, Wasmuth R, White DK, Yan P, Byrd JC, Lapalombella R, Woyach JA. Eμ-TCL1xMyc: A Novel Mouse Model for Concurrent CLL and B-Cell Lymphoma. Clin Cancer Res. 2019; 25(20): 6260-6273.
72. Kohlhaas V, Blakemore SJ, Al-Maarri M, Nickel N, Pal M, Roth A, Hövelmeyer N, Schäfer SC, Knittel G, Lohneis P, Nikolic M, Wiederstein JL, Franitza M, Georgomonolis T, Reinart N, Herling M, Herling C, Hartmann EM, Rosenwald A, Klapper W, Büttner R, Moia R, Rossi D, Boldorini R, Gaidano G, Frenzel LP, Reinhardt HC, Brüning JC, Hallek M, Krüger M, Peifer M, Pallasch CP, Wunderlich FT. Active Akt signaling triggers CLL toward Richter transformation via overactivation of Notch1. Blood. 2021; 137(5): 646-660.

doi: 10.20903/zpopov-2022-0029 UDK: 616.61:[602.4:577.112

Гоце СПАСОВСКИ[1], Ирена РАМБАБОВА БУШЛЕТИЌ[1],
Лада ТРАЈЧЕСКА[1], Сашо ДОХЧЕВ[2], Оливер СТАНКОВ[2],
Сотир СТАВРИДИС[2], Скендер САИДИ[2], Живко ПОПОВ[2,3]

УПОТРЕБА НА ПРОТЕОМИЦИ ВО УРОНЕФРОЛОГИЈАТА – РЕАЛНОСТ ИЛИ ФИКЦИЈА?

Апстракт

Хроничната бубрежна болест е светски проблем што постојано се зголемува, со здравствени и социоекономски импликации. Трансплантацијата на бубрег е најоптимален третман на терминална фаза на ХББ. Сè уште постои дискрепанца помеѓу потребите од органи за трансплантација и нивната достапноста. Неопходни се современи дијагностички методи за детектирање на раните промени на графтот со цел тој долгорочно да преживее. Уринарните протеомични анализи играат голема улога во полето на трансплантацијата на органи, детектирајќи ги промените пред тие да имаат клиничка презентација, овозможувајќи нивен соодветен ран третман.

Во полето на урологијата, како неинвазивни биомаркери за рана детекција на малигните заболувања, протеомичните анализи се сметаат за дијагностички пристап што ветува. Со нивната клиничка употреба би се избегнале повторувачките инвазивни дијагностички постапки и увидот во ефикасноста на применетата терапија. Поголеми клинички студии би овозможиле нивен преод од базичната во клиничката практика и индивидуален пристап за секој пациент.

[1] Клиника за нефрологија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Република Северна Македонија

[2] Клиника за урологија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Република Северна Македонија

[3] Македонска академија на науките и уметностите, Скопје; Медицински факултет, Универзитет “Св. Кирил и Методиј”, Скопје, Република Северна Македонија

Клучни зборови: уринарни протеомици, трансплантиран бубрег, уринарен карцином, рана дијагностика.

Вовед

Развиените европски земји со повисок стандард бележат тренд на стареење на популацијата како резултат на продолжениот животен век, а од друга страна се зголемува и бројот на пациенти со дијагностициран дијабетес и хипертензија како водечки причини за развој на хроничната бубрежна болест (ХББ). Податоците покажуваат дека ХББ претставува глобален светски проблем, со преваленца која варира од 760/милион жители во Исланд до 1612/милион жители во Португалија (1).

Се смета дека разбирањето на ХББ, како и свесноста и застапеноста на оваа состојба во големите промотивни кампањи, не се на задоволително ниво. Веројатно, една од причините за ваквата состојба се должи и на природата на болеста, текот на прогресијата која останува асимптоматска во раните стадиуми, меѓутоа со брз развој, но и со сериозно влијание (импакт) на пациентите во подоцнежните стадиуми (2). Потребно е преземање на повеќе активности, особено од непрофитната организација *European Kidney Health Alliance* – Европски сојуз за здравјето на бубрезите (ЕКНА/ЕСЗБ) (3), со цел да се укаже на актуелната состојба, но и да се надминат разликите во развојот на терапевтските процедури во нефрологијата наспроти брзиот развој на дијагностиката и третманот во областите како што се кардиологијата и онкологијата (4).

Препораките од 2012 година на организацијата Подобрување на глобалните резултати од бубрежните болести (*Kidney Disease Improving Global Outcome*) (KDIGO) ја дефинираат ХББ како нарушување во структурата или во функцијата на бубрезите, со импликации на здравјето на индивидуата, што трае подолго од 3 месеци, а ја вклучуваат и протеинуријата како маркер во дефинирањето на стадиумите на ХББ (5).

ХББ, особено терминалната фаза која бара сеопфатна терапија е во интерес на нефролозите во светот. Модалитети во лекување на терминалната фаза на ХББ се лекувањето со дијализа (хемодијализа и перитонеална дијализа) и трансплантацијата на бубрег (6).

Добиените резултати од публикуваните анализи покажуваат дека трансплантацијата на бубрег е модалитет на избор кој го подобрува квалитетот на животот и ги намалува смртноста и ризикот од кардиоваскуларните заболувања. Со тоа се оправдува напорот за зголемување на бројот на трансплантирани пациенти преку зголемувањето на корпусот на потенцијални дарители и реципиенти (7). Според добиените резултати, морталитетот кај трансплантираните пациенти е повисок во однос на оној кај пациентите на хронична хемодијализа само во периоперативниот период. Од друга страна, пак, вкупниот морталитет значително опаѓа, а ваквите резултати се должат на воведувањето нова имуносупресивна терапија, подобар третман на коморбидитетните состојби, како и подобра селекција на потенцијалните реципиенти (8). Во основа, за пролонгирано преживување на трансплантираниот бубрег, потребна е навремена дијагностика пред развојот на иреверзибилните хистолошки промени, како и можноста за следење на состојбата со неинвазивни методи. Уринарните протеомични анализи се биолошки маркери кои ги отсликуваат таквите промени во структурата на графтот исполнувајќи ги горенаведените карактеристики за анализа.

Слична состојба во однос на навремена дијагностика е можноста за откривање рани патолошки состојби во урологијата, заради нивно навремено дијагностицирање и следење на состојбата со и без терапија.

Протеомични анализи при трансплантацијата на бубрег

За успешното водење на пациентите со трансплантиран бубрег, неопходна е рана детекција на суптилните промени на графтот, кои се појавуваат и покрај соодветниот имуносупресивен третман. Рутинската клиничка практика подразбира добра клиничка процена, соодветна биохемиска анализа и преземање дијагностички и терапевтски постапки за рана детекција на сите промени што се случуваат. Според препораките на KDIGO за посттрансплантациско следење на пациентите, неинвазивни маркери се: серумскиот креатинин и протеинуријата, но тие не се ниту доволно сензитивни ниту, пак, специфични маркери и не ги детектираат раните промени кои сè уште немаат клиничка манифестација (9). Протеинуријата, секако, е докажан прогностички маркер за дисфункцијата на графтот и предиктор за негово рано губење. Појавата

на протеинурија кај пациенти со извршена трансплантација се должи на: рекурентна гломерулопатија, de novo трансплант-гломерулопатија или хронична алографт-нефропатија, како и можен несакан ефект од лек (на пример, на mTOR инхибиторите) (10).

Сè уште златен стандард за дијагностицирање на промените на функцијата на трансплантираниот бубрег е биопсијата на графтот (11). Секако, ова е процедура која во ретки случаи може да биде поврзана со одредени клинички компликации. Заради страв и дискомфорт кој го предизвикува оваа процедура кај пациентот, таа може да биде одложувана, со што би се пролонгирало добивањето навремена дијагноза (12). Со оглед на досегашните анализи, главно на полето на базичните истражувања, се смета дека протеомичните анализи би биле такана-речена „ликвидна – течна биопсија". Во тој контекст, уринарните протеомици се стандардна алатка во истражувањата, но исто така, и во фаза на транзиција кон клиничките анализи кои се поставени во центарот на многу мултидисциплинарни истражувања (13).

За разлика од манифестната протеинурија, малите протеински молекули со молекуларна тежина од 1,000 до 20,000 Da претставуваат геномски пептиди или пептидни вериги кои не се детектираат со стандарднокористените анализи во стандардните биохемиски лаборатории (14). Терминот протеомици првпат е презентиран во деведесеттите години и означува геномска експресија на протеините, односно нивна систематска анализа во врска со квантитетот, потеклото и функцијата (15, 16).

Нивната концентрација може да биде и до 1000-пати пониска од плазматската, и затоа се потребни посебни методи за нивна идентификација како што се: течната хроматографија – liquid chromatography (LC), капиларната електрофореза (CE) за сепарација, масспектрометријата (MS) за детекција и *time-of-flight* (TOF) за мерење на овие биомаркери. Предност на урината како извор на биомаркери е тоа што таа се собира неинвазивно, може да биде повторувана повеќепати, да се чува и да подготвува со специфични стандарди и протоколи, и користи мали количина изразени во микролитри. Ваквите анализи овозможуваат идентификација на голем број пептиди и протеински вериги. Во последната декада овие методологии доведоа до нови откритија за физиолошките и патолошките процеси, односно резултати од одредена терапија. Тие може да се истражат преку различни бази на податоци

како што се: GenBank, Protein Data Bank (PDB), SwissProt, Protein Information Resource (PIR), Protein Research Foundation (PRF) итн. (17).

Протеомичните анализи во полето на трансплантацијата можат многу рано да ги покажат првичните промени и да придонесат за долгорочно преживување на графтот со тоа што би направиле дистинкција на стабилната функција на графтот од акутните отфрлања, уринарните инфекции, акутната тубуларна некроза, калцинеуринската цитотоксичност, хроничната алографт-нефропатија, како и трансплант-гломерулопатијата (18).

Неколку уринарни биомеркери биле истражувани во насока на оштетувањето на графтот вклучувајќи ги CXCL9, CXCL10, CCL2, NGAL, IL-18, cystatin C, KIM-1 and Tim-3 (19).

Мертенс (Mertens) и соработниците направиле студија со цел да се идентификуваат уринарните биомаркери за хуморално отфрлање. Направените анализи од биопсијата на графтот биле користени за соодветна класификација. Анализата идентификувала 10 уринарни протеомици кои ги издвојуваат пациентите со акутни отфрлања наспроти групата без присутно отфрлање (20).

Значењето на уринарните протеомици во однос на откривањето на иницијалната нефропатија на графтот е покажано во студија со 75 реципиенти на бубрег и 20 здрави волонтери. Користејќи ја техниката *surface-enhanced laser desorption and ionization* (SELDI) MS, неколку уринарни протеини, социрани со напредната хронична алографт нефропатија (ХАН) како што се α1-микроглобулин, β2-микроглобулин, преалбумин, ендорепелин (endorepellin), антиангиогенетски Ц-терминален фрагмент на перлеканот (21).

Секако, значењето на неинвазивните биомаркери со висока сензитивност за калцинеуринска цитотоксичност е докажано преку идентификацијата на *neutrophil gelatinase-associated lipocalin* (NGAL) и *kidney injury molecule 1* (KIM-1) (22). Двата биомаркера се синтетизираат во проксималните тубули и може да се асоцирани со интерстициелната фиброза како резултат од цитотоксичноста на калцинеурининхибиторите (КНИ). Резултатите од клиничките студии покажуваат дека NGAL придонесува во класификацијата на оштетувањето и може да придонесе за предикција на акутното бубрежно оштетување, и тоа на неколку дена пред порастот на серумскиот креатинин (23).

Капиларната електрофореза удвоена со масспектрометрија (CE-MS) е користена за идентификација на панелот од 273 уринарни пептиди како единствен биомаркер за прогресијата на ХББ (CKD 273-класификатор), со споредба на уринарните протеомици кај 379 здрави и 230 учесници со ХББ со различна етиологија (24). Студијата „PRIORITY“ како мултицентрична интервентна студија која вклучува пациенти со дијабетес без ХББ, користејќи го CKD 273-класификаторот, овозможува идентификација на испитаниците со молекуларни показатели за дијабетска нефропатија, со позитивен скоринг за споменатиот класификатор (25).

Протеомични анализи во урологијата

Уролошките заболувања, вклучително и малигномите, а секако и бенигните тумори, се мошне комплексни и бараат навремена дијагностика и соодветен третман. Уринатa како извор на биомеркери носи информации не само од уринарниот тракт туку и од другите органи. На тој начин се добиваат информации не само за уринарниот мочен меур и простатата туку и за другите органи. Со молекуларна анализа и со увид во клиничката состојба на пациентот, уролозите преку протеомичните анализи имаат увид не само за степенување на болеста туку и за ефектот од дадената терапија (26, 27).

Во друга опсежна протеомична студија, 407 уринарни примероци од пациенти биле анализирани со MALDI-TOF MS. Два уринарни маркера, уромодулин (uromodulin) и семеногелин (semenogelin), можат да ги раздвојат пациентите со карцином на простата наспроти бенигната простатична хиперплазија (БПХ) со 71,2 % сензитивност и 67,4 % специфичност (28).

Друга студија за карцином на простата идентификувала β2M, PGA3, and MUC3 како нови уринарни маркери користејќи современи технологии (iTRAQ LC/LC/MS/MS) за дијагностика на карциномите. Оттука, со собирање на првата урина е можно да се идентификуваат пациенти со карцином на простата со висока сензитивност и специфичност (29).

Студијата на Давалиева и соработниците со цел да се најдат неинвазивни биомаркери за детекција на карциномот на простата идентификувала 20 протеини (CD14, AHSG, ENO1, ANXA1, CLU, COL6A1, C3, FGA, FGG, HPX, PTGDS, S100A9, LMAN2, ITIH4, ACTA2, GRN, HBB, PEBP1, CTSB, SPP1) како онкогени тумор-супресори, присутни кај пациенти со карцином на простата, додека 9 (AZU1, IGHG1, RNASE2, PZP, REG1A, AMY1A, AMY2A, ACTG2, COL18A1) биле асоцирани со други типови карциноми, но не и со карциномот на простата. LC-MS/MS податоците се достапни преку ProteomeXchange со идентификаторот PXD008407 (30).

Повеќе студии се фокусирани на наоѓање протеински молекули за рана детекција на карциномот на мочниот меур. Очекувано, предизвик за современата урологија е да се најдат неинвазивни биомаркери за карциномот на мочниот меур, со цел да се избегнат повторувачките цистоскопии што често се изведуваат кај пациентите со уролошки заболувања. Се смета дека карциномот на мочниот меур е на деветто место меѓу најзастапените карциноми во светот (31).

Американската агенција за храна и лекови (The Food and Drug Administration – FDA) одобрила за користење 5 уринарни тестови за протеомични анализи. Три од нив идентификуваат ДНК, РНК или протеински промени во уринарните клетки (UroVysion® Fluorescent In Situ Hybridization, Cxbladder™ и ImmunoCyt™), а останатите два ги квантифицираат протеините присутни во урината (NMP22® and BTA® tests) (32).

Креунин (Kreunin) и соработниците биле фокусирани на уринарните гликопротеини и опишале неколку потенцијални биомаркери вклучувајќи го алфа-1B-гликопротеинот (alpha-1B-glycoprotein). Дополнителниот извештај тој го идентификувал како најзначаен алфа-1-антитрипсин (alpha-1-antitrypsin) (A1AT) (33, 34).

Протеомичните анализи на егзомите се ветувачки пристап за биомаркерите кај карциномите, во најраните стадиуми. Вонклеточните везикули (Extracellular vesicles) (EVs) се генерираат од сите клеточни типови како дел од физиолошките процеси, и содржат информации за нуклеинските киселини и протеини. Во патолошките процеси, вклучувајќи ги и канцерите, можат да ги отсликаат клетките во однос на нивното потекло. Протеомичните анализи, базирани на масивната спектрометрија (MS) – и студиите на егзомите, можеби се ветувачки во областа

на урологијата. Нивниот придонес во патогенезата и прогресијата на карциномот на простата и на мочниот меур како најзастапени уролошки карциноми, во пораст во последните години, се предизвик за современата урологија (35).

Заклучок

Протеомичните анализи се сметаат за веќе докажан дијагностички пристап во базичната наука. Поопсежни и подетални студии од областите на урологијата и нефрологијата, особено во областа на трансплантацијата на органи, би овозможиле транзиција на овие неинвазивни анализи во клиничката практика. Крајна цел секако е раното откривање на патофизиолошките процеси кои се случуваат, со што би се продолжил животниот век како на пациентите така и долгорочното преживување на трансплантираните органи, односно рана дијагностика и навремен третман за соодветните патолошки состојби во урологијата.

РЕФЕРЕНЦИИ

1. Nephrology Global Kidney Health Atlas: structures, organization, and services for the management of kidney failure in Western Europe. *Kidney Int Suppl (2011)*. 2021;11(2): 106–118.
2. Hoste, E. A. J., Kellum, J. A., Selby, N. M. et al. (2018). *Global epidemiology and outcomes of acute kidney injury*. Nature reviews Nephrology; 14(10): 607–625.
3. http://ekha.eu/
4. Raymond Vanholder, Lieven Annemans, Aminu K. Bello, Boris Bikbov, Daniel Gallego, Ron T. Gansevoort, Norbert Lameire, Valerie A. Luyckx, Edita Noruisiene, Tom Oostrom, Christoph Wanner, Fokko Wieringa. (2021). Fighting the unbearable lightness of neglecting kidney health: the decade of the kidney, *Clinical Kidney Journal*.
5. KDIGO 2012. (2013). *Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease*, Kidney International Supplements.
6. Port, F. K., Wolfe, R. A., Mauger, E. A., Berling, D. P., Jiang, K. (1993). *Comparison of survival probabilities for dialysis patients vs cadaveric renal transplant recipients*. JAMA; 270: 1339–1343.

7. Tonelli, M. et al. (2011). *Systematic Review: Kidney Transplantation Compared With Dialysis in Clinically Relevant Outcomes.* Am J Transpl.; 11: 2093–2109.
8. Gill, J. S. (2008). *Cardiovascular disease in transplant recipients: Current and future treatment strategies.* Clin J Am Soc Nephrol; 3 (Suppl 2): S29–S37.
9. Kidney Disease: Improving Global Outcomes (KDIGO). (2009). *Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients*. Am J Transplant. 2009 Nov.; 9 Suppl 3:S1-155.
10. Roodnat, J. I., Mulder P. G., Rischen-Vos J. et al. (2001). *Proteinuria after renal transplantation affects not only graft survival but also patient survival*. Transplantation; 72: 438–444.
11. Robert B. Colvin. (1996). *The renal allograft biopsy*, Kidney International; 50 (3): 1069–1082.
12. Trajceska, L., Severova-Andreevska, G., Dzekova-Vidimliski, P., Nikolov, I., Selim, G., Spasovski, G., Rambabova-Busletik, I., Ristovska, V., Grcevska, L., Sikole, A. (2019). *Complications and Risks of Percutaneous Renal Biopsy*. Open Access Maced J Med Sci.; 7(6): 992–995.
13. Mischak, H. (2015). *Pro: urine proteomics as a liquid kidney biopsy: no more kidney punctures!* Nephrol Dial Transplant; 30: 532–537.
14. Julian, B. A., Suzuki, H., Suzuki, Y., Tomino, Y., Spasovski, G., Novak, J. (2009). *Sources of urinary proteins and their analysis by urinary proteomics for the detection of biomarkers of disease*. Proteomics.; 3(9): 1029–1043.
15. Wilkins, M. R.; Pasquali, C.; Appel, R. D.; Ou, O.; Golaz, O.; Sanchez, J. C.; Yan, J. X.; Gooley, A. A.; Hughes, G.; Humphery-Smith, I. et al. (1996). From proteins to proteomes: Large scale protein identification by two-dimensional electrophoresis and amino acid analysis. *Biotechnology (NY)*, *14*, 61–65.
16. Peng, J., Gygi, S. P. (2001). Proteomics: The move to mixtures. *J. Mass Spectrom.*, *36*, 1083–1091.
17. Mischak, H., Delles, C., Klein, J., Schanstra, J. P. (2010). *Urinary proteomics based on capillary electrophoresis-coupled mass spectrometry in kidney disease: discovery and validation of biomarkers, and clinical application*. Adv Chron Kidney Dis.; 17(6): 493–506.
18. Quintana, L. F., Solé-Gonzalez, A., Kalko, S. G. et al. (2009). Urine proteomics to detect biomarkers for chronic allograft dysfunction. *J Am Soc Nephrol*.;20(2): 428–435. doi:10.1681/ASN.2007101137
19. Kim, S. C., Page, E. K., Knechtle, S. J. (2014). *Urine proteomics in kidney transplantation*. Transplant Rev (Orlando). 2014 Jan; 28(1): 15–20.

20. Mertens, I., Willems, H., Van Loon, E. et al. (2020). *Urinary Protein Biomarker Panel for the Diagnosis of Antibody-Mediated Rejection in Kidney Transplant Recipients*. Kidney Int Rep.; 5(9): 1448–1458.
21. O'Riordan, E., Orlova, T. N., Mendelev, N., Patschan, D., Kemp, R., Chander, P. N., Hu, R., Hao, G., Gross, S. S., Iozzo, R. V., Delaney, V., Goligorsky, M. S. (2008). *Urinary proteomic analysis of chronic allograft nephropathy*. Proteomics Clin Appl. 2008 Jul; 2 (7-8): 1025–1035.
22. Yadav, B., Prasad, N., Agrawal, V., Jaiswal, A., Rai, M., Sharma, R. et al. (2015). *Urinary Kidney injury molecule-1 can predict delayed graft function in living donor renal allograft recipients*. Nephrology; 20: 801–806.
23. Mishra, J., Ma, Q., Kelly, C., Mitsnefes, M., Mori, K., Barasch, J. et al. (2006). *Kidney NGAL is a novel early marker of acute injury following transplantation.* Pediatr Nephrol.;21: 856–863.
24. Mischak, H., Schanstra, J. P. (2011). *CE-MS in biomarker discovery, validation, and clinical application.* Proteomics Clin Appl.; 5: 9–23.
25. Tofte, N., Lindhardt, M., Adamova, K., Bakker, S. J. L., Beige, J., Beulens, J. W. J. et al. (2020). *PRIORITY investigators. Early detection of diabetic kidney disease by urinary proteomics and subsequent intervention with spironolactone to delay progression (PRIORITY): a prospective observational study and embedded randomised placebo-controlled trial.* Lancet Diabetes Endocrinol.; 8(4): 301–312.
26. Meric-Bernstam, F., Johnson, A., Holla, V., Bailey, A. M., Brusco, L., Chen, K., Routbort, M., Patel, K. P., Zeng, J., Kopetz, S. et al. (2015). A decision support framework for genomically informed investigational cancer therapy. J Natl Cancer Inst.; 107(7).
27. Antunes-Lopes, T., Cruz, C. D., Cruz, F., Sievert, K. D. (2014). *Biomarkers in lower urinary tract symptoms/overactive bladder: a critical overview*. Curr Opin Urol.; 24(4): 352–357.
28. Yang, J., Roy, R., Jedinak, A., Moses, M. A. (2015). *Mining the human proteome: biomarker discovery for human cancer and metastases*. Cancer J.; 21(4): 327–336.
29. Jedinak, A., Curatolo, A., Zurakowski, D. et al. (2015). Novel non-invasive biomarkers that distinguish between benign prostate hyperplasia and prostate cancer. *BMC Cancer*.; 15: 259.
30. Davalieva, K., Kiprijanovska, S., Maleva Kostovska, I., Stavridis, S., Stankov, O., Komina, S., Petrusevska, G., Polenakovic, M. (2017). *Comparative Proteomics Analysis of Urine Reveals Down-Regulation of Acute Phase Response Signaling and LXR/RXR Activation Pathways in Prostate Cancer*. Proteomes. Dec 29; 6(1):1.
31. Siegel, R. L., Miller, K. D., Jemal, A. (2019). *Cancer statistics, 2019.* CA Cancer J Clin.; 69: 7–34.

32. Jorge Luis Wilson Jr., Mariana Pereira Antoniassi, Paula Intasqui Lopes, Hatylas Azevedo. (2021). *Proteomic research and diagnosis in bladder cancer: state of the art review*. 47 (3): 503–514, May – June, 2021.
33. Kreunin, P., Zhao, J., Rosser, C., Urquidi, V., Lubman, D. M., Goodison, S. (2007). *Bladder cancer associated glycoprotein signatures revealed by urinary proteomic profiling*. J Proteome Res. 2007 Jul; 6(7): 2631–2639.
34. Yang, N., Feng, S., Shedden, K., Xie, X., Liu, Y., Rosser, C. J., Lubman, D. M., Goodison, S. (2011). *Urinary glycoprotein biomarker discovery for bladder cancer detection using LC/MS-MS and label-free quantification*. Clin Cancer Res.; 17(10): 3349–3359.
35. Wang, Y-T., Shi, T., Srivastava, S., Kagan, J., Liu, T., Rodland, K. D. (2020). Proteomic Analysis of Exosomes for Discovery of Protein Biomarkers for Prostate and Bladder Cancer. *Cancers*.; 12(9): 2335.

Goce SPASOVSKI, Irena RAMBABOVA BUSLETIC,
Lada TRAJCESKA, SASHO DOHCHEV, Oliver STANKOV,
Sotir STAVRIDIS, Skender SAIDI, Zivko POPOV[4]

USE OF PROTEOMICS IN URONEPHROLOGY - REALITY OR FICTION?

Abstract

Chronic kidney disease is a growing global problem with health and socioeconomic implications. Kidney transplantation is the most optimal treatment for the terminal stage of CKD. There is still a discrepancy between the need for organs for transplantation and their availability. Modern diagnostic methods are needed to detect early graft changes for long-term survival. Urinary proteomic assays play an important role in the field of organ transplantation by detecting changes before they have a clinical presentation, enabling their appropriate early treatment.

In the field of urology as non-invasive biomarkers for early detection of malignancies, proteomic analysis is considered a promising diagnostic

[3] Macedonian academy of sciences and arts, Faculty of Medicine, SS Cyrill and Methodius University, Skopje, Republic of North Macedonia

approach. Their clinical use would avoid recurrent invasive diagnostic procedures and insight into the effectiveness of the applied therapy. Larger clinical studies would enable their transition from basic to clinical practice and an individual approach for each patient.

doi: 10.20903/zpopov-2022-0030 UDK: 616.611:602.9]-097

Gordana PETRUSHEVSKA[1], Slavica KOSTADINOVA-KUNOVSKA[1], Ladislava GRCHEVSKA[2], Momir POLENAKOVIC[2,3]

COMPARATIVE EXPRESSION OF PAX-2 AND OCT-4 IN FETAL, NORMAL ADULT, AND GLOMERULONEPHRITIC KIDNEYS

Abstract

Introduction: During organogenesis, the number and capacity of pluripotent stem cells capable of generating all types of kidney cells progressively decreases, and normal adult kidneys host a few immature multipotent cells with the capacity of self-regenerating.

Aims: We aimed to compare the expression of Pax-2, Oct-4, genes, which are responsible for the development and the differentiation of the embryonic stem cells in fetal, normal adult, and glomerulonephritic kidneys and compare these with CD133-main stem cell marker expression.

Material and Methods: Immunohistochemical analyses were performed with commercial antibodies against Pax-2, Oct-4 and CD133 on formalin-fixed, paraffin embedded tissue samples from 20 fetal kidneys with different gestational ages, 40 adult and 40 glomerulonephritic kidneys.

Results: The analyses showed a nuclear presence of both markers in fetal kidney structures, immature blastemic mesenchyme and early glomerular and tubular precursors, with decreasing to absent signals in imamture glomeruli, except in parietal cells. A weak Pax-2 signal was also present in the parietal cells and in some distal tubules of the adult normal

[1] Institute of pathology, Medical faculty - University "Ss Cyril and Methodius" - Skopje, North Macedonia

[2] Univeristy Clinic for Nephrology, Medical Faculty - University "Ss Cyril and Methodius" - Skopje, North. Macedonia

[3] Macedonian Academy of Sciences and Arts, Skopje, North Macedonia

kidneys, unlike Oct-4, which was entirely negative. An increased signal of both markers was observed in the glomerulonephritic kidneys: parietal glomerular cells and cellular crescents in cases of extra-capillary glomerulonephritis, and the number of positive atrophic tubules was higher ($p<0.05$) than the number of positive cortical tubules in normal adult kidneys. The interstitial cells in the three groups except rare cells were negative.

Conclusions: The presence of both markers in the immature fetal kidney points to the pluripotency of the mesenchymal-blastemic cells that precede the mature cells of all types, some of which undergo the process of mesenchymal-epithelial transition mediated by Pax-2. Their decreased expression in adult kidney tissue points to the differentiation and maturity of the cells that have lost their pluripotency. Since the hyperplastic crescent lesions are a result of the proliferation of parietal epithelial cells, Pax-2 and Oct-4 expression in cellular crescents indicates the presence of immature and undifferentiated cells which proliferate as a result of the impaired regeneration and differentiation of the damaged tissue. The positivity of the atrophic tubules can be attributed to the activation of the kidney's own potential stem cells in the process of defense and protection of its tissue.

Key words: Pax-2 and Oct-4, pluripotent kidney stem cells, glomerulonephritic lesions

Introduction

Traditionally, the kidneys have been considered to consist of stable cells with minimal capacity for regeneration. However, in the past decades investigations in this field have shown that the kidneys have a remarkable capacity for regeneration after damage has been done. This is due to the role of stem cells which are able to proliferate and differentiate in more than one terminally differentiated cell type (1, 2). Different authors have different ideas as to whether only immature structures might have progenitors capacity or mature differentiated tissues, in the conditions of injury might be activated and make use of the capacity of stem cells. Potential stem cells have been identified in the interstitium of normal adult kidneys (3) and at

the urinary pole of Bowmann's capsule (4) as well in fetal kidneys (5), and most of these had the characteristic expression of CD133 and CD24 (6). However, Oliver et al (2004) discovered that the renal papilla is a niche for adult kidney stem cells (9).

In our previous study on normal adult renal tissue and renal tissues with glomerulonephritic lesions we found the expression of CD133 in parietal epithelial cells. These were also found in rare proximal tubular epithelial cells and epithelial cells from the collecting ducts and Henle loop in cases from normal renal tissue as well as a higher expression of CD133 in the areas of the tubular lesions and interstitial inflammation.

It was postulated that the regeneration processes may recapitulate parts of the genetic program, which is evident during organogenesis, in order to reestablish proper tissue function after damage. During organogenesis, embryonal stem cells are the direct progenitors of kidney component cells, including podocytes, mesangial cells, and tubular epithelial cells, or via an additional appearance of kidney stem progenitor cells around mature kidney component cells. According to Saffirstain et al., third and fourth pathways are possible, including the role of extrarenal stem cells, either directly to mature kidney component cells or through the kidney stem progenitor cells to mature kidney component cells, including mesangial cells, podocytes and tubular epithelial cells.

Several genes have been shown to be modulated in response to kidney damage (7). Prominent among them is the expression of the immediate early genes that code for transcriptional factors that are rapidly and briefly expressed well before the onset of DNA synthesis. One of these genes is the transcription factor Pax-2, which is transiently expressed during nephrogenesis. This may be part of the genetic cascade leading to kidney regeneration in adulthood after kidney damage. It has been speculated that Pax-2 would show a biphasic expression pattern during kidney development and disease. M. Imgrund et al. (1999) in the study on experimentally induced ATN in adult mice, found transient temporally and locally restricted re-expression of Pax-2 in regenerating proximal tubular epithelial cells following kidney damage (8). During regeneration of the adult kidney, Pax-2 may play role by influencing proliferation.

Gupta et al. in her *in vitro* and *in vivo* differentiation of multipotent renal progenitor cells (MRPC-s) by ischemia-reperfusion experiment, found these cells expressed **vimentin**, **CD90**, **Pax-2** and **Oct-4,** but not cytokeratin, MHC class I and II and other markers of more differentiated cells. The authors proposed that MRPC participate in the regenerative response of the kidney to acute injury. **Oct-4** is required to prevent trophectodermal differentiation and, with the process of transcriptional repression, Oct-4, together with Nanog and Sox-2, are the key controllers of human embryonal stem cell pluripotency.

Data from the literature are mostly based on experimental animal models of embryonic tissues as well as in cell cultures. There are few data points for the studies done on human biopsy material, surgical, or autopsy material from fetal tissues.

The purpose of this study was to determine the expression of Pax-2 and Oct-4 genes responsible for the development and differentiation of embryonic stem cells in human fetal kidneys and to compare the results with the expression of Pax-2 and Oct-4 genes in in normal adult and glomerulonephritic kidneys. The final aim was to test the hypothesis that kidney stem cells exist in adult kidneys and that they participate in the processes of tissue reparation in damaged kidney tissue.

Material and Methods

The study included *100 cases divided in three groups:* (1) twenty fetal kidneys (FKs) received from autopsy archive material, (2) forty surgically extracted adult kidneys (Aks) due to diagnosed renal cell carcinoma, (3) forty renal biopsies taken in routine diagnostic procedures for glomerular disease (GD).

Tissue specimens from all cases from the three groups were fixed in 10% buffered formalin for 24 hours and paraffin embedded. Prepared paraffin sections were histochemically stained with H&E, PAS, PASM-Jones, and Trichrome Mason. Immunohistochemical analysis was done via the PT LINK immunoperoxidase technique for the following antibodies: Pax-2, Zymed, USA Polyclonal, 1:50, with nuclear staining in positive control cells

from Renal Cell Carcinoma; Oct-4, Santa Cruz, USA Polyclonal, 1:50, with nuclear staining in positive control cells in bone marrow, and CD133, Miltenyi, MoAC133, 1:11, with cytoplasmic staining in positive stem cells from bone marrow. The slides were then analyzed on a NICON Eclipse 2000 microscope, and the results were photographically documented.

The results have been statistically analyzed with Statistica 7.0, StatSoft Inc. software for descriptive and (non) parametric analyses.

Results

In the study of the first group, specimens from 20 fetal kidneys with a gestational age between 14th and 24th week were analyzed. In the second group, specimens from 40 surgically extracted adult kidneys (Aks) due to renal cell carcinoma, were analyzed. The mean age of the group was 59.4 years (minimum 34 years and maximum 79 years, and SD=8.4). In the third group, 40 renal biopsies taken in routine diagnostic procedures for glomerular disease were analyzed. The mean age of the group was 44.5years (minimum 21 years and maximum 73 years, SD=14.4).

Analysis for the Pax-2 expression in fetal kidneys revealed strong nuclear positivity in the S&comma shaped bodies from the 14th to the 24th gestational week, moderate positivity in the fetal glomeruli especially in the parietal epithelial cells in the 14th to the 16th gestational week and tubuli. In the medulla we found positivity in the cells from the collecting duct, Henle loop cells, and cells from the urothelial bud (Figure 1 A, B, C). Staining for Oct-4 in the fetal kidney specimens showed strong nuclear expression in the immature renal mesenchymal blastema, S&comma shaped bodies, parietal epithelial cells, tubuli and ureteral buds (Figure 1D). Immunohistochemical analysis for CD133 revealed positivity in the parietal cells of the immature glomeruli and rare cells in the proximal and distal tubuli, ureteral buds, and Henle loop cells. What is peculiar about this study is the finding of Oct-4 expression in rare podocytes of immature glomeruli and rare cells in the blood vessels of the fetal kidneys, as well as the low level of re-expression in GNKs.

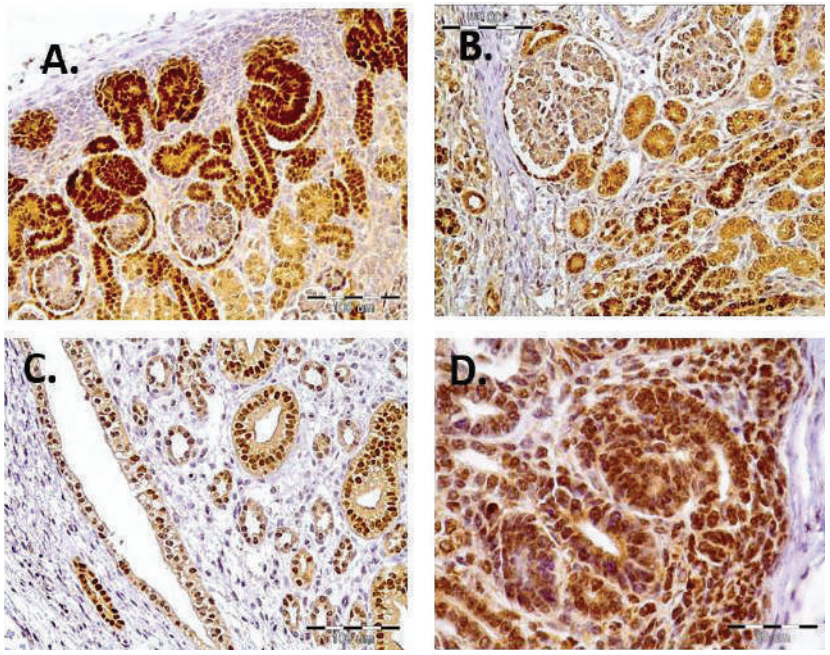


Figure 1 – (A) and (B) Pax-2 nuclear positivity in S & comma shaped bodies, positive parietal epithelial cells, and tubular epithelial cells; (C) Pax-2 positive epithelial cells in collecting ducts and urothelial bud; (D) Oct-4 nuclear positivity in renal mesenchymal blastema, S & comma shaped bodies and tubuli. (NIKON Eclipse 2000)

In the second group of adult mature kidney tissue, moderate Pax-2 positivity was found in the parietal cells of the glomeruli, collecting duct cells, and some of the distal tubules while Oct-4, was not present in any adult normal kidney structure (Figure 2A, B). Staining for CD133 revealed positivity in the parietal epithelial cells of the mature glomeruli and there was luminal positivity in the proximal tubuli and the cells of the Henle loop (Figure 2C, D and Table 1). In table 1 one can see that there is overlapping in the expression of CD133 with Pax-2 in parietal epithelial cells of the glomeruli, tubular epithelial cells, as well in the epithelial cells of the collecting duct.

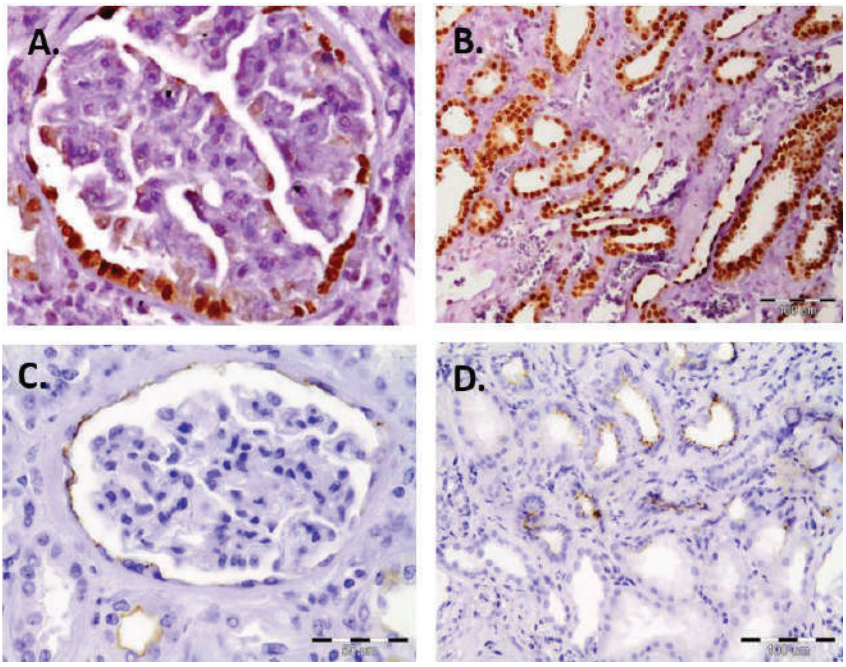


Figure 2 – (A) and (B) Pax-2 nuclear positivity in the parietal epithelial cells and epithelial cells of the collecting duct in adult kidney tissue; (C) and (D) CD133 expression in the parietal epithelial cells of mature glomeruli and tubular epithelial cells (NIKON Eclipse 2000).

Table 1

Expression of Pax-2 and Oct-4 in adult kidneys in relation with CD133 expression

	Expression of Pax-2 and Oct-4 in AKs Cortex			AKs Medulla
	Glomeruli	Atrophic tubules	Interstitium	
CD133	Parietal cells	Proximal	/	• Collecting • Henle loop
Pax-2	Parietal cells	Distal	/	• Collecting ducts
Oct-4	/	/	/	/

The third group consisted of tissues from 20 cases with diagnosed primary glomerular disease. Ten cases (25%) had mesangial glomeruloneph-

ritis, 9 cases (22,5%) had membranous glomerulopathy, 7 cases (17%) had IgA nephropathy, 8 cases (20%) had extracapillary glomerulonephritis, 4 cases (10%) had focal segmental glomerulosclerosis, and 2 cases (5%) had mesangioproliferative glomerulonephritis. The analysis of the results revealed a nuclear expression of Pax-2 similar to the expression in normal kidney specimens, i.e. expression in the parietal epithelial cells of the Bowman capsule, which was especially strong in the epithelial crescents of the extracapillary glomerulonephritis (Figure 3A). We also found positive expression in the areas of atrophic tubuli. Interestingly, the number of positive atrophic tubules was higher ($p<0.05$) than the number of positive cortical tubules in normal adult kidneys (Figure 3B). Higher level of expression has been found in the epithelial cells of collecting ducts. The interstitial cells were negative.

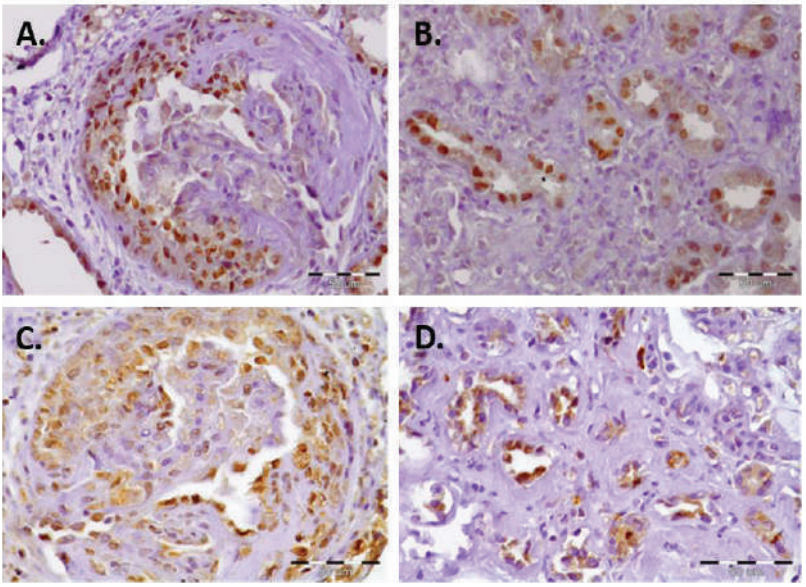


Figure 3 – (A) Pax-2 nuclear positivity in parietal epithelial crescents; (B) Pax-2 nuclear positivity in atrophic tubular epithelial cells; (C) Oct-4 nuclear positivity in parietal epithelial crescents (D) Oct-4 nuclear expression in atrophic tubular epithelial cells. (NIKON Eclipse 2000)

Cases with extracapillary glomerulonephritis and focal segmental glomerulosclerosis showed the strongest expression compared to other forms of glomerular lesions ($p<0.05$). This was confirmed via a strong correlation between the Pax-2 expression in tubular epithelial cells and interstitial fibrosis (Figure 4A). When we compared the level of expression in different forms of glomerular lesions and the control group, we found a significantly higher expression of Pax-2 positive tubuli between each of the entities and the control group (Figure 4B). One could speculate that this might be on account of a more extensive degree of damage of the glomeruli and the tubulointerstitial tissue in these forms of glomerulonephritic lesions.

Similar results were obtained when staining the specimens with Oct-4 (Figure 3C, D). We found a strong expression of Oct-4 in the epithelial crescents of the extracapillary glomerulonephritis, atrophic tubuli, and a small number of collecting duct cells. We also found low level of re-expression in glomerulonephritic kidneys in this group.

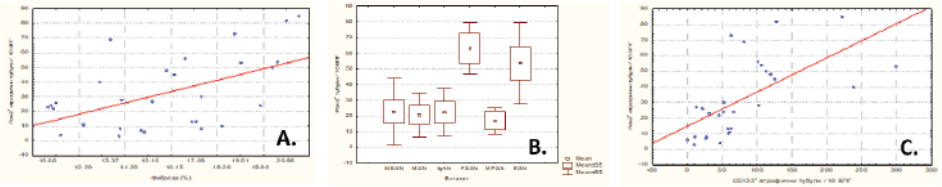


Figure 4 – (A) and (B) Pax-2 nuclear positivity in S & comma shaped bodies, positive parietal epithelial cells and tubular epithelial cells; (C) Pax-2 positive epithelial cells in collecting ducts and urothelial bud; (D) Oct-4 nuclear positivity in renal mesenchymal blastema, S & comma shaped bodies and tubuli.

CD133 expression in the glomerulonephritic group was found to be increased in the tubular epithelial cells in glomerulonephritic kidneys. Rare interstitial cells were also positive for CD133. We found a positive correlation between the Pax-2 and CD133 expression in the areas of atrophic tubuli (Figure 4C).

Table 2 displays the results from the comparative expression of Pax-2 and Oct-4 in the cortical and medullary tissue of glomerulonephritic lesions in relation to the expression of CD133 as the main stem cell marker. There is a strong overlap in the expression of Pax-2 and Oct-4 in the glomeruli and tubular compartments with partial overlapping with CD133 expression.

Table 2

Comparative expression of Pax-2 and Oct-4 in the cortical and medullary tissue of glomerulonephritic lesions in relation to the expression of CD133 as main stem cell marker.

	Comparative expression of Pax-2 and Oct4 in GNK - Cortex			Medulla
	Glomeruli	Tubules	Interstitium	
CD133	Parietal cells / Negative crescents	+++ (apico-lateral)	+	• Collecting ducts • Henle loop
Pax2	Parietal cells / Positive crescents	+++ (nuclear)	/	• Collecting ducts
Oct-4	Parietal cells / Positive crescents	+++ (nuclear)	/	• Collecting ducts

Discussion

We have presented a study on the expression of two transcriptional genes, Pax-2 and Oct-4, in kidney tissue from glomerulonephritic lesions and compared these with the expression of such genes in fetal kidneys and adult mature kidney tissue, having normal control groups of different ages. These results were compared with the expression of CD133, since it has been demonstrated the presence of CD133+ cells in a normal adult human kidney, which expressed Pax-2, is otherwise defined as an embryonic renal marker, suggesting their renal origin (3, 4, 5). These cells may respond to local environmental stimulation, with differentiation into endothelial or epithelial tubular cells, both *in vitro* and *in vivo*. The discovery of pluri-potent bone marrow-derived stem cells has raised the possibility that the stemness of the kidney tissue could be due to bone marrow derived stem cells, and there is a need to re-examine the cellular source further in the future (1, 5, 6). In our study, we showed the presence of cells that express activity of two immature transcriptional genes in both localizations – parietal epithelial cells as well in the cells of collecting ducts and ureteral epithelium. These results allow for the presence of at least two localizations in the kidney for cells with stemness capability – glomeruli and renal papilla.

During early embryogenesis, Oct-4 is a master transcriptional regulatory gene, and the presence of both markers in the immature fetal kidney structures points to the pluripotency of the mesenchymal blastemic cells that precede the mature cells of all types. Some of these cells undergo the process of mesenchymal-epithelial transition mediated by Pax-2. Their decreesed expression in adult kidney tissue points to the differentiation and maturity of the cells that have lost their pluripotency. Since hyperplastic crescent lesions are the result of proliferation of parietal epithelial cells, the presence of Pax-2 and Oct-4 in cellular crescents indicates that they are composed of immature and undifferentiated cells that proliferate as a result of impaired regeneration and differentiation of the damaged parietal epithelial cells. Another optional hypothesis is that the damaged parietal epithelial cells underwent a process of dedifferentiation with re-expression of immature transcriptional genes Pax-2 and Oct-2. The positivity of the atrophic tubules can also be attributed to the activation of the kidney's own resources, potential stem cells in the process of defense, and protection of its tissue. This ability of re-expression of immature transcriptional genes in the parietal epithelial cells, as well in some of tubular epithelial cells of damaged renal tissue, suggests their potential in the treatment of kidney damage.

Conclusions

Re-expression of Pax-2 and Oct-4 in the hyperplastic crescent lesions, which are result of proliferation of parietal epithelial cells, indicates that they are composed of immature and undifferentiated cells that proliferate as a result of impaired regeneration and differentiation of the damaged parietal epithelial cells.

The positivity of the atrophic tubules can also be attributed to the activation of the kidney's own resources, potential stem cells in the process of defense, and protection of its tissue in the process of epithelial mesenchymal transformation.

The findings of Oct-4 expression in rare podocytes of immature glomeruli and rare cells in the blood vessels of the fetal kidneys, as well as its low level of re-expression in GNKs displays that some bone marrow stem cells might also play a role in the process of regeneration of damaged kidney tissue.

Disclaimer: The authors declare that the research was conducted in the absence of any commercial or financial relationship such that the research cannot be constructed as a potential conflict of interest.

BIBLIOGRAPHY

1. Gupta S, Verfaille C, Chmielewski D, et al. Isolation and Characterization of Kidney-Derived Stem Cells. J Am Soc Nephrol 2006; 17: 3028-3040
2. Poulsom R, Alison M, Cook T, et al. Bone Marrow Stem Cells Contribute to Healing of the Kidney. J Am Soc Nephrol 2003; 14: S48-54
3. Bussolati B, Bruno S, Grange C et al. Isolation of Renal progenitor Cells from Adult Human Kidney. Am J Pathol 2005; 166: 545-555
4. Sagrinati C, Netti G S, Mazzinghi B, et al. Isolation and Characterisation of Multipotent Progenitor Cells from the Bowman's Capsule of Adult Human Kidneys. J Am Soc Nephrol 2006; 17: 2443-2456
5. Lazzeri E, Crescioli C, Ronconi E, et al. Regenerative potential of Embryonic Renal Multipotent Progenitors in Acute Renal Failure. J Am Soc Nephrol 2007; 18: 3128-3138;
6. Al-Awqati Q, Oliver J A. Stem Cells in the Kidney. Kidney International 2002; 61: 387-395
7. Safirstein R. Gene Expression in nephrotoxic and Ischemic Acute Renal Failure. J Am Soc Nephrol 1994; 4: 1387-1395
8. Imgrund M, Gröne E, Gröne HJ, et al. Re-expression of the developmental gene Pax-2 during experimental acute tubular necrosis in mice. Kidney International 1999; 56: 1423-1431
9. Oliver JA, Maarouf O, Cheema FA et al. The renal papilla is a niche for adult kidney stem cells. J Clin Invest 2004; 114/6: 795 – 804

Гордана ПЕТРУШЕВСКА, Славица КОСТАДИНОВА-КУНОВСКА,
Ладислава ГРЧЕВСКА, Момир ПОЛЕНАКОВИЌ

КОМПАРАТИВНА ЕКСПРЕСИЈА НА PAX-2 И OCT-4 КАЈ ФЕТАЛНИ, НОРМАЛНИ ЗРЕЛИ И ГЛОМЕРУЛОНЕФРИТИЧНИ БУБРЕЗИ

Абстракт

За време на органогенезата, бројот и капацитетот на плурипотентите матични клетки способни да ги продуцираат сите видови на клетки прогресивно се намалува, и нормалните зрели бубрези содржат малку незрели мултипотентни клетки со капацитет за само-регенерација.

Нашата цел беше да се спореди експресијата на Pax-2 и Oct-4, гени кои се одговорни за развојот и диференцијацијата на ембрионалните матични клетки кај фетални, нормални зрели и гломерулонефритични бубрези и да се споредат со експресијата на главниот маркер на матични клетки CD133.

Ние направивме имунохистохемиска анализа со комерцијални антитела за Pax-2, Oct-4 и CD133 на формалин фиксирани и парафин вклопени ткивни примероци од 20 фетални бубрези со различна гестациска старост, 40 зрели и 40 гломерулонефритични бубрези.

Анализата покажа јадрено присуство на двата маркери во феталните бубрежни структури, незрелиот бластемски мезенхим и раните гломеруларни и тубуларни прекурзори, со намалување до отсуство на сигналот во незрели гломерули, освен во париеталните клетки. Слаб Pax-2 сигнал беше присутен исто така во париеталните клетки и во некои дистални тубули на зрели нормални бубрези, за разлика од Oct-4, кој беше негативен во целост. Зголемен сигнал за двата маркери беше најден кај гломерулонефритични бубрези: париетални гломеруларни клетки и клеточни кресценци и во случаите со екстракапиларен гломерулонефритис, а бројот на позитивни атрофични тубули беше повисок ($p<0,05$) од бројот на позитивните кортикални тубули во нормалните зрели бубрези. Интерстицијалните клетки во трите групи освен ретки клетки беа негативни.

Присуството на двата маркери во иматурни фетални бубрези укажува на плурипотентност на мезенхималните-бластемски клетки кои претходат на зрелите клетки од сите видови, од кои некои подлежат на процес на мезенхимална-епителна транзиција посредувана со Pax-2. Нивната намалена експресија кај матурните зрели бубрези укажува на диференцијација и зреење на клетките кои ја изгубиле нивната плурипотентност. Бидејќи хиперпластичните кресцентни лезии се резултат на пролиферација на париеталните епителни клетки, Pax-2 и Oct-4 експресијата во клеточните кресценци индицира присуство на иматурни и недиференцирани клетки кои пролифериираат како резултат на оштетена регенерација и диференцијација на оштетеното ткиво. Позитивноста на атрофичните тубули може да се припише на активација на бубрежните сопствени потенцијални матични клетки во процесот на одбрана и заштита на ткивото.

Клучни зборови: Pax-2 и Oct-4, плурипотентни бубрежни матични клетки, гломерулонефритични лезии

doi: 10.20903/zpopov-2022-0031 UDK: 616.62-006.6-092
616.65-006.6-092

Скендер САИДИ[1], Живко ПОПОВ[1,2], Сашо ПАНОВ[3]

УЛОГАТА НА P53 ВО МОЛЕКУЛАРНАТА ПАТОГЕНЕЗА НА МАЛИГНИТЕ НЕОПЛАЗМИ НА УРИНАРНИОТ ТРАКТ

Апстракт

Карциномот на мочниот меур е значаен здравствен проблем и четврто најчесто малигно заболување во западните земји. Карциномот на простатата е еден од најчестите малигни заболувања кај мажите. Инциденцата рапидно расте во последните години поради продолжувањето на животниот век и поради воведувањето на пософистицирани дијагностички методи.

Тумор-супресорскиот ген *p53* го кодира истоимениот протеин кој дејствува како транскрипциски фактор и се активира при различни типови клеточни стресови, вклучувајќи ги оштетувањето на DNA и онкогенската активација, поттикнувајќи каскада од молекуларни слу-чувања кои доведуваат до прекин на клеточниот циклус, репарацијата на DNA и на апоптозата. Мутациите во генот *p53* се најчести генетски промени кај карциномите кај луѓето вклучувајќи го и карциномот на мочниот меур.

Во оваа студија беа анализирани ткивни примероци од 70 пациенти со хистопатолошки потврден, примарен уроепителен карцином на мочниот меур, како и од негативна контролна група која ја сочину-

[1] Универзитетска клиника за урологија, Клинички центар Скопје, ул. „Водњанска“ 17, 1000 Скопје, Република Северна Македонија

[2] Македонска академија на науките и уметностите, бул. „Крсте Мисирков“ бр. 2, 1000 Скопје, Република Северна Македонија

[3] Одделение за молекуларна биологија и генетика, Институт за биологија, Природно-математички факултет, Универзитет „Св. Кирил и Методиј“, ул. „Архимедова“ 3, 1000 Скопје, Република Северна Македонија

ваат примероци на хистолошки нормална мукоза од мочен меур на 40 пациенти со немалигни заболувања. Квантитативната детекција на протеинот p53 беше определена со имунофлуоресценција. Во студијата за карциномот на простатата се вклучени 83 пациенти, од кои испитуваната група се состои од 43 пациенти со хистопатолошки верификуван карцином на простата кај кои е направена радикална простатектомија, додека контролната група се состои од 40 пациенти со бенигна хиперплазија на простатата, кај кои е направена трансуретрална ресекција на простатата или трансвезикална простатектомија. Кај сите примероци, евалуирана е нуклеарната експресија на протеинот p53, со имунохистохемиска метода.

Кај примероците од карцином на мочниот меур, експресијата на протеинот p53 во хистолошките пресеци, изразена преку вредностите на коригираниот интензитет на флуоресценцијата, покажаа статистички високо сигнификантни разлики меѓу ниските и високите градуси и стадиуми, како и текот на болеста ($p < 0,01$). Кај карциномот на простатата, експресијата на протеинот p53 е во директна корелација со Глисон скорот (Gleason skor) ($p < 0,0001$), Глисон (Gleason) сумата ($p < 0,0001$), градусот на примарниот тумор ($p < 0,0001$), метастатските лимфни жлезди ($p < 0,0001$) и стадиумот на болеста ($p = 0,026$).

Резултатите од оваа студија индицираат дека протеинот p53 може да има практична употребна вредност во клиничко-патолошката евалуација и во прогностиката на пациентите со карцином на мочниот меур, како и малигниот потенцијал на туморот и прогнозата на карциномот на простатата.

Клучни зборови: карцином на мочниот меур, карцином на простата, протеин p53

Вовед

Карциномот на мочниот меур е значаен здравствен проблем и четврто најчесто малигно заболување во западните земји. Хистолошки,

карциномот на преодниот епител (Transitional cell carcinoma-TCC) е најчестиот тип и претставува речиси 90 % од сите карциноми на мочниот меур (КММ). Карциномот на простатата (CaP) е еден од најчестите малигни заболувања кај мажите. Инциденцата рапидно расте во последните години поради продолжувањето на животниот век на мажите и поради воведувањето на софистицирани дијагностички методи.

Во текот на последниве триесетина години откриени се голем број генски промени (супституциски мутации, делеции, инсерции, транслокации, инверзии и слично), како и епигенетски промени во примероците од пациенти со малигни неоплазми на уринарниот тракт вклучувајќи ги мочниот меур и простатата.

Мутациите на туморсупресорниот ген *TP53* (познат и како *p53*) се најчести генетски варијации на карциномите кај луѓето вклучувајќи го и карциномот на мочниот меур (1). Протеинот P53 е продукт на истоимениот ген и има улога на транскрипциски фактор инволвиран во клеточната машинерија за заштита од оштетувања на геномот, а со тоа и од малигните алтерации. Од тие причини, овој протеин во литературата е познат и како „чувар“ на геномот (2). Името потекнува од молекуларната маса на протеинот од 53 kDa. Генот *TP53* е лоциран на краткиот крак на хромозомот 17p13.1 (3–5). Функцијата на протеинот p53 е да ги активира протеините кои ја репарираат ДНА, да го блокира клеточниот циклус во точката на проверка G1/S фаза од клеточниот циклус, сè додека репарираторните протеини не ги поправат оштетувањата во ДНА-молекулата. Доколку репарацијата на ДНА е невозможна, протеинот p53 ја води клетката кон програмирана клеточна смрт – апоптоза.

Мутираниот протеин p53 не е во состојба ефикасно да ги извршува овие функции за привремено запирање на клеточната делба, па тоа ги води клетките до неконтролирана делба и придонесува во малигната трансформација на клетката (6).

Познато е дека полуживотот на мутираниот протеин p53 е значајно подолг од оној на дивиот тип протеин p53 (7). Акумулацијата на мутираниот протеин p53 во јадрата на малигните клетки е главната причина за зголемената стапка на детекција со имунохистолошките методи вклучувајќи ја и имунофлуоресценцијата.

Досегашните студии покажуваат сигнификантна корелација на експресијата на протеинот p53 со КММ. Нуклеарната експресија кај карциномот на мочен меур, според Попов со сор., изнесува 22 % (8), додека Еке (Ecke) и сор. (9), во својата студија изнесува податоци за мутација на TP53 од 44,6 % кај суперфицијалниот и 84,2 % инфилтративниот КММ.

Неколку досегашни испитувања покажаа дека и мутациите на генот p53 и имунохистохемиски детектираната експресија на p53 се независни прогностички биомаркери, укажувајќи дека стабилизацијата на p53, која не е кодирана од мутираниот ген, исто така, може да предизвика аберации во натамошните каскадни реакции на сигналните патишта, кои ја вклучуваат, но не се лимитирани, на експресијата на p21 (10).

Поради овие причини, сосем очекувано е што p53 и генот кој го кодира, се истражуваат екстензивно кај карциномот на мочен меур. Досега, заклучоците од многубројните студии се во насока да го дефинираат p53 како молекуларен маркер, со клинички, патолошки и предиктивни вредности кај оваа малигна неоплазма (8).

Мутацијата на протеинот p53 често се случува при раните фази на CaP која се манифестира со зголемување на клеточните онкопротеини. Квин (Quin) и соработниците проучувале 263 пациенти со CaP кај кои е направена RP. Тие утврдиле голема корелација помеѓу експресијата на протеинот p53 и прогнозата на пациентот. Кај сите шест пациенти умрени за време на студијата имало нуклеарна експресија на над 20 % од клетките со карцином. Исто така, оваа студија покажала висока корелација помеѓу нуклеарната експресија на протеинот p53 и релапсот на болеста (11). Исто така, и Лин (Lin), во својата студија во која биле вклучени 125 пациенти кај кои е направена RP, утврдил позитивна корелација помеѓу експресијата на протеинот p53 и PSA и екстензијата на туморот (12).

Материјал и методи

Во оваа статија, сублимирани се податоците од две студии спроведени врз примероци и податоци од независни групи на пациенти со

карцином на мочниот меур, на простатата и контролни групи на пациенти и за двете неоплазми.

Пациенти за истражувањето на карциномот на мочниот меур

Во студијата за КММ беа анализирани ткивни примероци добиени од вкупно 70 пациенти со хистопатолошки потврден примарен уроепителен карцином на мочниот меур, како и од негативна контролна група, која ја сочинувале примероци на хистолошки нормална мукоза од мочен меур на 40 пациенти со немалигни заболувања. Клиничко-патолошките параметри кои беа користени при статистичките анализи се следниве: хистопатолошкиот градус (I, II и III), стадиум (1. суперфицијален и 2. мускулно-инвазивен) и клиничкиот тек на болеста (во смисла на појавата на рецидиви, метастази или смрт, поврзана со карциномот во текот на 2-годишниот период на евалуација).

Пациенти за истражувањето на карциномот на простатата

Во студијата за карцином на простатата се вклучени 83 пациенти од кои испитуваната група се состои од 43 пациенти со хистопатолошки верификуван карцином на простата, кај кои е направена радикална простатектомија, додека контролната група се состои од 40 пациенти со бенигна хиперплазија на простатата кај кои е направена трансуретрална ресекција на простата или трансвезикална простатектомија. Кај сите примероци евалуирана е нуклеарната експресија на протеинот p53 со имунохистохемиска метода. За степенување на карциномот на простатата, во САД и во ЕУ се употребува Глисон-системот (Gleason). Глисон-системот се базира врз структурните карактеристики на туморот и се состои од пет степени: од градус I – добро диференциран, постепено до градус V – лошо диференциран. За попрецизен опис на малигните карактеристики се употребува Глисон-скорот (Gleason-скорот). При овој опис се употребуваат две карактеристики: примарна е карактеристиката на малигната клетка и секундарна е карактеристиката на зафатеноста на стромата. Врз основа на овој скор-систем, најнизок е 2 (1+1), кој е добро диференциран, а највисок 10 (5+5), кој е лошо диференциран.

Дигитална квантитативна имунофлуоресцентна детекција на p53

Квантитативната детекција на протеинот p53 беше определена со имунофлуоресценција во хистолошките пресеци на ткивните примероци од пациентите со ТСС на мочниот меур. Анализите беа извршени на примероци, претходно фиксирани во формалин и вкалапени во парафин, користејќи ја имунофлуоресцентната сендвич-техника со двојни антитела. Хистолошките ткивни исечоци од пациентите беа добиени од Институтот за патологија при Медицинскиот факултет при УКИМ. Тие беа депарафинизирани со ксилол и рехидрирани. За пристапување кон скриените епитопи беше користен методот на преттретман, со загревање 4-пати по 5 минути, во цитратен пуфер, во микробранова печка, на 800 W. Блокирањето на неспецифичните епитопи се вршеше со 1 % BSA (говедски серум албумин), растворен во PBS, во времетраење од 30 минути. По блокирањето, исечоците беа инкубирани со примерно моноклонално анти-TP53 антитело (Sigma-Aldrich) разредено 150 пати во 1 % BSA во PBS, преку ноќ, на 4 °C. По инкубацијата, препаратите беа исплакнати двапати по 15 минути во HS-PBS (хипертоничен раствор на PBS) и двапати по 15 минути во PBS. Исплакнатите препарати беа инкубирани со секундарно антитело конјугирано со флуоресцин (anti-mouse IgG – FITC conjugate, Sigma-Aldrich), разредено 150 пати со 1 % BSA во PBS, во времетраење од 1 час и 30 минути, на собна температура, во темница. Потоа тие беа исплакнати двапати по 15 минути во HS-PBS и двапати по 15 минути во PBS.

Подготвените хистолошки исечоци беа анализирани со епифлуоресцентнен микроскоп (Human, Germany), со филтерски сет за флуоресцин (ексцитациски максимум 450 nm/30 nm *bandpass*, емисиски максимум 540 nm/30 nm *bandpass*). Селекетирани делови од исечоците беа фотографирани со дигитална камера (*Canon*) интегрирана на епифлуоресцентниот микроскоп.

Дигиталните слики беа снимани и анализирани со софтверот ImageJ v.1.46r. Од секој пациент беа анализирани најмалку 50 малигни клетки. Средните заднински вредности потоа беа одземени од средната вредност на интензитетот на p53-специфичната флуоресценција на малигните клеточни јадра со цел да се добијат коригираните просечни вредности на интензитетот на флуоресценцијата (CFI) за секој пациент

одделно. Како негативна контрола се користеа хистолошки нормални примероци од мукозата на мочниот меур, и слични анализи беа направени на јадрата од епителните клетки.

Статистички анализи

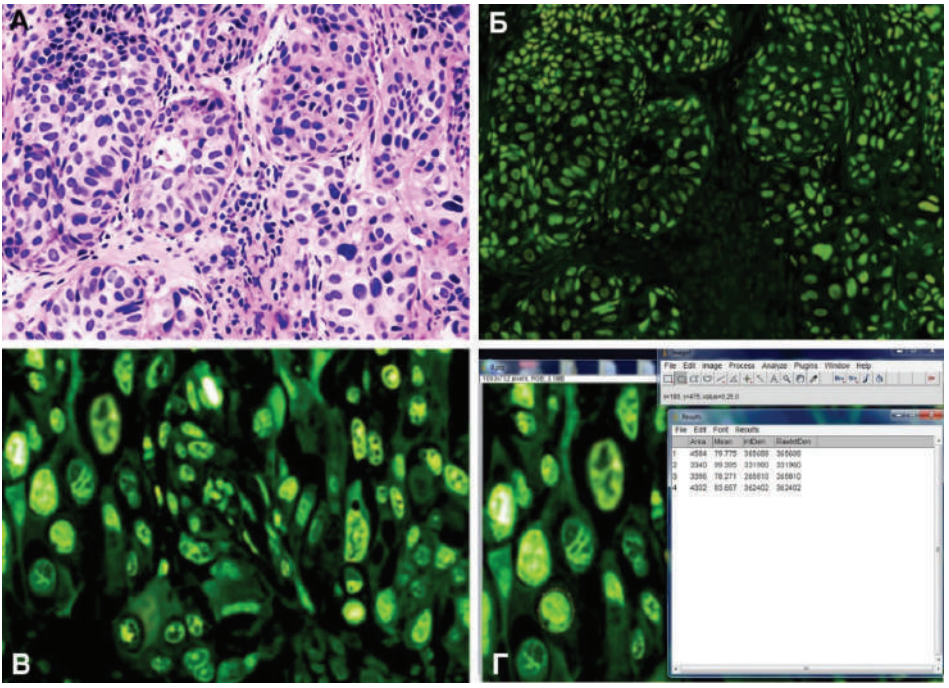
Компјутерската обработка беше направена со MS Office Excel 2016, со инсталиран софтверски додаток XLSTAT 2016. Основната обработка вклучуваше дескриптивни статистички анализи: дистрибуцијата на континуираните варијабли е прикажана со мерки на централна тенденција (средна вредност – mean, минимални и максимални вредности), како и вредност на варијабилноста (стандардна девијација – SD). Категоричките варијабли се прикажани како фреквенции (број и процент).

Корелациите меѓу молекуларните параметри добиени од определувањето на имунофлуоресцентната детекција на протеинот p53 во однос на хистопатолошкиот градус и стадиум, како и во однос на појавата на рецидиви, метастази и на смрт, поврзана со болеста, во текот на две години по добивањето на ткивото беа споредувани со користење параметарските и непараметриските анализи: Студентов *t*-тест и квадрат-тестот (со Yates-ова корекција).

Вредностите за *p* помали од 0,05 се сметаа за статистички значајни, а помалите од 0,01 за статистички високозначајни.

Резултати

Дигиталната анализа на јадрениот интензитет на флуоресценција во хистолошките пресеци на ткивните примероци од пациентите со КММ е презентирана на **слика 1**.



Слика 1 – Приказ на квантитативната дигитална анализата на застапеноста на p53 во хистолошки примероци на КММ. Заради ориентација и споредба, користени се стандардни хистолошки препарати обоени со хематокслин-еозин и анализирани под светлосен микроскоп (**А**). Дигитална фотографија од истиот регион на хистолошки пресек со имунофлуоресцентната визуализација на p53 под флуоресцентен микроскоп (објективно зголемување: 100 X) (**Б**). Поголемо зголемување на претходниот регион (објективно зголемување: 400 X) (**В**). Софтверска анализа на дигитална слика од имунофлуоресцентно прикажаниот p53 при која се мери интензитетот на флуоресценцијата на јадрата на малигните клетки (**Г**). Имунофлуоресценцијата беше дифузна и речиси униформна во јадрата на малигните клетки од испитаните ткивни примероци на сите испитани случаи.

Резултатите од статистичките анализи на споредбата на јадрениот интензитет на флуоресцентна детекција на p53 во хистолошките пресеци во однос на основните клиничко-патолошки параметри на КММ се прикажани во **табелите 1–3**.

Табела 1

Дистрибуција на флуоресцентниот интензитет на детектираниот p53 во јадрата на клетките во однос на градусите на КММ и контролната група

Параметри	**КММ градус**			**Контролна група**
Градус	**1**	**2**	**3**	**0**
n	5	42	23	40
Средна вредност	**47,11**	**54,00**	**104,21**	**0,92**
Стандардна грешка (SE)	6,11	1,74	2,45	0,07
t-тест (I и II)	0,330645769		/	/
t-тест (II и III)	/	**1,26139 x10^{-20}**		/
t-тест (I и III)	**0,0002**			/

Табела 2

Дистрибуција на флуоресцентниот интензитет на детектираниот p53 во јадрата на клетките во однос на стадиумите на КММ и контролната група

Параметри	**КММ стадиум**		**Контролна група**
Стадиум	**Супер-фицијален**	**Мускулно-инвазивен**	**0**
n	53	17	40
Средна вредност	**60,68**	**96,67**	**0,92**
Стандардна грешка (SE)	3,04	4,28	0,07
t-тест (суперф. и муск. иназивен)	**1,4009 x10^{-8}**		/
t-тест (суперф. и контрол. гр.)	**1,7775 x10^{-25}**		
t-тест (муск. иназивен и контрол. гр.)	/	**1,0333 x10^{-13}**	

Табела 3

Дистрибуција на флуоресцентниот интензитет на детектираниот p53 во јадрата на клетките во однос на текот на болеста на КММ и контролната група

Параметри	**Тек на болеста**		**Контролна група**
Тек на болеста	**Без рецидиви, метастази или смрт во текот на 2 години**	**Рецидиви, метастази или смрт во текот на 2 години**	**0**
n	44	26	40
Средна вредност	**0,93**	**2,37**	**0,58**
Стандардна грешка (SE)	0,20	0,54	0,03
t-тест (без и со рецидиви)	**0,0046**		/
t-тест (без и контрол. гр.)	0,1055		
t-тест (рецидиви и контрол. гр.)	/	**0,0001**	

Од прикажаните резултати е евидентно дека разликите на протеинската експресија на p53 се разликуваат со статистички висока значајност ($p<0,01$) меѓу нискиот и високиот градус на диференцијација на КММ, суперфицијалниот и мускулно-инвазивниот стадиум, како и клиничкиот тек на болеста (појавата на рецидиви и метастази во текот на 2-годишниот евалуациски период).

Имунохистохемиското боење на препаратите со CaP покажа дека нуклеарната експресија на p53 протеинот е забележена кај 28 %, додека кај контролната група, односно примероците со бенигна хиперплазија на простата, резултатите се негативни.

Резултатите од статистичките анализи во однос на истражувањето на протеинската експресија на p53 кај карциномот на простатата со [redacted]-тестот се прикажани во **табелите 4–6**.

Табела 4

Дистрибуцијата на имунохистохемиската детекција на p53 протеинот во однос на Gleason скорот

p53 протеин	**Gleason-скор**										**Вкупно**	***p***
	2+2	**2+3**	**3+2**	**3+3**	**3+4**	**4+3**	**4+4**	**4+5**	**5+4**	**5+5**		
Негативен	0	1	0	5	19	3	3	0	0	0	31	<0,00001
Позитивен	0	1	0	2	7	1	1	0	0	0	12	
Вкупно	0	2	0	7	26	4	4	0	0	0	43	

Табела 5

Дистрибуцијата на имунохистохемиската детекција на p53 протеинот во однос на Gleason-сумата

p53 протеин	**Gleason-сума**							**Вкупно**	***p***
	4	**5**	**6**	**7**	**8**	**9**	**10**		
Негативен	0	1	5	22	3	0	0	31	<0,00001
Позитивен	0	1	2	8	1	0	0	12	
Вкупно	0	2	7	30	4	0	0	43	

Табела 6

Дистрибуцијата на имунохистохемиската детекција на p53 протеинот во однос на Gleason-градусот

p53 протеин	**Gleason-градус**				**Вкупно**	***p***
	1	**2**	**3**	**4**		
Негативен		1	24	6	31	<0,00001
Позитивен		1	9	2	12	
Вкупно		2	33	8	43	

Прикажаните резултати индицираат дека дистрибуцијата на вредностите на Gleason скорот, сумата и градусот зависат статистички високосигнификантно од експресијата на p53 протеинот ($p<0,00001$).

Дискусија

Имунохистохемиските испитувања фокусирани на евалуација на експресијата на p53 и јадрената акумулација кај примероци од карцином на мочен меур се опишани одамна (13, 14). Всушност, генот *p53* и неговиот протеински продукт p53 се најчесто истражувани молекуларни маркери кај ракот на мочниот меур (15).

Иако поранешните истражувања покажаа дека p53 се детектира кај повисоките стадиуми и градуси на КММ (13, 14), други истражувања доведоа до различни заклучоци кои се делумно контрадикторни. Тие разлики меѓу истражувањата можат да се должат на разликите во популациите на пациентите, во различниот дизајн на студиите и на други фактори (16). Освен тоа, методолошкиот пристап употребен во различните студии може да биде барем делумно одговорен за разликите и слабата репродуктабилност на резултатите. Голем број фактори, вклучувајќи го, но не исклучиво, изборот на антителата, немањето стандардизиран метод за имунолошка детекција во хистолошките препарати, како и субјективностите при евалуацијата и интерпретацијата на резултатите од боењето, сè уште претставуваат проблем.

Повеќето претходни истражувања кај кои се проценувала експресијата на p53 во ткивни примероци се базирани на имунохистохемиска детекција преку создавање на обоен продукт од реакцијата ензим-суспстрат. Во тие случаи, квантитативната евалуација се сведува со броење на клетките или јадрата кои биле позитивни на специфичното боење.

Во ова истражување користевме пообјективна и порепродуктабилна методологија заснована на квантитативно мерење на флуоресцентниот сигнал од индивидуалните јадра на флуоресцентен микроскоп. Дигиталните податоци беа анализирани со посебен софтвер за анализирање на слики. Она што е значајно, заднинскиот сигнал-шум што претставува резултат од неспецифичното врзување на антителата и автофлуоресценцијата на ткивото беа одземени од секоја анали-

зирана индивидуална дигитална слика со цел да се намали непостојаноста која се должи на варијации во интензитетот на осветлувањето и оптичките несовршености на микроскопот и на препаратот. Овој методолошки пристап од неодамна е во употреба за квантитативна детекција на p53 и други протеини поврзани со карциноми (17) и е пософистициран и далеку пообјективен од нашироко распространетиот скоринг.

Во нашето истражување откривме дека средната вредност на коригираниот интензитет на флуоресценцијата значајно се разликува меѓу повисоките градуси на КММ (2 и 3), како и меѓу градусите 1 и 2. Освен тоа, најдовме статистички значајна разлика меѓу вредностите на флуоресценцијата на секој поединечен градус (1, 2 и 3) и вредностите CFI на негативната контролна група (хистолошки нормална мукоза на мочен меур). Анализата на нашите резултати покажа висока корелација на вредностите на интензитетот на флуоресценцијата на јадрениот p53 меѓу примероците од суперфицијален со тие од мускулно-инфилтративен КММ. Ваквите големи разлики во експресијата на p53 не се невообичаени. Имено, хистопатолошките и генетските испитувања доведоа до концептот дека површинските папиларни и инвазивни карциноми на мочниот меур имаат различен тек на болеста и прогноза. Општо е познато дека поголемиот број инфилтративни КММ немаат позната папиларна прекурсорна лезија (18). Овие резултати упатуваат на податокот дека генетските промени кои доведуваат до преголема експресија или акумулација на јадрениот p53 може да се појават релативно доцна во текот на процесот на малигна трансформација.

Што се однесува на корелацијата на вредностите на интензитетот на флуоресценцијата на јадрениот p53 со исходот кај пациентите, кај 44 од 70-те анализирани пациенти, не се појавија рецидив на туморот, далечни метастази или смрт поврзана со карциномот во текот на две години следење по добивањето на ткивниот примерок. Кај преостанатите 26 пациенти се појави локален рецидив на туморот, или метастаза или смрт од причини забележани во периодот на следење. Двете подгрупи пациенти не корелираа сигнификанто со нивните CFI-вредности ($p>0,05$). Од друга страна, постои статистички значајна разлика помеѓу вредностите кај секоја од овие подгрупи и вредностите на CFI кај контролната група ($p<0,001$).

Јасно е дека овие резултати не ја потврдија предиктивната вредност на јадрената акумулација на p53 кај ТСС во смисла на поја-вата на рецидиви, метастази или смрт поврзана со карцином, барем не во релативно кусиот период на следење од 2 години. Овој заклучок е сличен со многу претходни студии (19). Од друга страна, некои автори откриле дека p53 е значаен предиктор на прогресијата на ракот кај мочниот меур (13), а други заклучиле дека p53-имунохистохемијата се чини дека е предиктивна, но само кај високиот градус на ТСС (14). Значајно е да се потенцира дека корелацијата меѓу експресијата на p53 и прогнозата кај карциномот на мочниот меур била испитувана и публикувана во над 200 трудови (20). Подобрената и релативно новата методологија, како и краткиот период на евалуација на пациентите во нашата студија, може се значаен фактор што треба да се земе предвид кога се споредува со други слични студии.

Оттаму, со оглед на релативно малиот број и хетерогеноста на нашите пациенти, потребни се поопсежни испитувања за да се покаже клиничко-патолошката вредност на дигиталната квантитативна имунофлуоресцентна детекција на нуклеарниот p53 во примероци од ТСС.

Нашите резултати од имунохистохемиското боење на препаратите со CaP покажаа дека нуклеарната експресија на p53 протеинот е забележена кај 28 %, додека кај контролната група, т. е. примероците со бенигна хиперплазија на простатата, резултатите се негативни. Ова покажува цврста корелација на експресијата на протеинот p53 со клетките на CaP. Многу студии не даваат податок дали има експресија на протеинот p53 во бенигните клетки. Баскар (Bhaskar) случајно забележал p53 нуклеарно боење кај нормалните, т. е. кај бенигните базални клетки во близината на туморот (21). Во друга студија, Дермер (Dermer) ја проучувал појавата на експресијата на протеинот p53 во бенигната хиперплазија на простатата (22). Фокалната експресија во базалните клетки ја поврзал со премалигна лезија која ѝ дава на клетката предност во растењето.

Една од целите на оваа студија е да утврди дали експресијата на протеинот p53 би ја зголемила сигурноста во определувањето на стадиумот на примарниот тумор, т. е. дали болеста е ограничена на органот или не. Статистичката анализа со χ^2 –тестот покажа дека експресијата на p53 протеинот статистички не колерира со органската

ограниченост на примарниот тумор (p=0,647), т. е. нема статистичка разлика помеѓу експресијата на протеинот p53 во групата „organ confine“ (T1 и T2) и „orgnan nonconfine“ (T3 и T4). Овој резултат покажува дека одредувањето на протеинот p53 не ја зголемува сигурноста во клиничкиот стадиум (*staging*) на болеста, т. е. во определувањето на градусот на примарниот тумор. Во студијата направена е и корелација на нуклеарната експресија на протеинот p53 со неколку варијабли од клиничкиот и патолошкиот стадиум (staiging).

Томас (Thomas) со соработниците и Шурбаџи (Shurbaji) со соработниците, во одвоени студии, направиле имунохистохемиска евалуација на протеинот p53 кај CaP и на неговата вредност како прогностички фактор (23, 24). Резултатите покажале директна корелација со агресивноста на CaP. Падопулос (Papadopoulos) со соработниците, во студијата со примероци од CaP, кај пациенти третирани со RP, ги компарирале лабораториските резултати од биолошките маркери (p53, Ki-S5, плоидниот статус), со клиничките и хистопатолошките параметри од RP (25). Резултатите покажале дека високите вредности на протеинот p53 се наоѓаат во директна корелација со малигниот потенцијал на карциномот и со прогнозата на болеста.

Дека експресијата на протеинот p53 е во корелација со прогнозата на болеста потврдува и Шер (Scher) со соработниците во студијата со 46 пациенти кои се лекувале со радиотерапија (26). Во студијата ги евалуирале двата клучни регулатори на апоптозата bcl-2 и p53, со имунохистохемиско боење на примероците добиени со иглена биопсија кај 46 пациенти со CaP. Резултатите покажале дека примероците со експресија на bcl-2 и протеинот p53 асоцираат со слабата ефикасност на радиотерапијата, т. е. со преттерапиското одредување на овие два параметра би помогнале да се определи ефикасноста на дефинитивната радиотерапија.

Студијата на Навон (Navone) од Институтот за канцер „Андерсон“ во Тексас (Anderson Cancer Institute, Texas) наведува дека експресијата на протеинот p53 е во корелација со стадиумот на примарниот тумор и хистолошкиот градус (27). Тој, исто така, утврдил јака корелација помеѓу експресијата на протеинот p53 и случаите со лошо диференцирани карциноми и хормон-независни тумори. Зголемувањето на андрогените рецептори асоцира со андрогената резистентност на CaP.

Алимира, Ф. (Alimirah, F.), во објавената студија, изнесува податоци дека намалувањето на функцијата на p53 супресор генот резултира со експресија на андрогените рецептори со што се објаснува хормоналната резистентност на CaP (28).

Заклучок

Резултатите од нашите студии индицираат дека експресијата на протеинот p53 може да има практична употребна вредност во клиничко-патолошката евалуација и во прогностиката кај пациентите со карцином на мочниот меур, како и за евалуација на малигниот потенцијал и во прогнозата на карциномот на простатата. Потребни се дополнителни истражувања со вклучување на поголем број испитаници, валидација на постојните и воведување нови молекуларни методи.

ЛИТЕРАТУРА

1. Ecke, T. H., Schlechte, H. H., Schulze, G., Lenk, S. V., Loening, S. A. (2005). *Four tumour markers for urinary bladder cancer--tissue polypeptide antigen (TPA), HER-2/neu (ERB B2), urokinase-type plasminogen activator receptor (uPAR) and TP53 mutation*. Anticancer Res.; 25(1B): 635–41.
2. Vogelstein, B., Lane, D., Levine, A. J. (2000). *Surfing the p53 network*. Nature.; 408(6810): 307–10.
3. Matlashewski, G., Lamb, P., Pim, D., Peacock, J., Crawford, L., Benchimol, S. (1984). *Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene.* EMBO J.;3(13): 3257–62.
4. Isobe, M., Emanuel, B. S., Givol, D., Oren, M., Croce, C. M. (1986). Localization of gene for human p53 tumour antigen to band 17p13. Nature.;320(6057): 84–5.
5. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C., Vogelstein, B. (1991). *Identification of p53 as a sequence-specific DNA-binding protein*. Science.;252 (5013): 708–11.
6. Konstantakou, E. G., Voutsinas, G. E., Karkoulis, P. K., Aravantinous, G., Margaritis, L. H., Starvopodis, D. J. (2009). *Human bladder cancer cells undergo cisplatin-induced apoptosis that is associated with p53-dependent and p53-independent responses.* Int J Oncol.;35; 401–16.

7. Finlay, C. A., Hinds, P. W., Tan, T. H., Eliyahu, D., Oren, M., Levine, A. J. (1988). *Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life*. Mol Cell Biol.;8(2): 531–9.
8. Popov, Z., Hoznek, A., Colombel, M., Bastuji-Garin, S., Lefrere-Belda, M. A., Bellot, J., Abbou, C. C., Mazerolles, C., Chopin, D. K. (1997). *The prognostic value of p53 nuclear overexpression and MIB-1 as a proliferative marker in transitional cell carcinoma of the bladder*. Cancer.; 80(8): 1472–81.
9. Ecke, T. H., Schlechte, H. H., Gunia, S., Lenk, S. V., Loening, S. A. (2008). *Body mass index (BMI) and mutations of tumor suppressor gene p53 (TP53) in patients with urinary bladder cancer*. Urol Oncol.;26(5): 470–3.
10. George, B., Datar, R. H., Wu, L., Cai, J., Patten, N., Beil, S. J., Groshen, S., Stein, J., Skinner, D., Jones, P. A., Cote, R. J. (2007). *p53 gene and protein status: the role of p53 alterations in predicting outcome in patients with bladder cancer*. J Clin Oncol.;25(34): 5352–8.
11. Quin, D. I., Henshall, S. M., Head, D. R. et al. (2000). *Prognostic significance of p53 nuclear accumulation in localized prostate cancer treated with radical prostatectomy*. Cancer Res.; 60(6): 1585–94.
12. Lin, J. T., Wang, J. S., Jiann, B. P. et al. (2005). *Correlation of p53 protein accumulation and Bcl-2 overexpression with histopathological features in prostatic cancer*. J Formos Med Assoc.;104(11): 864–7.
13. Davidson, D., Bostwick, D. K., Qian, J. et al. (1995). *Prostatic intraepithelial neoplasia is a risk factor for adenocarcinoma: predictive accuracy in needle biopsies*. J Urol; 154: 1295–9.
14. Sobin, L. H. (2002). *TNM classification of Malignant Tumours*. 6th ed. Sobin L. H., Wittekind C. H. (ad.).; Wiley-Liss: New York pp. 184–7.
15. Office for national Statistics, Cancer Statistics registrations: Registrations of cancer diagnosed in 2002, England.series MB1 no.33. 2005, Nacional statistics: London.
16. Forman, D., Stockton, D., Moller, H. et al. (2003). *Cancer prevalence in the UK; results from the EUROPREVAL study*. Ann Oncol 2003; 14: 648–54.
17. Bray, F., Sankila, R., Ferlay, J. et al. (2002). *Estimates of cancer incidence and mortality in Europe in 1995*. Eur J Cancer.; 38: 99–166.
18. Black, R. J., Bray, F., Ferlay, J. et al. (1997). *Cancer incidence and mortality in the European Union: cancer registry data and estimates of national incidence for 1990*. Eur J Cancer.; 33: 1075–107.
19. Sandblom, G., Dufmats, M., Nordenskjöld, K. et al. (2000). *Prostate carcinoma trends in three counties in Sweden 1987-1996: Results from a population based national cancer register*. South-East Region Prostate Cancer Group. Cancer.; 88: 1445–53.

20. Amling, C. L., Blute, M. L., Bergstralh, E. J. et al. (2000). *Long-term hazard of progression after radical prostatectomy for clinically localized prostate cancer: continued risk of biochemical failure after 5 years*. J Urol. 164: 101–5.
21. Kallakury, B. V., Figge, J., Leibovich, B. et al. (1994). *Association of p53 Immunoreactivity With Hight Gleason Tumor Grade in Prostatic Adenocarcinoma*. Hum Pathol.; 25: 92–7.
22. Dermer, G. B. (1978). *Basal cell proliferation in benign prostatic hyperplasia*. Cancer.; 41: 1857–62.
23. Thomas, D. J., Robinson, M., King, P. et al. (1993). *p53 expression and clinical outcome in prostate cancer*. Br J Urol.; 72: 778–81.
24. Shurbaji, M. S., Brooks, S. K., Thurmond, T. S. et al. (1995). *Immunohistochemical detection of p53 protein as a prognostic indicator in prostate cancer*. Hum Pathol.; 26(1): 106–9.
25. Papadopoulos, I., Rudolph, P., Wirth, B. et al. (1996). *p53 expression, proliferation marker Ki-S5, DNA content and serum PSA: possible biopotential markers in human prostatic cancer*. Urology.; 48: 261–8.
26. Scher, D. S., Vaughan, E. D., Wei, J. et al. (1999). *BCL-2 and p53 expression in localized prostate cancer predicts respons to external beam radiotherapy*. J Urol.; 3: 119–27.
27. Navone, N. M., Troncoso, P., Pisters, L. L. et al. (1993). *p53 Aculmulation and gene mutation in the progression of human prostate carcinoma*. J Natl Cancer Inst.; 85: 1657–69.
28. Alimirah, F., Panchanathan, R., Chen, J. et al. (2007). *Expression of androgen receptor Is Negatively Regulated By p53*. Neoplasia.; 9(12): 1152–59.

Skender SAIDI, Zivko POPOV, Sasho PANOV

THE ROLE OF P53 IN MOLECULAR PATHOGENESIS OF MALIGNANT NEOPASMES OF THE URINARY TRACT

Abstract

Bladder cancer is a significant health problem and is the fourth most common malignancy in the Western world. Prostate cancer is among the most frequent malignant neoplasms in males. The incidence is rapidly growing in the recent years due to the prolonged live span and due to the introduction of more sophisticated diagnostic methods.

The tumor-suppressor gene *p53* encode the same-named protein which acts as a transcription factor that is activating under various types of cellular stress, including DNA damage and oncogene activation. In those cases, it induces a cascade of molecular events leading to cell-cycle arrest, DNA repair and apoptosis. Mutations in the *p53* gene are among the most frequent genetic alterations in human malignant neoplasms, including the urinary bladder cancer.

In this study we have investigated tissue samples of histopathologicaly confirmed cancer of the urinary bladder derived from 70 patients. Normal urinary bladder mucosa obtained from 40 patients with nonmalignant diseases was used as a negative control group. Quantitative immunofluo-rescent detection of p53 protein was used. Prostate cancer study included 83 patients, 43 of which underwent radical prostatectomy and a control group of 40 patients with benign hyperplasia of the prostate to which a transurethral resection or a transvesical prostatectomy was undertaken. The nuclear expression of p53 protein was evaluated using immunohistochemistry method.

In bladder cancer study, the detection of p53 protein in histological samples in the terms of the corrected fluorescence intensity values reveals statistically highly significant differences between the samples with low and high grades, stages and clinical outcome ($p<0.01$). In prostate cancer study, the results have shown that the expression of the p53 protein is in direct correlation with the Gleason score ($p<0.00001$), the Gleason sum (<0.00001), primary tumor grade (<0.00001), metastatic lymph nodes (<0.00001) and the disease stadium ($p=0.026$).

The result of this study indicates that p53 protein could have a considerable value in clinical and pathological evaluation and prognosis of patients with urinary bladder cancer. The p53 protein can also be used for determination of malignant potential of the tumor and prognosis of the prostate cancer.

Keywords: urinary bladder cancer, prostate cancer, p53 protein.

ПОГЛАВЈЕ VII

ТРУДОВИ ПО СПЕЦИЈАЛНА ПОКАНА

doi: 10.20903/zpopov-2022-0032 UDK: 061.12(497.7)

Mirko SPIROSKI, MD, PhD[1,2], Zivko POPOV, MD, PhD, FEBS, Acad[2,3]
Ljupcho KOCAREV, PhD, Acad[3]

CURRENT IMPACT OF THE MACEDONIAN ACADEMY OF SCIENCES AND ARTS IN THE SCOPUS DATABASE (2021 YEAR)

Abstract

Background: The mission of the Macedonian Academy of Sciences and Arts is to provide its full contribution to the inclusion of the state of Macedonian science and arts with regards to modern European and world-wide scientific trends. These crucial spheres of the human spirit and civilizational existence, reflected in the work of the Macedonian Academy of Sciences and Arts, are supported by the scientific and research work at the Academy and through its constant care for the preservation and affirmation of Macedonian artistic and cultural treasures and heritage. As these aspects are important premises for the overall development of the Republic of Macedonia. The vision of the Macedonian Academy of Sciences and Arts is to advance the Republic of Macedonia, thereby becoming a more advanced society, based on science and knowledge.

Aim: We aimed to investigate the current impact of the Macedonian Academy of Sciences and Arts has had in the Scopus database (2021).

Methods: On June 06, 2021**,** we performed an affiliation search of the Scopus database in order to identify published papers from the Macedonian Academy of Sciences and Arts (MASA) and from Ss Cyril and Methodius University, Skopje (UC&M), both located in the Republic of Macedonia.

[1] Scientific Foundation SPIROSKI, Skopje, North Macedonia
[2] Medical faculty - University "Ss Cyril and Methodius" - Skopje, North Macedonia
[3] Macedonian Academy of Sciences and Arts, Skopje, North Macedonia

Results: We found 820 articles published by 120 authors, or 8.83 documents per author compared with 9927 articles with 3357 authors, or 3.36 documents per author published from those at the SS Cyril and Methodius University, Skopje. The majority the published works are scientific articles (81.2%) and the majority of the published documents are from the fields of physics and astronomy, medicine, as well as from biochemistry, genetics and molecular biology. The majority of publications is from Janev RK. The most frequent affiliation for these works is Macedonian Academy of Sciences and Arts. Based by country in the region, North Macedonia and Macedonia rank first place. The most frequent sponsor for the published papers is the Seventh Framework Programme. The first three authors ranked according to the Hirsh-Index are Janev Ratko K (h-index = 37), Kocarev L (h-index = 30), and Polenaković Momir H (h-index = 23).

Conclusions: There is a larger proportion of authors affiliated with the MASA (around 2.6 times more) than authors affiliated to the SS Cyril and Methodius University in Skopje who publish scientific papers.

Keywords: Macedonian Academy of Sciences and Arts; Ss Cyril and Methodius University, Skopje; Scopus database; Republic of Macedonia.

Introduction

The Macedonian Academy for Science and Arts (MASA) was established on February 23, 1967, by decree, proclaimed by the Law of the Macedonian Academy of Sciences and Arts (Statute No.18/67 from February 23, 1967) and the Assembly of the Socialist Republic of Macedonia and it later adopted the law regarding the Macedonian Academy of Sciences and Arts. Based on the law, a Register Committee was established, which was tasked with preparing the groundwork for the Macedonian Academy of Sciences and Arts. The election of the first 14 full members of MASA was completed on August 18, 1967 at the National Museum in Ohrid. The opening ceremony of the Macedonian Academy of Sciences and Arts was on October 10, 1967 in the Great Hall of the Assembly of the Republic of Macedonia. On that day, the Academy began its scientific and cultural activities, as mandated by the legal act for its establishment (1).

The Academy was established as the highest scientific and artistic institution in our country which monitors and stimulates the development of the sciences and the arts and strives for their advancement. The Academy surveys the situation of cultural heritage and natural resources, collaborates in preparing national policy regarding the sciences and the arts. The Academy also stimulates, coordinates, organizes, and conducts scientific research and artistic achievements, especially those particularly relevant to the Republic of Macedonia. In addition, the Academy facilitates the scientific and artistic work of its members and encourages the use of the most advanced methodologies, scientific knowledge, and results in the field of scientific research, and establishes, maintains. Finally the Academy also facilitates international cooperation in the fields of the sciences and arts (2).

As the highest independent scientific and artistic institution in the Republic of Macedonia, the Academy achieves its objectives by organizing basic, developmental, and applied research, with a focus on comprehensive and inter-disciplinary research. This is accomplished by organizing scientific meetings and artistic presentations and by publishing the results of said scientific research and scientific meetings and artistic works. The Academy collaborates with the universities, scientific and cultural institutions, scientific and artistic societies, and other organizations in the fields of the sciences and the arts throughout the Republic of Macedonia. It also collaborates with academies of sciences and arts and other scientific and artistic institutions abroad. After the Macedonian state was established at the First Antifascist Assembly of the People‘s Liberation of Macedonia (ASNOM) in 1944, 1967 was decided as the year of the official foundation of the Macedonian Academy of Sciences and Arts (2).

Candidates for the academicians prepares list of papers published in scientific journals with impact factor (IF), list of the papers published in other international and national journals, list of papers published in international foreign and national scientific conferences, as well as a list of prominent papers for the best presentation of the candidate (list of 10 the most important publications in international journals with impact factor) (3).

We aimed to investigate the current impact of the Macedonian Academy of Sciences and Arts in the Scopus database (2021).

Methods

The affiliation search of the Scopus database was performed on July 16, 2021. The goal was to identify published papers from the Macedonian Academy of Sciences and Arts (MASA) and from Ss Cyril and Methodius University, Skopje (UC&M), Republic of Macedonia.

Affiliation ID (AF-ID) of MASA is 60072633, but the affiliation ID of UC&M is not unique and contains 5 different affiliation identification numbers (Ss. Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Skopje, AF-ID 60072629; SS Cyril and Methodius University, Faculty of Medicine, Skopje, AF-ID 60072630; SS Cyril and Methodius University, Faculty of Pharmacy, Skopje, AF-ID 60072638; Clinic for Children's Diseases, Skopje, AF-ID60072628; and University Clinical Center, Skopje, AF-ID 60072639).

The articles selected for analysis date from 1989 until July 16, 2021. MASA provided a total number of 820 articles from 120 authors UC&M had 9927 articles with 3357 authors (Ss. Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Skopje, 7893 articles from 2389 authors; SS Cyril and Methodius University, Faculty of Medicine, Skopje, 1542 articles from 812 authors; SS Cyril and Methodius University, Faculty of Pharmacy, Skopje, 256 articles with 70 authors; Clinic for Children's Diseases, Skopje, 16 articles from 43 authors; and University Clinical Center, Skopje, 72 articles with 43 authors).

Results

The number of publications in the period from 1989-2005 was exceedingly small (1-20 papers per year). Starting in 2007, the number of published papers increased sharply with a maximum of 60 papers in 2011 (Fig. 1 upper). Most of the articles were published in the Balkan Journal of Medical Genetics (44 articles); Journal of Physics B Atomic Molecular and Optical Physics (37 articles); Physical Review A Atomic Molecular and Optical Physics (32 articles); Hemoglobin (29 articles); and Prilozi Makedonska Akademija Na Naukite I Umetnostite Oddelenie Za Bioloski I Medicinski Nauki - Contributions Macedonian (Academy Of Sciences And Arts Section of Biological And Medical Sciences) (18 articles) (Fig. 1 down).

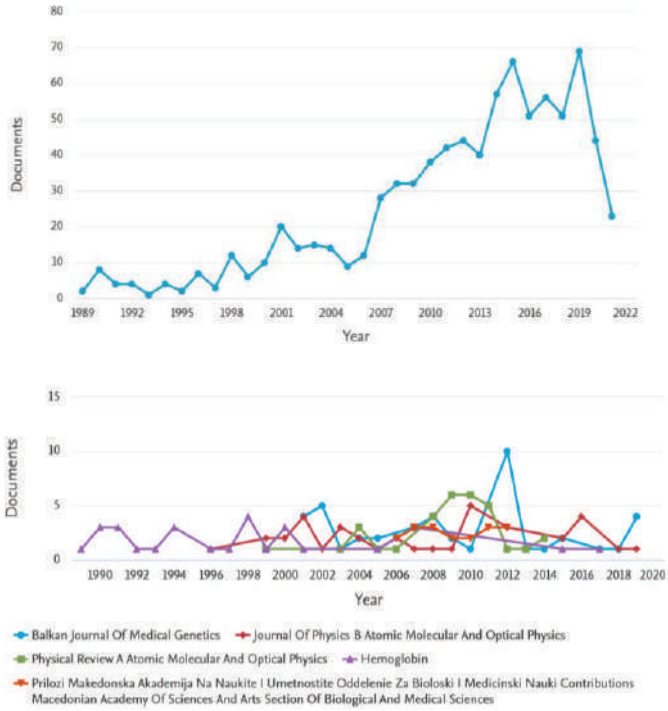


Figure 1 – Documents by year (upper) and documents per year by source (lower) published by the Macedonian Academy of Sciences and Arts included in the Scopus database from 1989 until July 16, 2021.

Most of the published papers are articles (81.2%), conference papers (9.4%), review articles (3.0%), and other types of articles (Fig. 2). The majority of the published documents are from Physics and Astronomy (252); Medicine (231); Biochemistry, Genetics and Molecular Biology (159); Engineering (95); Chemistry (88); Mathematics (73); Energy (49); Materials Science (45); Computer Science (44); and Agricultural and Biological Sciences (43). The rest of the subjects are presented in 15.7% (Fig. 2).

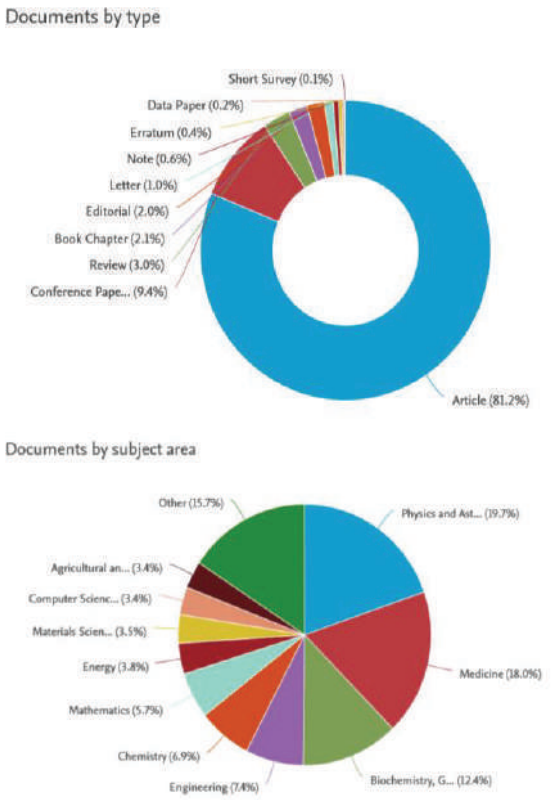


Figure 2 – Documents by type (upper) and documents by subject area (lower) published by the Macedonian Academy of Sciences and Arts included in the Scopus database from 1989 until July 16, 2021.

The most frequent publications are from Janev RK, Efremov GD, Plaseska-Karanfilska D, Kocarev L, Wang JG, Polenakovic M, Markovska N, Sandev T, Jovanovski G, and Liu L (Fig. 3a). Documents by the 10 most frequent affiliations are from the Macedonian Academy of Sciences and Arts (820), SS Cyril and Methodius University (327), Beijing Institute of Applied Physics and Computational Mathematics (77), SS Cyril and Methodius University, Faculty of Medicine (76), Institute of Chemistry, SS Cyril and Methodius University 74), University of California, San Diego (61), Forschungszentrum Jülich FZJ (42), Columbia University (40), University of Belgrade (33), and BioCircuits Institute (32) (Fig. 3b). Based on country or territory, North Macedonia (635) and Macedonia (173) are on first place followed by United States (161), Germany (133), China (93), Italy (69), Bulgaria (53),

Serbia (50), United Kingdom (50), and Croatia (44) (Fig. 3c). The most frequent sponsors for the published papers from the MASA are Seventh Framework Programme (35), National Institute of Mental Health (32), National Natural Science Foundation of China (30), Deutsche Forschungsgemeinschaft (25), National Institutes of Health (25), European Commission (20), Alexander von Humboldt-Stiftung (16), National Key Research and Development Program of China (13), Canadian Institutes of Health Research (11), and Horizon 2020 Framework Programme (11) (Fig. 3d).

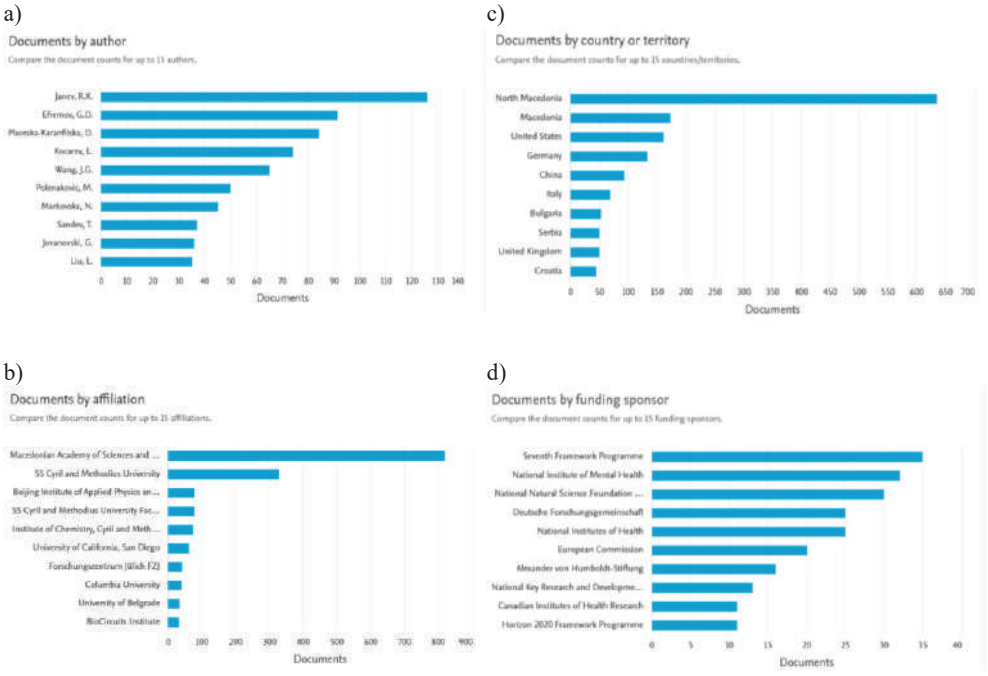
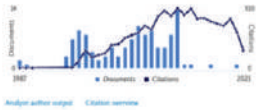
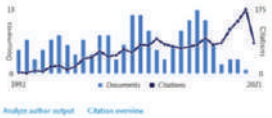
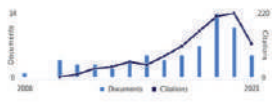
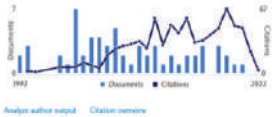
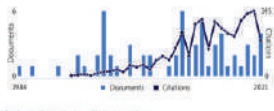


Figure 3 – Documents by author (a), documents by affiliation (b), documents by country or territory (c), and documents by sponsor (d) published by the Macedonian Academy of Sciences and Arts included in the Scopus database from 1989 until July 16, 2021.

Figure 4 displays the analysis of the articles of the first 10 authors published from the Macedonian Academy of Sciences and Arts which are included in the Scopus database from 1989 until June 06, 2021 ranked according to the Hirsh-Index. First three authors are Janev Ratko K (h-index = 37), Kocarev L (h-index = 30), and Polenaković Momir H (h-index = 23). The rest of the academics have an h-index of 20, 16, 15, 14, and 13, respectively. Documents per author range from 299 (Janev Ratko K) to 16

(Ristov M). The number of citations is 5264 to 593 and is not equally distributed from the first to the tenth place. Citation trends are very heterogenous among the first ten academicians (Fig. 4)

Author Scopus Author ID:	Document & citation trends	Documents by author	Citations	h-Index
1. Janev, Ratko K. 7007174243		299	5264	37
2. Kocarev, L. 7005782701		122	4978	30
3. Polenaković, Momir H. 8122907000		194	1862	23
4. Sandev, Trifce 36619822900		68	1056	20
5. Stefov, Viktor 6601978749		62	775	16
6. Grčev, Leonid D. 55952986500		64	1660	15

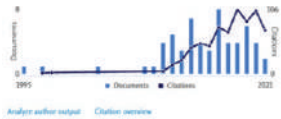
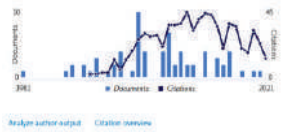
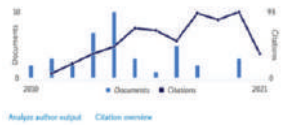
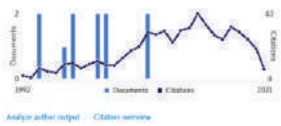
7. Markovska, Nataša 22994164800		59	693	15
8. Popov, Zivko 7004730664		69	736	15
9. Bačeva Andonovska, Katerina 57115361000		38	593	14
10. Ristov, M. 6601912303		16	846	13

Figure 4 – Analysis of the articles from the first 10 authors published by the Macedonian Academy of Sciences and Arts included in the Scopus database from 1989 until July 16, 2021.

Table 1 compares published papers from MASA and UC&M in the Scopus database. We can see that authors from MASA published 820 papers in the period of 1989-2021 which are included in the Scopus database. The 120 authors have an average of 8.83 documents per author. Comparatively, Ss. Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Skopje published 7893 articles with 2389 authors with an average 3.30 documents per author. SS Cyril and Methodius University, Faculty of Medicine, Skopje published 1542 articles with 812 authors o6 1.90 documents per author. SS Cyril and Methodius University, Faculty of Pharmacy, Skopje published 256 articles from 70 authors with 3,65 document/author, Clinic for Children's Diseases, Skopje published 164 articles from 43 authors

with an average 3.81 documents per author. Finally, the University Clinical Center, Skopje published 72 articles from 43 authors with an average of 1.67 documents per author. The cumulative Results for all Affiliations from SS Cyril and Methodius University, Skopje reveal 9927 published articles from 3357 (authors?) with an average 3.36 documents per author (Table 1).

Table 1
Comparison of the published papers from MASA and UC&M in the Scopus database

Affiliation	Affiliation ID	No of Documents	No of Authors	Documents/ author
Macedonian Academy for Sciences and Arts, Skopje (MASA)	60072633	820	120	8.83
Ss. Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Skopje	60072629	7893	2389	3.30
SS Cyril and Methodius University, Faculty of Medicine, Skopje	60072630	1542	812	1.90
SS Cyril and Methodius University, Faculty of Pharmacy, Skopje	60072638	256	70	3.65
Clinic for Children's Diseases, Skopje	60072628	164	43	3.81
University Clinical Center, Skopje	60072639	72	43	1.67
Cumulative Results for all Affiliations from SS Cyril and Methodius University, Skopje (UC&M)	Should be officially corrected	9927	3357	3.36

Discussion

In this paper we conducted the initial analysis of the published papers from the Macedonian Academy of Sciences and Arts in Skopje from 1989 until July 16, 2021 via the Scopus database. We found 820 articles published form 120 authors affiliated with MASA, or 8.83 documents per author compared to 9927 articles with 3357 authors, or 3.36 documents per author published from the SS Cyril and Methodius University, Skopje. This suggests a higher level of publishing productivity among the authors affiliated with MASA (around 2.6 times more productive) than authors from SS Cyril and Methodius University in Skopje. Affiliation ID of SS Cyril and Methodius University, Skopje is not complete, but is distributed among the five affiliations. This should be officially corrected. A similar situation was discussed in 2014, in the plans for the structure of SS Cyril and Methodius, Skopje. The problem, however, was not solved, and this is still present in the Scopus database, and thus could negatively influence the Institutional ranking of the SC&M University (4).

The majority of the published papers from the authors affiliated with MASA are scientific articles (81.2%) and the biggest number of the published documents are from the fields of physics and astronomy, medicine, as well as from biochemistry, genetics and molecular biology. The most prolific publisher is Janev RK, and the most frequent affiliation is the Macedonian Academy of Sciences and Arts. Based on country or territory, North Macedonia and Macedonia are first place. The most frequent sponsor for the published papers is Seventh Framework Programme. The first three authors, ranked according to the Hirsh-Index, are Janev Ratko K (h-index = 37), Kocarev L (h-index = 30), and Polenaković Momir H (h-index = 23).

The results of the scientific journals indexed in Scopus from IX Section of the Hungarian Academy of Sciences show that 80 percent of the journals have their own website, half of them are published on time. One fifth have an archiving policy and one tenth have a code of ethics. On average, 42 months have passed since the publication of the most recent issue. These indicators depend on the quality categories, and there is a significant correlation between the category, the number of papers and the number of citations per paper [5].

A comparison of the average h-index of members of the Brazilian Academy of Sciences (BAS) and of the National Academy of Sciences of the USA (NAS-USA) was carried out for 10 different areas of science. The comparison, however, was unfavorable towards the members of the BAS; the imbalance was distinct in different areas. Since these two academies represent, to a significant extent, top quality science produced in each country, the comparison allows the identification of the areas in Brazil that are closer to the international participants of scientific excellence. The areas of physics and mathematics are of particular interest. The heterogeneity of the h-index in the different areas, estimated by the median dispersion of the index, is significantly higher in the BAS than in the NAS-USA [6].

Details for the journals with an Impact Factor (IF) and journals listed in the Scopus (SJR, Q number) are mandatory mostly in the scientific fields of natural sciences, biotechnical, technical, and medical sciences and partly for social and humanitarian sciences. These help to focus on national questions and national strategies published mostly in national journals and monographs which are equally important but not present in the indexed journals.

Several papers are published in the Republic of Macedonia using the Scopus database and other resources connected to it (4-7). The SCImago database was used for the analysis of country rank, journal rank and H-index in the Republic of Macedonia and other former Yugoslav countries (Slovenia, Croatia, Serbia, Bosnia and Herzegovina, and Montenegro) for the period of 1996-2008, as they are presented in the Scopus database. Of a total number of 222 countries for the period of 1996-2008, the Republic of Macedonia, with an H-index of 20, is placed at 118th position, the percentage of citable documents in the field of medicine is 88.92%, and the percentage of relative production of documents in the world is below 0.01. In 2008, the Macedonian biomedical journal Prilozi was ranked 2484th with 0.048 SJR citable documents in the last three years. The field of nephrology in Macedonia had the highest H-index of 10 for the period between 2007 and 2008, this is followed by medicine (miscellaneous), with H-index of 7; hematology and endocrinology, diabetes and metabolism, with H-index of 6; and finally, transplantation, oncology and pathology, and forensic medicine, with H-index of 5. There is only one Macedonian biomedical journal (Prilozi, Macedonian Academy of Sciences and Arts, Section of Biological and Medical Sciences) included in the Scopus database for the period between 1996 and 2008. This might be due

to error, as it is listed among the journals as being from Serbia, instead of from the Republic of Macedonia. The primary task of the Editorial Boards of other Macedonian medical journals is to include their journals in the Scopus database (7).

We also performed an analysis of h-index for the full members of the Brazilian Academy of Sciences (BAS). We then determined the h-index of 402 members listed in 10 distinct categories by the BAS, cross-checked with the curriculum vitae of each of the members listed on the Plataforma Lattes database (CVL), and then compared these with each other. Despite the large production, mostly in journals without an impact factor, the h-indexes among the BAS members are comparatively low and show a large variation in all of the 10 categories, particularly in biomedical and physical sciences. The highest average of h-index values was found in biomedical, health and chemical sciences; the lowest values were found in human sciences where this index is meaningless (8).

A search of the Scopus database was performed on February 23, 2013 in order to identify published papers from the field of medical sciences affiliated with Macedonia. A total number of 967 articles were selected for analysis and the h-index was calculated for these documents. The papers were published in a total of 160 journals. The largest number of papers were published in domestic journals. The published papers have been cited 4380 times (mean citation of 4.5 per paper) with a Hirsh index (h-index) value of 27 (9).

An affiliation search of the Scopus database was performed on November 23, 2014 in order to identify published papers from the Ss Cyril and Methodius University of Skopje (UC&M), Republic of Macedonia. A total number of 3960 articles were selected for analysis (1960-2014). The largest number of papers were published in the Macedonian Journal of Medical Sciences, Journal of Molecular Structure, Lecture Notes in Computer Science, Acta Pharmecutica, and Macedonian Journal of Chemistry and Chemical Engineering. The first three places at the top ten authors belong to Dimirovski GM, Gavrilovska L, and Gusev M. Top three places based on the Scopus h-index (total number of published papers) belong to Kocarev L, Stafilov T, and Polenakovic M. Most papers originate from UC&M, but a significant number of papers are affiliated with the Faculty of Medicine, Faculty of Pharmacy, and Institute of Chemistry as members of UC&M, as well as the Macedonian Academy of Sciences and Arts. Articles

are the most dominant type of documents followed by conference papers, and review articles. Medicine is the most represented subject (4).

The country rankings of Macedonia were analyzed with SCImago Country & Journal Rank (SJR) for subject area of medicine in the years of 1996-2013 and then ordered by H-index value. Medicine in the Republic of Macedonia, according to the SCImago Journal & Country Rank (SJR), is 110th in the world, and 17th in Eastern Europe. Of the 20 universities in Macedonia, only Ss Cyril and Methodius University, Skopje, and the University St. Clement of Ohrid, Bitola, are listed in the SCImago Institutions Rankings (SIR) for 2013. An exceedingly small number of Macedonian scholarly journals is included in the Web of Sciences (2), PubMed (1), PubMed Central (1), SCOPUS (6), SCImago (6), and Google Scholar metrics (6). The Hirsh index (h-index) ranking was different from the rank of number of abstracts indexed in PubMed for the top 20 authors from Macedonia (10).

The analysis in Bosnia and Herzegovina showed a significant correlation between the Academy and the country of origin of the academician. AMNuBiH and ANUBiH mainly represent academics from Bosnia and Herzegovina, while in ANURS 71.4% of the members are academics with a background from Serbia. There is no significant correlation between the observed parameters (Scopus parameters–number of papers, H-index, number of citations) according to membership in Academies. By analyzing the correlation between the country of residence, the number of papers, H-index and the number of citations, the correlation appears significant between the state and the number of papers, but not significant in the other two observed parameters. We concluded that criteria for admission into the main academic communities are highly questionable. Progress in the academic hierarchy must be more stringent, and the criteria must be set to the highest possible level, as this is the only path which leads to progress (11).

The African Academy of Sciences (AAS) is the preeminent science academy on the African continent. The study investigated the bibliometric parameters of the AAS medical and health sciences fellows was published (12). The demographic information of the 80 medical and health sciences fellows were obtained from the AAS website. Subsequently, the bibliometric information (total number of publications, H-index scores, citation, and co-authorship counts) were extracted from the Scopus database. Most of

the fellows were from the Eastern (36%) and Western (33%) African regions; the Northern (6%) and Central (4%) regions were vastly underrepresented. Although only 34% of the AAS fellows were women, there was no statistically significant difference in the bibliometric parameters for both genders. The year of induction as a fellow and region of employment in Africa significantly influenced the bibliometric parameters. The fellows from the West African region had the highest number of publications, citations, and co-authorship count, and the South African fellows had the highest H-index score. The data presented provide insight into the biblio-metric productivity of African scientists compared with their peers from other science academies around the world. Similarly, the data may assist burgeoning scientists aspiring to be an AAS fellow in setting realistic goals toward achieving the stipulated H-index benchmarks (12).

Determination of the current state of international scientific cooperation in the context of the scholarly research periodicals of the National Academy of Sciences of Ukraine in the world of scientometric databases was published. The information framework of the research includes the legislative acts, resolutions of the Cabinet of Ministers of Ukraine, and the orders of the Ministry of Education and Science of Ukraine. This analysis included paper versions of the journals of the National Academy of Sciences of Ukraine and their websites, scholarly research periodicals of Ukraine by the Vernadsky National Library of Ukraine, Web of Science, SCOPUS, Google Scholar, Index Copernicus, Open Access Journals (DOAJ), and ERIH PLUS. The introduction of scholarly research periodicals to the international abstract and scientometric databases and their actual state has been described. The introduction of information technology, access to databases and the stimulation of their development of magazines open up ways to increase (intensify) international scientific cooperation in the scientific domain by broadening the presentation of the results of research activity in scholarly research periodicals (13).

We have to say that the academics working in the fields of literature, arts, and music are examined differently with other quality indexes and cannot therefore be compared with the identical indexes of other fields.

There are several limitations of this investigation. There are several authors included with affiliation in the Macedonian Academy of Sciences and Arts, but they work outside of the academy. Affiliation in the Macedo-

nian Academy of Sciences and Arts is for the academics as well for scientific collaborators in different centers of the Macedonian Academy of Sciences and Arts. Thus, all results are cumulative from all academics plus their collaborators, not only from the academics. In the future, a differentiation between the academics and other employees at the Macedonian Academy of Sciences and Arts should be made, an investigation of other databases, as well as a comparison with other similar institutions.

In conclusion, we can say that there is more academic publishing productivity on behalf of the authors affiliated with MASA (around 2.6 times) than by authors affiliated with SS Cyril and Methodius University in Skopje.

REFERENCES

1. http://manu.edu.mk/en/home-2/%d0%be%d1%81%d0%bd%d0%be%d0%b2%d0%b0%d1%9a%d0%b5/ [Accessed July 05, 2021]
2. http://manu.edu.mk/en/home-2/%d0%bc%d0%b8%d1%81%d0%b8%d1%98%d0%b0/ [Accessed July 05, 2021]
3. http://manu.edu.mk/wp-content/uploads/2017/05/%D0%A1%D1%82%D0%B0%D1%82%D1%83%D1%82-%D0%BD%D0%B0-%D0%9C%D0%90%D0%9D%D0%A3-2.pdf [Accessed July 05, 2021]
4. Spiroski M. Current Scientific Impact of Ss Cyril and Methodius University of Skopje, Republic of Macedonia in the Scopus Database (1960-2014). Open Access Maced J Med Sci. 2015 Mar 15; 3(1): 1-6.
5. Sasvári P, Urbanovics A. The journals on the domestic lists of the ix section of the Hungarian academy of sciences in light of the requirements of international journal selection. Pénzügyi Szemle = Public Finance Quarterly. 2019; 64(3): 369–92.
6. Mugnaini R, Packer A, Meneghini R. Comparison of scientists of the Brazilian Academy of Sciences and of the National Academy of Sciences of the USA on the basis of the h-index. Brazilian Journal of Medical and Biological Research. 2008; 41(4): 258-262.
7. Spiroski M. Country rank, journal rank and H-index in the field of Medicine in the Republic of Macedonia (1996-2008) using data from Scimago. Macedonian Journal of Medical Sciences. 2010 Jun 15; 3(2): 99-108.

8. Kellner AW, Ponciano LC. H-index in the Brazilian Academy of Sciences: comments and concerns. Anais da Academia Brasileira de Ciências. 2008; 80: 771-81.
9. Spiroski M. Analysis of Macedonian Medical Scientific Papers in the Scopus Database. Macedonian Journal of Medical Sciences. 2013 Mar 1; 6(1): 5-10.
10. Spiroski M. Current biomedical scientific impact (2013) of institutions, academic journals and researchers in the Republic of Macedonia. Prilozi. 2015 Mar 1; 35(3): 67-80.
11. Masic I. Evaluation of the Medical Academic community of Bosnia and Herzegovina Based on Scopus Parameters. Medical Archives. 2017; 71(3): 164.
12. Balogun J, Mamuzo E, Okonofua F, Balogun A, Oyeyemi A. Bibliometric profile of the African Academy of Sciences medical and health sciences fellows. The Pan African Medical Journal. 2021; 38.
13. Sivova TYu. International Scientific Cooperation of Ukraine: Periodical Publications of the National Academy of Sciences of Ukraine in Scientific Bases. Sci Innov. 2020; 16(3): 14-24.

doi: 10.20903/zpopov-2022-0033 UDK: 338.124.2:[616.98:578.834}-036.21(100)"2019/2021"

Таки ФИТИ*

ОПШТЕСТВЕНИ ТРОШОЦИ ОД ЕПИДЕМИИ И ПАНДЕМИИ

Кратка историја на епидемиите и пандемиите

Од најстари времиња, па сè до денешни дни, во светот се јавиле голем број епидемични болести (чума, маларија, туберкулоза, колера, жолта треска, мали сипаници, инфлуенца, сифилис и др.) кои одзеле милиони човечки животи. Се разбира, сите споменати епидемични болести немаат потенцијал да продуцираат пандемии[1]. Таков потенцијал поседуваат чумата, колерата и болестите на инфлуенца. „Од античко време до сега, светот поминал низ повеќе од десет пандемии на инфлуенца, со три чуми, седум колери и пандемијата низ којашто проаѓаме денес" (Kiliç 2020: 15).

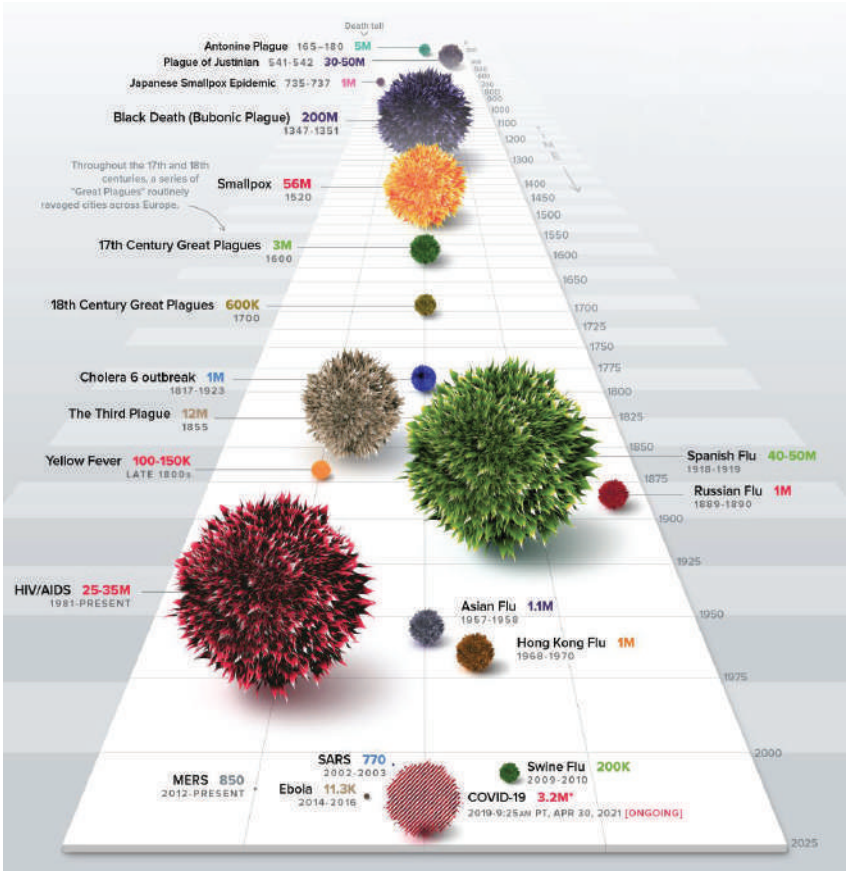
Постои широко распространето верување дека голем број епидемични болести, кои се појавиле од најстари времиња, потекнуваат од Египет, поради неговата специфична позиција на подрачје низ кое минувале значајни трговски рути и подрачје кое нуди поволни услови за избувнување и ширење на болестите.

Сликата што ја даваме во прилог претставува **визуелна илустрација (по хронолошки редослед) на пандемиите во светот** – почнувајќи од антонинската (Галеновата) чума, па сè до пандемијата предизвикана од корона-вирусот (ковид-19).

---

* Редовен професор на Универзитетот „Св Кирил и Методиј" во Скопје, Економски факултет – Скопје и редовен член на МАНУ; fiti@manu.edu.mk

[1] Светската здравствена организација ја дефинира пандемијата како „Ширење на болеста, инфекцијата или на поврзаните здравствени проблеми во рамките на голем број земји или континенти, или во пошироки области, како што е светот во целина, погодувајќи значаен дел од населението". *(The Assessment Report on COVID -19 Global Outbreak*, Turkish Academy of Sciences (TUBA), Ankara, July 2020, p. 19).
Извор: www.visualcapitalist.com



Од илустрацијата се гледа дека најкатастрофални последици предизвикала чумата. Професорот Орхан К'л'ч (Orhan Kılıç), од Универзитетот „Фират" во Турција, истакнува дека за чумата, во исламскиот свет се употребуваат термините ***waba*** и ***ta'un,*** а во западниот свет, термините ***Black Death (црна смрт), Black Plague (црна чума),*** односно термините ***plague, peste, pestik,*** итн., во други јазици. Термините во западниот свет потекнуваат од инфекцискатa бактерија ***Yersinia pestis.*** Чумата, од времето на византискиот император Јустинијан (527 – 565), па до почетокот на XX век, се манифестирала во три големи бранови. Првиот голем бран на чумата е познат како Јустинијанова чума. Според некои извори, чумата потекнува од Египет, а според други, од Азија. Како и да е, таа силно се манифестирала во

Северна Африка, Европа и во Централна и Јужна Азија. Во рамките на овие региони, т. е. во нивните најпогодени делови, таа уништила над 50 % од популацијата. Вториот бран, наречен „Црна смрт“, се појавува во Европа, Средниот Исток, Анатолија, Азија и во Северна Африка, при што најпогодени делови биле Египет, Сирија и Анатолија. Третиот бран почнал во Индија, во 1868 година, предизвикувајќи смрт на 10 милиони Индијци до 1918 година, односно 13 милиони до 1948 година (Kılıç 2020).

Во текот на XX век, најсмртоносните пандемии се поврзани со шпанскиот грип и со сидата. Шпанскиот грип (1918 – 1919), во период од неполни осум месеци, убил милиони луѓе – проценитe за жртвите од оваа епидемија се движат од 40 до 50 милиони. Во секој случај, шпанскиот грип предизвикал поголем број човечки жртви отколку војната (Првата светска војна). Големата смртност во случајот на шпанскиот грип (и пошироко во случајот на епидемиите и пандемиите кои му претходеле) се објаснува со дејството на повеќе фактори: неразвиеноста на медицинската наука (во тоа време, лекарите знаеле дека постојат вируси, но нивните знаења за потеклото на вирусите, за нивната природа, за вакцините и сл., отсуствувале или биле сосема ограничени); голем број од заболените, во услови на непостоење на антибиотици, умирале од секундарни инфекции; условите за живеење биле мизерни и дополнително влошени од крвавата војна (Првата светска војна) поради што вирусот брзо се ширел меѓу војниците, трасите и регионите низ кои минувала војската (види: Robson 2018). Сидата, од 1981 година до денес, уби повеќе од 30 милиони луѓе. Вакцина за сидата сè уште не е пронајдена, но заболените од сида, денес, имаат пристап до прилично ефикасна антивирусна терапија, која дејствува врз забавувањето, па дури и запирањето на напредокот на болеста која го напаѓа имунолошкиот систем на луѓето.

Во XXI век се јавиле следниве епидемии: САРС – тежок акутен респираторен синдром (2002 – 2003), птичји грип (2003/2004 година), свински грип (2009 – 2010), МЕРС – Блискоисточен респираторен синдром – коробана-вирус (2012) и ебола (2013 – 2016). Иако некои од овие епидемии имаат висока смртност (смртноста кај еболата и мерсот е околу 40 %), вкупниот број жртви од овие болести е многу помал во споредба со епидемијата од ковид-19 која досега однесе во смрт над 3 милиони луѓе.

Економските импликации од пандемијата од ковид-19во глобални рамки

На преодот меѓу 2019 и 2020 година, се појави пандемијата детерминирана од корона-вирусот која резултираше со вонредно силна глобална и високосинхронизирана рецесија која ги зафати сите современи економии. Оваа рецесија влегува во редот на трите најголеми економски и финансиски кризи во долгата светска историја на бизнис-циклусите – Големата депресија од 1930-тите години, Големата рецесија 2007 – 2009 година и актуелната криза условена од ковид-19 (The Great Depression, The Great Recession и The Great Lockdown).[2] Кризата од ковид-19 има чудна, невообичаен природа, и многу е поразлична од претходните две големи кризи, пред сè, поради фактот што е детерминирана од фактори од неекономска природа – пандемија предизвикана од опасен вирус. Кризата најпрвин услови силен шок на *страната на агрегатната понуда* – поради потребата од физичка и социјална дистанца, тоа доведе до затворање и престанување со работа на цели економски сектори: туризам и угостителство, авионска индустрија, јавен транспорт и такси-услуги, делови на преработувачката индустрија и сл., затворање на образовните институции, затворање на институциите од областа на културата, намалена економска активност во речиси сите други сектори, а ги прекина трговските врски меѓу земјите, меѓу регионалните и глобалните синџири на понуда. Шокот на страната на агрегатната понуда, симултано и брзо се пренесе на страната на *агрегатната побарувачка*, и предизвика намалување на побарувачката и на потрошувачката на финални добра и услуги во секторот на домаќинствата – од храна и облека, преку трајни потрошни добра (станови, автомобили, апарати за домаќинство и сл.), до многу различни услуги – образовни, туристички, рекреативни, културолошки и сл. Шокот на страната на агрегатната побарувачка резултираше и со силен пад на инвестициската активност на бизнис-секторот – нама-

[2] За економските импликации од трите големи кризи, во светски размери, во одделни региони и групи на земји и во Република Северна Македонија, заинтересираните ги упатувам на мојот труд *Макроекономија на големите кризи,* изд. Македонска академија на науките и уметностите, Скопје 2020.

лување на вложувањата во фабрички згради и деловни простори, во машини, опрема, инсталации и во човечки капитал. Така, пандемијата ги втурна современите економии во тешка рецесија којашто, според професорот Кенет Рогоф, најверојатно, ќе го услови најсилниот краткорочен пад на економската активност во историјата на бизнис-циклусите во последниве 150 години (види: Kose and Ohnsorge 2019).

Економските загуби од кризата условена од ковид-19, во глобални рамки, ќе ги проследиме преку *падот на бруто-домашниот производ* (БДП), како најсинтетички показател на вкупната економска активност, преку *изгубените работни часови* (пораст на невработеноста) и преку *дисторзиите во јавните финансии* (пораст на буџетските дефицити и јавните долгови).

Загуби на БДП – бруто-домашен производ (збирот на вредноста на вкупнопроизведените финални добра и услуги на една земја во текот на една година) е најсинтетички показател за вкупната економска активност. Шокот на страната на агрегатната понуда, кој, овој пат, многу брзо се пренесе и на страната на агрегатната побарувачка, ја намалија економската активност во светски размери и особено во најпогодените земји од пандемијата уште во првиот квартал на 2020 година. Голем пад на економската активност се случи во вториот квартал на 2020 година. Меѓународните финансиски институции (ММФ и Светска банка), други проминентни истражувачки институции (Виенскиот институт, Секторот за проектирање на економската активност на *The Economist*, Европската комисија и др., како и многубројни национални институции, излегоа со прогнози за идната економска активност во светот, по региони и по земји, а владите и централните банки со широка палета на мерки за спас на економиите, т. е. за ублажување на негативните последици од пандемијата врз економиите. На економистите и на експертите за проектирање на економската активност во светот, најверојатно, никогаш не им било потешко, отколку што е тоа случај денес, да излезат со релативно издржани проекции за идниот економски раст. Тоа е така, бидејќи станува збор за многу специфична криза, која носи со себе многубројни неизвесности и ризици. Економистите, уште од пред 100 години (од времето на славните економисти

Френк Најт и Џон Мејнард Кејнз) знаат дека ризиците може, барем до некаде, и да се предвидат и да се квантификуваат, но тоа не е случај со неизвесноста (Фити 2011). Оттука, и прогнозите за движењето на БДП во различни земји во светот се различни и крајно неизвесни, а зависат од претпоставките што се вградени во моделите. Сите прогнози поаѓаа од две различни сценарија, главно, условени од должината на траењето на пандемијата и од опфатот и ефикасноста на мерките на владите за надминување на кризата. Фактички, честата, многу брза промена на предвидувањата на импликациите од кризата предизвикана од ковид-19 врз вкупната економска активност и врз другите макроекономски агрегати, во глобални рамки, во рамките на регионите и во рамките на одделни економии, стана една од препознатливите карактеристики на кризата со ковид-19. Толку брзи промени, ревидирања и актуализирање на проекциите не се случиле ниту во една претходна глобална рецесија од Втората светска војна досега.

Во **април 2020 година**, Меѓународниот монетарен фонд прогнозираше пад на светскиот БДП во 2020 година, во однос на 2019 година, од –3,0 %, на БДП на групата развиени земји од –6,1 %, на БДП на еврозоната од –7,5 %, на БДП на САД од –5,9 %, на БДП на Германија од –7,0 %, на БДП на Јапонија од –5,2 %, на БДП на Кина раст од само 1,2 %, итн. (*IMF* 2020a: 4/5). Прогнозите на ММФ за последиците од кризата врз економиите во светски размери, претрпеа значителни корекции, главно, надолу, во текот на **јуни 2020 година,** откако стана јасно дека очекувањата за намалувањето на силината на епидемијата во втората половина на 2020 година се нереални. Понатаму, **во јануари 2021 година,** следи нова проекција на IMF за светскиот економски раст по региони и по одделни земји. Појавата на вакцините против корона-вирусот ги зголеми надежите за побрзо надминување на пандемијата и забрзување на растот на економиите. Меѓутоа, појавата на новите мутации на вирусот, како и неможноста на производителите на вакцини навремено да одговорат на зголемената побарувачка за вакцини, повторно ја врати неизвесноста во проекциите на идниот економски раст.

Табела 1

Одразот на пандемијата од ковид-19 врз светскиот економски раст
– по региони и по одделни земји –
Проекции на IMF, јуни 2020 и јануари 2021

	Проекции на IMF, јуни 2020		**Проекции на IMF, јануари 2021**	
	2020	**2021**	**2020**	**2021**
Свет	**– 4,9**	**5,4**	**– 3,5**	**5,5**
Развиени земји	**– 8,0**	**4,5**	**– 4,9**	**4,3**
САД	– 8,0	4,5	– 3,4	5,1
Еврозона	– 10,2	6,0	– 7,2	4,2
Германија	– 7,8	5,4	– 5,4	3,5
Франција	– 12,5	7,3	– 9,0	5,5
Италија	– 12,8	6,3	– 9,2	3,0
Шпанија	– 12,8	6,3	– 11,1	5,9
Јапонија	– 5,8	2,4	– 5,1	3,1
Велика Британија	– 10,2	6,3	– 10,0	4,5
Канада	– 8,4	4,9	– 5,5	3,6
Други развиени економии	– 4,8	4,2	– 2,5	3,6
Земји во подем и земји во развој	**– 3,0**	**5,9**	**– 2,4**	**6,3**
Азиски земји во подем и во развој	– 0,8	7,4	– 1,1	8,3
Кина	1,0	8,2	2,3	8,1
Индија	– 4,5	6,0	– 8,0	11,5
АСЕАН 5	– 2,0	6,2	– 3,7	5,2
Европски земји во подем и во развој	– 5,8	4,3	– 2,8	4,0
Русија	– 6,6	4,1	– 3,6	3,0
Латинска Америка и Кариби	– 9,4	3,7	– 7,4	4,1
Бразил	– 9,1	3,6	– 4,5	3,6
Мексико	– 10,5	3,3	– 8,5	4,3
Среден Исток и Централна Азија	– 4,7	3,3	– 3,2	3,0
Саудиска Арабија	– 6,8	3,1	– 3,9	2,6
Супсахарска Африка	– 3,2	3,4	– 2,6	3,2
Нигерија	– 5,4	2,6	– 3,2	1,5
Јужна Африка	– 8,0	3,5	– 7,5	2,8
Земји во развој со низок доход	– 1,0	5,2	– 0,8	5,1

Извор/Source: IMF (2020c), *World Economic Outlook, Update, June* 2020, p. 7 and IM F (2021), *World Economic Outlook, Update, January* 2021, p. 4.

Ревидираните прогнози на **Светска банка за глобалниот економски раст** предвидуваат стапка на раст на светскиот БДП за 2020 година, од – 4,3 %, стапка на раст на развиените земји од – 5,4 %, на САД од – 3,6 %, на еврозоната од – 7,4 %, на Јапонија од – 5,3 % итн. (World Bank Group 2021a: 4).

Како и да е, сега станува извесно дека најголемите и најсилните економии во светот, со големо учество во светската продукција, во светскиот доход и во светската трговија, во 2020 година, ќе забележат силен пад на економската активност, што фактички се сведува на енормно големи загуби на БДП кои се мерат во илјади милијарди долари.[3]

Загуби на работни часови – последиците од кризата предизвикана од пандемијата од корона-вирусот **врз пазарот на труд с**е крајно неповолни. Проценитe на Меѓународната организација на трудот (ILO), во мај 2020 година, предвидуваа дека 94 % од работниците во светот живеат во земји во кои се применуваат одредени видови мерки на затворање на работните места. Износот на изгубени работни часови во светот, во вториот квартал на 2020 година, во однос на четвртиот квартал на 2019 година, ILO го проценуваше на 10,7 %, што е еквивалент на 305 милиони работни места со полно работно време (ILO 2020, Monitor, 27 May).

Поновите процени на ILO за изгубените работни часови во 2020 година, во светски размери, и во третиот и во четвртиот квартал на 2020 година, релативно во однос на четвртиот квартал на 2019 година, се поместени во следнава табела:

[3] Иако Кина, како втора најсилна економија во светот, ќе забележи економски раст од 1 % до 2 %, таа, исто така, ќе изгуби енормно висок износ на БДП ако се има предвид фактот дека во преткризниот период бележеше многу високи стапки на раст (14,2 % во 2007 година, просечни стапки на раст од околу 9,5 % во периодот 2008 – 2011 и просечни стапки на раст од околу 7 % во периодот 2012 – 2018 година) (data.worldbank.org).

Табела 2

Изгубени работни часови во светот во целина
и по доходовни групи земји

Групи земји	Вкупно 2020	Трет квартал на 2020	Четврт квартал на 2020
Свет	**8,8**	**7,2**	**4,6**
Земји со низок доход	**6,7**	**7,6**	**3,3**
Земји со средно низок доход	**11,3**	**9,3**	**4,5**
Земји со средно висок доход	**7,3**	**5,6**	**3,9**
Земји со висок доход	**8,3**	**7,3**	**7,0**

Извор: ILO (2021) Monitor: COVID – 19 and the world work, Seventh edition, 25 January, p. 6.

Во Извештаите на Меѓународната организација на труд (ILO), посебно се истакнува дека кризата од ковид-19 најсилно ја погодува младата популација (нивните работни места и нивниот доход) на возраст од 15 до 24 години, па и оние до 30 години, поради неколку причини: затворање на образовните институции и намалени можности за едукација и тренинг, голема застапеност на младите работници во најпогодените сектори на економијата, пониски примања на младите работници во однос на повозрасните, потешко вработување на младите и во нормални услови и сл.

Невработеноста е секогаш проциклична категорија и таа, во услови на рецесија, се зголемува со опаѓањето на производството, но редовно со одредено задоцнување (time lag). Кај овој тип криза, невработеноста расте многу брзо, т. е. задоцнувањето (time lag) е пократок. Побрзиот пораст на невработеноста за време на актуелнава криза најдиректно е поврзан со основната разлика во импликациите на двете кризи врз пазарот на труд. Според професорот Лари Самерс (Larry Summers), додека за време на Големата рецесија од 2007 до 2009 година, економиите беа соочени со *перманентно губење на работни места*, за време на Големиот пад на економиите (Great Shutdown), условен од пандемијата од корона-вирусот, економиите се соочени не

со перманентно туку со *привремено отпуштање од работа* (gregmankiw.blogspot.com: Larry Summers Interview, May 8, 2020). Ваквиот заклучок е валиден само под претпоставка дека бизнис-циклусот ќе ја добие траекторијата на латиничната буква В (V) (брзо опаѓање на економиите и нивно брзо заздравување). Но, доколу рецесијата се пролонгира (поради новите мутации на вирусот, отежнатиот пристап до вакцини на помалку развиените земји и сл.), последиците врз пазарот на труд ќе бидат понеповолни. Од друга страна, според моето мислење, во овие анализи не смее да се запостави и ефектот на т.н. хистереза (hysteresis) – високата невработеност денес условува висока невработеност во иднина, висок пад на БДП денес условува пониски стапки на економски раст во иднина) – кој, во крајна линија, продуцира подолгорочни негативни импликации на пазарот на труд и ја потенцира т.н. секуларна стагнација (опаѓање на стапките на економскиот раст на долг рок) (Phelps 2006; Blanchard 2018; Cerra and all 2020; Фити 2020).

Сериозни нарушувања на јавните финансии – со оглед на тоа што сите влади во засегнатите земји се определија за нагласено експанзивна фискална и експанзивна монетарна политика за надминување на кризата, се очекува силен пораст на буџетските дефицити и кумулирање на јавниот долг во одделните економии и во светската економија во целина. Така, сега станува јасно дека оваа криза ќе им создаде проблеми на сите земји, посебно на оние кои го изгубија фискалниот простор – веќе кумулираа висок јавен долг во претходниот период и особено при надминувањето на Големата рецесија од 2007 до 2009 година. Експертите на IMF, во април 2020 година (IMF 2020b: 5), прогнозираа дека во 2020 година, ќе се случи енормен пораст на буџетските дефицити по земји, региони и во светски размери, пораст без преседан во досегашната светска економска историја. Имено, буџетските дефицити (искажани преку показателот General Government Fiscal Balance, како процентуално учество во БДП) во 2020 година, во однос на 2019 година, во светски размери, ќе се зголемат за дополнителни 6,2 процентни поени – од – 3,7 % на – 9,9 %. Во групата развиени земји, во истиот период, буџетските дефицити дополнително ќе се зголемат за 7,7 %, во еврозоната за 6,8 %, во земјите во подем и во развој за 4,3 процентни поени и во земјите во развој со низок доход за 1,6 % процентни поени. Се разбира дека кумулирањето на буџетските дефицити ќе донесе нов, вонредно брз и голем пораст на јавниот долг.

Според проекциите на ММФ, во 2020 година, јавниот долг во светот, во однос на претходната, 2019 година, ќе се зголеми за дополнителни 14 процентни поени и ќе достигне износ од 96,4 % како процент во светскиот БДП. Во истиот период, во групата развиени земји, долгот ќе забележи пораст од дополнителни 17 процентни поени и ќе достигне учество од 122,4 % во БДП, во економиите со среден доход ќе се зголеми за дополнителни 9 процентни поени и ќе достигне учество од 62 % во нивниот БДП, итн. (IMF 2020b: 6). Фискалната поддршка на економиите погодени од пандемијата од ковид-19 продолжи и се засили во втората половина на 2020 година. Во развиените земји (на пример, во САД, ЕУ, Јапонија, Канада, Велика Британија и во други земји), во текот на 2020 година, беа предвидени дополнителни пакети за фискална поддршка, а тоа се случи и во Кина, Индија, Бразил итн.

Поновите прогнози на ММФ упатуваат на уште побрз раст на буџетските дефицити и на јавниот долг во светски размери и по региони и земји (види: IMF, Fiscal Monitor 2021).

Енормното зголемување на буџетските дефицити и на јавниот долг, ги соочува економиите со директни и индуцирани загуби и со проблеми во одржливоста на јавните финансии поради многубројни резони: големите буџетски дефицити значат негативно национално штедење; отплатата на огромните долгови во иднина паѓа на товар на генерациите што доаѓаат; големите јавни долгови го истиснуваат приватниот сектор од економската активност (т.н. crowding-out ефект) и го забавуваат економскиот раст во иднина. Понатаму, значајните потенцијални загуби на економиите од постоењето на високите буџетски дефицити и на високите долгови се поврзани и со изгубениот фискален простор на економиите и со неможноста политиките (фискалната и монетарната) успешно да се изборат со евентуална појава на нови длабоки рецесии во иднина. Во овој контекст не треба да се забораваат и ризиците од „проработување“ на инфлацијата и зголемување на каматните стапки во иднина. Дури и мало зголемување на каматните стапки на меѓународниот финансиски пазар, може да биде проследено со сериозни нарушувања на јавните финансии во одделни земји, групи земји и во светски размери. Иако се проценува дека овој тип ризик (од зголемување на инфлацијата и на каматните стапки) засега е мал, тој не смее да се потцени. На крајот, треба да се има предвид дека по завршувањето на актуелнава криза во најголемиот број земји во светот ќе

мора да се отвори процесот на фискална консолидација, т. е. на сведување на буџетските дефицити и јавните долгови на „нормално“ ниво. Овој процес е долгорочен, мачен, поврзан со периоди на реверзибилност, со опасност од зголемување на даночното оптоварување на граѓаните и претпријатијата во иднина итн. (Fiti and Tashevska 2012).

Можно ли е да се пресметаат општествените трошоци од ковид-19?

Калкулацијата на вкупните општествени трошоци од големите епидемии и пандемии воопшто не е едноставна работа.

Економистите, при утврдувањето на *економските импликации* од пандемиите, најчесто ги калкулираат трошоците поврзани со губењето на БДП и зголемената невработеност. Меѓутоа, овие пристапи ги запоставуваат трошоците на општествата и на економиите поврзани со загубите на човечките животи (Fan, Jamison and Summers 2018). Моето мислење е дека, ако направиме аналогија со начинот на кој економистите ги пресметуваат вкупните трошоци од постоењето на висока невработеност, можеме да дефинираме пристап за утврдување на вкупните општествени трошоци од појавата на големи епидемии и пандемии. Имено, синтетички гледано, вкупните трошоци од високата невработеност опфаќаат две категории (групи) на трошоци: економски трошоци и социјални трошоци. Економските трошоци од невработеност, економистите ги калкулираат преку Окуновиот закон, кој упатува на релацијата според која секое 2-процентно намалување на актуелниот БДП под потенцијалниот, ја зголемува стапката на невработеност за 1 процентен поен. Според тоа, ако претпоставиме дека стапката на невработеност во една економија изнесувала 6 % и дека, поради појавата на рецесија, актуелниот БДП во однос на потенцијалниот БДП се намалил за 2 %, стапката на невработеност ќе се зголеми од 6 % на 7 %. Притоа, односот 2:1 не е фиксен, туку е различен од земја до земја, па дури и во иста земја, во различни периоди, и се движи во опсег меѓу 2:1 до 3:1. Доколку релацијата на која упатува Окуновиот закон (2:1) се чита во обратна насока, тогаш уште појасно се согледуваат загубите во БДП од зголемувањето на невработеноста – порастот на невработеноста за 1 процентен поен предизвикува намалување на БДП за 2 %. Во контекстот на економските трошоци од невработеност можат да се

третираат и трошоците на буџетот – невработеноста му носи загуби на буџетот по две основи: од една страна, невработените не плаќаат даноци и социјални придонеси, а од друга страна, државата на невработените им исплатува социјални трансфери.

За да се пресметаат вкупните општествени трошоци од пандемиите мора во калкулациите да се вклучат и *социјалните трошоци.* Оваа криза покажа, повеќе отколку во случајот на претходната голема криза – Големата рецесија 2007 – 2009, дека станува збор за ***вистинска општествена криза***, бидејќи испорачува тешки последици не само за економиите (за финансискиот и реалниот сектор на економиите) туку и за здравствениот сектор, за образованието, науката, културата итн., а е проследена и со сериозни психолошки последици – страв од заразата од смртоносниот вирус кај сите групи на популацијата, неспокојство и нервоза предизвикана од самоизолацијата на луѓето, прекинување на комуникациите и дружењето на луѓето и суштински нарушувања на природниот постулат, одамна потенциран од страна на Аристотел, дека човекот, како мисловно суштество, е *zoon politikon*, кое живее и комуницира во заедница со други луѓе. Социјалните трошоци имаат широк опфат и се тешко мерливи. Во еден свој дел, тие се поврзани со хуманите и психолошките последици што ги трпат луѓето кои се невработени или го изгубиле своето работно место – чувство на пониженост и инфериорност, депресии, разни видови заболувања, разводи, влегување во матните води на криминалот, дрогата, проституцијата и сл. Но, негативните социјални импликации од кризата не произлегуваат само од брзото затворање на работни места и порастот на невработеноста, иако токму порастот на невработеноста е фактор со најголемо значење за социјалните последици од кризата. Имено, зголемувањето на невработеноста ги намалува доходите на луѓето, ги зголемува нееднаквостите во распределбата на доходот, посебно во помалку развиените земји, и се заканува, во светски размери, дополнително да втурне во екстремна сиромаштија 40 до 60 милиони луѓе. Понатаму, крајно неповолни социјални последици од кризата произлегуваат и од затворањето на огромен број образовни институции насекаде во светот. За неполни три месеци (февруари, март и април), речиси 1,6 милијарди ученици и студенти во светот беа засегнати од затворањето на предучилишните установи, основните училишта, средните училишта и високообразовните институции. Затворањето на образовните институции, заедно со

изгубените човечки животи, имаат силни негативни импликации и за човековиот и социјалниот капитал. Кризата предизвикува и мноштво други неповолни социјални и хуманитарни импликации: загрозување на човечките права, какви што се правото на живот и здравствена заштита (се случуваат кога здравствените системи немаат доволен капацитет да ги третираат сите заболени лица, при што доаѓа и до дискриминација на болните по различни основи), загрозување на правото на слобода на говорот, дискриминација и отсуство на услови за третман на ранливите категории од популацијата (лица со инвалидитет, лица со ретки болести, сиромашни лица и сл.), пораст на насилството во рамките на семејството (особено над жените и девојчињата) итн.[4]

Оттука, вкупните општествени трошоци од појавата на епидемии и пандемии се еднакви на **економските трошоци** и на **социјалните трошоци** што тие ги предизвикуваат.

Во ***економските трошоци од пандемиите***, три позиции се посебно значајни:

- Загубите во аутпутот (БДП) во контекстот на аналитичката рамка што ја дава Окуновиот закон (Okun'law);
- Загубите на работни места и зголемувањето на невработеноста;
- Загубите на буџетот – зголемената невработеност во услови на рецесии детерминирани од пандемии, на буџетот му носи загуби по две основи: од една страна, невработените не плаќаат даноци и социјални придонеси, а од друга страна, државата им исплатува на невработените социјални трансфери. Постоењето на т.н. *трејд оф* меѓу буџетските алокации за здравство (спасување човечки животи) и буџетските алокации за спасување на економијата во време на пандемии, упатува и на постоењето на опортунитетен трошок – поголемите буџетски трошоци за здравството ги намалуваат буџетските алокации за спасување и поддршка на бизнисите;

Социјалните трошоци од пандемиите – овие трошоци треба да ги опфатат:

[4] Заинтересираните за овие аспекти на кризата ги упатувам на студијата на Комитетот за координација на статистичките активности при ООН, под наслов: *How Covid -19 is changing the World: a statistical perspectives,* CCSA, UN, 2020.

- Загубите поврзани со хуманитарните и психолошките последици, споменати погоре, кои ги трпат невработените и целото општество;
- Трошоците поврзани со зголемувачките диспаритети во распределбата на доходот и продлабочувањето на сиромаштијата;
- Трошоците од губењето човечки животи и нивните импликации врз човечкиот капитал. Нобеловците Пол Семјуелсон и Вилијам Нордхаус, повикувајќи се на студиите на еден од најголемите експерти во оваа област, Харви Бренер, ќе констатираат дека според неговите процени „...растот на невработеноста од само еден процентен поен за период од 6 години доведува до 37.000 случаи на прерана смрт во САД“, и понатаму дека според психолошките студии, губењето на работното место е „...еднакво трауматски настан како и смртта на близок пријател или неуспехот на училиште“ (Samuelson, Nordhaus 1992: 575). Изгубените човечки животи како последица на пандемиите имаат сериозни негативни импликации за економиите, главно, преку зголемените стапки на морталитет кои, на краток, среден и долг рок, влијаат врз понудата на трудот. Иако смртноста за време на епидемиите е повисока кај возрасните групи на популацијата (дел од нив веќе не се економски активни, односно се пензионери), пандемијата со ковид-19 покажа дека во групата со висок ризик од комплицирање на болеста и со нејзин фатален крај влегува популацијата над 60 години, па дури и лица од пониската старосна група, чиј состав опфаќа и високоедуцирани кадри, со експертски знаења и вештини во различни профили на занимања – членови на топ-менаџментот на корпорациите, инженери, лекари, научници итн., што од чисто економски аспект (се разбира, овде не ги потценувам хуманитарните аспекти на проблемот бидејќи човечкиот живот нема цена) упатува на губење животи од најкреативниот дел од работната сила. Овој факт ги зголемува загубите на економиите и на општествата во целата сфера на расположливиот човечки капитал. Тука треба да се имаат предвид и загубите во формирањето на човечкиот капитал, предизвикани од привременото затворање на образовните институции, во сите степени на образование, штети врзани за одвивање на наставата поради проблеми со слабата дигитали-

зација во земјите со среден и низок доход, прекинати и одложени специјализации и студиски престои во странство, проблеми во реализацијата на програмите за обука и тренинг на вработените во бизнис-секторот итн.

- Сето ова упатува на сложеноста на калкулацијата на социјалните трошоци од пандемиите – тие, речиси, и да не можат да се искажат во монетарна вредност, за разлика од економските трошоци кои мошне полесно можат да се квантификуваат и да се искажат во денари, евра, долари итн.

Наместо заклучок – лекции од кризата од ковид-19 за македонското општество

Република Северна Македонија, мерено според бројот на инфицирани случаи на 100.000 жители и според бројот на смртни случаи на 100.000 заболени, влегува во редот на земјите кои се силно погодени од пандемијата од ковид-19.[5]

[5] Траекторијата на пандемијата од ковид-19 во Република Северна Македонија помина низ три фази. Првата фаза, т. е. првиот бран на пандемијата се случи во периодот од март 2020 – до почетокот на октомври 2020 година. Во текот на март 2020 година, бројот на новоинфицирани случаи во Република Северна Македонија беше релативно мал и само во два наврата надмина 50 нови случаи на дневна основа. На 16 април, се случи еден поголем пик, со 107 нови случаи, а потоа бројот на новозаразени остана низок до крајот на месец мај. Тоа е период кога здравствените власти добро се бореа со пандемијата. Меѓутоа, со олабавувањето на рестрикциите и почетокот на работата на дел од бизнисите, и во Република Северна Македонија, како што тоа беше случај и со земјите во Регионот, а подоцна и со европските земји, дојде до засилување на бранот на новоинфицирани случаи. Во текот на јуни и првата половина на јули, бројот на новозаразените, на дневна основа, се одржуваше на трицифрен износ, со два поголеми пика, едниот на 5 јуни, кога бројот на новозаразени изнесуваше 179 лица, а другиот на 17 јуни, со 194 новозаразени случаи. И покрај ваквата епидемиолошка ситуација, Владата, во текот на јули, не воведе нови рестрикции, а фокусот на заштитата беше префрлен на одговорноста на граѓаните, со континуирани апели на здравствените власти за строго почитување на пропишаните мерки. До крајот на август, вкупниот број инфицирани надмина 14.000 лица, бројот на излечени надмина 11.000 лица, а бројот на починати се доближи до 600. Втората фаза почна на преодот од октомври/ноември 2020 и траеше до почетокот на март 2021 година. Особено брз раст на новоинфицирани лица (втор бран на пандемијата) е забележан во периодот од крајот на октомври 2020 година до средината на декември 2020, со два големи пика на новоинфицирани лица на дневна основа (едниот на 18 ноември, со 1.402 случаи, а другиот на 3 декември, со 1342 лица). Владата дополнително ги заостри рестриктивните мерки и, од средината на декември 2020 година, па досега, бројот на новоинфицираните лица покажа тренд на значајно опаѓање.

Овде, во најсинтетичка форма, ќе укажам на економските и социјалните трошоци, т. е. на општествените трошоци што земјата ги претрпи од кризата детерминирана од пандемијата од корона-вирусот.

– Според Државниот завод за статистика на РС Македонија, падот на БДП на земјата во 2020 година ќе изнесува околу – 4,5 %. Ако во 2019 година, БДП на Република Северна Македонија изнесуваше 11,2 милијарди евра, по тековен курс (ДЗС 2020), тоа значи дека загубата мерена според апсолутната големина на БДП, како најсинтетички показател за вкупната економска активност, ќе изнесува околу 500 милиони евра;

– Првите процени за ударот на кризата врз македонскиот пазар на труд беа дека стапката на невработеноста ќе се зголеми за 2 до 3 процентни поени – оваа процена е конзистентна со релациите меѓу БДП и стапката на невработеноста, што ги опишува Окуновиот закон. Поновите анализи потврдуваат дека последиците од кризата врз пазарот на труд се релативно благи, меѓу другото, благодарение на мерките што ги презеде државата во рамките на владините пакети за надминување на кризата: ослободување од плаќање на дел од социјалните придонеси кои се врзани за цената на трудот, субвенционирање на плати во приватниот сектор и низа мерки за зголемување на ликвидноста на фирмите (види: https://vlada.mk/node/21431). Повнимателната анализа

Ковид-статистиката за РС Македонија покажува дека на 19 февруари 2021 година, вкупниот број заболени лица изнесуваше 98.584, вкупниот број оздравени лица 89.022, а вкупниот број смртни случаи 3.037 лица. Нов (трет) бран на пандемијата во Република Северна Македонија почна во почетокот на март 2021 г., со појавата на англиската варијанта на ковид-19 која има потенцијал на побрзо ширење и потешки последици врз здравјето на луѓето. Бројот на новоинфицирани случаи, на дневна основа, се одржуваше на четирицифрен износ (над 1.000 случаи), така што, на 29 март 2021 година,,вкупниот број на случаи на заболени од корона-вирусот (Corona virus cases) достигна 126.938 лица, бројот на умрени лица (deaths) 3.675 лица и бројот на оздравени (recovered) 104.356 лица.
(https://www.worldometers.info/coronavirus/country/macedonia).
Силината на третиот бран почна да се намалува кон крајот на април 2021 година. На 1 мај 2021 година, бројот на вкупно заболени лица изнесуваше 152.867, бројот на излечени 133.798, а бројот на починати 4.936 (koronavirus.gov.mk). Поради проблемите со Ковакс-системот, вакцините во Македонија почнаа да пристигнуваат со задоцнување. Се очекува масовната вакцинација на населението да почне во мај.

на податоците што доаѓаат од ДЗС покажува дека структурата на регистрираната невработеност ја сочинуваат три категории на активната работна сила: 16.000 лица кои ја изгубиле работата како директна последица од ковид-19, 11.000 лица (ученици, студенти и сл.) кои првпат се пријавиле (регистрирале) како баратели на работа и околу 20.000 лица кои влегуваат во категоријата на т.н. обесхрабрени работници – овие претходно биле избришани од евиденцијата на невработени, бидејќи активно не барале вработување, а по избувнувањето на кризата повторно се регистрирале како невработени. Во меѓувреме, до крајот на декември 2020 година, бројот на евидентирани невработени дополнително се зголеми, но само дел од нив го изгубиле вработувањето како директна последица на кризата. Во првиот квартал на 2020 година, стапката на невработеност во Република Северна Македонија достигна 16,2 %, односно го забележа историски најниското ниво во периодот по осамостојувањето на земјата, а на крајот на вториот квартал на 2020 година, достигна 16,7 %. Процените се дека стапката на невработеност во 2020 и во 2021 година, ќе изнесува малку над 17 %, а потоа повторно ќе се намали под 17 %.

– **Јавни финансии** – состојбата во областа на јавните финансии**,** како последица од кризата детерминирана од пандемијата од ковид-19, дополнително се усложнува. Македонија, во периодот по 2008 година, за шест години, го дуплираше својот јавен долг и значајно го стесни фискалниот простор, за жал, со непродуктивни јавни инвестиции, кои не само што не генерираат приходи за сервисирање на долговите туку се и негативно корелирани со економскиот раст. Владата донесе пет пакети мерки за ублажување на последиците од кризата во износ од околу 950 милиони евра, што чини 9 % од македонскиот БДП. Владата мобилизираше значителни средства од странски извори (од ММФ, од ЕУ и од емисија на нова еврообврзница), но и од домашни извори (позајмување на домашниот пазар на капитал – дел преку емисија на државни обврзници и дел од комерцијалните банки). Буџетскиот дефицит, во 2020 година, ќе достигне 8,4 %. На крајот на 2020 година, јавниот долг на земјата ќе надмине 60 % (како учество во БДП), а бруто-надворешниот долг (овој го опфаќа и долгот на корпоративниот сектор), на крајот на првиот квартал на 2020 година, надмина 76 %. Иако Министерството за финансии на Република Северна Маке-

донија планира да почне процес на фискална консолидација (континуирано намалување на буџетските дефицити во периодот 2020 – 2025 година), јавниот долг на земјата ќе продолжи да расте во 2021, 2022 и 2023 година. Во 2023 година, јавниот долг ќе достигне ниво од околу 64 %, а во наредните две години тоа ќе се намали (на 62 % во 2024 година и на 58,8 % во 2025 година) (Бесими 2020). Притоа, не е исклучено, финансиската консолидација во иднина да бара и зголемување на даночното оптоварување на граѓаните и на бизнис-секторот, со сите негативни последици кои произлегуваат од овој пристап (Alesina, Favero Giavazzi 2019). Очевидно, последиците на кризата врз одржливоста на јавните финансии се сериозни – во овој момент, земјата е доведена до ситуација постојано да се задолжува на домашниот и меѓународниот финансиски пазар за да го осигура нормалното сервисирање (отплатата) на долговите. Стабилизацијата на јавниот и, пошироко, на бруто-надворешниот долг, не е можна доколку македонската економија, во иднина, не успее да креира високи стапки на раст, на нивото од потенцијалниот бруто-домашен производ (4,5 % до 5,2 % просечно, годишно).

– Социјалните трошоци од кризата се тешко мерливи и не можат да се квантификуваат. Но, квалитативната анализа укажува на значајни дисторзии во оваа сфера: (1) буџетските алокации кон Министерството за здравство значајно се зголемија. За да го амортизира т.н. опортунитетен трошок (поголемите буџетски средства за здравство условуваат помали алокации на средствата за поддршка на економијата), Владата мораше значајно да го зголеми буџетскиот дефицит, т. е. да мобилизира речиси 1 милијарда евра од домашни и странски извори – товарот за отплата на долгот паѓа на идните генерации; (2) очекуваното намалување на странските директни инвестиции во македонската економија во 2020 година, во однос на 2019 година (од 2,6 % во 2019 г., на 1,4 % во 2020 година, како учество во БДП), како и намалувањето на **дознаките** и на приходите од работа, според проценитe на Светската банка, ќе ја вратат сиромаштијата во Македонија на нивото од 2017 година – од 17 % во 2017 година, на 20 % до 23 % во 2020 година (World Bank 2020b); (3) кризата, поради потребата од одржување физичка и социјална дистанца предизвика сериозни проблеми во сферата на образованието – го отежна нормалното одвивање на наста-

вата и проверката на знаењата, особено во руралните средини, ја оневозможи практичната настава и обука на учениците и студентите, ги оневозможи или ги одложи специјализациите во странство итн. Сето ова ќе испорача негативни импликации во сферата на формирањето на човечкиот капитал. Република Северна Македонија, до почетокот на мај 2021 година, изгуби 5.000 човечки животи. Прекинатите комуникации на луѓето преку физичко присуство со поширокото семејство, со родниниte и со пријателите, продуцираа негативни психолошки последици, разочараност, депресии, пораст на насилството во рамките на семејствата, полош третман на другите заболувања на луѓето, итн.

Во продолжение ќе изнесам неколку сугестии до креаторите на политиките кај нас (посебно на фискалната политика) за подобро наоѓање решенија, сега и во иднина, за длабоките рецесии, без оглед дали тие ќе бидат предизвикани од евентуална појава на нови пандемии или од економски фактори:

– Оваа криза недвосмислено потврди дека здравствениот сектор има клучна улога, не само во превенцијата на болестите и лечењето на болните туку и во функционирањето на економијата и општеството во целина. Оттука, инвестициите во здравството (во инфраструктурата, во модерната опрема, во кадрите – едукација, специјализации, дома и во странство итн.) мора да биде перманентен, а не инцидентен процес;

– На фискалната политика ѝ припаѓа централно место во надминувањето на тешки, длабоки рецесии. Затоа е неопходно: големината и структурата на фискалните стимули, во време на големи рецесии, да кореспондира со тежината на кризата и да не се дозволи нивно предвремено укинување; позначаен дел од фискалните стимули да се насочуваат кон слоевите од популацијата со понизок доход, т.е. со повисока маргинална склоност кон потрошувачка. Оваа сугестија има посебно значење за РС Македонија бидејќи автоматските стабилизатори на фискалната политика кај нас, меѓу другото, и поради постоењето на рамен данок, се слаби; бидејќи Република Северна Македонија, во периодот по 2008 година, го изгуби фискалниот простор, а јавниот долг дополнително се зголеми за време на оваа криза, неопходно е во иднина да почне и процесот на фискална консолидација. Овој процес, пред сè, треба да се заснова на кратење на сите непродук-

тивни владини трошоци, а пристапот за финансиска консолидација преку зголемување на даноците да се примени во услови на крајна потреба; во „добри времиња“ (експанзии на економската активност) треба да се води политика на одржување ниски буџетски дефицити, па дури и на креирање на буџетски суфицити, за да се овозможи создавање фискален простор за „лоши времиња“ (т.н. The tax smoothing principle); македонската економија ќе почне да заздравува во текот на 2021 година, иако не со очекуваната динамика. Рационализацијата на буџетските трошоци, подобрувањето на среднорочното и долгорочното буџетско планирање и на менаџирањето со јавните финансии, ќе овозможат проширување на просторот за јавни инвестиции кои, главно, треба да бидат насочени кон крупната економска инфраструктура (патишта, модернизација на железницата, гасификација, обновливи извори на енергија, зелени технологии и сл.), како и во здравството, образованието и научноистражувачката дејност.

– По завршувањето на рецесијата условена од ковид-19, клучните макроекономски политики ќе треба доминантно да се концентрираат на својата стабилизациска функција. Монетарната политика ќе дејствува со својот проширен (двоен) мандат (покрај за ценовната стабилност, ќе треба да се грижи и за стабилноста на вкупниот финансиски систем и за поддршка на економскиот раст), а високите фискални стимули треба да бидат заменети со структурни реформи, бидејќи токму тие можат да осигураат долгорочен раст и развој на економијата. Во случајот на македонската економија, структурните промени треба приоритетно да бидат насочени кон: изградба на силни, кредибилни и ефикасни институции, владеење на правото и доброто управување, квалитативно подобрување на најзначајните сегменти на бизнис-климата (заштита на сопственичките права, добра економска и општествена регулација на бизнисите, ефикасен и поправеден даночен систем, пријателска инвестициска клима за домашните и за странските инвеститори, борба против корупцијата и сл.), јакнење на конкурентниот притисок во економијата, поддршка на иновативноста во општеството и, посебно, во македонскиот бизнис-сектор, особено преку фокусирање врз т.н. зелен раст итн., со крајна цел, изградба на одржлива пазарна економија која ќе испорачува економски прогрес.

КОРИСТЕНА ЛИТЕРАТУРА

1. Alesina, A., Favero, C. and Giavazzi, F. (2019). *Austerity: When It Works and When It Doesn't,* Princeton University Press.
2. *The Assessment Report on COVID-19 Global Outbreak.* (2020). Turkish Academy of Sciences (TUBA), Ankara, July.
3. Blanchard, O. (2018). "Should We Reject the Natural Rate Hypothesis?", in *Journal of Economic Perspectives*, Volume 32, Number 1 –Winter – Pages 97–120.
4. Бесими, Ф. (2020). „Предлог-буџет на Република Северна Македонија, со рамка, за 2021 – 2025", презентација, Министерство за финансии на Република Северна Македонија, Скопје, ноември.
5. Cerra, V., Fatas, A. and Saxena, S. (2020). "Husteresis and Business Cycles", IMF Working Paper, WP/20/73, May.
6. Fan, V., Jamison, D. and Summers, L. (2018). „Pandemic risk: how large are the expected losses?", Bulltin of the World Health Organization, 96:129-134Idoi:http://dx.doi.org/10.2471/BLT.17.199588.
7. Државен завод за статистика на Република Северна Македонија. (2020). Соопштение бр.3.1.20.16, 5 октомври.
8. Фити, Т. (2011). *Кејнзијанската економска филозофија – од Кејнз до денешни дни,* изд. Македонска академија на науките и уметностите и Економски факултет, Скопје.
9. Fiti, T. and Tashevska, B. (2012). "Problems of Fiscal Consolidation in the Post-Crisis Period - Lessons for the Republic of Macedonia", *Contributions,* Section of Social Sciences, XLIII 1-2, Macedonian Academy of Sciences and Arts, Skopje, pp. 127–149.
10. Fiti, T. (2013). "The Phenomenology of the European Debt Crisis", in *Crossroads, The Macedonian foreign policy journal,* Skopje, July, pp. 95–106.
11. Фити, Т. (2020). *Макроекономија на големите кризи,* изд. Македонска академија на науките и уметностите, Скопје.
12. ILO Monitor (2020), *COVID-19 and the world work,* Fourth edition, 27 May.
13. ILO Monitor (2021), *COVID-19 and the world work,* Seventh edition, 25 January.
14. IMF (2020a), *World Economic Outlook:* "The Great Lockdown", April, p. 4/5.
15. IMF: (2020b), *Fiscal Monitor* "Policies to Support People During the COVID-19 Pandemic", April.
16. (IMF(2020c), *World Economic Outlook* "A Crises Like No Other, An Uncertain Recovery", June.

17. IMF. (2021a). *World Economic Outlook:* "Policy Support and Vaccine Expected to Lift Activity", January.
18. IMF *Fiscal Monitor.* (2021b). "Government Support Is Vital as Countries Race to Vaccinate", January.
19. Kılıç, O. (2020). „Pandemics Throughout History and Their Effects on Society Life", in *Reflections on the Pandemic in the Future of the World,* Turkish Academy of Sciences (TUBA), 2020, pp.15–53.
20. Kose, M. A. and Ohnsorge, F. (Editors). (2019). *A Decade After the Global Recession: Lessons and Challengers for Emerging and Developing Economies,* Advance Edition,Wachington, DC: World Bank. Licence: Creative Commons Attribution CC BY 3.0 IGO.
21. Mankiw, G. (2020). "Larry Summers Interview", (gregmankiw.blogspot.com, May 8.).
22. Ozturk, R. (2020). „Outbreak Control and Preparatory Actions in the New Normal Period" in *Reflections on the Pandemic in the Future of the World,* Turkish Academy of Sciences (TUBA), Ankara, pp. 57–73.
23. Phelps, E. (2006). *Seven Schools of Macroeconomic Thought*, Clarendon Press, Oxford.
24. Robson, D. (2018). "The Spanish flu killed quiclly, and it killed in huge numbers. Other flu pandemics in modern times have been for less deadly. Why?", bbc.com/future/article/20200325-covid-19-the –history-of-pandemic, 31 October.
25. Samuelson, P. and Nordhaus, W. (1992). *Ekonomija,* 14 izdanje, Mate, Zagreb (Economics, 14.ed., Mate Zagreb).
26. World Bank Group. (2021). *Global Economic Prospects,* January.
27. World Bank Group. (2020b). "Western Balkans Regular Economic Report*",* No17/Spring 2020.
28. Walsh, B. (2020). „COVID-19: The History of Pandemics", bbc.com/future/article/20200325-covid-19-the –history-of-pandemic, 26 March.

Taki FITI

SOCIAL COSTS FROM EPIDEMICS AND PANDEMICS

Abstract

The aim of this paper is to offer a methodology for measuring the total social costs of the crises determined by pandemics and epidemics. According to the author, the calculation of the total social costs caused by major epidemics and pandemics is not a simple matter, primarily due to the fact that the social impact of crises is difficult to quantify. The economic costs of the Crisis coused by the COVID-19 pandemic are analyzed globally and in the Macedonian economy through the movement of key macroeconomic variables: Gross Domestic product (GDP), Unemployment rates and Public finance (the large increase in budget deficits and public debt). Lost in output (GDP) and increase of unemployment are analyzed through the regularities described by Okun's Low. There is a duble negative impact on the Budget - the unemployed do not pay taxes and social contributions, but receive social transfers. In this type of crisis, the total social costs consist of the economic costs increased by the losses associated with the humanitarian and psychological consequences suffered by the unemployed and sick persons, costs correlated with growing disparities in the income distribution and the deepening of poverty as well as the costs of the losses of human lives and their implications for human capital. The concluding part of the paper contains recommendations to the policy makers in the Republic of North Macedonia (especially fiscal policy) to better deal with deep recessions, now and in the future.

Keywords: epidemics, pandemics, economic costs of pandemics, social costs of pandemics, total costs of pandemics, COVID-19 pandemic, fiscal policy.

doi: 10.20903/zpopov-2022-0034 UDK: 616.831-005-073.763.5

Миодраг ВРЧАКОВСКИ[1]

МОЗОЧНО ХЕРНИРАЊЕ ВО ТРАНСВЕРЗАЛЕН ВЕНСКИ СИНУС (Приказ на случај)

Пред неколклу месеци, во „Неуромедика“ дојде 32-годишна пациентка која веќе две години добива напади на силна главоболка на левата страна на главата, почесто навечер, во сон, но исто така, и при стресни состојби и зголемување на крвниот притисок.

Болките се остри, како убод со шило.

Од силината на главоболките кои се појавуваат во серија, повеќепати, ѝ се случувало да изгуби свест, но тоа траело десетина секунди. Во тие моменти ѝ се случувал и спонтано да измокри.

Во описите на претходните, во други дијагностички центри направени МР-прегледи, стоеше дека е дијагностициран поголем дефект во луменот на левиот трансверзален синус, сфатен како коагулум настанат поради тромбоза на синусот.

Врз основа на овој наод, ординирана е антикоагулантна терапија.

Пациентката е упатена кај нас за контролен МР-преглед на мозокот.

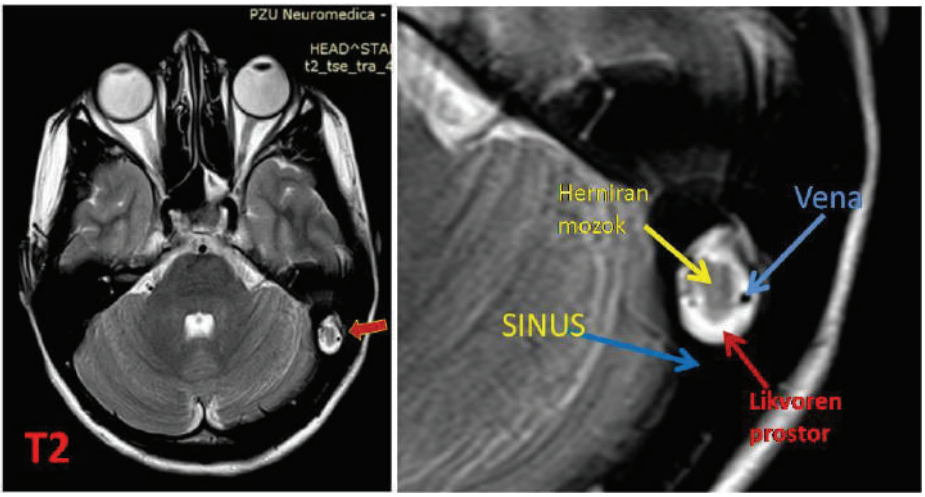
Тој е направен на апаратот „Магнетом Ессенца од 1.5 Т“ на фирмата „Сименс“.

МР наод

Трансверзалните Т2,Т1 и Флаир пулс-секвенции покажуваат присуство на јасно ограничен супстрат во десниот трансверзален

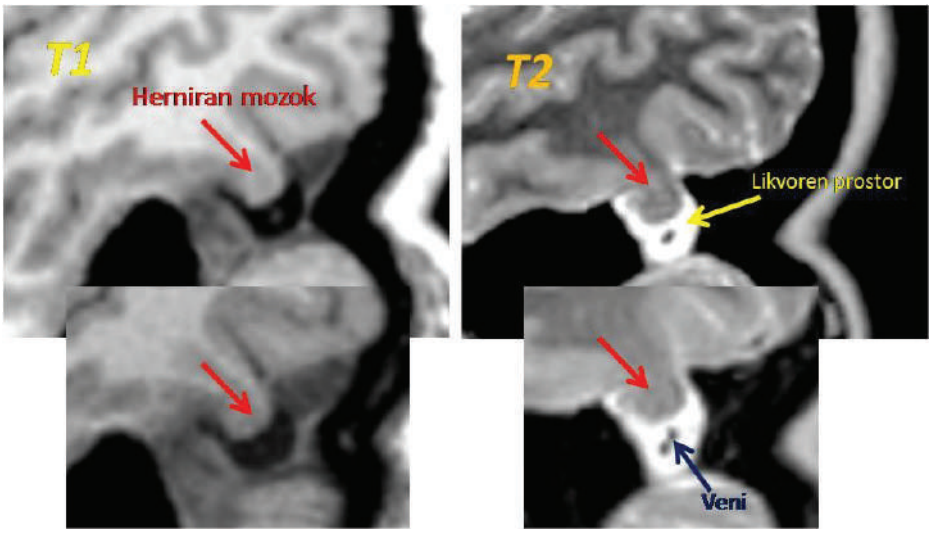
[1] Редовен професор по радиологија, Медицински факултет, Универзитетот „Св. Кирил и Методиј“, Скопје; Болница Неуромедика-Скопје, РС Македонија

синус, со дијаметар од 8 мм, со сигнални карактеристики слични на оние на нормалниот мозочен паренхим во сите пулс-секвенции. Околу промената постои добро ограничено подрачје со карактеристики на ликвор во кое во Т2 пулс-секвенцијата се забележуваат две тубуларни формации со воид-феномен (слика 1).



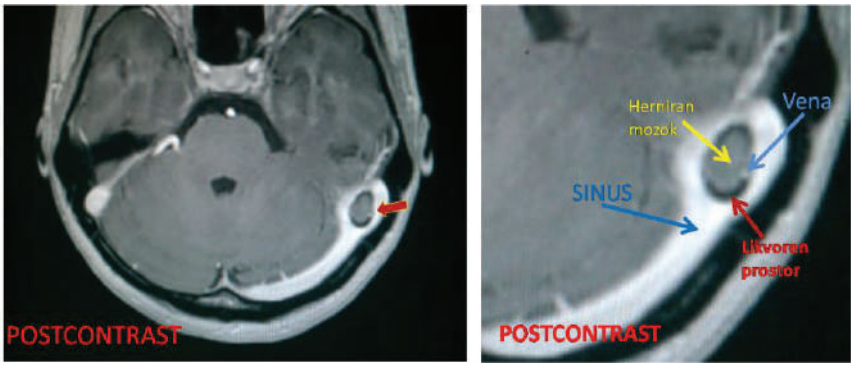
Слика 1 – Две тубуларни формации со воид-феномен

На направените 3Д-скенови со висока резолуција се гледа дека супстратот во левиот трансверзален синус има исти карактеристики со нормалниот мозочен паренхим. Јасно се разграничуваат сивата и белата мозочна маса, а во околината се забележува јасно демаркиран простор со карактеристики идентични со оние на ликворот. Добро се прикажани и тортуозните структури за кои нема сомневање дека се крвни садови. Тие се несоборлив доказ дека овде не станува збор за тромб, туку за мозочно хернирање кое со себе ги повлекло и околните вени (слика 2).



Слика 2 – Мозочно хернирање кое со себе ги повлекло и околните вени

На оваа пациентка, пред три месеци, во друг дијагностички центар, ѝ била направена и постконтрастна серија (слика 3).



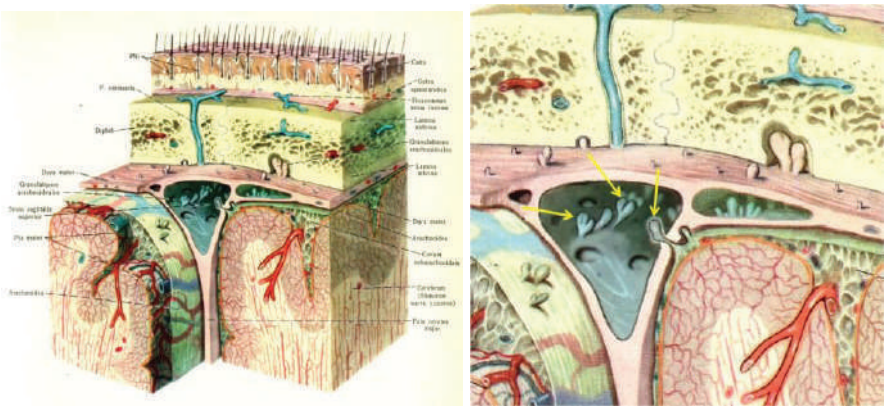
Слика 3 – Постконтрастна серија

Таа сосема го потврдува претходно опишаниот наод. Во левиот трансверзален синус, по даденият контраст, лесно го воочуваме дефектот со сите компоненти на мозочното хернирање. За жал, овој наод е погрешно дијагностициран како тромб.

Дискусија

Каква состојба би можело да направи дефект на ѕидот на синусот?

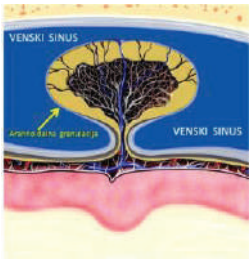
Погледнете го цртежот на слика 4, позјмен од анатомскиот атлас на Синелников, од каде што јас учев анатомија во 1965 година. Во луменот на централниот мозочен венски синус (Синус сагиталис суперирор) гледаме повеќе полипоидни анатомски структури (жолтите стрелки).



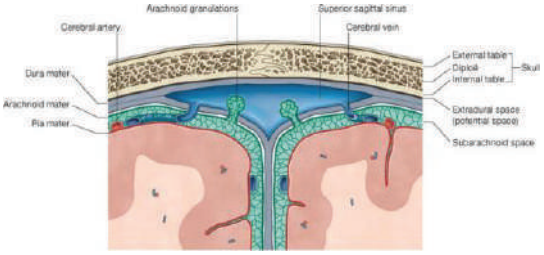
Слика 4 – Централниот мозочен венски синус (Синус сагиталис суперирор)

Тоа се арахноидалните гранулации.

Мали се и речиси незабележливи со голо око, но преку нив се врши трансферот на ликворот, од мозочниот конвекситет во дуралните венски синуси, всушност тие се вентилите што го овозможуваат тоа (слика 5 и слика 6).



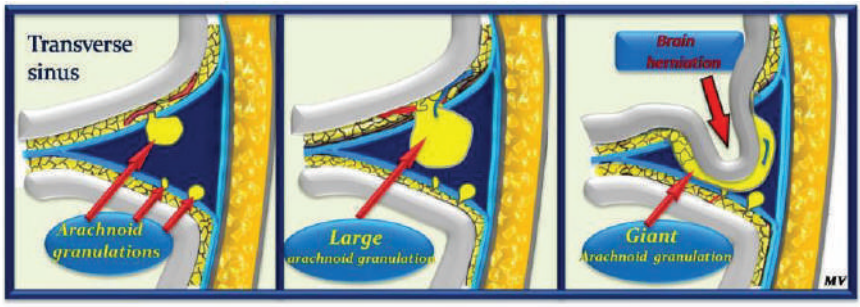
Слика 5 – Венски синус



Слика 6 – Арахноидални гранулации

Малите арахноидални гранулации со текот на времето може да се зголемуваат или, едноставно кажано, тие во текот на животот може да растат до тој степен што стануваат гигантски, при што силно го прошируваат и отворот на sидот од венскиот синус создавајќи услови, преку овој голем дефект, мозочниот паренхим да хернира во синусот.

На мојот цртеж на слика 7 се обидов да ви објаснам како настанало мозочното хернирање во левиот трансверзален венски синус во нашиот случај.



Слика 7 – Како настанува мозочното хернирање
во левиот трансверзален венски синус

Значи, финалниот заклучок би бил дека мозочното хернирање во венски дурален синус е, всушност, хернирање во една гигантска арахноидална гранулација.

Друго објаснување не постои.

Зошто растат и хипертрофираат арахноидалните гранулации?

Растот на арахноидалните гранулации и нивната хипертрофија, најверојатно се резултат на зголемувањето на притисокот, односно на волуменот на ликворот.

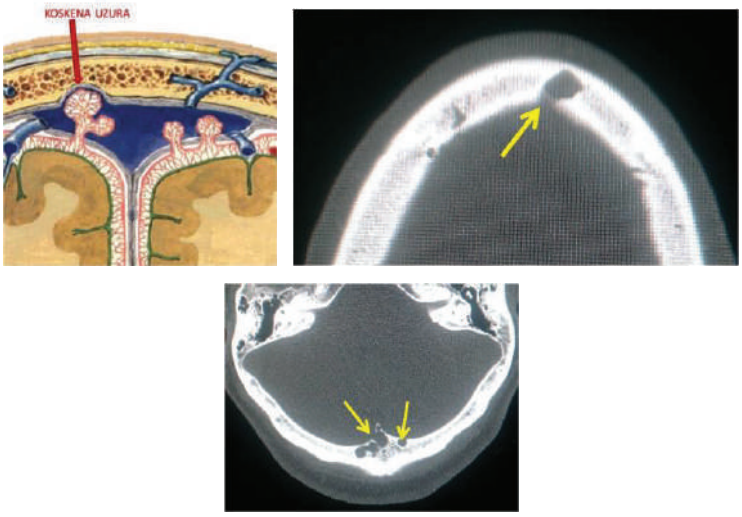
Просечниот ликворен притисок изнесува околу ***10 mmHg***.

Притисокот во дуралните венски синуси се движи околу ***0 mmHg***, па оваа разлика овозможува ликворот, преку арахноидалните гранулации, да премине во венскиот систем.

Зголемениот ликворен притисок придонесува тие да се дилатираат, да хипертрофираат, да растат, проширувајќи ги постепено, како што претходно веќе ви објаснив, и дуралните отвори низ кои тие мину-

ваат, до тој степен, тие да станат доволно широки за низ нив да хернира мозочниот паренхим.

За моќта на арахноидалните гранулации говорат и обемните остеолитични коскени промени на калваријата (дефекти на табула интерна и диплоето) како резултат на пробивот на арахноидалните гранулации во коската (слика 8).



Слика 8 – Обемни остеолитични коскени промени на калваријата (дефекти на табула интерна и диплоето) како резултат на пробивот на арахноидалните гранулации во коската

Заклучок

1. Мозочното хернирање во дурален венски синус е ретка состојба.

2. Во најголем број случаи тоа е асимптоматско.

3. Понекогаш (како во нашиот случај) хернирањето е проследено со главоболки, па дури и со епи-напади.

4. МР ни овозможува прецизна дијагностика на овие состојби, но стандардните Т1, Т2 и Флаир пулс-секвенциите не се доволни за целосна евалуација и дијагностицирање на мозочните хернирања во

дуралните венски синуси. Неопходни се 3Д T1 и T2 градиент ехо-пулс секвенции со висока резолуција, при што, во нашиот случај, драгоцени информации добивме и со специјално направените реконструкции по, од нас зададени, криви рамнини. Тие ни овозможија одлична визуализација на хернираното мозочно ткиво, како и приказ на проширените околни ликворни простори и васкуларни структури.

КОРИСТЕНА ЛИТЕРАТУРА

1. Kan, P., Stevens, E. A., Couldwell, W. T. (2006). Incidental giant arachnoid granulation. *American Journal of Neuroradiology*; 27(7): 1491–1492.
2. Potts, D. G., Reilly, K. F., Deonarine, V. (1972). Morphology of the arachnoid villi and granulations. *Radiology*; 105(2): 333–341.
3. Trimble, C. R., Harnsberger, H. R., Castillo, M., Brant-Zawadzki, M., Osborn, A. G. (2010). "Giant" arachnoid granulations just like CSF?: NOT!! *American Journal of Neuroradiology*; 31(9): 1724–1728.
4. Battal, B., Castillo, M. (2014). Brain herniations into the dural venous sinuses or calvarium: MRI of a recently recognized entity. *Neuroradiology Journal*; 27(1): 55–62.
5. Chin, S. C., Chen, C. Y., Lee, C. C. et al. (1998). Giant arachnoid granulation mimicking dural sinus thrombosis in a boy with headache: MRI. *Neuroradiology*; 40(3): 181–183.
6. Turner, L. (1961). *The structure of arachnoid granulations with observations on their physiological and pathological significance.* Ann R Coll Surg Engl 29: 237–264.

ПОГЛАВЈЕ VIII

КОЛЕГИ И ПРИЈАТЕЛИ ЗА АКАДЕМИК ЖИВКО М. ПОПОВ

doi: 10.20903/zpopov-2022-0035 UDK: 929Попов, Ж.

академик Луан СТАРОВА*

МАКЕДОНСКИОТ КАРТЕЗИЈАНЕЦ АКАДЕМИК ЖИВКО ПОПОВ

Размислувајќи како да се вклучам со свој прилог во оваа книга – омаж по повод 70-годишнината од животот на нашиот академик Живко Попов, хирург уролог со светски глас, иако не сум од неговата „бранша“, најдов едно обележје од неговиот живот и научно вишнеење, кое ми се стори најпригодно за да се доближам, на еден посебен начин, до неговата научна мисија од француска провениенција.

Академикот Живко Попов е нашиот најуспешен картезијанец.[1] Овој поим со ретка употреба кај нас потекнува од името на големиот француски филозоф Рене Декарт (1596 – 1650), познат по својата филозофија „за потрагата по вистината преку разумот“. Европското картезијанство ќе доминира во целиот XVIII век и своето значење ќе го задржи до денес.

По што е нашиот академик Живко Попов нов картезијанец? Првин, постои една случајност (фрацузите велат: „Случајноста не постои!“, односно „L'hasard n'existe pas!“), академикот Попов своите најголеми научни и академски успеси ќе ги постигне во престижниот универзитетски и научен француски и европски центар со името на славниот француски и европски научник Рене Декарт, меѓу француски универзитетски професори достојни на името на големиот мислител.

Покрај несомнените резултати што ќе ги постигне академик Попов и за нив ќе добие мериторни признанија, тој ќе се збогати и со картезијанските парадигми: строгост, јасност, прегледност, ред, дисцип-

* Македонска академија на науките и уметностите, Скопје, Р Македонија.

[1] Да се биде картезијанец, тоа значи да се следи методата на филозофот Рене Декарт, односно централниот став „за потрагата на вистината преку разумот“, и од неа да се изведат фундаменталните принципи кои потоа се применуваат во различни домени на знаењето.

лина, логичност, прецизност, методичност, проникливост. Според картезијанската логика, душата постои по тоа што ништо не ни останува непознато во човековото постоење.

Картезијанството на нашиот академик Живко Попов, во неговата мисија на животот има пошироко значење и во една наша национална и пошироко балканска мисија. Тој е човекот кој успешно ја пренесува универзалната медицина од еден од нејзините центри, во својата земја, во многубројните животи чиј неуморен спасител е токму тој...

* * *

Навикнати сме во освојувањето на европскиот Пантеон на вредностите од страна на балканските интелектуалци, најчесто да се задржуваме пред уметничките дострели на видни личности, особено од доменот на книжевноста, ликовните уметности, музиката и други. Посебно Париз бил и останува еден од европските центри кон кои не престануваат и натаму да конвергираат носителите на вредности, врвни национални дарби во различни области од науката и уметностите.

Честопати носителите на овие вредности, затечени пред вратите на напредната европска цивилизација, во овие центри, во Париз посебно, ќе бидат носители на ренесансна мисија за својот народ. Кога се има предвид историјата на носителите на овие мисии, посебно кај македонскиот народ, кој се здобива со своја држава и независност, институции од областа на економскиот и општествениот развој, се појавуваа силни креативни личности кои стануваат луѓе мостови помеѓу врвните институции на своите земји и европските.

Во овој домен како карактеристичен пример може да се истакне поврзаноста на неколку генерации македонски студенти по медицина, лекари, магистранти и докторанти, со француската медицина, која во светот е една од најпрестижните, со голем број нобеловци, со медицинските факултети во Париз, Лион, Ница и други.

Академикот Живко Попов, македонскиот самостоен трансплантациски хирург од европски и светски глас, комплетно едуциран и подготвен да ја спроведе компликуваната хируршка методологија во пресадувањето, признат експерт во доменот урогениталната хирургија, лидер во воведувањето тешки процедури во онколошката урологија, познат по многубројните операции во областа на пелвичната хирургија

на егзереза со илио-обтураторни лимфни дисекции и уринарна реконструција, модерните ендоуролошки методи (перкутаната нефролитолапаксија, уретерореноскопијата и др.) за лекување на уринарната калкулоза, лидерството во воведувањето на лапароскопијата во урологијата, со посебно насочено внимание кон лапароскопската хирургија на надбубрежните жлезди, како и многубројните други специјалности, во голема мера, ѝ ги должи на големата „париска медицинска школа“.

Откако ја завршил четиригодишната специјализација по урологија на тогаш престижниот државен Медицински факултет во Скопје, започнуваат неговите повеќегодишни, успешни и животворни „француски универзитети“: откако завршил и специјализација по трансплантациска хирургија на Универзитетско-клиничкиот центар „Хенри Мондор“, XII Париски универзитет, и по одбранетата докторска дисертација на Медицинскиот факултет во Скопје, своето образование го продолжил во Париз, при што во 1994 год. се здобил со универзитетска диплома за продлабочени студии во хируршките науки на Парискиот универзитет „Рене Декарт“ (V), а во 1998 год., во Париз, на истиот универзитет, „Рене Декарт“, под менторство на познатиот професор Доминик Шопен, ја одбранил својата втора докторска дисертација во Франција, високо оценета од комисијата, а потоа во 2011 г., во рамките на Европската Унија, на медицински специјалности (EUMS), се здобил со европска диплома за трансплантациска хирургија.

Здобивањето со високи француски признанија од угледни француски и европски универзитетски и академски авторитети, здобивајќи се со нивната апсолутна доверба, како рамен на рамни, академик Живко Попов ќе се здобие со престижни работни и академски титули на Парискиот универзитет: во 1993/94 г., тој беше назначен за клинички шеф при Универзитетскиот клинички центар „Хенри Мондор“, на парискиот Универзитет XII, додека во учебните години од 1997 до 2003 год., се здобил со титулата универзитетски професор по уролошка хирургија, во рамките на францускиот национален контингент на професори од прва класа. За време на овие престои на Парискиот универзитет, академик Живко Попов е редовно вклучен во изведувањето и во менторството на универзитетска диплома за продлабочени знаења во хируршките науки.

За истакнување се и високите професионални врски и угледот што ги ужива академик Живко Попов меѓу величините на хируршките истражувања од Центарот за хируршки истражувања, како што се про-

фесорите Клод Абу (Claude Abbou) и Доминик Шопен (Dominique Chopin). Овде ќе изврши двегодишна суплементарна едукација во полето на бубрежната и мултиорганската трансплантација, онколошката урологија, како и научноистражувачка работа во полето на имунологијата на урогениталниот канцер. Академик Живко Попов (автор и коавтор на над 220 научни трудови, објавени во реномирани списанија), ќе се појави во коавторство со професорите Клод Абу и Доминик Шопен во низа значајни трудови...[2]

* * *

Ја доживеав среќната околност да го запознаам и да почнам блиско и ненаметливо пријателство со академик Живко Попов, во средината на 90-тите години, кога бев амбасадор на Република Македонија во Франција. Тој често доаѓаше во Амбасадата, најчесто, при административно посредување на македонски пациенти кои се лекуваа во париските клиники со посредство на македонскиот хирург трансплантолог.

Уште од првите контакти пленеше неговата дискретна и ненаметлива добрина, искрениот пристап на пренесување на сложените прашања од медицинска природа до пациентите. Иако според своето меѓународно реноме, научните постигнувања, признатата хируршка моќ во најкомпликуваните зафати, признанијата од врвни хирурзи уролози од Париската медицинска школа, академик Живко Попов можеше лесно да се најде во еден од европските, американските и во други научни и универзитетски центри, тој, со недвосмислено и проникнато патриотско чувство во својата човечка длабина, реши да ја надградува својата медицинска и научна кариера во својата родна и сакана Македонија станувајќи еден од столбовите на македонската медицина...

[2] Поопширно за врските на француските универзитети и клиничките центри и влијанието на париската школа во развојот на медицината и хирургијата во Македонија од: Académicien Zivko Popov, „Les liens avec les universités et les centres hospitaliers parisien et l' influence de l' école française sur le développement de la Macedoine et de la chirurgies macédoniennes" (Expériences et perspectives actuelles), in: La cooperation franco-macédonienne dans l'esprit des nouveaux defies de la francophophonie,Institut français, Académie macédonienne des sciences et des arts, Skopje, 2017, pp. 55–88.

doi: 10.20903/zpopov-2022-0036 UDK: 929Попов, Ж.

Таки ФИТИ[1]

КАЖУВАЊЕ ЗА АКАДЕМИК ЖИВКО ПОПОВ

Поканата со свој прилог да учествувам во подготовката на еден Зборник на трудови посветен на акад. Живко Попов, по повод 70 години од неговото раѓање, ја прифатив со големо и нескриено задоволство, од повеќе причини. Прво, Живко Попов е проминентен универзитетски професор, врвен хирург – уролог, врстен научник и истражувач, редовен член на Македонската академија на науките и уметностите, човек со неоспорни заслуги за развојот и афирмацијата на хируршките и на медицинските науки, не само во нашата земја туку и на европските простори и пошироко. Второ, ваквите атрибути на Живко Попов, му донесоа висок личен престиж и афирмација во меѓународни размери, но придонесоа и за афирмација на македонската медицинска наука, на Македонската академија на науките и уметностите, чиј член е од 2009 година, и на земјата, во целина. Трето, со акад. Живко Попов споделуваме многугодишно искрено пријателство, пријателство што трае повеќе од 30 години, пријателство исполнето со високо заемно почитување и пријателство кое, со текот на годините, континуирано се зацврстува и се продлабочува. Кога ќе се погледаат научните и професионалните перформанси на акад. Попов, паѓа во очи неговата импозантна и неверојатно широка едукација – четиригодишна специјализација по урологија на Медицинскиот факултет во Скопје, специјализација по трансплантациска хирургија на Универзитетскиот клинички центар „Хенри Мондор“, XII париски универзитет, кај професорите со светски глас, Клемент – Клод Абу и Жан Овер, докторат по медицински науки на Универзитетот „Св. Кирил и Методиј“, Медицински факултет – Скопје, диплома за продлабочени студии по хируршки науки

[1] Редовен професор на Универзитетот „Св Кирил и Методиј“ во Скопје, Економски факултет – Скопје и редовен член на МАНУ; fiti@manu.edu.mk

на престижниот V париски универзитет „Рене Декарт“, втор докторат, опција канцерологија, исто така, на V париски универзитет „Рене Декарт“, под менторство на професорот Доминик Шопен, европска диплома за трансплантациска хирургија, издадена од Европската унија на медицински специјалисти, титулата универзитетски поканет професор по уролошка хирургија, во рамките на францускиот национален контингент на професори од права класа, итн. итн. Академик Живко Попов во континуитет реализирал и реализира голем број студиски престои, тренинзи и наобразби во многу земји во светот: Србија, Словенија, САД, Франција, Швајцарија, Австрија, Израел итн., на престижни универзитети и реномирани универзитетски клиники. Како врвен и афирмиран хирург, Попов се вбројува во редот на пионерите на бубрежната трансплантација во Република Северна Македонија. Имено, Живко Попов го оформил првиот самостоен македонски тим за трансплантација на бубрези и го основал Центарот за трансплантација на бубрези при Медицинскиот факултет во Скопје. Тој извршил околу 260 успешни бубрежни трансплантации (кадаверични и од живи донатори), спасувајќи огромен број пациенти со тешка бубрежна инсуфициенција и подарувајќи им нов, многу подостоинствен и поквалитетен живот. Ова потврдува дека токму овој вид хируршки ангажман на Попов има длабока човечка, хумана димензија. Но, вистинските хирурзи не се одликуваат само со своите врвни вештини и способности да изведат комплицирани хируршки зафати и да спасат човечки животи. Комплетниот хирург мора да биде и врвен научник, со особен и специфичен фонд на знаења од неговата област, за да може да се соочи со сите компликации и ризици кои нужно ги следат тешките операции, какви што се оние, поврзани со трансплантацијата на човечки органи. Двете компоненти се интегрирани во личноста на акад. Живко Попов. За тоа сведочи и неговата импозантна научна библиографија – повеќе од 220 научни трудови (национални и меѓународни научноистражувачки проекти, научни статии објавени во респектирани медицински списанија, главно, со висок фактор на влијание (импакт-фактор), научни реферати поднесени на престижни меѓународни научни собири – конгреси, симпозиуми, советувања итн.), во голем број земји во светот. Сето ова придонело акад. Живко Попов да стане познато и респектирано име, дома и во странство, хирург и научник со кој може да се гордеат македонската медицинска наука и нашата највисока научна и

уметничка институција, МАНУ, чиј актуелен потпретседател е акад. Живко Попов.

Ова е добра пригода да кажам нешто за Живко Попов одблиску, за нашето повеќегодишно пријателство, за неговите човечки доблести и личниот интегритет. Моето познанство со Живко Попов датира од раните 1990-ти години. Како што тоа обично се случува, кога е во прашање лекарската фела, Живко Попов го запознав преку мои пријатели кои беа негови пациенти. Тоа беше период кога кариерата на Живко Попов одеше по нагорна линија и кога неговите чести престои, специјализации и работа, на престижните француски и европски универзитетски клинички центри, придонесоа тој да се формира како уролошки хирург и научник со високо реноме. Бев импресиониран од неговите постигнати резултати на професионален план, но и од неговата комуникативност, отвореноста, длабоката хуманост и од подготвеноста секогаш да разговара со своите пациенти, да ги охрабри и да ги мотивира заедно да се соочат и да се борат против болеста. Благодарение на нашите чести заеднички дружења, брзо сфатив дека во личноста на акад. Попов има една нагласена интелектуална нитка, остроумност и моќ за логично размислување и промислување на нештата, не само во доменот на неговата професионална преокупација туку и многу пошироко. Како универзитетски професор и интелектуалец тој се интересираше и за значајни општествени прашања и теми. Периодот кога се запознавме коинцидира со прогласувањето на независноста на Република Македонија и со подготовките за нејзиното монетарно осамостојување. Монетарното осамостојување беше комплексен процес, проследен со голем број ризици и неизвесности. Тогаш постоеше објективна опасност, процесот, ако добро не се обмисли, да се компромитира уште на почетокот – особено поради присуството на банки од поранешните југословенски републики кои можеа да ги „наполнат“ своите сметки со југословенски динари, кои требаше да се заменат со македонските бонови, пред официјално да се воведе националната валута – денарот. Кога ги пишувам овие редови, се присетувам дека, во една пригода, пред Живко го коментирав овој проблем. Неколку денови потоа, ми се јави Живко и ме информираше дека негов гостин ќе биде господинот Даниел Лакур, негов пријател од Париз, професор по монетарен систем на I париски универзитет и директор на Париска банка, познат француски експерт од монетарната сфера и дека е добро

да го запознаам и да поразговарам со него – ’ќе оцениш‘, ми вели Живко, ’дали Даниел може да помогне со совети поврзани со монетарното осамостојување‘. Набрзо потоа, во станот на Живко, се сретнав со Даниел Лакур, продискутиравме околу процесот на монетарното осамостојување и сфатив дека тој, со неговите знаења и искуство, може да биде корисен, односно да испорача значајни совети за оваа комплицирана монетарна операција. Веднаш потоа го информиравме тогашниот премиер, акад. Никола Кљусев, кој беше пријател и на Живко. Премиерот, кој воедно беше и претседател на Комисијата за монетарно осамостојување, брзо организираше состанок со својот стручен тим, на кој го покани и Даниел Лакур. Состанокот траеше долго, дискусијата беше конструктивна и вонредно корисна. Се сеќавам дека набрзо потоа го одведов Даниел Лакур и кај акад. Ксенте Богоев, тогаш, претседател на МАНУ и член на Комисијата за монетарно осамостојување. Професорот Богоев, исто така, оцени дека Даниел Лакур одлично ги познава најзначајните прашања и предизвици на монетарното осамостојување и дека, треба да се размисли за тоа, Даниел Лакур, во иднина, да се ангажира како советник на Владата. Неколку години потоа, за време на еден мој престој во Париз, се сретнав со акад. Попов, кој тогаш работеше како универзитетски клинички шеф во реномираната француска клиника „Хенри Мондор“, во Кретеј (Париз). Живко ме покани да ја посетам клиниката „Хенри Мондор“. Бев импресиониран и многу горд кога видов какви репутација и почит стекнал акад. Попов меѓу своите колеги и медицинскиот персонал на клиниката. Тогаш, во Париз, случајно, на една прошетка низ градот, го сретнавме реномираниот професор и уролошки хирург Клемент – Клод Абу, сега член на МАНУ надвор од работниот состав, кој, исто така, искрено и топло зборуваше за нашиот Живко, за неговите високи постигнувања во областа на уролошката хирургија и во областа на медицинските науки. Подоцна, во Мавровo, го сретнав Попов, со неговиот француски ментор, професорот Доминик Шопен, за жал, сега покоен, со кој поминавме неколку прекрасни денови на мавровските скијачки терени. Професорот Шопен имаше само пофални зборови за Живко, посебно нагласувајќи дека благодарение нему, Македонија е вброена во редот на земјите во кои се изведуваат успешни трансплантации на бубрези според највисоките европски медицински стандарди. Сите изречени факти за Живко Попов, по логиката на нештата, резултираа со негов избор за редовен член на

МАНУ во 2009 година. Со тоа, Одделението за медицински науки на МАНУ и нашата Академија, добија во своите редови врвен научник и уролошки хирург, човек кој во целост ги исполнува строгите критериуми за членство во највисоката научна и уметничка институција на нашата земја – Македонската академија на науките и уметностите. По изборот за редовен член на МАНУ, Живко Попов, со несмален интензитет, со ентузијазам и со голема посветеност, продолжи да работи и да реализира значајни национални и меѓународни научноистражувачки проекти, во соработка со врвни странски универзитети и научни институции, со што придонесува за подигање на реномето и меѓународната афирмација на МАНУ и на македонската медицинска наука. Во 2019 година, акад. Живко Попов беше избран за потпретседател на МАНУ, со што се покажа дека тој ужива висок авторитет и почит меѓу членовите на Академијата.

На крајот, сакам да му го честитам 70-тиот роденден на нашиот Живко Попов, да му посакам добро здравје и уште многу години успешна и плодотворна творечка активност.

doi: 10.20903/zpopov-2022-0037 UDK: 929Попов, Ж.

Јордан ПЛЕВНЕШ[1]

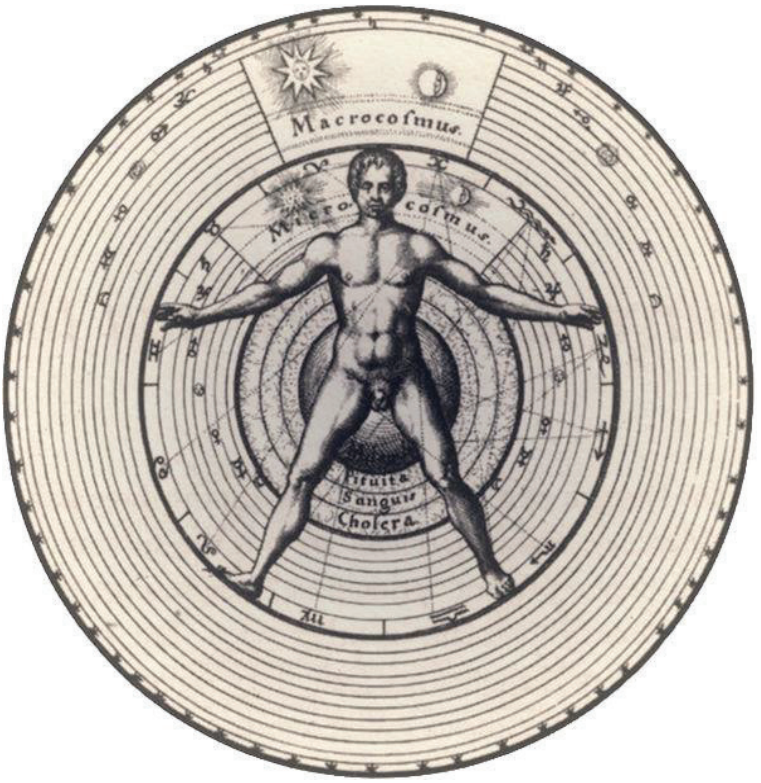
ЖИВКО ПОПОВ – НА ВРВОТ НА СВЕТСКАТА МЕДИЦИНА, СО МАКЕДОНИЈА ВО СРЦЕТО

Академик Живко Попов сонуваше
За врвовите на светската медицина
Уште во својата рана младост
И бидејќи ние оттогаш се знаеме
Од Битола, франкофонската метропола на Балканот
Во нашите прошетки од античкиот амфитеатар на Хераклеја
Преку „Широк Сокак“ до „Св. Димитрија“
Всушност, како да ја поминувавме историјата
На Европа и на светот во последните најмалку 3.000 години
Академик Живко Попов ја љубеше реченицата дека
„Франкофонијата е Империја на духот во која сонцето никогаш не заоѓа“
И во таа империја на духот тој секогаш знаеше да ги поврзе
Историјата на медицината со историјата на уметноста со
Историјата на политичката хронологија
Како бескрајно поле на бесмртни сознанија
Во смртниот човечки живот
Тука, во Хераклеја Линкестис, се појавуваа сенките на Хипократ и Асклепиос
Целата историја на Античка Европа
И римските истражувачи на медицината
Од Corpus Hipocraticus до De humani corporis fabrica

[1] Македонски писател драматург, поранешен амбасадор на Република Македонија во Париз, Франција, ректор на Меѓународниот универзитет *Europa Prima* – Скопје


ANDREAE VESALII
BRVXELLENSIS, SCHOLAE
medicorum Patauinæ professoris, de
Humani corporis fabrica
Libri septem.
CVM CAESAREAE
Maiest. Gallorum Regis, ac Senatus Venti gra-
tia & priuilegio, ne in diplomatis eorundem continetur.
BASILEAE.

И сето тоа продолжи во Париз во деценииите што доаѓаа
Како во магичен лет во којшто градот Битола ни ги подари
Основите на францускиот јазик
И се најдовме во Градот на светлината
Што Данте Алигиери пред 700 години го нарекол
Универзална татковина на духот
Се сеќавам на едно од првите предавања што ги слушавме заедно
Во College de France, највисоката институција на Сорбона
Имавме можност младешки да ги впиваме зборовите на
Членот на Француската академија Жан Бернар (1907 – 2006)
Еден од најлуцидните визионери на светската медицина
Што останаа запишани во вечноста
Во неговото дело „Grandeur et tentation da la medicine“ (Buchet/Chastel, Paris 1973)
Одекнуваше гласот на академик Бернар со зборовите
Коишто станаа знак на распознавање на нашето пријателство од младоста:
„Предметот на медицината се луѓето, човекот, воопшто, и најпосле
Еден единствен човек.
Нејзината вредност не произлегува само од биологијата
Туку, исто така, и од љубовта“.
И тука, академик Жан Бернар ја изговара дефиницијата на
Легендарниот Парацелзиус (1493 – 1541) кој вели:
„**Сеопштата и сеопфатната медицина е, всушност, љубов**“.
Истата таа вечер, по оваа дефиниција цитирана од Парацелзиус
За когошто се тврди дека ја прошетал цела Европа уште во 16 век



Па, можеби, и Балканот и Македонија
Ние направивме со академик Попов едно ходочастие низ Париз
Во чест на сите македонски духови, научници, лекари, сликари, писатели
Кои поминале низ Париз, како што се
Најстариот ресторан на светот, „Прокоп“
Каде што се собирале Гемиците
Академиите на Личеноски и Мартиноски
Издавачката куќа „Cujas“
Што во 1921 година посмртно ја објавува книгата
„Македонија и Македонците“ на Edmond Bouchier de Bell
Кој загинал за време на Првата светска војна во Македонија
Ја посетивме редакцијата на првиот весник на македонската
Франкофонска интелигенција – „Mouvement macedonien“
Којшто излегувал од 1902 до 1903 година

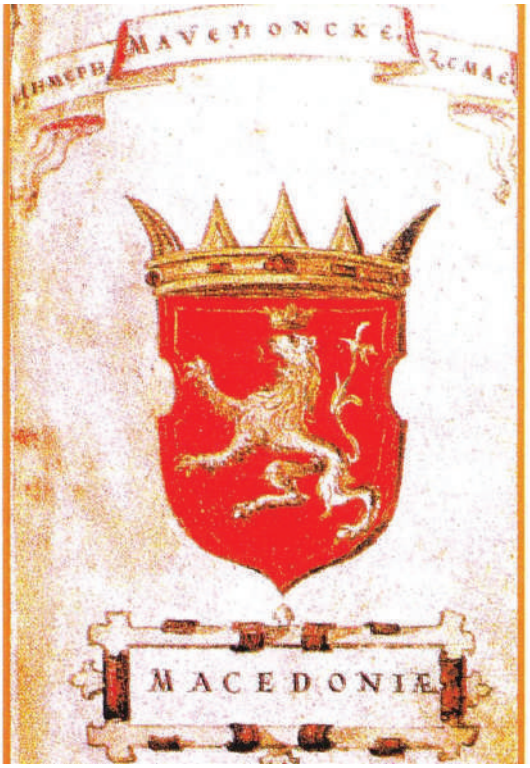
И по повод стогодишнината од неговото излегување на „Булеварот Распај 206“
Беше поставена мермерната плоча со која
Македонскиот народ им се заблагодаруваше на сонувачите за
Што би рекол Виктор Иго, Независна Македонија во
Соединетите држави на Европа
Всушност, пред тие легендарни прозорци на „Распај“
Академик Живко Попов ја изговори знаменитата реченица на нашето пријателство
„Јас имам две љубови, едната е Медицината и сонот за нејзините врвови во светот
А другата е Македонија и нејзините вечно неразгатнати тајни во историјата“

Чудна беше таа синтеза на нашите театарски освојувања и
Преплетувањето на научните презентации на академик Попов
И моето присуство на тие академски свеченост
И неговото присуство на премиерите на моите драми
На премиерите на моите драми во Франција и насекаде во светот
И посебно мојот авторски текст во угледниот париски „Монд“
„Le people phantom“ („Народот фантом“), во март 1992 година
Јас бев сведок на неговото научно понирање во
Тајните на хирургијата – трансплантологијата во
Универзитетскиот клинички центар „Анри Мондор“
И лично ги запознав професорите Clement-Claude Abbou и Jean Auvert
Во исто време, додека тој го внесуваше својот медицински генијв во
„Реактивноста на локорегионалната туморска лимфаденопатија“
Отпатувавме заедно во градот на Корнеј и Молиер, Руан

Каде што во Националниот театар се изведуваше „Еригон“
Како прва македонска драма во Франција и Западна Европа, во мај 1991 година
Чијшто француски наслов беше „Мој драг убиецу“
И во кој основната идеја беше: Да се биде Македонец е естетски чин.
Во 1994 година, исто така, бев сведок на неговите ексклузиви
Продлабочени знаења во хируршките науки на Петтиот париски универзитет „Рене Декарт“
И неговите професори Didier Houssin и Dominique Chopin
Станаа, исто така, и мои пријатели
Пролетта 1994 година, кога се изгради архитектонското чудо
Театарот „Силвија Монмфор“ во Париз
Во инаугуралната сезона беше изведена мојата драма „Р“
Во режија на славниот Жак Селер, современик на Жан Вилар
И академик Попов на таа премиера се појави
Со целата медицинска француска елита
А познатиот албански писател Исмаил Кадаре
Им се обрати на една група француски писатели
„Ако нешто се разболите во Париз, барајте го Плевнеш
Бидејќи тој е пријател на академик Попов и на најпознатите во француската медицина“
Всушност, академската одисеја на Попов и
Врвот на светската медицина стануваат реалност на
Неговата интерконтинентална позиција
FEBS (Fellow of European Board of Surgery)
И неговата присутност на меридијаните на сите пет континенти
Веќе минува низ *Mayo Medical Medical School* во Рочестер, Минесота
Потоа минува низ *Cornell University* во Њујорк
Кај професорот Neal Bander
Во откривање на најскриените мистерии на уролошката патологија
И кога повторно се наоѓаме во 1998 година, во
Театарот „Миранда“ на „Оф Бродвеј“ во Њујорк каде што се одвива
Премиерата на француско-американската копродукција
На мојата драма „Среќата е нова идеја во Европа“

За која „Њујорк тајмс“ напиша дека античките трагедии можат да се препознаат
Во сегашните состојби на Балканот и Македонија на крајот на 20 век.
Брилијантната кариера на академик Попов продолжува во Универзитетскиот клинички центар во Виена, кај професорот Marberger и
Универзитетската „Елизабетска болница“ во Линц, кај професорот Јанечек
Посебно место во неговата академска и научна кариера се
Неговите престои во Берн, Швајцарија, кај професорот Urs Studer
И нашата средба во Женева, каде што во 1998 година
Излегува првиот весник на француски јазик на македонските анархисти „Effort“
И нашето ходочастие во Лозана, каде што излегувал весникот „Independence Macedonienne“
Од исклучително научноистражувачко значење се неговите конекции со Израел
Во *Rabin Medical Centre Beilinson Hospital, Department for organ transplantation* и *Shiba Hospital* во Тел Авив
И светски познатиот професор Amram Ayalon
Секогаш кога ќе се сретневме по моите враќања од светските метрополи
Ако јас му раскажував за реченицата на Крсте Петков Мисирков во Санкт Петербург
„Ако не ми кркореа цревата од глад никогаш не ќе научев санскрит“
Тој ќе ми кажеше дека поминал во Фрајбург, во Швајцарија
Каде што Чернодрински го откривал европскиот театар
Или, пак, ќе го цитираше Димитар Влахов и неговото пријателство со Основачот на Израел, Бен Гурион
И нивните говори во Отоманскиот парламент
Во кои беа визионери на создавањето на модерен Израел и модерна Македонија
Ако зборувавме за Виена, тој ќе ми кажеше дека поминал кај

Хотелот „Ерц Херцог Рајнер“ и редакцијата на весникот „Балканска Федерација“
Што го цитирав во летото 2014, кога објавив еден политички есеј
Во виенскиот дневен весник „Стандард“
Во врска со балканскиот и македонскиот сон за Европа
Во исто време додека тој се издигнуваше во
Врвните познавачи на кадаверичните трансплантации
И урогениталната хирургија
Се допишувавме во врска со нашето заедничко откритие на американските универзитети
На книгата „За балканските војни“ од Леон Давидович Тротски
Кој како воен дописник од Балканот
Напишал во весникот „Киевска мисл“:
„Со Балканските војни, живото тело на Македонија беше раскинато на три дела“
Ја предадовме книгата на академик Гане Тодоровски и
Тој ни изрази посебна благодарност со неговите познати
Поетски фусноти во научните интерпретации за „Сонцељубивите“
Како што ги викаше македонските и светските духови што ја љубеа Македонија
Во дечениите што следеа продолжува одисејата на академик Попов
Со учество во многубројни меѓународни симпозиуми и конгреси со
Над 200 објавени трудови во најреномирани светски списанија
Во исто време тој почнува и една иницијатива за
Усвојување од страна на Македонскиот парламент на
На нов македонски грб пронајден во хералдичките архиви на Европа
Тој анимираше голем број поддржувачи, но
Историската инерција на нашиот политички амбиент за жал
Не ја разбра прекрасната идеја на академик Попов



Во 1995 година, со изборот на Жак Ширак за претседател на Франција
Најблискиот пријател и ментор на Попов, Доминик Шопен
Што ги знаеше сите култни места на европските трезори на Македонија
Беше избран за претседател на Националната комисија за онколошки истражувања
И почна неговата мисија на европско и светско ниво во која
Академик Попов имаше привилегирано место во научната елита
Собрана околу професорот Шопен
Иронијата на судбината сакаше во 2000 година
Јас да бидам избран за вонреден и полномошен амбасадор на Франција
И да ја организирам Првата официјална тридневна посета на
Претседателот Борис Трајковски која се одвиваше во Елисејската палата
Во официјалната делегација беше и академик Попов со епохален предлог
Од здравствената елита на Македонија:

На местото на некогашната фабрика „Цветан Димов“
Која по Првата светска војна била фабрика за фармакологија
Да се изгради Француски институт за Македонија и Југоисточна Европа
За истражувања во областа на онкологијата.
До тогашниот министер за здравство на Франција
Беше анимиран еден висококомпетентен круг на
Француски и македонски експерти на чело со академик Попов
На прес-коминикето кое двајцата претседатели, Ширак и Трајковски
Го презентираа заедно пред големиот број медиуми од Европа и светот
Беше изговорена од двата претседатели историската реченица:
„Македонија е една единствена и неделива!!!“
Но, ветриштата на историјата, наспроти доминацијата на нуклеарната надмоќ
И геополитичката поделба на интересни сфери
Ја надвладеаја визијата на интернационалниот хуманизам
И во бесмртноста на науката и во смртноста на човечкиот живот
Што многугодишниот ректор на Универзитетот во Саламанка „Мигел де Унамуно“
Ги изговори и тие важат и до ден-денес:
Во историјата со горчлив вкус осознава секој од нас дека ќе биде победен
Од ***Del sentimientotrágico de la vida***
Во Македонија почна воената криза и беа разнишани нејзините темели
Неочекувано, многу млад, од истата болест за која беше претседател на
Националната научноистражувачка комисија за онкологија
Почина Доминик Шопен, на 53 години, и
Во погребната поворка додека чекоревме се појави пред нас
Идејата на Розата на Парацелзиус како единствена континуирана
Па макар и илузорна верба дека медицината е сеопфатна љубов
Бидејќи заедно со академик Попов го посетивме гробот на
Хорхе Луис Борхес во Женева кој по инспирација на
Францускиот мислител Жан Бодријар го
Напиша краткиот расказ со истото име – „Розата на Працелзиус“

Се случува невозможното за историјата и за медицината како плод на љубовта.
Имено, младичот го прашува знаменитиот лекар Парацелзиус
Дали ако ја фрли розата во Огништето на неговиот кабинет
Ќе се создаде истата роза со интервенција на неговиот медицински гениј
Младичот во очите на легендарниот лекар ја гледа
Незгасливата светлина во вербата на медицината дека невозможното е возможно
Но розата беспомошно е претворена во пепел.
Кога младичот излегува од неговиот дом во
Десната рака на научникот што веруваше дека медицината е љубов
Ќе се појави бесмртната роза
Тоа е симболичката приказна на научната и истражувачката одисеја на академик Попов
Veram Medicinam, Veram Macedoniam

Јордан Плевнеш,
15 мај 2021, Скопје

doi: 10.20903/zpopov-2022-0038 UDK: 929Попов, Ж.

Методи ЧЕПРЕГАНОВ[2]

БЕСЕДА ЗА ПРИЈАТЕЛОТ ОД ПАРИЗ

Фундаментот на променадата низ цивилизациските психо-емотивни основи на животот, почнува и завршува со една основна животна сентенца: умот царува, а моќта и снагата гнијат, при што цивилизациското мисловен дуктус продолжува и ни порачува: да живееш и да постоиш, значи да работиш, да твориш, да креираш, да создаваш, да умееш, да почитуваш, да те красат емпатија, флексибилност и толерантност, значи, да имаш свест и совест, свеста како физиолошка функција, а совеста како еволутивен процес при кој на психофизичката обдареност од мајката природа, со текот на времето, личноста аквирира и инкорпорира воспитание, едукација, знаења, искуство, социјални, морални, етички и духовни норми, таа постепено матурира во милјето во кое постои.

Тоа се насушните норми кои ја формираат цитоархитектурата на една личност, кои го креираат и го формираат нејзиниот психопрофил.

Модерната психологија, за една матурирана личност вели дека станува збор за зрелост со социјална когницијa, како конгломерат од психичките функции, инкорпорирани, или хармонично, со капацитетот на висока емоционална интелигенција, или дисхармонично, со карактерни настраности и асоцијален модел на однесување во интерперсоналните односи.

Во овој контекст, во нашето *ratio* постојат две спротивставени структури кои се наоѓаат во постојана спрега, од една страна, амигдалното јадро, нашиот емотивен мозок и носител на емоционалната интелигенција и на нашите психоемотивни напливи, значи нашите емоции и емотивниот живот кај секого од нас и структурите на префрон-

[2] Редовен професор по невропсихијатрија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје; Болница „Неуромедика“, Скопје, РС Македонија

талниот лобус, нашиот рацио, кој ја контролира амигдалата и емоциите и ја донесува децизијата, одлуката за нашиот мисловен дуктус, одговорност и професионалност, со каков вокабулар, но и како и на кој начин и со каква мимичка гестикулација, гримаса, боја на глас и движење ќе биде нашиот воспоставен однос кон соговорникот, дома, на улица, на работа, секаде и на секое место.

Ова значи дека во нашите интерперсонални односи ништо не е случајно, сè е предодредено и зависи од тоа колку сме созреале и колку сме ја надградиле нашата личност, со норми на воспитание и едукација, поточно со богатство од знаење и искуство.

Практично, овие две структури се нашето огледало кон надвор, нашата карактерна експресија на особините на психопрофилот на нашата личност, тоа сум јас, тоа сме ние, со сето она што го носиме и што сме го инкорпорирале во нас како норма и параметар, социјален, морален, етички и духовен, на фонот на богатството од знаења, умешност, креација и искуство, особини што или ги имаме или ги немаме.

Со една таква личност, би рекол исклучителна и несекојдневна, животните патишта ни се составија токму во Париз, поради што и оваа животна беседа ја нареков беседа за пријателот од Париз.

Од многу порано го познавав колегата Живко Попов, можеби уште кога беше студент, но едно е да познаваш некого, а друго е карактерно и стручно да го запознаеш и да го втемелиш во себе својот пријател. Париз, со целата своја убавина, со големината и начинот на живот, нè беше голтнал и двајцата, тој во една, а мојата *маленкоста* во друга установа. Медицински научни огништа, високопрофесионални, со долг век на постоење, во кои можеше многу да се збогати резервоарот на знаења.

Оддадени на својата едукација, во слободното време имавме можност да се дружиме, да се запознаеме, да си поприкажуваме, да се зближиме.

Нашето пријателство продолжи со уште поголема почит и со достоинство и во нашите матични установи, овде, во Македонија, а посебно во последната година, кога случајот сакаше да работиме заедно, секој на својата специјалност во иста медицинска установа.

Мислам дека не ќе можам да најдам зборови со кои ќе ги насликам личноста и психопрофилот на мојот драг пријател од Париз. Можеби и затоа ја повикав, на почетокот, психологијата на нашето постоење на помош.

Крајно професионална личност, со висока емоционална интелигенција, максимално одговорен и стручен за професијата на која ѝ го посветил целиот живот, вреден, трудољубив, креативен, полн со енергија и со визионерство, секогаш подготвен да помогне, да даде од себе, да створи нешто ново и да гледа со огромен оптимизам во иднината.

Многу едукација, многу трудови, многу операции, многу нови методи, висока посветеност на специјалноста, многу признанија од секаде, личност за почит и со достоинство, повеќе од реткост.

Но, она што посебно ја краси личноста на мојот драг колега е скромноста, едноставноста, секаде и со секого, мирен, тивок, спокоен, реален, објективен, креативен и подготвен да помогне и да поучи. Особини на матуриран психопрофил, кој ѝ го посветил својот животен пат на стручноста и во неа го достигнал корифејот, а сепак, во екологијата на духот, поточно во социјалните когнитивни односи, останал крајно природен и обичен, секојдневен, ненаметлив, одмерен и одговорен.

Лично му благодарам на животот што ми подари таков пријател кој умее да ја чува, да ја негува и да ја милува колегијалноста, од која извира енергија, доброта, милост, почитување и она на што му се восхитувам, висока стручна професионалност.

Нашата животна сказна е менлива и минлива, тече, се менува и продолжува, но познавајќи го мојот драг и почитуван пријател и колега Живко, длабоко сум убеден дека тој има во себе уште многу духовна енергија, знаења и доблести, да посее, да помогне и да остави, за доброто на оние кому им го посветил животот, на пациентите.

март, лето Господово 2021
проф. М. Чепреганов, невропсихијатар

doi: 10.20903/zpopov-2022-0039

ПИСМО ОД ОДДЕЛЕНИЕТО ЗА МЕДИЦИНСКИ НАУКИ ПРИ БУГАРСКАТА АКАДЕМИЈА НА НАУКИТЕ

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ

ДО

АКАДЕМИК, ПРОФЕСОР, Д-Р
ЖИВКО М. ПОПОВ, MD, PHD, FEBS
ЗАМ. ПРЕДСЕДАТЕЛ
НА МАКЕДОНСКАТА АКАДЕМИЯ
НА НАУКИТЕ И ИЗКУСТВАТА

УВАЖАЕМИ АКАДЕМИК ПОПОВ,

За нас, членовете на Отделението по "Медицински науки" при Българската Академия на Науките (БАН) и лично за мен е чест и удоволствие да Ви поздравим с Вашия Юбилей.

Вие сте известен учен и изследовател в областта на Онкогенезата на рака на пикочния мехур и откривател на молекулярни прогностични маркери; въвел сте в урологичната практика множество диагностични и лечебни методи; пръв на Балканите извършвате лапароскопска нефректомия на донорски бъбрек от жив дарител и много други приноси. Поради това за нас Вие сте учен от много високо ниво с международно признание.

Ние адмирираме Вашата настойчивост за осъществяване на Международния проект „Интегриран имуногенетичен анализ на молекулярни маркери при карцином на пикочния мехур за оценка на рецидив и прогресия на болестта", на който сте ръководител от Северно-Македонска страна, и в който участват членове на нашето отделение (Акад. Дамян Дамянов и чл.кор.проф. Чавдар Славов). С този проект е положено началото на Българо-Северномакедонска ДНК банка, съдържаща туморни ДНК, изолирани от тумори на пикочния мехур в различни стадии и степени на развитие.

Пожелаваме Ви много здраве, дълголетие и нови професионални и научни достижения и Ви уверяваме в нашата добронамереност и желание за по-нататъшно сътрудничество.

23.06. 2021 г.
София

ПРЕДСЕДАТЕЛ НА ОМН
ПРИ БАН:

Акад.проф.д-р Богдан Петрунов, дмн

Издавач:

Македонска академија на науките и уметностите

Јазична редакција:

Софија Чолаковска-Поповска
Николинка Нолевска

Компјутерска обработка:

Драгица Топузоска

Печат:
„Европа 92“ – Кочани

Тираж:

200 примероци

Зборникот е подготвен за печатење во мај 2022 година