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NON-HODGKIN'S LYMPHOMAS: IMMUNOLOGIC PROGNOSTIC STUDIES

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Abstract: Non-Hodgkin's lymphomas are malignant lymphoproliferative disorders that originate from B or T-lymphocytes and rarely from NK cells. They represent an extremely heterogeneous group of diseases regarding histologic subtypes, clinical presentations, immunophenotypic profiles, cytogenetic and molecular features and suitable mode of treatment.

A clinical indicator of prognosis, the International Prognostic Index, takes into account factors that are mostly linked to patient characteristics (age, performance status) and to disease extension and growth (disease stage, s.lactate dehydrogenase level and extent of extranodal involvement). However, it is clear that differences in clinical features and in treatment responses are a result of the marked genetic, immunophenotypic and molecular heterogeneity that underlie disease aggressiveness and tumour progresssion. We applied IPI (based on pretreatment clinical characteristics) in our group of 136 patients that identified a subgroup of clinical features that remained independently significant in multivariate analyses.

In our results IPI turned out to be of prognostic significance for response rate and survival percentages. Based on their number of "poor risk" factors, patients were placed into four IPI risk groups: low (one or no risk factors), low-intermediate (two risk factors), intermediate-high (three risk factors), and high (four or five risk factors) with five years survival rates of 88%; 82%; 18% and 0% respectively.

However, one limitation of this prediction strategy is that IPI does not encompass molecular abnormalities of tumour cells, which may play a critical role in determining profoundly different clinical outcomes in patients within the same group as defined by IPI.

The aim of this study was to assess the clinical significance and potential prognostic value of the expression of the new immunologic prognostic markers including nuclear proliferating antigen, suppressor and oncogenic proteins, HLA-DR surface antigens, tumour infiltrating T-lymphocytes, lymphocyte homing receptor and angiogenic molecules.

Immunohistochemistry was used to examine paraffin-embedded tumour tissues for determining the expression of immunologic prognostic markers.

Survival analysis showed that serum-high lactate dehydrogenase level, poor performance status (ECOG 3, 4 and 5), high proliferative activity defined as nuclear Ki67 expression greater than 60% of malignant cells and high tumour invasive potential defined by discontinued or loss of Collagen IV were found as strong predictors of poor survival among these patients. These four prognostic parameters determined the New-PI with three risk groups: good (0–1 risk factors), intermediate (2 risk factors) and poor (3 and more risk factors), with predicted five-years survival rates of 88%, 64% and 0%. The New-PI more accurately predicts the outcome than the standard IPI ($p < 0{,}001$ vs. $p < 0.0001$).

Based on a single institution series of 136 patients the IPI has proved to be a useful prognostic tool for NHL patients. Addition of new cellular markers into the standard IPI significantly improves risk stratification in NHL.

Key words: malignant lymphomas, prognosis, monoclonal antibody, immunophenoltype, immunohistochemical analysis, Ki67, cyclinD1, type IV collagen, CD44, p53, bcl2, bcl6, vimentin, IPI, N-PI.

Introduction

Non-Hodgkin's lymphomas are malignant lymphoproliferative disorders that originate from B or T-lymphocytes and rarely from NK cells. They represent an extremely heterogeneous group of diseases regarding histologic subtypes, clinical presentations, immunophenotypic profiles, cytogenetic and molecular features and suitable mode of treatment. This heterogeneity has important prognostic and therapeutic implications.

The prognosis of patients with NHL generally depends on the histologic sub-type and the stage of disease. However, even within histology and clinical stage groupings there is a remarkable variability in outcome [28]. For this reason a clinical indicator of prognosis, the International Prognostic Index (IPI) was developed [6, 15]. As a predictive model based on patients' clinical characteristics before treatment, IPI incorporates factors that are mostly linked to patients' characteristics, including clinical features that reflect the growth and invasive potential of the tumour (tumour stage, serum Lactate Dehydrogenase [LDH] level and number of extranodal sites), the patient's response to the tumour (Performance Status [PS]), and the patient's ability to tolerate intensive therapy (age, PS).

This prognostic model identifies four prognostic groups: low, low-intermediate, intermediate-high and high risk groups and is much more accurate than the Ann Arbor staging system.

However, one limitation of this prediction strategy is that IPI does not encompass molecular abnormalities of tumour cells, which may play a critical role in determining profoundly different clinical outcomes in patients within the same group defined by IPI [26].

In addition to the clinical characteristics, many new immunologic and molecular factors have been identified associated with prognosis. These new prognostic factors include markers, which reflect profound changes in the nature of the lymphomatous cells, particulary those involving the control of mitosis and the host response to tumour. Beyond their predictive value, these markers have identified phenotypes that may serve as new phenotype-directed therapies [1, 2, 26, 28, 29]. To determine whether immunologic markers can be combined with IPI to more accurately predict outcome, we analysed proliferative status, cell adhesion molecule status, histocompatibility antigens, tumour infiltrating T-lymphocytes, oncogenic alterations and angiogenic molecules.

The monoclonal antibody Ki-67 detects a human nuclear antigen present in proliferating cells. The determination of the growth fraction with Ki-67 identifies patients who might benefit from more aggressive therapy [2, 12, 13, 14, 21].

Cell-adhesion molecules are critical in the control of lymphocyte homing and migration, and their expression may influence lymphoma dissemination. Several studies suggest that the cell-adhesion molecule, Lymphocyte Homing Receptor (LHR), CD44 identifies lymphomas with high metastatic potential and poor prognosis. The correlation between LHR expression and disease stage suggests that LHR could be a "stage specific" phenotype [17, 18].

Hystocompatibility Antigens (HLA) are cell surface antigens relevant to cell self-recognition, cell-cell interaction, immunosurveillance and tumour containment [28]. It was noted that low HLA-DR expression or loss is associated with poor outcome compared with intact HLA-DR molecules [3, 25]. In the case of HLA loss, successful treatment requires restoration of tumour immunogenicity with IFN-α and INF-γ stimulating HLA antigen expression.

Rosenberg *et. al*. have emphasized the importance of tumour –infiltrating T-lymphocytes (T-TIL) in tumour containment. Lymphoma relapse and patient survival have been related to the type and number of specific T-cell subsets that infiltrate B-cell lymphoma [4].

The malignant transformation of lymphoid cells is triggered by genetic changes including chromosomal translocations, deletions, gene rearrangements,

mutations and genetic alterations. These alterations frequently involve oncogenes that are critical to control of lymphoid cell proliferation, differentiation and survival. The oncogene protein products of altered genes are now detected with monoclonal antibodies making them important immunologic markers.

p53 inhibit growth in their wild form. These genes serve as a natural check on unbridled proliferation, unless their function is lost through mutation or alotypic deletion.

The presence of p53 mutation and overexpression in some lymphomas is consistent with the hypothesis that the p53 gene could play a role in the later stages of lymphomagenesis or in the progression of the disease.

CyclinD1 protein expression is essential for reliable diagnosis of Mantle Cell Lymphoma (MCL). MCL and its cytologic variants are associated with poor prognosis and its distinction from other small B-cell lymphomas is clinically important [7].

Bcl-2 is unique among protooncogenes being localized to mithochondria [9]. Bcl-2 protein expression blocks programmed cell death extending cell survival. High bcl-2 protein expression is more frequently associated with an advanced stage of disease with reduced survival [10, 11].

Bcl-6 protooncogen is localized in the nuclei of most of the germinal centre B-cells. The Bcl-6 gene expression is regulated during B-cell differenttiation and suggests a role for Bcl-6 in germinal centre development and function. Deregulated Bcl-6 expression may contribute to lymphomagenesis by preventing postgerminal center differentiation [16].

Tumour angiogenesis is a complex process involving growth factors and receptors. A particular member of the extracellular matrix is the basement membrane, a thin layer which ensheaths blood vessels. Collagen type IV is a major constituent of basement membranes. Benign lesions showed intact basement membranes with strong linear staining of collagen type IV. The disruption or loss of basement membranes by invasive tumours but not by their preinvasive or benign changes represents a fundamental morphologic and biologic difference between malignant and benign tumours [20, 22].

CD31/PECAM1, integral membrane glycoprotein expressed on endothelial cells, platelets and leucocytes and Cd34, membrane phosphoglycoprotein expressed by hematopoietic stem cells, vascular endothelial cells and tissue fibroblasts are important endothelial cell markers in the study of malignant neovascularisation. Staining for CD31 and CD34 is used to measure tumour angiogenesis and to predict tumour recurrence.

The perivascular expression of the intermediate filament protein, vimentin**,** is assumed to be an important marker for tumour neovascularisation.

Aims of the study

The aim of this study was to assess the clinical significance and potential prognostic values of the expression of the new immunologic prognostic markers including nuclear proliferating antigen, suppressor and oncogenic proteins, HLA-DR surface antigens, tumour infiltrating T-lymphocytes, lymphocyte homing receptor and angiogenic molecules.

The other aims, if there are significant clinical and prognostic values for these markers, were:

– to determine the variables that would predict an asymptomatic period of the disease and survival.

– to include these prognostic parameters in the IPI parametric list, increasing the prognostic value of IPI.

Material and methods

Patients

In this study 136 patients were included with diagnosed non-Hodgkin`s lymphoma, treated and followed up at the Haematology Clinic in the period between June 1997 and December 2003. The criteria for including the patients were complete clinical data from the Clinic archives and representative histologic material at the Institute of Pathology, Medical Faculty, Skopje.

The patients were diagnosed according to accepted standards for diagnosis of malignant lymphomas. Further investigations that were undertaken in all patients included: physical examination, peripheral blood analysis, biochemical analysis, radiography of the chest, ultrasound of the abdomen, CT of the chest and abdominal cavity, bone marrow biopsy and biopsies of other tissues invaded by malignant lymphomas.

Special attention was paid to the variables that consistuted the IPI.

Diagnosed malignant lymphomas were classified according to the criteria of the REAL classification. Twenty-four patients had small lymphocytic lymphomas, twenty-eight patients had follicular lymphomas, fourteen patients had marginal zone lymphoma, 63 had diffuse large B cell lymphoma and 8 patients had lymphoblastic lymphomas, one of which was of T cell origin.

Histological and Immunohistochemical analysis

Histological analysis included nodal and extranodal tissues affected by lymphomatous infiltration, taken by biopsy or by surgical procedure. Ten lymph nodes with diagnosed reactive changes were used as a control group. The tis-

sues were fixed in 10% neutral formaline for 18–24 hours and paraffin embedded. Paraffin sections of 4–6 microns thickness were stained for haemalaun eosin, Giemsa, PAS and reticulin-Gomori. Immunohistochemical analysis for the immunological markers was done by modified biotin-strepatvidin immunoperoxidase reaction in three steps. We used the following panel of monoclonal antibodies:

– pan B cell markers: anti-human CD20cy, B cell, Clone L26 and CD79a, B cell, Clone JCB117;

– pan T cell markers: anti-human CD45RO, T cell, Clone UCHL1; anti-human CD43, T cell, Clone DF-T1; anti-human CD3, T cell, Clone F7.2.38; anti-human CD8 T cell, Clone C8/144B;

– markers with possible diagnostic and prognostic values: anti-human Cyclind1, Clone DCS-6; anti-human Bcl2 Protein, Clone 124; anti-human Bcl6 Protein, Clone PG-B6p; anti-human HLA-DR, alpha-chain, Clone TAL.1B5;

– oncogen prognostic marker – anti-human p53 Protein, Clone DO-7;

– adhesive molecules: anti-human CD44 Clone 2A5; anti-human Collagen IV Clone CIV 22;

– Angiogenic molecules: anti-human CD31, Endothelal cell (PECAM-1), Clone JC/70A; anti-human Hematopoietic Progenitor Cell, CD3e4 (human endothelial venules), ClassII, Clone QBEnd 10; Anti-Vimentin, Clone Vim 3B4.

– Proliferative index was determined on slides stained for anti-human Ki-67 antigen, Clone MIB-1.

In the control group we included positive control on the reactive lymph nodes and negative control omitting the primary antibody with PBS.

The proliferative index was determined as a percentage of the cells that express Ki67 nuclear positivity out of the whole of the tumour cells [13]. There were three groups dependent on the estimation of the proliferative index: less than 20%; 20-60% (Figure 1) and more than 60%.

The expression of the bcl1, bcl2 and bcl6 had diagnostic value in the mantle cell lypmphoma, follicular lymphoma and diffuse large B cell lymphoma respectively. Their expression was defined as nuclear and/or cytoplasmic [7].

Expresion of p53 was semiquantitatively estimated and expressed as a percentage of the positive cells in three groups: negative expression, 10–30% and more than 30%. Nuclear expression was taken as a specific expression.

CD44 is an adhesive molecule that helps lymphocytes in the homing of determined tissue compartments [17]. CD44 expression was defined as diffuse expression when more than 60% of the cells showed membrane expression and focal expression of up to 10% and more than 10% with perivascular localization or randomly located expression. Staining of reactive macrophages in these sections was taken as an additional internal positive control.

HLA-DR expression was defined as cytoplasmic expression from 10-30%, 30-60% and more than 60%.

Tumour infiltrating T cells were semiquantitatively determined by analysis of more than 10 HPF in sections stained with CD8, as well as CD3 and CD43. Analysed cases were divided into four groups: absence of TIL, presence of less than 10%, 10 to 30% (Figure 2) and more than 30% [26].

Immunohistochemical staining for Collagen IV enabled us to visualize endothelial basal membrane. Depending on the degree of tumour invasion we found different morphological expressions (Figure 3). In the control group we found a continuous basement membrane while the analysis of the Collagen IV in the analysed group showed changes such as thinning, discontinuous expression and complete disappearance [22].

Angiogenesis in the analysed group was defined as the mean number of blood vessels determined at 10 HPF, possibly in so-called 'hot' areas. We used two monoclonal antibodies that label vascular endothelium: CD31/PECAM-1/ECM and CD34/ECM. We defined three grades of neovascularisations (Figure 4): five blood vessels, 5 to 10 blood vessels and more than 10 blood vessels.

Vimentin staining expressed cytoplasmic staining in lymphoid, histiocytic and vascular endothelial cells as well as perivascular expression, isolated or combined with fragmented stromal expression [23].

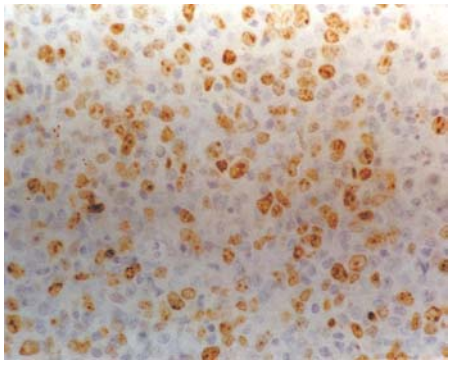

Figure 1 – Proliferative index (Ki67 > 60%), Ki67 : 400x
Слика – Пролиферативен индекс (Ки67 > 60%), Ки67 : 400x

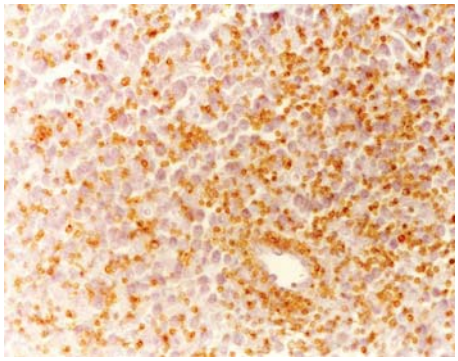

Figure 2 – Tumour infiltrating T-lymphocites (> 30%), CD3 : 400x
Слика 2 – Тумор на инфилтрирачки Т-лимфоцити (> 30%), CD3 : 400x

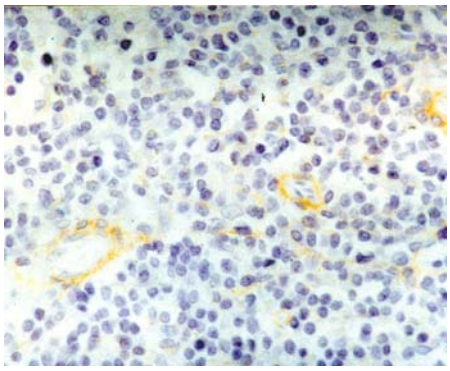

Figure 3 – Discontinuous Collagen type IV expression, Collagen: 400x
Слика 3 – Дисконтинуирана експресија на колаген тип IV, Колаген: 400x

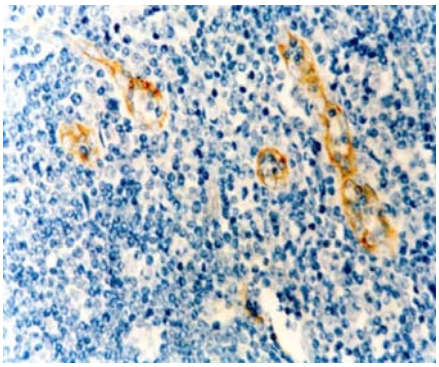

Figure 4 – Endothelial CD31 expression (up to 5 blood vessels), CD31 : 400x
Слика 4 – Ендотелијална CD31 експресија (до 5 крвни садови), CD31 : 400x

Results

The clinical and pathological characteristics of 136 patients were included in the study.

We have used the widely accepted clinical characteristics that reflect tumour extension and growth (level of serum LDH, clinical stage, tumour size, number of extranodal sites, bone marrow involvement), patient's response to the tumour (performance status, B-symptoms) and the patient's ability to tolerate intensive treatment (age, performance status, bone marrow involvement).

The median age of the patients was 49 years at diagnosis, with 95 patients aged up to 60 years and 41 patients older than 60 years. Patients included 54 females and 82 males (F : M ratio 1 : 1.5).

The International Prognostic Index (IPI) was tested on the entire group based on the clinical characteristics at the time of diagnosis: the age, tumour stage, serum lactate dehydrogenase concentration, performance status and number of extranodal sites.

The results showed that an advanced stage of the disease, elevated concentration of serum LDH, age over 60 years and poor performance status are significantly associated statistically with shortened survival and shortened disease-free survival. The number of extranodal sites at the time of diagnosis did not show any significant influence on the survival period.

These four characteristics remained independently significant in the analysis of the study group. The inclusion of these characteristics in the IPI offers a model for predicting the individual patient's risk of death.

The following graphs show IPI related to survival and disease-free survival respectively (Figure 5).

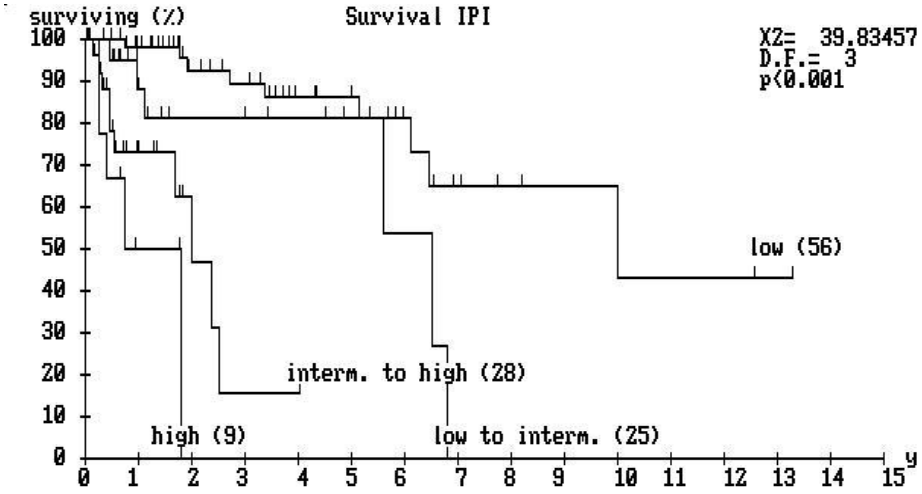

Figure 5 – Survival according to IPI[*]
Слика 5 – Pre`ivuvawe vo odnos na IPI

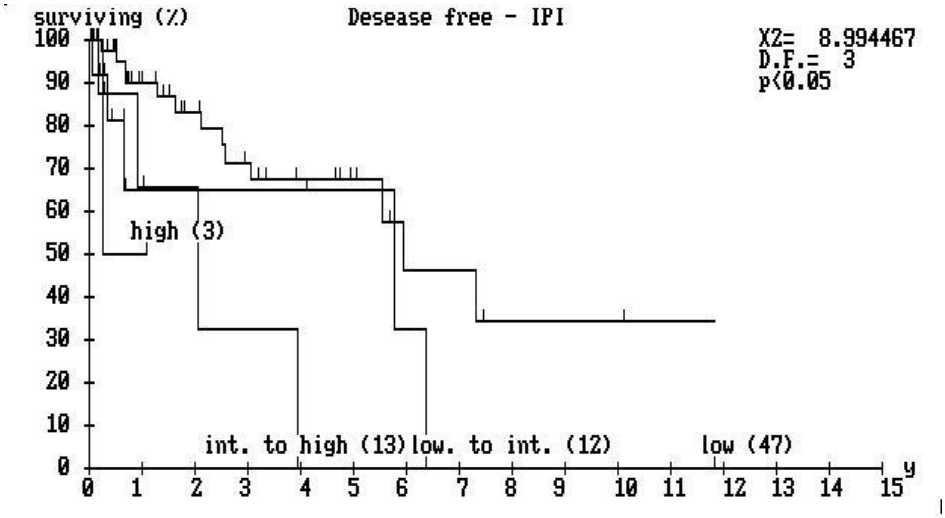

Figure 6 – Disease free survival according to IPI[**]
Слика 6 – Период без болеш̄ во однос на ИПИ

IPI identified four risk groups of patients: low risk, low to intermediate, intermediate to high and high-risk group (Figure 5). Analysis of the data confirmed that disease-free survival is directly correlated to IPI (Figure 6).

Application of this widely accepted model in our study confirmed the clinical importance of this index. This prognostic model identified four groups of patients with low, low to intermediate, intermediate to high and high risk, with predicted five-year survival rates of 87%, 82%, 18% and 0% respectively, and five-year disease free survival rates of 68%, 66%, 30% and 0%.

IPI survival curves for all four groups of patients showed high statistical significance ($p < 0.001$). The prognostic significance for disease-free survival is also high ($p < 0.05$).

The following results show relations between immunological markers and both survival and disease-free survival:

Ki67 protein expression was classified into three proliferative subgroups: high (> 60%), intermediate (20–60%) and low proliferative index (< 20%). The patients with an intermediate proliferative index had five-year survival in 64%, but the pragmatically favourable group with a low proliferative index (<20%) showed five-year survival in 90% of cases. Five-year survival for the prognostically unfavourable group (Ki67 expression more than 60%) is only 38%.

Cyclin D1 protein expression was confirmed in all three patients with mantle cell lymphoma, in two of 24 patients with small lymphocytic lymphomas and in only one patient of 63 with diffuse large cell lymphoma. This oncogen protein did not show any expression in cases with follicular and marginal zone cell lymphomas, or in patients with reactive lymphoid hyperplasia. Thus, cyclin D1 remains a specific marker for mantle cell lymphomas. The rare expression of cyclin D1 in other types of lymphomas can be explained as one of its cytological variants.

bcl-2 protein expression was found in 87% of the patients with follicular lymphomas and in a relatively high percent (38%) of cases with diffuse large cell lymphomas. Our study confirmed that the expression of bcl-2 is significantly more present in advanced compared with early stages of the disease ($p < 0.001$). Bcl-2 expression did not show any significant influence either on total survival or on disease-free survival.

Analysis of **bcl-6** expression in patients with diffuse large cell lymphomas showed no statistically significantly prolonged survival and disease-free survival compared with patients with loss of bcl-6 expression.

Expression of nuclear protein **p53** was equally present in different histologic subtypes. According to the clinical stage, this oncogen protein showed significantly higher positivity in advanced stages (70%) compared to early stages of the disease (30%).

The leukocyte homing receptor **CD44** was analysed in relation to lymphoma dissemination (clinical stage). High association was confirmed between

CD44 and clinical stage as 71% of the patients were in an advanced stage of the disease at diagnosis.

HLA-DR molecules expression *v* patient survival showed that a high expression (> 50%) is significantly related to better survival compared with cases with low expression or with none. The expression of HLA-DR molecules did not show any significant relation to disease-free survival in patients with previously achieved complete remission.

The number of tumour infiltrating T-lymphocytes greater than 10% did not appear statistically significant concerning the length of survival in our patients. However, a high percent of **T-TIL** confirmed the significant positive effect on disease-free survival in patients with achieved complete remission ($p < 0.05$).

The grade of tumour invasion, expressed by immunomorphological analysis of Collagen type IV was divided into three invasive degrees: high, intermediate and mild. High grade tumour invasion, histochemically defined as loss of Collagen type IV on the level of basal membranes, showed significantly shortened survival ($p < 0.05$) compared with remaining degrees, intermediate and mild, defined as discontinued or thin expression of Collagen type IV. The loss of Collagen IV (the highest grade of tumour invasion) confirmed significantly shortened survival, with 54% of patients surviving a period of five years, compared with the group with intermediate grade tumour invasion with a 78% five-years survival and the group of patients with mild tumour invasion with 100% five years survival.

The high grade of tumour invasion is in positive correlation to the advanced stage of the disease.

Expression of endothelial cell markers **CD31/PECAM-1** and **CD34** defined three grades of neovascularisation. Low degree vascularisation, determined as the presence of fewer than 5 blood vessels on a large microscopic field (LMF), intermediate degree vascularisation – 5–10 vessels on LMF, and high grade tumour vascularisation as more than 10 vessels on LMF. Vascular degrees were analyzed in relation to survival, but statistical significance was not confirmed. Analysis of the degree of vascularisation and disease-free survival showed that patients with greater vascularisation have a longer duration of remission. Although the statistical significance was not confirmed, we consider that vascularisation of tumour tissue is in direct positive correlation with the concentration of chemotherapeutic agents reaching the malignant cells. However, as the number of patients in our study with high vascularisation was relatively small, the consideration of the connection of the degree of vascularisation of malignant tissue and expected therapeutical and clinical response requires further clinical investigation.

The intermediate filament protein **Vimentin** showed cytoplasmatic or perivascular expression, or cytoplasmatic and perivascular expression at the same time. As an angiogenic molecule that marks the perivascular area it did not show a statistically significant influence on the survival of the patients and on the degree of tumour dissemination.

Univariant analysis of all investigated prognostic parameters showed that statistically significant factors in our study group were: age, ECOG, LDH, clinical stage, Ki67, Collagen IV, HLA-DR, T-TIL and bcl-6. Of these nine highly significant prognostic factors, the multivariant analysis confirmed four parameters as significant: LDH, ECOG, Collagen IV and Ki67. These four prognostic factors are suggested as a New Prognostic Index: N-PI.

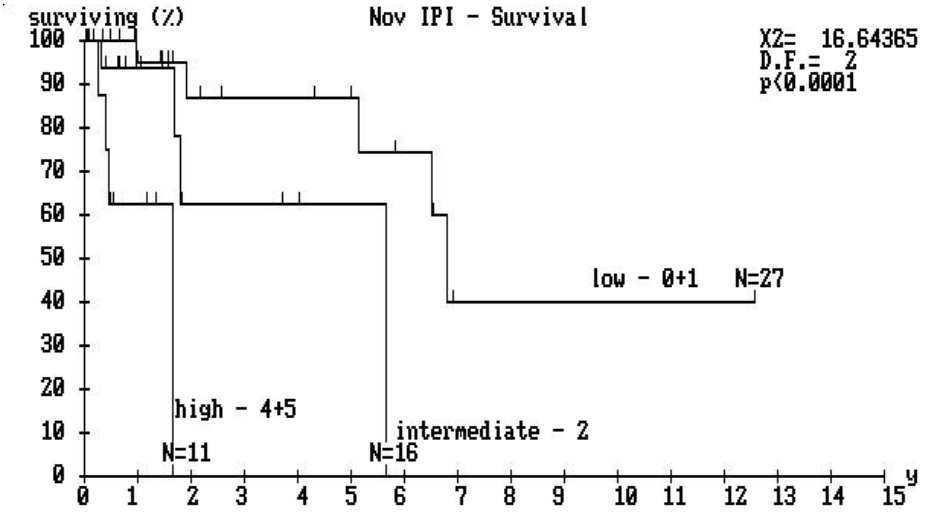

Figure 7 – Survival according to N-PI
Слика 7 – Pre`ivuvawe vo odnos na N-PI

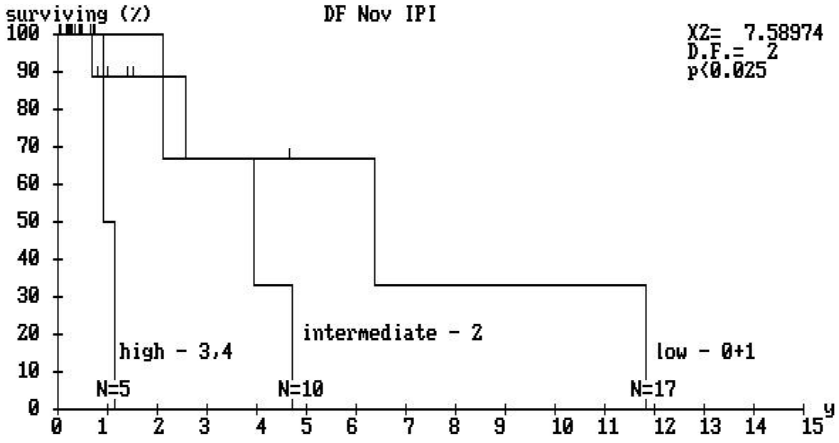

Figure 8 – Disease free survival according to N-PI
Слика 8 – Период без болести во однос на Н-ПИ

Figure 7 and Figure 8 present the results of total survival and disease-free survival of our study group according to the new prognostic index, NPI, that confirm its higher statistical significance ($p < 0.0001$) compared with the standard IPI model ($p < 0.001$). N-PI constitutes three groups of patients: a low risk group (score 0.1) with 5 year survival of 88%, an intermediate risk group (score 2) with 5 year survival of 63%, and a high risk group (score 3.4) with 5 year survival of 0%.

* Seventeen patients were excluded because of uncompleted data.
** Seventy-five patients achieved CR after the treatment

Discussion

The new prognostic index includes highly significant prognostic factors that enable better defining of patients with respect to survival.

Discovering new prognostic parameters which will define patients at high risk of recurrent disease is becoming an urgent issue. The International Prognostic Index contains widely accepted criteria for predicting the evolution of lymphomas, including factors closely connected to patients' characteristics or to the dissemination of the disease and tumour growth. However, it has appeared that this predicting strategy has some limitations. It does not include the molecular abnormalities of tumour cells which may play a critical role in discovering deep changes leading towards different clinical outcomes in patients within the same group as defined by IPI.

Important contributions made by this study are the results showing that immunological prognostic markers, combined with the clinical parameter of IPI, provides a new prognostic model with higher prognostic value. Addition of Ki67 and collagen type IV into the IPI significantly improves risk stratification in our NHL patients.

Today's investigations of NHL show tendencies towards unification of different prognostic models. One of the most widely accepted is IPI and its recent modification FLIPI. Our new prognostic model is a result of a union of clinical prognostic factors and exact, immunohistochemically defined, immunologic factors in representive samples for clinical application.

Conclusions

- The results of this study show that the prognosis of patients with Non-Hodgkin lymphomas mainly depends on their score on the International Prognostic Index, expression of the proliferative antigen Ki67, and the level of tumour invasion as defined by Collagen type IV.

• Inclusion of Ki67 and Collagen IV within the new prognostic model, together with the clinical parameters, performance status and the serum concentration of the lactate dehydrogenase, constitutes a new prognostic index with a higher prognostic value than IPI.

• Non-Hodgkin lymphomas as a biologically extreme heterogeneous group of malignant lymphoproliferative diseases require further assessment of the immunologic factors which, together with the clinical parameters, will serve as a means for predicting disease outcome and the choice of an optimal therapeutic approach.

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Р е з и м е

НЕ-ХОЧКИНОВИ ЛИМФОМИ: ИМУНОЛОШКИ ПРОГНОСТИЧКИ СТУДИИ

Хаџи-Пецова Л.,[1] Петрушевска Г.,[2] Стојановиќ А.[1]

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Не-Хочкиновите лимфоми претставуваат малигни лимфопролиферативни заболувања кои појавуваат екстремна хетерогеност во поглед на хистолошкиот подтип, клиничката презентација, имунофенотипскиот профил, цитогенетските и молекуларни карактеристики и можниот начин на лекување.

Потребата од одредување прогностички параметри кои ќе одредат пациенти со висок ризик од неуспех на стандардната хемотерапија, а кои можат да имаат корист од ризик-адаптирана терапија станува се поургентна. Клиничкиот прогностички индикатор, интернационалниот прогностички индекс (ИПИ), вклучува фактори кои се претежно поврзани со какрактеристиките на пациентите и со распространетоста на болеста и туморниот раст.

Неговата примена на нашата група пациенти (врз основа на предтераписките клинички својства) во зависност од бројот на неповолните ризик фактори ги разграничи пациентите во четири ИПИ ризични групи: ниска, ниска спрема средна, средна спрема висока, и висока ризична група со пет-годишна рата на прживување од 88%, 82%, 18% и 0%.

Меѓутоа, постои ограничување на оваа стратегија на предвидување. Таа не ги вклучува молекуларните абнормалности на туморните ќелии кои можат да играат критична улога во одредувањето на различни клинички исходи кај пациенти во рамките на една иста група дефинирана со ИПИ.

Цел на оваа студија беше процена на клиничката сигнификантност и потенцијалната прогностичка вредност на експресијата на новите имунолошки прогностички маркери како нуклеарниот пролиферативен антиген, супресорните и онкогени протеини, HLA-DR површинските антигени, туморните инфилтрирачки Т-лимфоцити, лимфоцитниот хоминг рецептор и ангиогените молекули.

За таа цел беше користена имунохистохемиска анализа на туморни ткива вкалапени во парафин.

Анализата на резултатите покажа дека абнормални вредности на лактат дехидрогеназа, лош перформанс статус, висока пролиферативна активност и висок инвазивен потенцијал на туморот се статистички значајни претскажувачи на лошото преживување кај овие пациенти. Овие четири прогностички параметри одредија нов прогностички индекс (Н-ПИ) со три ризични групи: добра, средна, и лоша ризична група со претскажани рати на петгодишно преживување од 88%, 64% и 0%. Резултатите покажаа дека Н-ПИ попрецизно го претскажува исходот на болеста во споредба со стандардниот ИПИ ($p < 0.001$ спрема $p < 0.0001$).

Клучни зборови: малигни лимфоми, прогноза, моноклонални антитела, имунофенотип, имунохистолишка анализа, Ki67, ciclinD1, тип IV collagen, CD44, p53, bcl2, bcl6, vimentin, ИПИ, Н-ПИ

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Figure 1. Proliferative index (Ki67>60%), Ki67: 400x.
Слика 1. Пролиферативен индекс (Ки67>60%), Ки67: 400ц.

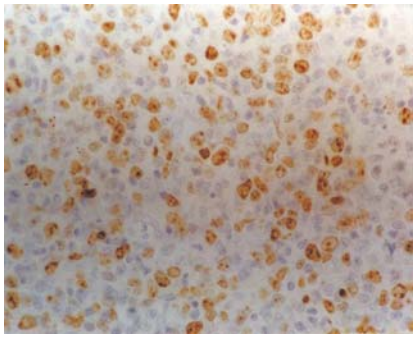


Figure 2. Tumour infiltrating T – lymphocites (>30%), CD3: 400x
Слика 2. Тумоур нфилтрирачки Т – лимфоцити (>30%), CD3: 400x.

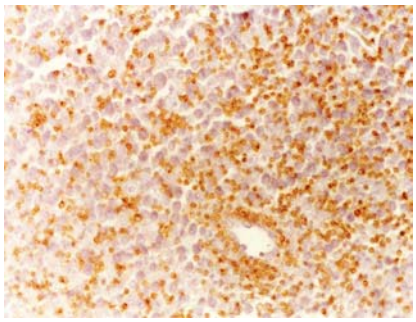


Figure 3. Discontinuous Collagen type IV expression, Collagen: 400x.
Слика 3. Дисконтинуирана експресија на колеген тип IV, Колаген: 400x.

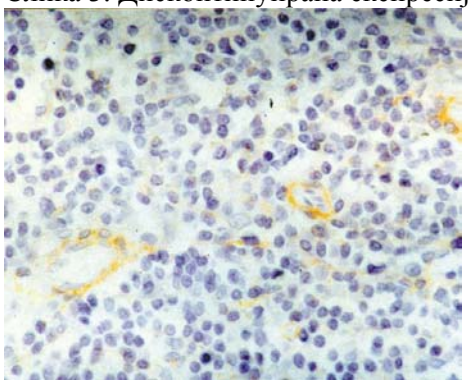


Figure 4. Endothelial CD31 expression (up to 5 blood vessels), CD31: 400x.
Слика 4. Ендотелијална CD31 експресија (до 5 крвни садови), CD31: 400х..

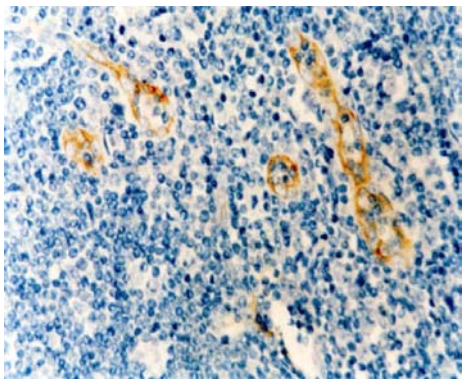


Figure 5. Survival according to IPI*
Слика 5. Pre`ivuvawe vo odnos na IPI.

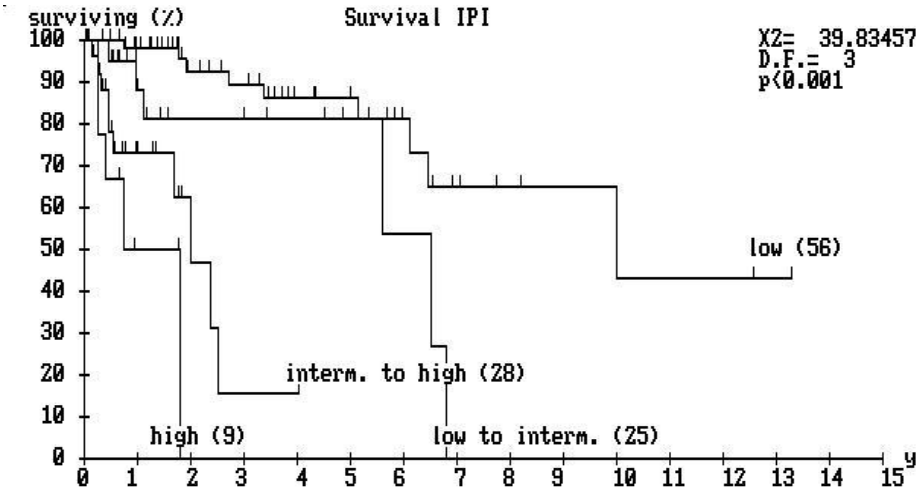
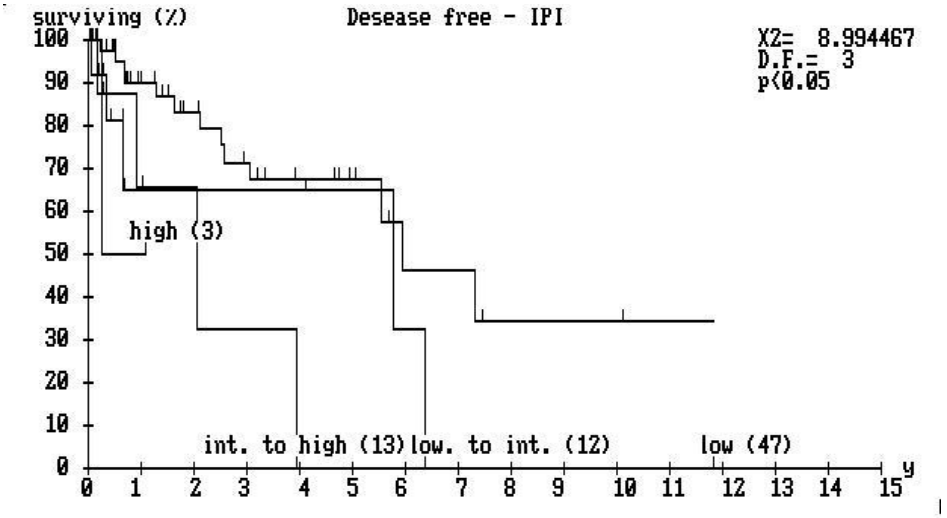


Figure 6. Disease free survival according to IPI**
Слика 6. Период без болест во однос на ИПИ.



* Seventeen patients were excluded because of uncompleted data.
** Seventy-five patients achieved CR after the treatment
Figure 7. Survival according to N-PI
Слика 7. Pre`ivuvawe vo odnos na N-PI.

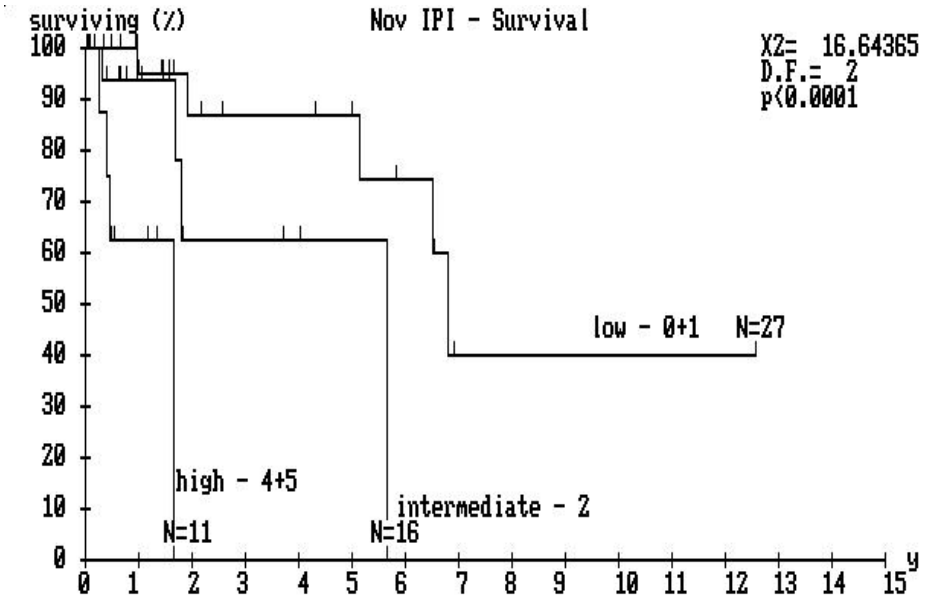
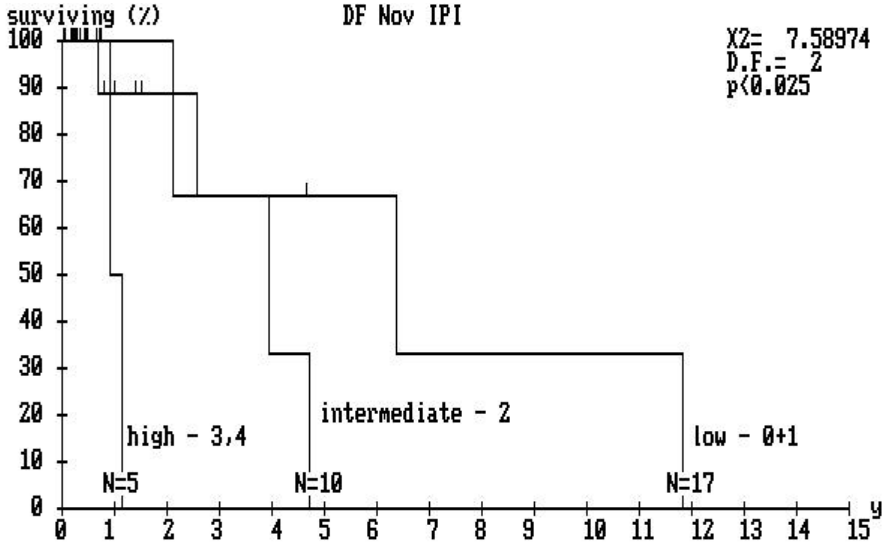


Figure 8. Disease free survival according to N-PI.
Слика 8. Период без болест во однос на Н-ПИ.



Р е з и м е

НЕ-ХОЧКИНОВИ ЛИМФОМИ: ИМУНОЛОШКИ ПРОГНОСТИЧКИ СТУДИИ

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