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ANCA-GBM DOT-BLOT TEST IN DIAGNOSIS OF PATIENTS WITH GLOMERULONEPHRITIS

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A b s t r a c t: Patients with rapidly progressive glomerulonephritis who are positive for anti-neutrophil cytoplasmic antibody (ANCA) or anti-glomerular basement membrane (GBM) antibodies may develop chronic renal failure leading to end-stage renal disease (ESRD) within days or weeks. The early serologic detection of auto-antibodies associated with ANCA and anti-GBM diseases will be helpful in preventing ESRD. We evaluated the combined ANCA-GBM dot-blot strip assay (Biomedical Diagnostics, Brugge, Belgium) in 30 consecutive patients with biopsy proven glomerulonephritis (GN). MPO- and PR3-ANCA were detected in 5 and 2 samples, respectively. Three samples were positive for both MPO- and PR3-ANCA (all 3 had focal segmental necrotizing GN). One patient was diagnosed as having Goodpastures' syndrome (the only anti-GBM positive result) and two had Wegener's granulomatosis (the two PR3-ANCA positive results). Two additional samples were equivocal: positive for MPO-ANCA and PR3-ANCA, respectively. Patients positive only for MPO-ANCA had only limited extrarenal organ manifestations. Anti-PR3 positive patients with necrotizing glomerulonephritis had a more dramatic deterioration of their renal function at diagnosis. Radiographically, these patients had nodular or pneumonia-like lesions. Acute respiratory failure necessitating mechanical ventilation was developed in one GBM positive patient. In conclusion, the

ANCA-GBM dot-blot is a useful screening tool in situations where conventional ANCA testing is not readily available.

Key words: rapidly progressive glomerulonephritis, anti-neutrophil cytoplasmic antibodies, anti-glomerular basement membrane antibodies, diagnosis.

Introduction

Rapidly progressive glomerulonephritis (RPGN) is a condition leading to irreversible renal failure within days or weeks and is potentially life-threatening. The shared glomerular lesion of the RPGN is a necrotizing glomerulonephritis, usually with resultant cellular crescent formation within most glomeruli [1]. The histologic counterpart of RPGN is crescentic GN: the proliferative cellular response outside the glomerular tuft but within Bowman's space that is known as a crescent. Typically, the glomerular tuft shows segmental necrosis – focal segmental necrotizing GN – and this is particularly characteristic of the vasculitis syndromes. RPGN can be primary or secondary. Primary RPGN is an autoimmune disease caused by immune-complexes, anti-neutrophil cytoplasmic antibodies (pauci-immune RPGN) or by anti-glomerular basement membrane antibodies (anti-GBM GN). Secondary RPGN can be any form of glomerulonephritis (lupus nephritis, IgA nephropathy, poststreptococcal GN, membranoproliferative GN). This emphasizes the need to obtain histologic confirmation of the clinical diagnosis. Treatment consists of disease-specific, immunosuppressive therapy. The aim of the therapy is to prevent further deterioration of the renal function as well as in part to reverse the damage [2, 3]. The likelihood of success of long-term maintenance of the renal function is inversely correlated with the serum creatinine when therapy begins, which indicates the importance of early diagnosis. Other predictors of the outcome are dialysis dependency and the percentage of non-sclerotic glomeruli at diagnosis [4]. Taken together, there are strong indications to start the therapy as soon as possible. For that reason early and quick diagnosis and serologic detection of auto-antibodies (ANCA, anti-GBM) involved in the pathogenesis of the diseases is obligatory.

Laboratory testing for ANCA should include both indirect immunofluorescence microscopy assay (IFA) and enzyme immunoassay (EIA). IFA using normal human neutrophils as substrate produces two major staining patterns, cytoplasmic (cANCA), and perinuclear (pANCA). By EIA, most cANCA have specificity for proteinase 3 (PR3-ANCA) and most pANCA have specificity for myeloperoxidase (MPO-ANCA) [5, 6]. For adequate diagnosis accuracy, all testing for ANCA should include an immunochemical analysis for antigen specifi-

city, such as an EIA. Anti-GBM GN is caused by autoimmunity to a specific component of the GBM that has been identified as the carboxyl-terminal, non-collagenous (NC1) domain of a type IV collagen chain, alpha 3(IV)NC1. Detection of anti-GBM antibodies in serum can also be evaluated by both IFA and EIA. The indirect immunofluorescence onto frozen sections of normal kidney is too insensitive for routine use[7].

For small laboratories with a not so frequent need for serologic testing EIA is expensive. Both forms of testing, IFA and EIA, require an adequately equipped immunologic laboratory with trained staff. Recently, the combined ANCA-GBM dot-blot strip assay (Biomedical Diagnostics, Brugge, Belgium) has been developed. This assay is easy to use, a fluorescence microsope or an optical density meter is unnecessary. The duration of the test is about two hours and results are obtained in one single assay.

We set up this study to evaluate the number of ANCA and anti-GBM positive patients with dot-blot assay in well-defined, i.e. with diagnosed renal biopsy patients with glomerulonephritis, at the Department of Nephrology in Skopje.

Patients and methods

Patients

Thirty consecutive patients with biopsy-proven glomerulonephritis at the Department of Nephrology, Skopje were included in the study. All serum samples were obtained at the time of renal biopsy and before treatment was initiated. The demographic, clinical and serological features of our patients were obtained. The indications for RB were follows: 1. proteinuria greater than 1g/d, 2. haematuria (>10 dismorphic red blood cells or one or more red blood cells casts, in the absence of a urinary tract infection or an indwelling catheter), and 3. abnormal renal function reflected by increasing serum creatinine (sCr) or declining glomerular filtration rate (GFR). Renal biopsy specimens were prepared for light and immunofluorescence microscopy. Pulmonary disease was defined as the presence of pulmonary infiltrates, coin lesions, or alveolar haemorrhage in the absence of other diseases in relation to those manifestations. Respiratory failure was defined as severely impaired gas exchange necessitating mechanical ventilation. Anti-GBM mediated glomerulonephritis was diagnosed based on the linear deposition of immunoglobulin, fluorescence of IgG, along with the GBM in the renal biopsy. Wegener's granulomatosis was defined as a granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting

small to medium-sized vessels including the kidney. Classification criteria for Wegener's granulomatosis are somewhat arbitrary [8].

Detection of ANCA and anti-GBM antibodies by ANCA-GBM dot-blot

The ANCA-GBM dot-blot assay is a qualitative assay. Purified antigens are blotted onto nitrocellulose strips as present spots. PR3 and MPO antigens used in this assay are produced from human leukocytes. GBM preparations contain the NC1 domain of type IV collagen and are produced from bovine kidneys. The producer provides directions for the test procedure. The strip was incubated with patient serum diluted 1:50 for 45 minutes. After washing with a standardized wash buffer, the bound antibody was visualized by incubation of the strip with an alkaline phosphatase-protein A conjugate for 20 minutes. Then the strip was incubated with the substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium for 10 minutes. Finally, the strip was allowed to dry completely for 30 minutes before reading the results. The dot-blot kit provides all the necessary reagents. Positive results were recorded when a grey/blue coloured spot was observed with a well-defined outline. Three observers independently evaluated all dot-blots. We had no inter-observer differences.

Results

PR3-ANCA were detected in 2 and MPO-ANCA in 5 serum samples (Table 1). Three additional samples were positive for both PR3- and MPO-ANCA; all three patients had focal segmental nectotizing GN. One patient was diagnosed as having Goodpasture's syndrome. This was the only positive anti-GBM result. Two patients were diagnosed as having Wegener's granulomatosis; they were positive for PR3-ANCA. Two serum samples were equivocally positive for PR3- and MPO-ANCA.

Patients positive for MPO-ANCA had only limited extrarenal organ manifestations (Table 2). Anti-PR3 positive patients with necrotizing glomerulonephritis had a more dramatic deterioration of their renal function at diagnosis, and they both continued on dialysis. Radiographically, these patients had nodular or pneumonia-like lesions. The only anti-GBM positive patient developed acute respiratory failure necessitating mechanical ventilation for a limited time. Later, the patient recovered with immunosuppressive therapy and plasma exchange but remained dialysis dependent.

Table 1 – Табела 1

Pathohistological diagnosis of thirty consecutive patients with biopsy proven glomerulonephritis.

Патохистолошки дијагнози на триесет последователни пациенти со гломерулонефритис докажан со биопсија

Patient No.	ANCA-GBM dot-blot positivity
1. Membranous nephropathy	
2. Lupus nephritis IV-V	
3. **Endo and extracapillaris GN (vasculitis)**	MPO-ANCA
4. **Endo and extracapillaris GN (WG)**	PR3-ANCA
5. **Endo and extracapillaris crescentic RPGN**	MPO-ANCA
6. Membranous nephropathy	
7. **Mesangial proliferative focal and segmental**	MPO-ANCA
8. **anti-GBM crescentic GN**	anti-GBM antibodies
9. **Endo and extracapillaris GN (WG)**	PR3-ANCA
10. End stage chronic GN	
11. IgA nephropathy	
12. Chronic GN	
13. Membranoproliferative GN	
14. Mesangial proliferative GN	
15. **Mesangial proliferative focal and segmental**	equivocal MPO-ANCA
16. **Focal segmental glomerulosclerosis**	MPO and PR3 -ANCA
17. IgA nephropathy	
18. **Endo and extracapillaris, RPGN**	MPO-ANCA
19. Mesangial proliferative GN	
20. End stage chronic GN	
21. **Focal segmental glomerulosclerosis**	MPO and PR3 -ANCA
22. **Focal segmental glomerulosclerosis**	MPO and PR3 -ANCA
23. IgA nephropathy	
24. End stage chronic GN	
25. Acute tubulointerstitionephritis	
26. IgA nephropathy	
27. Mesangial proliferative GN	
28. **Mesangial proliferative focal and segmental**	equivocal PR3-ANCA
29. IgA nephropathy	
30. **Endo and extracapillaris, RPGN**	MPO-ANCA

GN: glomerulonephritis; WG: Wegener´s granulomatosis; RPGN: rapidly progressive glomerulonephritis;

Table 2 – Табела 2

Clinical findings and organ involvement in patients positive on ANCA-GBM dot-blot.
Клинички наоди и орѓанско зафаќање кај пациентите позитивни на АНЦА-ГБМ дот-блот.

	Patient No.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
ANCA													
- MPO	+		+	+			+/-		+				+
- PR3		+				+						+/-	
- MPO and PR3								+		+	+		
Anti-GBM					+								
Age	54	64	16	21	20	40	48	46	64	37	51	26	48
Sex	M	F	M	M	M	M	F	F	M	F	F	M	F
Initiate													
- more chronic	+			+			+	+			+	+	
- more acute		+	+		+	+			+	+			+
Presenting symptoms...													
- constitutional	+	+	+		+	+			+	+			+
- arthralgia	+										+		
- dyspnea		+			+	+			+				+

	Patient No.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
- haemoptysis		+			+								
- sinusitis	+			+									
- conjuctivitis	+												
Organ involvement...													
- lung		+	+		+	+							
- ENT				+		+						+	
- joints	+												
- eye					+								+
- skin	+												
- others	+								+				
GFR (ml/min)	93	HD	HD	96	HD	HD	32	85	84	60	49	105	HD
ESR (mm/hr)	60	138	55	47	126	100	32	30	77	30	43	38	150
Proteinuria g/24h	2.2	0.5	2.0	0.7	1.0	0.9	1.7	2.7	2.2	8.4	1.5	1.8	ND
BP (mmHg)	230/ 120	140/ 80	160/ 110	190/ 130	120/ 70	200/ 120	150/ 90	140/ 95	140/ 90	160/ 120	180/ 110	120/ 80	110/ 70

ANCA: antineutrophil cytoplasmic autoantibodies; MPO: myeloperoxidase; PR3: proteinase 3; GBM: glomerular basement membrane; ENT: ear, nose, and throat; GFR: glomerular filtration rate; HD: haemodialysis; ESR: erythrocyte sedimentation rate;ND: not done, anuric; BP: blood pressure.

Discussion

The variable prognosis of glomerulonephritis has frustrated a unified approach to its treatment. This applies both to the severity of the presentation and to the subsequent relapse risk. There may be a need for rapid disease control to restrict renal and other vital organ damage in some, while in other cases progression is slower. The standard and most widely recommended therapy, steroids and cyclophosphamide, while very effective at suppressing disease activity, has proved too toxic. For this reason, rapid and clear identification of the type of RPGN, including ANCA-associated crescentic GN and anti-GBM nephritis, is mandatory. Severe renal disease is a hallmark of ANCA associated disease, most studies report renal involvement in 75%–90% of patients [9].

The more severe renal damage and necessity of dialysis in both our PR3-ANCA positive patients can be explained by the pathohistological findings. PR3-ANCA associated glomerulonephritis reveals acute tubular necrosis and more acute glomerular necrosis and cellular crescents. Renal lesions in MPO-ANCA patients develop more slowly. This, in turn, results in increased chronic lesions, and more glomerular and interstitial fibrosis [10, 11, 12].

MPO-ANCA positive patients generally have fewer extrarenal organs involved in the disease process than PR3-ANCA patients. In MPO-ANCA positive patients the upper respiratory tract involvement is milder and characterized by nasal polyps, rhinitis and/or sinusitis. These patients less frequently have an aggressive, destructive inflammatory process with nodular and/or pneumonia-like lesions typical of PR3-ANCA patients on their chest x-rays and have more patchy lesions [13]. The absence of nodular lesions is probably due to the absence of granulomatous inflammation in most of the MPO-ANCA patients. Granulomas are almost exclusively found in PR3-ANCA patients (14).

Rutgers *et al*. found that the performance of the dot-blot is excellent for the detection of PR3-ANCA, with a high sensitivity and specificity (15). MPO-ANCA were detected with a high specificity as well, but a reduced sensitivity was observed: 14–20% of patients were missed. In the case of a negative dot-blot result in patients with a high suspicion of MPO-ANCA associated RPGN, they advise that conventional analysis for MPO-ANCA should be performed. Specificity in their study was 100%, i.e. no patients were wrongly diagnosed as having MPO-ANCA associated vasculitis. They also found that the sensitivity of the anti-GBM dot-blot was excellent. They suggested that a negative anti-GBM dot-blot makes anti-GBM disease very unlikely, but positive samples should be re-evaluated by conventional anti-GBM tests.

The ANCA-GBM dot-blot is a practical and useful screening tool in situations where standard ANCA testing is not available. For small laboratories

and in situations where rapid diagnosis is required, the ANCA-GBM dot-blot has the advantage in terms of simplicity, time and price.

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Резиме

АНЦА-ГБМ ДОТ-БЛОТ ТЕСТ ВО ДИЈАГНОЗАТА НА ПАЦИЕНТИТЕ СО ГЛОМЕРУЛОНЕФРИТИС

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Пациентите со брзо прогредирачки гломерулонефритис кои се позитивни за антитела насочени кон цитоплазмата на неутрофилите (АНЦА) или антитела насочени кон гломеруларната базална мембрана (ГБМ) може да развијат хронична бубрежна слабост која води до краен стадиум на бубрежна болест во тек на неколку денови или недели. Раното серолошко откривање на автоантителата асоцирани со АНЦА или анти-ГБМ болест е корисно во превенција на крајниот стадиум на бубрежната болест. Ние го евалуиравме АНЦА-ГБМ дот-блот стрип есејот кај 30 консекутивни пациенти со биопсија докажан гломерулонефритис (ГН). МПО-АНЦА беа најдени кај 5, а ПР3-АНЦА кај 2 примерока. Три примероци беа позитивни и за МПО- и за ПР3-АНЦА, сите тројца пациенти имаа фокално сегментен некротизирачки ГН. Еден пациент беше дијагностициран како синдром на Goodpastures (тоа беше единствениот анти-ГБМ позитивен резултат) и двајца пациенти имаа Wegener-ова грануломатоза (двата ПР3-АНЦА позитивни резултати). Два додатни примерока беа несигурно позитивни за МПО-АНЦА и ПР3-АНЦА. Пациентите позитивни само за МПО-АНЦА имаа само ограничени екстра-

ренални манифестации на другите органи. Анти ПР3-АНЦА позитивните пациенти со некротизирачки гломерулонефритис имаа подраматична детериорација на реналната функција при дијагностицирањето. Радиографски, овие пациенти имаа нодуларни лезии или лезии кои личат на пневмонија. Акутна респираторна слабост која бара механичка вентилација се развви кај ГБМ позитивниот пациент. Во заклучок, АНЦА-ГБМ дот-блот есејот е корисен инструмент за скрининг во ситуации каде што конвенционалното АНЦА тестирање не е расположиво.

Клучни зборови: брзо прогредирачки гломерулонефритис, антитела кон цитоплазмата на неутрофилите, анти-гломеруларна базална мембрана антитела, дијагноза.

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