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Adress: Univerzitetski trg 2, 18000 Niš, YU, Tel: +381 18 547-095 Fax: +381 18 547-950
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GLYCOSAMINOGLYCANS AND MYOFIBROBLASTS MODULATE THE GLOMERULAR AND TUBULOINTERSTITIAL INJURY IN PRIMARY TYPE I MESANGIOCAPILLARY GLOMERULONEPHRITIS

Ligia Petrica[1], Marius Raica[2], Adalbert Schiller[1], Silvia Velciov[1], Gheorghe Gluhovschi[1], Virginia Trandafirescu[1], Gheorghe Bozdog[1], Cristina Gluhovschi[1], Flaviu Bob[1]

University of Medicine and Pharmacy
[1]Department of Nephrology and [2]Department of Histology, County Hospital, Timişoara, Romania

Summary. *Glycosaminoglycans (GAGs) play a crucial role in the layout of the electronegative filter of the glomerular basement membrane (GBM) and have a possible involvement in renal fibrosis. The expression of α-smooth muscle actin (α-SMA) amongst other cytoskeletal proteins, such as vimentin (Vi), in the glomerulus and the intestitium correlates with glomerulosclerosis (GSL) and tubulointerstitial fibrosis (TI). The purpose of the study was to evaluate clinical, biochemical and histological aspects of primary type I mesangiocapillary glomerulonephritis (MCGN) related to alterations of GAGs deposits and to the expression of α-SMA and Vi by glomerular and interstitial cells.*
A group of 28 patients (p) with primary type I MCGN was assessed concerning blood pressure, proteinuria and serum creatinine. Kidney biopsies were performed in all patients and were processed in light microsopy, immunofluorescence and immunohistochemistry in order to reveal GAGs deposits, Vi-and α-SMA / PCNA (proliferating celll nuclear antigen) positive cells in the glomerulus and the interstitium.
Of the 28 p, 19 p (67.85%) - group B presented with significant alterations of GAGs in the capillaries, mesangium and interstitium, while 9 p (32.15%) - group A, had a mild impairment of the distribution of GAGs only in the capillary walls.
Linear regression analysis showed in both groups an inverse correlation between GAGs staining in the capillaries and proteinuria and Ig deposits in the capillary walls. In group B, we found an inverse correlation between the GSL score and Vi expression in the glomerulus, and a direct correlation between this score and glomerular α-SMA and PCNA expression. TI injury correlated positively with interstitial α-SMA and PCNA. In group A, the GSL score showed an inverse correlation with glomerular Vi, but did not reveal a significant correlation with glomerular α-SMA and PCNA. TI lesions correlated weakly with intersititial α-SMA alone. In group B, proteinuria (P) and serum creatinine (SC) correlated inversely with glomerular Vi and directly with glomerular and interstitial α-SMA / PCNA. In group A, P showed an inverse correlation with glomerular Vi, but no correlations with glomerular and interstitial α-SMA / PCNA; SC correlated inversely with glomerular Vi and weakly with interstitial α-SMA.
In conclusion, the alterations of GAGs deposits are significant in primary type I MCGN and they correlate with the glomerular and interstitial expression of α-SMA and Vi. The biochemical picture of MCGN is far more severe in these p and correlates with GSL and TI fibrosis.

Key words: *Glomerulosclerosis, glycosaminoglycans, interstitial fibrosis, mesangiocapillary glomerulonephritis, myofibroblasts, proteinuria*

Introduction

Over the last years, basic and clinical research has brought new insights into the pathogenesis of renal fibrosis. Attention has focused on the mechanisms of glomerulosclerosis and of tubulointerstitial lesions. There is a large body of evidence that highlights the primary importance of proteoglycans, as well as of myofibroblasts in the initiation and perpetuation of these processes.

Proteoglycans (PG), as a class of molecules belonging to the non-collagen's matrix components, are considered a heterogeneous group of macromolecular glycoconjugates composed of a variable number of N- and O-linked sulfated polysaccharides, known as glycosaminoglycans (GAG_s) chains, covalently linked to a core protein. The most important types of chains involved in renal pathology are heparan sulfate (HS),

chondroitin sulfate (CS), and dermatan sulfate (1).

From the practical standpoint, two major roles of GAG_s have raised heated debate: their contribution to the electronegative charge of the glomerular basement membrane (GBM) and their possible involvement in renal fibrosis.

Of the major GBM components, GAG_s play a crucial role in the layout of the electronegative filter of the GBM, thus conferring its filtration properties: permeability and selectivity. Heparan sulfate proteoglycans, (HS-PG), especially perlecan, are increasingly studied due to their main localization in the GBM (2). Alterations of the HS-PG disposition along the GBM, which correlated to the proteinuria, have been demonstrated in membranous glomerulonephritis (3), in lupus nephritis (4), in diabetic nephropathy (5,6), as well as in essential hypertension (6). These aspects were not found in IgA nephropathy and Alport's syndrome, entities which present with heavy proteinuria, but with normal disposition of HS - PG in the GBM (4).

The presence of GAG_s in the mesangium and the renal interstitium is strongly correlated with their involvement in the complex process of renal fibrosis. Several studies have underlined potential pro-fibrogenic actions of GAG_s: – they control extracellular matrix expansion and cell proliferation; – interact with growth factors (mainly transforming growth factor β-TGF β), thus controlling cell behavior within the glomerulus and the interstitium (proliferation, migration) and influencing fibrogenesis and extracellular matrix remodeling (1, 8).

Myofibroblasts represent a type of cells expressing α-smooth muscle actin (α-SMA) which possess characteristics of both fibroblasts and smooth muscle cells (9). Their origin is still incompletely known, three potential sources being forwarded: cortical (10) and medullary (11) fibroblasts, tubular cells (12) and pericytes (13).

The presence of α-SMA expression has been recorded within the glomerulus, as well as within the interstitium. Glomerular α-SMA immunostain has been associated with glomerulosclerosis, ascribed to phenotypic changes of mesangial cells secondary to their activation or proliferation (14). Also, it has been assumed that the presence of α - SMA expression within the glomerulus may reflect their invasion by periglomerular and interstitial myofibroblasts (15,16).

More recently, α - SMA expression within the glomerulus has been demonstrated in the mesangium in IgA nephropathy (17), diabetic nephropathy (18) and mesangiocapillary glomerulonephritis (19), in the fibrotic crescents in rapidly progressive glomerulonephritis (20,21) and in mesangial, endothelial and epithelial cells in experimental renal scarring (22).

The presence of α-SMA cells in the tubulointerstitium has been claimed as responsible for initiating and perpetuating renal fibrosis in experimental (15) and clinical (17) glomerulonephritis. Their possible role in the progression of renal interstitial fibrosis has been postulated in experimental studies in immune-mediated (12,23) and in non-immunologically induced renal diseases (22,24,25).

Apart from the α-SMA expression, activated fibroblasts or myofibroblasts express intermediate filament proteins (such as vimentin and desmin), fact that implies their differentiation during scarring (26).

Similar changes have been described in the phenotype of glomerular mesangial and visceral and epithelial cells which express intermediate filaments in diseased human glomeruli (27).

Progression towards chronic renal failure of various types of chronic glomerulonephritis strongly correlated with the extent of the tubulointerstitial injuries, as demonstrated in mesangial proliferative glomerulonephritis, diabetic nephropathy, renal amyloidosis and mesangiocapillary glomerulonephritis (28).

The purpose of our study was to evaluate particular clinical and biochemical aspects of primary type I mesangiocapillary glomerulonephritis (MCGN) related to alterations of glomerular and interstitial GAG_s , as well as to the extent of tubulointerstitial injury. A special attention was focused on the expression of cytoskeletal proteins (vimentin, α-SMA) by glomerular and interstitial cells. Also, we assessed a possible relationship between GAG_s and α-SMA/PCNA expression at the glomerular and interstitial level.

Subjects and Methods

Study design

The study was conducted on a group of 28 patients with primary type I MCGN. All patients were assessed concerning blood pressure, proteinuria and serum creatinine. Kidney biopsies were performed in all patients and specimens were processed as presented below. Blood pressure was determined in mmHg, proteinuria was evaluated by the Biuret method (g/24h) and serum creatinine was measured by standard autoanalyser technique Axsym (mg%).

Histology

Specimens of core needle biopsies were fixed in buffer formalin and embedded in paraffin. Five-micrometer paraffin sections were dewaxed, hydrated and stained with hematoxylin-eosin and Masson's trichrome staining methods.

Histochemistry

Additional sections were stained with the following staining methods:

- PAS (periodic acid Schiff) reaction and PAS-control reaction in order to differentiate deposits of glycoproteins from other components of GBM (Hotchkiss-Mc Manus technique);
- Gordon-Sweet silver staining which identifies tubular and glomerular basement membranes in black;

Glomerulosclerosis (GSL) was assessed on PAS-stained sections (5 μm 30 consequent sections) using a

semi-quantitative arbritary score (0–3), where 0 = normal glomeruli, 1 = glomeruli with < 25% sclerosis, 2 = glomeruli with 25–50% sclerosis, 3 = glomeruli with > 50% sclerosis. Each glomerulus was scored and the mean score for each biopsy was deducted on 12 glomerular cross-sections (18, modified). Tubulointerstitial (Ti) lesions were evaluated on Masson's trichrome stained sections and were quantified morphometrically by point count analysis. Renal biopsies were examined with a light microscope [a × 20 flat field objective (× 200)]. A squared lattice of 25 points with a total surface area of 0.016 mm^2 was superimposed on the tissue and the data collected from 10–12 adjacent fields along the biopsy specimen. Points falling on stained tissue were counted and their percentage of the total number of measured points was estimated (15, 17,18).

- Toluidin blue staining in phosphate buffer pH 4.0 in order to detect metachromasia induced by sulfated GAG_s;
- Alcian blue – $MgCl_2$ staining in critical electrolyte concentration (CEC) was performed to identify the type of the sulfated GAG_s stored in the capillary loops, mesangium and the interstitium: CEC-1 for HS-GAG_s and CEC-0.7 for CS-GAG_s. The intensity of scoring in the capillary loops, mesangium and interstitium was assessed by a semiquantitative score on a 0 to 4+ scale in glomeruli: *-in the capillary loops* no staining = 0; staining of 25% of all capillary loops and /or lack of staining of 75% of the capillary loops = 1; staining of 50% of all capillary loops and /or lack of staining of 50% of the capillary loops = 2; staining of 75% of the capillary loops and /or lack of staining of 25% of the capillary loops = 3; normal staining of all capillary loops = 4; – *in the mesangium and interstitium:* mild staining = 1; moderate staining = 2; strong staining = 3; severe staining = 4.

Immunofluorescence. (IF) was performed in two-micrometer cryosections to reveal Ig deposits in the capillary loops and mesangium. Direct immunofluorescence was undertaken with fluorescein labelled rabbit anti human Ig for IgG, IgA and IgM (Cantacuzino Institute,Bucharest, Romania). Examinations were performed with a Nikon fluorescence microscope. A semiquantitative scoring scaled 0–4+ was used in order to appreciate the intensity of staining: no staining = 0; mild staining = 1; moderate staining = 2; strong staining = 3; severe staining = 4.

Immunohistochemistry The immunohistochemical procedure was performed only in specimens fixed no longer than 24 hours.

Three-micrometre sections from each case were immunostained with the following antibodies provided by Dako Ltd., Glostrup, Denmark:

- Anti-smooth muscle cell actin, Clone 1 A_4 – highly specific for α- mooth muscle actin;
- Anti-vimentin, Clone $Vim3B_4$;
- Anti-proliferating cell nuclear antigen, Clone PC-10, which recognizes cells in G_1, S and G_2 phases.

The visualization of the final reaction product was performed with diaminobenzidine dyhidrochlorid (DAB). All immunoreactions were performed with the EPOS system, introduced in practice by Dako.

Labelled cells in tissue sections were evaluated as described at the evaluation of histological tubulointerstitial injury. Data related to Vi^+, α-SMA^+ and $PCNA^+$ cells are presented as means ± SD per glomerular cross-section and scored for Vi (3 = 6 – 8 mesangial cells (MC); 2 = 4 – 5 MC; 1 = 1 – 3 MC; 0 = absent); α-SMA (0 = absent; 1 = 1 – 2 cells; 2 = 3 – 5 cells; 3 ≥ 5 cells); PCNA (0 = absent; 1 = 5%; 2 = 5 – 15% stained nuclei).

Statistical analysis

Clinical, biochemical, histological, histochemical amd immunohistochemical data were evaluated by statistical methods using the EPI INFO 5 and INSTAT statistical packages : comparison betweeen clinical, biochemical, histological, histochemical and immunohistochemical data (expressed as means ± SD) by Student's unpaired t-test; -correlations between histological and immunohistochemical data, histological and histochemical HS staining in the capillary walls – proteinuria and HS staining in the capillary walls – immunofluorescence data, by the non-parametric Spearman's rank order test; – correlation between clinical, biochemical and immunohistochemical data by the parametric single Pearson's test .

Correlation coefficients (r values) of the linear regression analyses are presented in relationship with P values. Statistical significance was considered as $P < 0.05$; weak significance was accepted as $0.01 < P < 0.02$ and very weak significance as $0.02 < P < 0.05$, respectively.

Results

A comparison between clinical, biochemical, histological histochemical and immunohistochemical data in groups A and B is presented in Table 1.

Glycosaminoglycans

Of the 28 patients with MCGN, 9 patients – group A (32.15%) had a distribution of HS-GAG (scored 1–3) in the capillary loops and did not present GAG_s deposits (HS, CS) in the mesangium and the interstitium; 19 patients –group B (67.85%) presented with significant alterations of GAG_s deposits, with different patterns and combined aspects in each patient: HS-present (score 1–3) /absent (score 0) in the capillaries; HS and/or CS deposits (score 1–4) in the mesangium and the interstitium.

Linear regression analysis (Spearman's rank order test) was performed in group A and B, and showed a strong inverse correlation between HS straining in the capillaries and proteinuria (group A – $r = -0.81$,

Table 1. Comparison between clinical, biochemical, histological, histochemical and immunohistochemical data in groups A and B

Groups of patients	Blood pressure (mmHg)	Protei-nuria (g/24 h)	Serum creatinine (mg%)	Glomerulo-sclerosis (GSL) score (0-3)	Tubuloin-terstitial injury (TI) %	GAG (0-4)			Vi (0-3)	α-SMA	
						CW	M	I	M	M	I
Group A	148.36±15.42	2.73±1.24	1.41±0.11	0.66±0.03	2.3±0.41	2.31±0,06[c]	-	-	2.56±0,62[c]	0.66±0,03	0.71±0,08
Group B	154.82±28.31	5.81±1.56[b]	2.95±0.23[a]	1.68±0.25[c]	28.6±2.83[c]	1.73±0.21	2.74±0.63	3.21±0.86	1.06±0.03	2.36±0.41[c]	2.48±0.73[c]

Statistical analysis - Student's unpaired t-test ([a]P < 0.05; [b]P < 0.01; [c]P < 0.001); data are expressed as means ± SD; CW-capillary walls; M-mesangium; I-interstitium.

P < 0.001; group B – r = –0.86, P < 0.001). Also, the linear regression analysis revealed in both groups an inverse correlation between HS staining in the capillaries and Ig deposits in the capillary walls (group A – r = –0.79, P < 0.001; group B – r = –0.91, P < 0.001).

The correlation between histological and histochemical data in groups A and B is presented in Table 2.

Table 2. Correlation between histological and histochemical data in groups A and B

		Glomerulus GAG (0-4)		Interstitium GAG (0-4)
		CW	M	
Group A	GSL score (0-3)	-0.63[a]	-	-
	TI %	-	-	-
Group B	GSL score (0-3)	-0.91[b]	0,89[b]	-
	TI %	-	-	0.79[b]

The histological index was compared with the histochemical data by Spearman's rank order test. Correlation coefficients (r_s values) are presented in relationship with P values ([a]P < 0.01; [b]P < 0.001; CW-capillary walls; M-mesangium; GAG-glycosaminoglycans).

α-Smooth -muscle actin (α-SMA)

α-Smooth -muscle actin (α-SMA) was detected within the media of renal arterioles, glomeruli and interstitium. The glomerular immunostain was confined only to mesangial cells in a granular pattern, and did not involve epithelial or endothelial cells.

In group B, GSL correlated inversely with GAG_s in the capillary (r = –0.91; P < 0.001) and directly with GAG_s in the mesangium (r = 0.97; P < 0.001) Ti lesions correlated significantly with interstitial GAG_s.

In group A, GSL presented an inverse correlation with GAG_s in the capillary loops (r = –0.63; P < 0.001).

α-SMA was noted in a diffuse pattern in the periglomerular and peritubular areas , as well as in the interstitium. α-SMA positive cells were identified as myofibroblasts infiltrates.

Proliferating cell nuclear antigen (PCNA)

Proliferating cell nuclear antigen (PCNA) was expressed by α-SMA – positive mesangial cells within the glomerulus and by α-SMA positive peritubular and interstitial cells.

Vimentin (Vi)

Vi immunostain was detected in mesangial cells and it appeared neither within visceral epithelial and endothelial cells, nor within peritubular and interstitial cells.

Histological and immunohistochemical data were correlated in groups A and B as showed in Table 3. The correlation analysis revealed in group B a strong inverse correlation between the GSL score and Vi expression within the glomerulus (r = –0.81; P < 0.001) and a direct correlation between this score and glomerular α-SMA (r = 0.96; P < 0.001) and PCNA expression (r = 0.48; P < 0.05). Also, Ti injury correlated positively with interstitial α-SMA (r = 0.98; P < 0.001) and PCNA expression (r = 0.61; P < 0.01).

Table 3. Correlation between histological and immunohistochemical data in groups A and B

		Glomerulus			Interstitium	
		Vimentin	α-SMA	PCNA	α-SMA	PCNA
Group A	GSL score (0-3)	-0.62[b]	0.17	0.08	-	-
	TI %	-	-	-	0.45[d]	0.11
Group B	GSL score (0-3)	-0.81[c]	0.96[c]	0.48[a]	-	-
	TI %	-	-	-	0.98[c]	0.61[b]

The immunohistochemical index for glomerular and interstitial Vi, α-SMA and PCNA cells was compared with the histological index by the non-parametric Spearman's rank order test. Correlation coefficients (r_s values) are presented in relationship with P values ([a]P < 0.05; [b]P < 0.01; [c]P < 0.001; [d]0.01 < P < 0.02).

In group A, the GSL score showed an inverse correlation with glomerular Vi expression (r = –0.62; P < 0.01), but did not reveal a significant correlation with glomerular α-SMA and PCNA expression. Moreover, TI lesions correlated weakly with interstitial α-SMA (r = 0.45; 0.01 < P < 0.02), while the correlation with interstitial PCNA was absent.

The clinical, biochemical and immunohistochemical data were correlated in both groups as presented in Table 4 and Table 5. Blood pressure did not correlate with the immunohistochemical aspects, neither in group A, nor in group B.

In group B, proteinuria presented an inverse correlation with glomerular Vi expression (r = –0.93; P < 0.001) and a direct correlation with glomerular and interstitial α -SMA (r = 0.96, P < 0.001) and PCNA (r = 0.49; P < 0.05) straining. Serum creatinine assessed in this group had an inverse correlation with glomerular Vi expression (r = –0.67; P < 0.01) and a direct correlation with glomerular α-SMA (r = 0.61; P < 0.01) and PCNA (r = 0.51; P < 0.05). Also, serum creatinine correlated directly with interstitial α-SMA (r = 0.97; P < 0.001) and PCNA (r = 0.57; P < 0.01) expression.

On the contrary, in group A, proteinuria showed an inverse correlation with glomerular Vi straining (r = –0.51; P < 0.05), but no correlation with glomerular

and interstitial α-SMA and PCNA. Serum creatinine presented an inverse correlation with glomerular Vi ($r = –0.49$; $P < 0.05$) and no correlations with glomerular α-SMA /PCNA and interstitial PCNA, while interstitial α-SMA had a very weak correlation with this biochemical parameter ($r = 0.41$; $0.02 < P < 0.05$).

Table 4. Correlation between clinical, biochemical and immunohistochemical data in group A

	Glomerulus			Interstitium	
	Vimentin	α-SMA	PCNA	α-SMA	PCNA
Blood pressure	0.16	0.03	0.07	0.12	0.08
Proteinuria	-0.51[a]	0.20	0.05	0.11	0.18
Serum creatinine	-0.49[a]	0.03	0.09	0.41[b]	0.19

The immunohistochemical index for glomerular and interstitial Vi, α-SMA and PCNA cells was compared with the clinico-biochemical data by the parametric single Pearson's test. Correlation coefficients (r values) are presented in relationship with P values ([a]$P < 0.05$; [b]$0.02 < P < 0.05$).

Table 5. Correlation between clinical, biochemical and immunohistochemical data in group B

	Glomerulus			Interstitium	
	Vimentin	α-SMA	PCNA	α-SMA	PCNA
Blood pressure	0.19	0.08	0.03	0.12	0.07
Proteinuria	-0.93[c]	0.96[c]	0.49[a]	0.86[c]	0.64[b]
Serum creatinine	-0.67[b]	0.61[b]	0.51[a]	0.97[c]	0.57[b]

The immunohistochemical index for glomerular and interstitial Vi, α-SMA and PCNA cells was compared with the clinico-biological data by the parametric single Pearson's test. Correlation coefficients (r values) are presented in relationship with P values ([a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$).

Immunohistochemical aspects evidenced in group B are presented in Fig. 1 and Fig.2.

Discussion

The results of this study show significant alterations of GAG_s deposits in the histological features of primary type I MCGN. The biochemical picture as well as the histological parameters of the glomerular and tubulointestitial lesions are far more severe in patients with an impaired distribution of GAG_s, especially HS-GAG.

Proteinuria correlated significantly with the loss of HS staining in the capillary walls, fact that is consistent with data presented by previous studies (3,4,5,6,7).

The impairment or the absence of HS staining in the GBM is of strong positive predictive value for a poor prognosis in the long run, due to the subsequent alterations of the filtration properties of the GBM.

The reduced distribution and the lack of HS deposits along the capillary loops may be attributed to several causes, such as:

– undersulfation of GAG chains, especially for HS, fact that is related to an overexpression of collagen IV and laminin in the GBM (4,5,6); –increased enzymatic degradation of GAG_s (4,5,29); – GAG_s may be masked by immune deposits (4,5); – decreased synthesis of GAG_s (1,5).

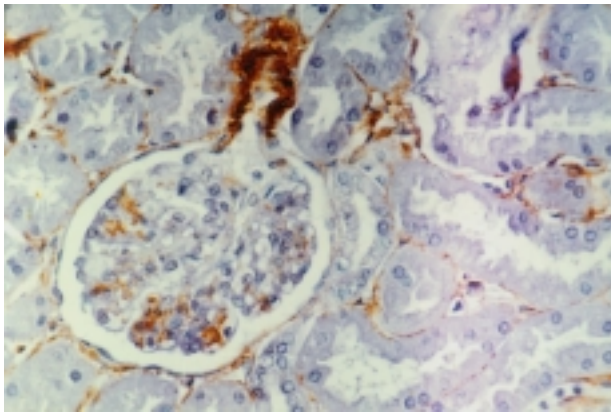

Fig. 1. Immunohistochemistry showing α-SMA expression (brown stain) within the glomerulus in type I MCGN. Magnification ×400.

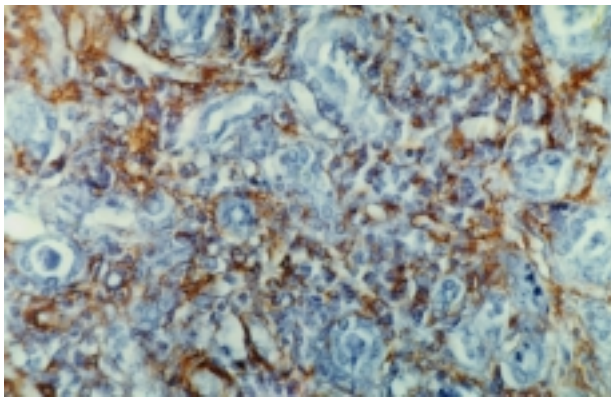

Fig. 2. Immunohistochemistry showing α-SMA expression (brown stain) identified as myofibroblasts in the peritubular areas and interstitium in type I MCGN. Magnification ×400.

This last aspect is consistent with our results, which show an inverse correlation between HS staining in the capillary loops and Ig deposits in the capillary walls.

In addition, it is worth pointing out that renal function assessed by serum creatinine was significantly impaired in patients with severe glomerular and tubulointerstitial lesions who presented extended mesangial, peritubular and interstitial GAG_s deposits. The presence of GAG_s in the mesangium, peritubular areas and interstitium has been correlated with their implication in cell proliferation and extracellular matrix remodeling, thus contributing to renal fibrosis and progression of renal diseases towards chronic renal failure [1,30].

The second purpose of this study was to reveal a possible relationship between GAG_s deposits and cytoskeletal proteins expression at the glomerular and interstitial level.

It is of note that in patients who presented significant mesangial, peritubular and interstitial GAG_s deposits (group B) not only were the histological lesions severe, but they also correlated with the severity of the histochemical and immunohistochemical data. However, our

data show a close association between the distribution of GAG_s deposits and cytoskeletal proteins expression, namely Vi and α -SMA.

Glomerulosclerosis and mesangial GAG_s deposits correlated inversely with mesangial Vi expression and directly with mesangial α-SMA and PCNA expression. The co-localization of mesangial GAG_s deposits and proliferating mesangial α-SMA positive cells strongly suggests a common involvement of GAG_s and myofibroblasts (derived from phenotypically modified mesangial cells) in the process of glomerulosclerosis in MCGN.

Furthermore, similar aspects were found in the tubulointerstitium in patients with important peritubular and interstitial GAG_s deposits. α -SMA /PCNA positive cells, identified as proliferating myofibroblasts, were encountered within the peritubular areas and the interstitium. Also, these cells were organized in the periglomerular areas, surrounding like a 'pseudo-capsule' glomeruli with advanced aspects of glomerulosclerosis.

There is a debate as to whether these immunohistological findings might predict the outcome of chronic glomerulonephritis. It has been generally assumed that amongst other factors perpetuating damage and promoting progression of chronic renal failure, severe interstitial myofibroblasts infiltrates are an important hallmark of renal fibrosis and, therefore, they predict a poor outcome of renal diseases.

Several studies highlight the fact that glomerular mesangial cells exhibit de -novo α-SMA expression when they transdifferentiate into myofibroblasts, which is associated with progressive glomerulosclerosis. Also, the same studies reveal the appearance of α-SMA positive myofibroblasts within the tubulointerstitium as a characteristic of interstitial fibrosis in glomerulonephritis (15).

Another important issue would be the relationship between the above mentioned immunohistochemical findings and the biochemical data. Proteinuria correlated significantly with glomerular Vi and α-SMA /PCNA expression in group B, while this correlation was found only for Vi in group A. Studies that focused on MCGN showed an important glomerular expression of Vi which correlated, in untreated patients, with the severity of proteinuria (19). The same authors underlined the fact that Vi immunostain co-localized to a large extent with that of glomerular α-SMA and the expression of these cytoskeletal proteins had a predictive value for a severe glomerulosclerosis when the renal biopsies were repeated (19).

We did not encounter tubular and interstitial cells expressing Vi, observations made by Adam and coworkers in patients with MCGN, who later developed tubulointerstitial fibrosis (19).

The severity of the interstitial and peritubular myofibroblasts infiltrates correlated strongly with the impairment of renal function in group B, while in group A, serum creatinine correlated weakly with the interstitial α-SMA immunostain. These data support the idea that the progression of chronic renal failure is closely related to the extent of tubulointerstitial lesions. Bohle et al suggested in their works that progressive glomerulosclerosis, as well as renal failure are seldom recorded in patients who do not present severe tubulointerstitial changes (28).

Apart from their contribution to interstitial fibrosis, α-SMA myofibroblasts are considered responsible for initiating alterations of glomerular filtration and matrix production by glomerular and tubular epithelial cells (31).

Taking into account the severe alterations of mesangial, peritubular and interstitial GAG_s deposits noted in group B, associated with a significant Vi and α-SMA/PCNA expression in the glomerulus and with α-SMA/PCNA positive cells within the interstitium, we attempted to forward an unifying hypothesis with regard to the mechanisms of glomerulosclerosis and interstitial fibrosis. We assume that co-localization of GAG_s deposits and the described immunostains may initiate, amplify and perpetuate extensive damage of glomerular filtration and extracellular matrix production by glomerular and tubulointerstitial cells. This hypothesis could be discussed in relationship with common pathways that involve both GAG_s and glomerular and interstitial myofibroblasts.

One aspect is related to the cooperation between certain GAG molecules and interstitial cells, such as fibroblasts. Syndecan 4, a cell surface HS-PG , which possesses extracellular matrix receptor properties, is a component of focal adhesions of fibroblasts and extracellular matrix substratum [30].

This is demonstrated as an important factor that activates kidney fibroblasts in order to form focal adhesion after binding to heparin - binding domains of extracellular matrix components (30). Another aspect worth mentioning is the capacity of interstitial myofibroblasts to express receptors for fibrogenic cytokines, of which TGFβ (8,21,26) and platelet -derived growth factor (PDGF) are of considerable importance (21,32). Also, it has been shown that TGFβ (26), PDGF (32) and the more recently described connective tissue growth factor (33) increase α-SMA expression of myofibroblasts. TGFβ and PDGF, as major pro-fibrogenic cytokines interact with proteoglycans, modulating cell surface and extracellular $HS\text{-}PG_s$ and thus influencing cell behavior and renal fibrosis (1). It becomes patently obvious that in the complex process of renal fibrosis, PG and activated myofibroblasts play their key role via a common pathogenic pathway, namely TGFβ. At present we perform a study in order to demonstrate that TGFβ modulates GAG_s and the expression of cytoskeletal proteins by glomerular and interstitial cells in primary type I MCGN (unpublished data).

In conclusion, our study reveals significant alterations of GAG_s deposits in the histological features of primary type I MCGN. The biochemical picture and the histological parameters of the glomerular and tubulointerstitial lesions are severe in patients with an impaired

distribution of GAG_s, especially HS-GAG. In patients with significant mesangial, peritubular and interstitial GAG_s deposits, apart from the severity of the histological lesions, we found important corresponding immunohistochemical aspects. Furthermore, our data show a strong relationship between the distribution of GAG_s deposits and cytoskeletal proteins expression, such as Vi and α -SMA within the glomerulus and the interstitium.

Therefore, it should be kept in mind that in severe forms of glomerulonephritis, such as MCGN, an important therapeutic goal should focus on those therapies that aim at re-establishing the filtration properties of the GBM, and at inhibiting mesangial cells proliferation, thus correcting the abnormalities of the extracellular matrix /GBM ratio.

Also, it is of note that therapies should be directed against crucial glomerular and interstitial deleterious events, such as the expression of cytoskeletal proteins (especially of α-SMA), which contribute to glomerulosclerosis and interstitial fibrosis.

Based on the foregoing discussion, one might conclude that, in the future, therapeutic strategies should target on TGFβ, in order to reduce these processes in the course of glomerulonephritis.

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GLIKOZAMINOGLIKANI I MIOFIBROBLASTI MODULIŠU OŠTEĆENJE GLOMERULA I TUBULOINTERSTICIJUMA U PRIMARNOM MEZANGIOKAPILARNOM GLOMERULONEFRITISU TIP I

Ligia Petrica[1], Marius Raica[2], Adalbert Schiller[1], Silvia Velciov[1], Gheorghe Gluhovschi[1], Virginia Trandafirescu[1], Gheorghe Bozdog[1], Cristina Gluhovschi[1], Flaviu Bob[1]

[1]*Klinika za nefrologiju i* [2]*Institut za histologiju, Fakultet medicine i farmacije, Temišvar, Rumunija*

Kratak sadržaj: *Glikozaminoglikani (GAGs) igraju ključnu ulogu u održavanju elektronegativnog naboja glomerulske bazalne membrane (GBM), i moguća je njihova uloga u nastanku fibroze bubrega. Ekspresija α-glatkomišićnog aktina (α-SMA) u glomemlu i intersticijumu, uy druge citoskeletne proteine, kao što je vimentin (Vi), koreliza sa glomerulosklerozom (GSL), tubulointersticijskom fibrozom (TI). Cilj ovog rada je da se procene klinički, biohemijski i histološki aspekti primarnog mezangiokapilarnog glomemlonefritisa (MCGN) tip I u odnosu na promene GAGs deposita i ekspresiju α-SMA i Vi u glomenilskim i intersticijskim ćelijama.*

Grupi od 28 bolesnika sa primarnim MCGN tip I određeni su krvni pritisak, proteinurija i serumski kreatinin. U svih bolesnika učinjena bubrežna biopsija pripremljena je za svetlosnu mikroskopiju, imunofluorescenciju i imunohistohemiju u cilju otkrivanja GAGS depozita, Vi- i α-SMA/PCNA (nuklearni antigen proliferišućih ćelija) pozitivnih ćelija u glomerulu i intersticijumu.

Od 28 bolesnika, 19 bolesnika (67,85%) - grupa B, imalo je značajne promene GAGs u kapilarima, mezangijumu i intersticijumu, dok je 9 bolesnika (32,15%) - grupa A, imalo umerene promene distribucije GAGs, samo u zidovima kapilara. Analiza linearne regresije pokazala je, u obema grupama, inverznu korelaciju između GAGs bojenja u kapilarima i proteinurije te Ig depozita u zidu kapilara. U grupi B nađena je inverzna korelacija između GSL skora i ekspresije Vi u glomerulu, a direktna korelacija između ovog skora i ekspresije u glomerulu α-SMA i PCNA. Oštećenje TI koreliralo je pozitivno sa ekspresijom u intersticijumu α-SMA i PCNA. U grupi A, GLS skor je pokazao inverznu korelaciju sa glomerulskim Vi, ali nije bilo značajne korelacije sa glomerulskim α-SMA i PCNA. Oštećenje TI koreliralo je jedino ali slabo sa ekspresijom α-SMA u intersticijumu. U grupi B, proteinurija (P) i serumski kreatinin (SC) korelirali su inverzno sa vimentinom u glomerulima, a direktno sa α-SMA i PCNA u glomerulima i intersticijumu. U grupi A, P je inverzno korelirala sa vimentinom u glomerulima, ali nije bilo korelacije sa α-SMA i PCNA u glomerulima i intersticijumu. SC je korelirao inverzno sa Vi u glomerulima, a vrlo slabo sa α-SMA u intersticijumu.

U zaključku, GAGs depoziti su značajni u primarnom PICGN tip I i korelišu sa ekspresijom α-SMA i Vi u glomerulu i intersticijumu. Biohemijski pokazatelji su mnogo više oštećeni u ovih bolesnika, i korelišu sa GSL i TI fibrozom.

Ključne reči: *Glomeruloskleroza, glikozaminoglikani, intersticijska fibroza, mezangiokapilarni glomerulonefritis, miofibroblasti, proteinurija*

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