



Influence of the infiltrate density in the interstitium on the prognosis of primary glomerulonephritis

Uticaj gustine infiltrata u intersticijumu na prognozu primarnog glomerulonefritisa

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Abstract

Background/Aim. Development of inflammatory changes, fibrosis and loss of morphological structures of the interstitium have an important role in pathogenesis of primary glomerulonephritis, affecting the development, course and prognosis of the disease. The aim of this study was to determine the influence of changes in the interstitium on the prognosis of primary glomerulonephritis. **Methods.** The research included 216 patients suffering from different types of primary glomerulonephritis treated at the Clinic for Nephrology and Clinical Immunology of the Clinical Center of Vojvodina, Serbia who were being monitored on average for 77.5 months. After determining on pathohistological diagnosis of the type of glomerulonephritis, renal changes in the interstitium were quantified. Numerical density in the tissue volume unit and structure of infiltrates of the interstitium were established by using the Weibel system (M42) incorporated into light microscope. Routine analyses were performed by using standard laboratory procedure. **Results.** During the research period the highest numerical density of infiltrates was verified in extracapillary glomerulonephritis ($147,869 \times \text{mm}^{-3}$), slightly less in membranoproliferative

glomerulonephritis ($116,800 \times \text{mm}^{-3}$) and focal segmental glomerulosclerosis ($96,147 \times \text{mm}^{-3}$), and the least being in glomerulonephritis with minimal changes ($11,416 \times \text{mm}^{-3}$). In all types of glomerulonephritis, apart from glomerulonephritis with minimal changes, there was a significantly ($p < 0.0005$) higher numerical density and incidence of infiltrate cells in relation to the control group. By comparing the numerical density of infiltrates of all cells to the parameters of renal function, a significant ($p < 0.01$) correlation of these phenomena was established. In order to get a better insight into the speed of progression of renal failure by setting a numerical limit of the density of infiltrates $< 100,000 / > 100,000 \text{ cells/mm}^3$, regardless of the type of glomerulonephritis, a prognostic predictor was established on the basis of which the patients with lower infiltration of the interstitium had significantly ($p < 0.005$) lower progression of renal failure. **Conclusion.** Density of infiltrates in the interstitium in primary glomerulonephritis is an important early prognostic predictor of progression of renal failure.

Key words: glomerulonephritis; renal insufficiency; connective tissue; prognosis; histological techniques.

Apstrakt

Uvod/Cilj. Razvoj inflamatornih promena i ožiljavanja i gubitak morfoloških struktura intersticijuma zauzimaju značajno mesto u patogenezi primarnih glomerulonefritisa, što utiče na nastanak, tok i prognozu ove bolesti. Cilj istraživanja bio je da se ispita uticaj promena u intersticijumu na prognozu primarnih glomerulonefritisa. **Metode.** Ispitivanjem je bilo obuhvaćeno 216 bolesnika sa različitim tipovima primarnih glomerulonefritisa lečenih na Klinici za nefrologiju i kliničku imunologiju Kliničkog centra Vojvodine koji su praćeni prosečno 77,5 meseci. Nakon utvrđivanja patohistološke dijagnoze tipa glomerulonefritisa, kvantifikovane su promene u intersticijumu bubrega. Određivana je

numerička gustina u jedinici zapremine tkiva i struktura infiltrata korišćenjem Weibel-ovog sistema (M42) inkorporisanog u svetlosni mikroskop. Rutinske analize rađene su standardnom laboratorijskom procedurom. **Rezultati:** Tokom ispitivanog perioda najveća numerička gustina infiltrata verifikovana je kod ekstrakapilarnog glomerulonefritisa ($147\ 869 \times \text{mm}^{-3}$), nešto manja kod membranoproliferativnog glomerulonefritisa ($116\ 800 \times \text{mm}^{-3}$) i fokalnosegmentne glomeruloskleroze ($96\ 147 \times \text{mm}^{-3}$), a najmanja kod glomerulonefritisa sa minimalnim promenama ($11\ 416 \times \text{mm}^{-3}$). Kod svih tipova glomerulonefritisa, osim glomerulonefritisa sa minimalnim promenama, ustanovljena je značajno ($p < 0,0005$) veća numerička gustina i zastupljenost ćelija infiltrata u odnosu na kontrolnu grupu. Upoređujući

numeričku gustinu infiltrata svih ćelija sa parametrima bubrežne funkcije, utvrđena je značajna ($p < 0,01$) povezanost ovih pojava. Radi boljeg uvida u brzinu progresije bubrežne insuficijencije postavljenjem numeričke granice gustine infiltrata $< 100\ 000 / > 100\ 000$ ćelija/mm³ nezavisno od tipa glomerulonefritisa, ustanovljen je prognostički prediktor na osnovu kojeg su bolesnici sa manjom infiltracijom intersticijuma imali značajno ($p < 0,005$) sporiju progre-

siju bubrežne insuficijencije. **Zaključak.** Gustina infiltrata u intersticijumu kod primarnih glomerulonefritisa je važan, rani prognostički prediktor progresije bubrežne insuficijencije.

Ključne reči:
glomerulonefritis; bubrež, hronična insuficijencija; vezivno tkivo; prognoza; histološke tehnike.

Introduction

Primary glomerulonephritis (GN) is a significant group of diseases which can lead to chronic renal failure (CRF). The prognosis of these diseases, apart from changes on glomeruli, is also affected by inflammatory changes in interstitium which are almost always more or less present¹. In previously conducted studies, the authors analyzed cellular infiltrates in interstitium by using monoclonal antibodies². All types of primary GN reported a significant increase in the number of T lymphocytes in the interstitium from 6 to 11 times per area unit comparing to healthy kidneys in which there is a smaller number of leukocytes intraglomerularly and in the interstitium^{3,4}. The experimental models showed that cellular infiltrate always appears first perivascularly around the hilar glomerular arteriole in the first 12 hours since nephrotoxic serum has been administered, with a tendency to spread periglomerularly and only after 7 days diffusely on the interstitium⁵. Hu et al.⁶ indicated that the dominant change of infiltration of the interstitium is around the glomeruli which is positively correlated with the frequency of ruptures of the Bowman capsule. It is known that infiltration of the interstitium is a prerequisite of formation of fibrosis of the interstitium and tubular atrophy whose main promoters are macrophages including a large number of mechanisms as well as the possibility for direct a macrophage-myofibroblastic change⁷⁻⁹. Therefore, the development of inflammatory changes, fibrosis and loss of morphological structure of these parts of nephrons leads to CRF progression¹⁰.

Previous studies showed that cellular infiltrates of the lowest density can be found in postinfective GN, slightly denser in focal and diffuse mesangioproliferative GN (MzPGN foc., MzPGN dif., respectively), even denser in membranoproliferative GN (MPGN) and the densest infiltrates in extracapillary GN (RPGN). In all types of GN a positive correlation of density of cellular infiltrates with a disease prognosis was established⁴. According to the data in literature, decreasing of renal function correlates more significantly with the changes in the interstitium (infiltration, fibrosis, tubular atrophy) in comparison with the glomerular changes^{11,12}. Changes in the interstitium are an independent determinant of the development of CRF as well as primary changes on glomeruli which was confirmed in the study of Ihm¹³ who have shown that hypertension is also significantly more frequent and more prevalent in the developed changes in the interstitium. Based on the abovementioned, it is important to quantify changes in the interstitium in order to provide better treatment to these patients¹⁰.

The aim of this research was to quantify cellular infiltration in the interstitium in primary GN, compare the verified changes in relation to the control group and determine the correlation of cellular infiltration in the interstitium with the relevant parameters of the renal function.

Methods

The research involved 216 patients with different types of primary GN who were treated at the Clinic for Nephrology and Clinical Immunology of the Clinical Center of Vojvodina, Serbia. The patients were being monitored in the period of 10 years. The beginning of monitoring patients was based on establishing a histopathological diagnosis of the GN type. The end of monitoring patients was defined by the last clinical control or the diagnosed end-stage renal disease and initiation of dialysis.

In the first part of the research, demographic, biochemical parameters (concentration of urea and creatinine) and endogenous creatinine clearance were analyzed. The concentration of creatinine in the blood and urine were measured by the Jaffé method – alkaline picrate reaction. Creatinine clearance (CrCl) was calculated by using the formula: $CrCl = [U_{cr} \times 24 \text{ h volume (mL)}] / [S_{cr} \times 1440 \text{ (min)}]$; Note: U_{cr} - urine creatinine ($\mu\text{mol/L}$), S_{cr} - serum creatinine ($\mu\text{mol/L}$). After that, the CrCl was calculated, and the obtained value was normalized in comparison to the body surface of 1.73 m². The calculated values were compared to the expected values with respect to the gender and the age of the patient.

In order to get a better insight into the speed of decreasing of renal function during the research period in comparison with the GN type, we defined an average monthly decline of CrCl (mL/min) in our patients. The calculated value was the result of the difference in creatinine clearance at the beginning and at the end of the research divided by the number of months of monitoring the patients.

In the second part of the research, density of cellular infiltrates of the interstitium was determined by using the quantitative method. The study included the patients who provided sufficient material for both types of microscopy and whose findings of light and immunofluorescence microscopy were compatible with regard to definitive diagnosis.

Kidney samples were taken by performing a classical percutaneous renal biopsy by using Tru-Cut needles with diameters of 1.6 and 2.0 mm. One part of the material was sent for immunofluorescence microscopy. The other part of the material was fixed in 70% alcohol, processed for hematoxylin-eosin staining and embedded in paraffin. In order to de-

termine the numerical parameters of the interstitium, the material was cut with microtome in 20 incisions at thickness of 5 μm , taking every even number incision to avoid an error of double counting the same cell, which means that 10 alternating incisions were done. Within these incisions, the kidney interstitium was being observed and interstitium infiltration was being quantitatively determined. In other words, the numerical density is a relative stereological dimension which shows how many particles there are per volume unit. In our research, for the reason of having the particle size considerably smaller than the thickness of the incision which was 5 μm , we used a thick incision and applied the Abercrombie¹⁴ method of determining the numerical density for the thick incisions. According to this method the thickness of the incision on the side is to be observed, and the particles whose center is in the incision itself and the ones whose center is in the layer above or below the thickness of the incision, i.e., in one of the over incisions, are to be counted. In order to calculate the numerical density we used the formula according to the aforementioned author: $N_v = N_A / (t+D)$; Note: N_v – numerical density, N_A – the number of cells at the cross-section, t – thickness of the incision, D – the average diameter of the cells.

According to Weibel and Gomez¹⁵, for the purpose of counting cells, a multipurpose system M42 was used and it was introduced in order to avoid a large number of cross-sections of outlines or contours of the studied structure with the test lines. Number 42 represents the number of the test lines which can be struck. The advantage of the system is transparency during counting although the mesh does not cover the entire observed field. The view of the Weibel and Gomez system on the interstitium of our patients is shown in Figure 1.

Quantifying the numerical density was performed at magnification 400, with an incorporated “mesh“, i.e., a testing system, into the eyepiece (Carl Zeiss GF-Pw 10 \times). A system incorporated into the microscopic system with a video camera was also used. In both cases there was a calibrated distance between two adjacent lines at 1.04 or 2.08 μm ¹⁶. At least 10, but no more than 30 counts were done in all the patients. The patients who did not have at least 10 representative fields for determining the numerical density were excluded from the study. Lymphocytes, monocytes/macrophages, plasmocytes, fibrocytes and polymorphonuclear granulocytes were counted.

The control group consisted of 30 deceased persons under various accidental circumstances from the Center for Forensic Medicine, who had not been suffering from CRF. The renal tissue was analysed on the same counting conditions as in our patients. It was analyzed in 10 alternate incisions and always in 30 counts, which was possible due to sampling a larger amount of tissue.

The numerical data of the research are shown above the mean value and standard deviation, and descriptive variables above absolute and relative numbers. The data processing was performed by using the Student's t -test with a level of significance ($p < 0.05$), the Kaplan-Meier analysis and the Gehan-Wilcoxon test.

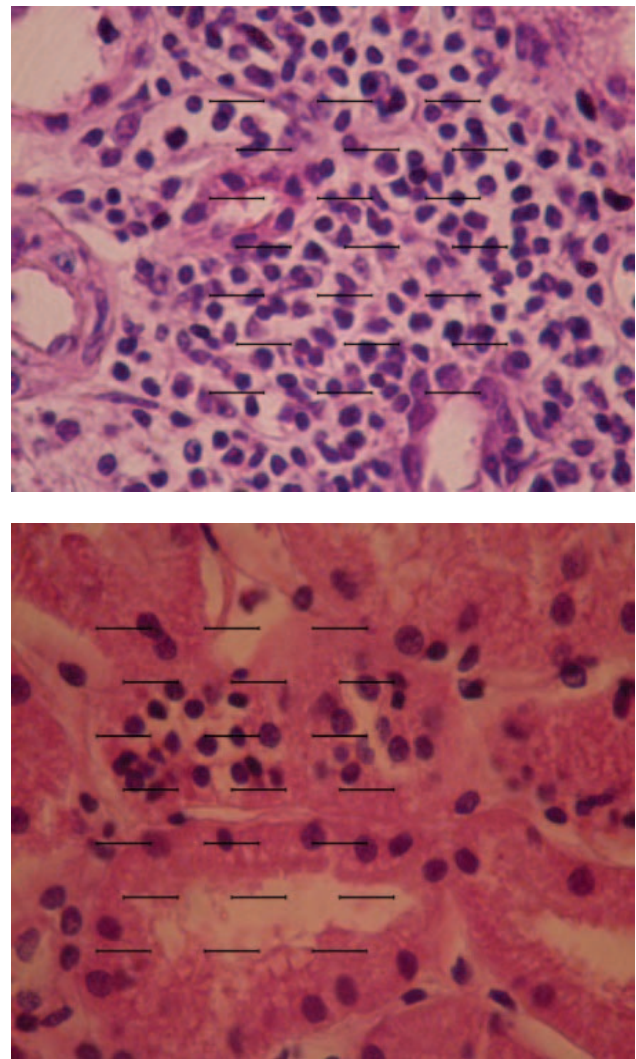


Fig. 1 – The Weibel and Gomez¹⁵ system applied to the kidney interstitium (our material).

Results

The total number of patients involved 127 (59%) men and 89 (41%) women, the average age of 40.2 ± 11.8 and 37.9 ± 11.8 years respectively, who were diagnosed with primary GN based on the histopathological findings (Figure 2). With regard to the clinical manifestations of GN, the nephrotic syndrome was the most prevalent in membranous glomerulonephritis (MGN) 88.9% and glomerulonephritis with minimal changes (MCGN), 87.5%; persistent microscopic haematuria (with or without proteinuria) in IgA nephropathy (IgA), 81.25% and MzPGN foc., 80%; chronic nephritis syndrome in focal segmental glomerulosclerosis (FSSH), 33.3%; and the rapidly progressive nephritis syndrome was most frequent in RPGN, 85.71%.

The longest monitoring period was performed in the IgA patients whereas the shortest monitoring period was in the RPGN patients (109.75 ± 38.34 and 18.6 ± 19.17 months, respectively).

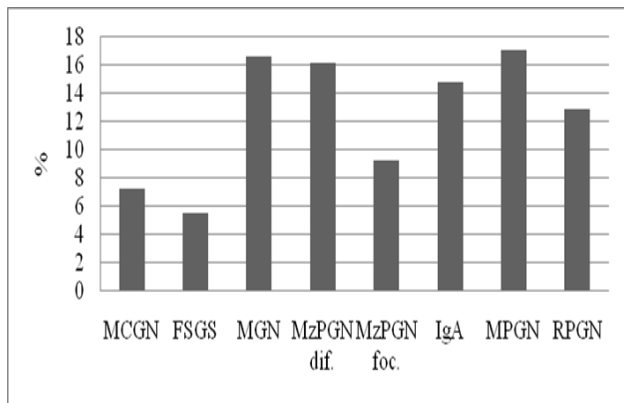


Fig. 2 – Frequency of primary glomerulonephritis.

MCGN – minimal change glomerulonephritis; FSGS – focal segmental glomerulosclerosis; MGN – membranous glomerulonephritis; MzPGNdif – diffuse mesangioproliferative glomerulonephritis; MzPGNfoc – focal mesangioproliferative glomerulonephritis; IgA – IgA nephropathy; MPGN – membranoproliferative glomerulonephritis; RPGN – rapidly progressive (crescent) glomerulonephritis.

The numerical density and infiltrate structure of the interstitium were determined and a significantly ($p < 0.001$) higher total density of infiltrates as well as individual cells of infiltrates were established in the patients comparing to the control group. The infiltrate structure of the patients suffering from GN abounded with lymphocytes, followed by the number of monocyte while less prevalent were other types of cells (fibrocytes, plasmocytes and polymorphonuclear granulocytes). However, in the patients with healthy kidneys, apart from significantly less density of infiltrates, the incidence of lymphocytes and monocytes was almost equal (Table 1). In the patients diagnosed with a worse type of GN (RPGN, MPGN, FSSH) a higher density of infiltrates comparing to other types of GN was verified (Table 2). A significant ($p < 0.0005$) difference among the patients regarding the GN type and the control group for the numerical density and structure of infiltrates in all GN, apart from MCGN, was established. In order to have a more transparent view of the results in Table 3 only the values of the structure and density of cellular infiltrate in RPGN, MCGN and the control group are shown.

Table 1
The total numerical density and structure of interstitial infiltrate in the patients compared to the control group

Structure of infiltrate	Patients (n = 216)	Control group (n = 30)	p
	mean ± SD	mean ± SD	
Lymphocytes	57682.3 ± 10816.0	5955.7 ± 2652.5	< 0.001
Monocytes	18783.6 ± 11360.9	5132.20 ± 1447.9	< 0.001
Fibrocytes	3246.71 ± 2388.8	282.03 ± 119.1	< 0.001
Plasmocytes	1886.0 ± 2249.7	183.33 ± 85.1	< 0.001
PMN	1384.9 ± 879.4	239.50 ± 73.6	< 0.001

All values are meaning cell density/mm³; PMN – polymorphonuclear granulocytes; SD – standard deviation.

Table 2
The numerical density of cells in the interstitium in relation to the type of glomerulonephritis

Type of GN	Structure infiltrate (mm ³)					Total density mean ± SD
	Lymphocytes mean ± SD	Monocytes mean ± SD	Fibrocytes mean ± SD	Plasmocytes mean ± SD	PMN mean ± SD	
MCGN	8,049.9 ± 2,901.7	2,567.3 ± 936.6	365.1 ± 122.2	254.3 ± 92.4	180.3 ± 71.8	11,416.9 ± 4,095.7
FSGS	59,990.8 ± 10,816.0	29,141.8 ± 4,804.8	3,847.3 ± 657.1	1,872.2 ± 408.0	1,295.2 ± 290.4	96,147.3 ± 16,238.8
MGN	33,012.4 ± 13,170.3	10,930.6 ± 4,498.3	1,856.1 ± 1,008.3	1,016.8 ± 368.4	772.2 ± 354.3	47,588.1 ± 19,259.0
MzPGd.	66,571.2 ± 39,194.6	20,905.9 ± 11,692.0	3,890.4 ± 3,229.0	2,065.1 ± 1,211.9	1,475.9 ± 889.6	94,908.4 ± 55,822.9
MzPGf.	39,882.9 ± 24,497.7	3,068.1 ± 7,545.7	1,939.5 ± 1,211.6	1,226.0 ± 727.9	1,255.5 ± 1,178.4	57,372.0 ± 34,576.7
IgA	42,204.5 ± 15,807.0	13,319.6 ± 5,222.3	2,275.1 ± 1,112.5	1,319.3 ± 702.5	1,620.0 ± 824.6	60,738.4 ± 23,243.1
MPGN	81,813.4 ± 26,290.8	26,480.9 ± 8,269.0	4,635.5 ± 1,884.9	2,214.8 ± 605.5	1,655.5 ± 543.7	116,800.1 ± 37,032.5
RPGN	104,176.6 ± 27,070.3	31,210.5 ± 8,028.7	5,828.2 ± 1,865.8	4,402.6 ± 5,170.1	2,251.9 ± 690.5	147,869.9 ± 35,998.4
C. G.	5,955.7 ± 2,652.5	5,132.2 ± 1,447.9	282.0 ± 119.1	183.3 ± 85.1	239.5 ± 73.5	11,792.7 ± 4,154.3

GN – glomerulonephritis; MCGN – minimal change glomerulonephritis; FSGS – focal segmental glomerulosclerosis; MGN – membranous glomerulonephritis; MzPGNd. – diffuse mesangioproliferative glomerulonephritis; MzPGNf. – focal mesangioproliferative glomerulonephritis; IgA – IgA nephropathy; MPGN – membranoproliferative glomerulonephritis; RPGN – rapidly progressive (crescent) glomerulonephritis; C.G. – control group; PMN – polymorphonuclear granulocytes.

All values are meaning cell density/mm³.
SD – standard deviation.

Table 3

The numerical density and structure of interstitial infiltrate in MCGN and RPGN compared to the control group

Structure of infiltrate (mm ³)	MCGN/control group		RPGN/control group	
	mean ± SD	<i>p</i>	mean ± SD	<i>p</i>
Ly.	8,049.9 ± 2,901.7/5,955.7 ± 2,652.5	0.0171	104,176.6 ± 27,070.3/5,955.7 ± 2,652.5	< 0.0005
Mon.	2,567.3 ± 936.6/5,132.0 ± 1,447.9	< 0.0005	31,210.5 ± 8,028.7/5,132.2 ± 1,447.9	< 0.0005
Fibr.	365.1 ± 122.2/282.0 ± 119.1	0.0307	5,828.2 ± 1,865.8/282.03 ± 119.1	< 0.0005
Plaz.	254.4 ± 92.4/183.3 ± 85.1	0.0121	4,402.6 ± 5,170.1/183.33 ± 85.1	< 0.0005
PMN	180.3 ± 71.8/239.5 ± 73.5	0.0119	2,251.9 ± 690.5/239.5 ± 73.5	< 0.0005
T. D.	11,416.9 ± 4,095.7/11,792.73 ± 4,154.3	0.7703	147,869.9 ± 35,998.4 / 11,792.73 ± 4,154.3	< 0.0005

MCGN – minimal change glomerulonephritis; RPGN – rapidly progressive (crescent) glomerulonephritis; Ly. – lymphocytes; Mon. – monocytes; Fibr. – fibrocytes; Plaz. – plasmocytes; PMN – polymorphonuclear granulocytes; T.D. – total density. All values are meaning cell density/mm³.

SD – standard deviation.

**p* < 0.05.

Table 4 shows an average monthly decline of creatinine clearance as a parameter of deterioration of renal function in comparison with the GN type. The difference values of serum creatinine and CrCl at the beginning and at the end was 130 mmol/L and 21.3 mL/min, respectively in all patients. By comparing the numerical density of infiltrates of all cells to the creatinine increase, the CrCl decline and the average monthly decline of CrCl, a significant correlation between these phenomena was determined ($r = 0.4484$, $r = 0.2244$, $r = 0.5055$, respectively; $p < 0.01$).

In the final part of the research a numerical limit of the infiltrate density < 100.000 / > 100.000 cells/mm³ was set regardless of the GN type. Comparing these two groups of patients by using the Kaplan-Meier analysis and comparing the two curves by using the Gehan-Wilcoxon test, a prognostic predictor according to which the patients suffering from less infiltration of the interstitium had a signifi-

cantly ($p < 0.005$) slower progression of CRF (Figure 3) was established.

Table 4

The average monthly decline of creatinine clearance (CrCL)

Type of glomerulonephritis	CrCl (mL/min) mean ± SD
Focal segmental glomerulosclerosis	0.017 ± 0.042
Minimal change glomerulonephritis	0.734 ± 0.228
Membranous glomerulonephritis	0.376 ± 0.453
Diffuse mesangioproliferative glomerulonephritis	0.189 ± 0.220
Focal mesangioproliferative glomerulonephritis	0.102 ± 0.164
IgA nephropathy	0.227 ± 0.282
Membranoproliferative glomerulonephritis	0.547 ± 0.383
Extracapillary glomerulonephritis	0.765 ± 0.669

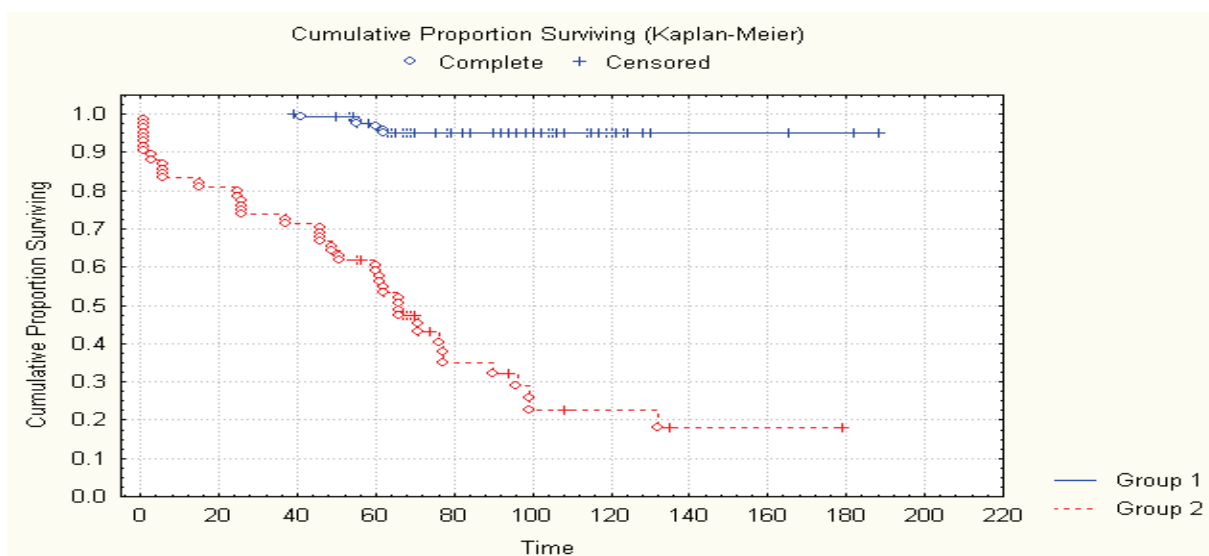


Fig. 3 – Renal function in relation to the numerical density of interstitial infiltration. Group 1 – numerical density < 100,000 cells/mm³; Group 2 – numerical density > 100,000 cells/mm³. **p* < 0.005 (Gehan-Wilcoxon test).

Discussion

Glomerular and tubulointerstitial lesions cause a certain level of damage of renal function and affect the outcome of the disease in patients suffering from primary or secondary GN¹⁰.

In our study, the patients we were diagnosed with all types of primary GN manifested in different clinical syndromes during different periods of monitoring, which matches the results in several published studies. Considering the fact that the clinical work revealed histopathological findings of kidney biopsy without glomerular sclerosis, and that the clinical signs of CRF were already present in the patients and vice versa, and considering the fact that the available literature had not provided us with the knowledge of measured numerical density of infiltrates in primary GN, we think, as well, it is necessary for an early prognosis of the disease to examine the changes in the interstitium over several years of monitoring.

By using a stereological method of determining numerical density of cellular infiltrates, the changes in the interstitium were verified and quantified. A multipurpose system according to Weibel and Gomez¹⁵ was used and the cell density per volume unit of the interstitium tissue was determined^{17,18}. Apart from the total cell infiltration in the interstitium, the types of cells which infiltrate contains were also analyzed individually. The results obtained were compared to the finding of the same parameters in the interstitium in the control group because their minimal cell infiltrates were also verified. Our patients had significantly higher numerical density and infiltrate structure in comparison with the control group, as expected. In primary GN, the cellular infiltrate abounded with lymphocytes, which contributed almost 2/3 to the total number of cells, followed by a three times smaller number of monocytes, whereas other cells were less prevalent. Several authors indicated that the presence of a large number of lymphocytes and monocytes/macrophages was responsible for the occurrence of changes in the interstitium in primary GN, which was confirmed in this research as well^{1,4,19}. Unlike our results, Schena et al.²⁰ showed a slightly higher density of polymorphonuclear granulocytes in IgA in relation to other types of GN. However, the healthy kidneys had a ratio between lymphocytes and monocytes almost 1:1, which caused the infiltrate structure to be completely different.

The highest numerical cell density was in the types of GN in which the expected prognosis was worse (RPGN, FSSH i MPGN). The average values of the numerical density were from 11,417/mm³ in MCGN to 147,870/mm³ in RPGN, unlike the healthy kidneys whose value was 11,792/mm³. The total number of cells in the interstitium in MCGN was slightly smaller than in the healthy kidneys. Comparing to the control group, the numerical density and infiltrate structure in all types of GN, apart from MCGN, were significantly higher, which is in accordance with the results presented in one study, although different techniques of cell quantification in the interstitium were used²¹.

While examining the changes in the interstitium in primary GN, some authors used different methods of quantify-

ing changes. Hooke et al.³ quantified cells in the interstitium by using the monoclonal antibodies typical of certain cells. Although the way is accurate, cross-sections are possible in only one geometrical plane, thus the results were shown as a number of verified cells per square millimeter. Li et al.²² used a similar technique to prove the distinct changes in the IgA patients who had more rapid progression of CRF. The given studies reported similar results to our results.

The authors of previous studies indicated that infiltration of the interstitium, density and the number of cells correlate significantly with deterioration of renal function, even more effectively than the changes in glomeruli^{11,12}. At the beginning of the research a decrease of renal function in most of our patients was verified, therefore they were monitored in different periods of time depending on the GN type and the course of the disease, clinical and laboratory parameters. Through relevant indicators of renal function at the beginning and the end of the research a corresponding stage of CRF was verified. However, for the purpose of clearer understanding of the decline speed of renal function during the examining period related to the GN type, we determined an average monthly decline of creatinine clearance, whose obtained values were the lowest in MCGN, and the highest in RPGN (0.017 and 0.765 mL/min/month, respectively).

We established a significant correlation among the increase of creatinine, the decline of CrCl, the average monthly decline of CrCl and the numerical cell density in the interstitium, thus showing that denser cellular infiltrates appeared considerably more frequently in the patients who had more rapid progression of CRF. Similar results are revealed in some studies^{10,23}.

The research showed that infiltration of the interstitium had an impact on renal function and therefore on the GN prognosis. In order to get a better insight into the speed of the CRF progression, by setting a numerical limit of the infiltrate density < 100,000 / > 100,000 cells/mm³, regardless of the GN type, a prognostic predictor was set according to which the patients suffering from a lower infiltration of the interstitium had a significantly slower progression of CRF. According to the predictor, the numerical density higher than 100,000/mm³ indicated more rapid progression of CRF towards the end stage renal disease and simultaneous deterioration of the pathohistological findings through verified changes characteristic of fibrosis, which was proven in several studies²⁴. Therefore, over the last few years, a considerable effort was made to discover appropriate inflammation inhibitors, primarily endogenous, which would decrease infiltration of the interstitium, modify the role of proximal tubular cells to infiltration and fibrosis and slow down the progression of CRF²⁵⁻²⁷.

Conclusion

Our research established that the quantitatively determined changes in the interstitium are an important early predictor of the GN prognosis, therefore, by using the prognostic predictor, they could represent an additional criterion when deciding on the treatment of patients, taking into con-

sideration not only the GN type as a basic parameter for determining a treatment protocol. The given method of determining the numerical density requires use of light micro-

scope with few adaptations, which makes it available and inexpensive.

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