Pol J Pathol 2007, 58, 2, 65–71 PL ISSN 1233-9687

Krzysztof Okoń[1], Władysław Sułowicz[2], Olgierd Smoleński[3], Antoni Sydor[4], Barbara Chruściel[2,3], Agnieszka Kirker-Nowak[3], Zbigniew Rosiek[5], Krzysztof Sysło[6], Jerzy Stachura[1]

Predicting Kidney Function from Renal Biopsy. Semiquantitative Versus Quantitative Approach

[1] Chair and Department of Pathomorphology and [2] Chair and Department of Nephrology, Collegium Medicum, Jagiellonian University, Kraków
[3] Department of Nephrology, "L. Rydygier" Regional Hospital, Kraków
[4] Department of Nephrology, "St Lucas" Hospital, Tarnów
[5] Department of Nephrology, Province Hospital, Nowy Sącz
[6] Department of Nephrology, "E. Szczeklik" Hospital, Tarnów

The term glomerulonephritis encompass a heterogeneous group of diseases; these are a important cause of end stage renal disease. Although several evidence exist, that the main prognostic factors are extraglomerular lesions, no quantitative assessment is usually done. In nephropathological practice a semiquantitative approach is preferred. However, most of work on extraglomerular lesions significance was done with quantitative methods.

The aim of the study was to compare the effects of quantitative and semiquantitative assessment of extraglomerular lesions in glomerulonephritis. The material consisted of 120 renal biopsies. On inspection, percentage of sclerosed glomeruli, degree of interstitial fibrosis, degree of interstitial infiltration, degree of tubular atrophy were and degree of mesangial matrix expansion assessed. For quantitative measurements AnalySIS 3.0 pro image analysis system was used. Relative interstitial volume, volume of interstitial infiltrate, with their variability – ross sectional areas of proximal and distal tubules were assessed by point counting method.

Relative interstitial volume was significantly correlated to percentage of sclerosed glomeruli (R = 0.33 p<0.001), degree of tubular atrophy (gamma = 0.57 p<0.00001), degree interstitial fibrosis (gamma = 0.31 p<0.0002) and mesangial matrix expansion (gamma = 0.24 p<0.001). Semiquantitative and quantitative assessment of interstitial infiltrate was significantly correlated as well (gamma = 0.81 p<0.001). Semiquantitatively assessed degree of tubular atrophy showed significant relation to total proximal tubular area (gamma = –0.30 p = 0,004). Percentage of sclerosed glomeruli was significantly correlated to creatinine level (R=0.24 p=0.03), but not to urea level (R = 0.09, NS). Semiquantitatively assessed degree of interstitial fibrosis showed only marginal correlation to creatinine level (gamma = 0.18 NS), however degree of interstitial infiltration was significantly correlated to creatinine (gamma = 0.34 p = 0.002) and urea level (gamma = 0.22 p=0.06). Degree of tubular atrophy was significantly correlated to creatinine (gamma = 0.43 p < 0.001) and urea level (gamma = 0.28 p=0.015). Relative interstitial volume was the very most important correlate of creatinine (R=0.47 p<0.0001) and urea level (R=0.30 p<0.01).

In conclusion, it was confirmed, that the strongest correlate of renal function is relative interstitial volume. Some, but not all of semiquantitative parameters are also significantly correlated to kidney function.

Introduction

The principal categories of medical renal diseases include vascular and tubulointerstitial nephropathies, as well as glomerulonephritis (GLN). In view of the importance of renal biopsy in their diagnostic management, GLN is the focus of interest for a nephropathologist. As the term "glomerulonephritis" indicates, the lesions are primarily situated within the glomerulus. Although the disease course is usually prolonged, and in many cases there is a risk of chronic renal failure (CRF) development, its behaviour is difficult to predict. And so, to use an example, in membranous glomerulopathy, the incidence of CRF after 10 years approximates 20–30%. In patients with GLN, any attempts at establishing a prognosis based on glomerular parameters alone are unreliable and often overly optimistic. On the other hand, even advanced glomerular lesions seen in a biopsy do not necessarily have to be associated with a major impairment of renal function. Therefore, it is necessary to search for morphological and functional parameters that might facilitate the prediction for a further development of the disease. In 1968, Risdon, Sloper and Wardener studied the associations between morphological parameters and renal function in patients with persistent glomerulonephritis and found a strong relation between the level of renal function and the degree of tubular loss and interstitial fibrosis [21]. In subsequent years, Bohle et al. published a series of reports [e.g. 2, 3, 12, 13], where they emphasised the importance of tubulointerstitial lesions.

Extraglomerular lesions observed in patients with GLN include widening of the interstitial layer accompanied by its fibrosis and myofibroblast proliferation, inflammatory infiltration and tubular loss. The basic mechanism underlying the development of these lesions may be summarised as follows:

1) proteinuria activates the tubular epithelial cells and triggers changes in mediator and surface antigen production,
2) within the interstitium, inflammatory infiltrate cells appear, mostly as macrophages and T lymphocytes, which also produce specific mediators,
3) activity of myofibroblasts in the interstitium increase,
4) myofibroblasts produce components of the extracellular matrix, what is manifested by a histologically visible interstitial expansion,
5) hemodynamic changes occur in the kidney parenchyma, causing impaired renal function [5, 12, 17, 20].

The evaluation of changes in the extraglomerular elements of the kidney in the course of a routine assessment of a biopsy specimen is done in a qualitative or semiquantitative way. Quantitative methods, however, allow for obtaining much more precise results that are more closely associated with the prognosis. Indeed, most observation on the significance of extraglomerular lesions in GLN used a quantitative approach. The aim of the present study was to compare of the results of a semiquantitative and a quantitative assessment.

Material and Methods

The material consisted of 120 renal biopsies. Cases of systemic diseases and non-representative biopsies were excluded. Representative biopsies were defined as such where ultrastructural studies and immunofluorescence tests were performed, while the portion of material to be studied under light microscopy contained at least 6 glomeruli that did not manifest advanced sclerosis. Such cases were reclassified with uniform criteria [4]. The controls consisted of slides originating from macroscopically normal renal parenchyma of surgically resected tumour-containing kidneys (9 cases) and from 2 autopsies. Specimens with histologically evident autolysis or any significant pathologies were excluded.

In both the experimental and control groups the material was fixed in 10% buffered formalin, processed and embedded in paraffin using routine methods. Paraffin blocks served for the preparation of 4 μm-thick sections; their thickness was verified using the method described by Weibel [22].

While reviewing the slides, the following parameters were evaluated with a semiquantitative scale from 0 to 3:

1. the total number of glomeruli in the biopsy seen in the section containing the highest number of glomeruli,
2. the percentage of totally sclerosed glomeruli (PSG),
3. renal interstitial expansion,
4. tubular atrophy (TA),
5. the presence and intensity of cellular infiltration within the interstitium,
6. the increase of mesangial matrix.

Image acquisition was done with Zeiss Axioscop microscope (Zeiss GmbH, Germany) and ZVS-47DE camera (Optronics Inc, USA), connected with a RGB line with a GraBIT PCI graphic card (Soft Imaging System GmbH, Germany) installed on a standard PC. The image processing software , operated under the Windows NT 4.0 operating system (Microsoft Corp, USA) and consisted of the AnalySIS 3.0 pro image analysis system (Soft Imaging System GmbH, Germany) and a custom-made applications that implemented the measuring process and was developed by one of the authors (K. O.) in Imaging C language.

The measurements of the relative interstitial volume (RIV), interstitial inflammatory infiltrate, mean total surface area of proximal and distal tubule cross-section, as well as the cross-section of tubular cells were performed by point counting method with a 72-point grid. PAMS-stained slides were used (Fig. 1). To asses the variability of in interstitial volume, its variance between fields of vision was used.

Degree of small vessels hyalinosis was evaluated in PAS-stained slides, using the small vessel hyaline change index [9] according to the formula:

$$SVHI = \frac{n_1 + 2n_2 + 3n_3 + 4n_4}{n_1 + n_2 + n_3 + n_4}$$

where

n_1 – number of vessels without hyaline changes
n_2 – number of vessels with hyaline changes involving <50% of vessel circumference
n_3 – number of vessels with hyaline changes involving >50% of vessel circumference
n_4 – number of vessels with hyaline changes involving entire vessel circumference

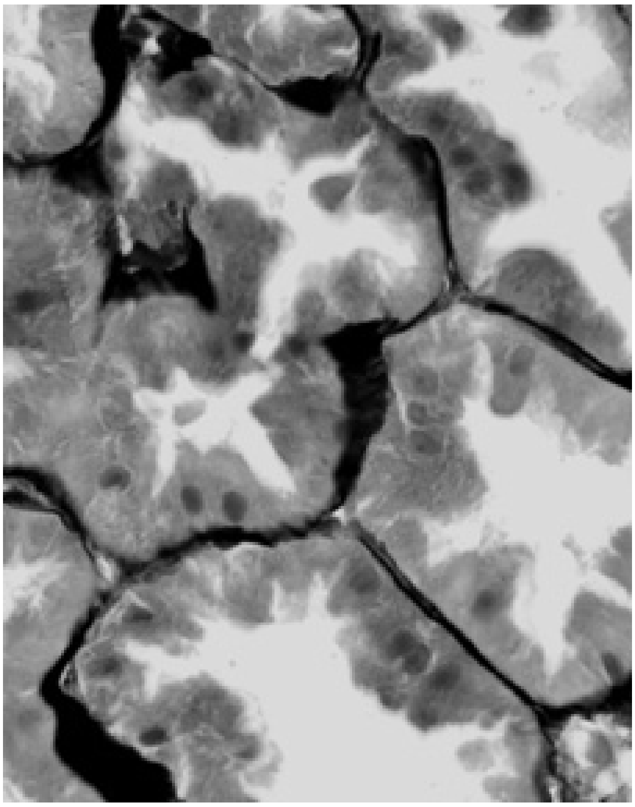

Fig. 1. PAMS stain renders definition of tubulo-interstitial borders straightforward. Silver methenamine – trichrome stain. Lens magnification 60×

The following clinical data at the time of the biopsy were also included into the analysis: creatinine and urea serum levels, daily proteinuria and blood pressure. The date of the eventual beginning of chronic renal replacement was used as the final point for survival analysis.

The statistical analysis was performed with Statistica for Windows v. 5.5 PL (StatSoft Inc., USA). The differences between groups were analysed with Kruskall-Wallis ANOVA, Mann-Whitney U test and Wald-Wolfowitz test. The correlation between variables were assessed with Spearman's, gamma and Pearson's correlation coefficients. The significance level was set to p=0.05.

For reference, the expected relative interstitial volume and the expected percentage of sclerosed glomeruli were calculated with the formulas by Kappel [7]:

Expected percentage of sclerosed glomeruli (ePSG) = –1.20+0.06 *age in years

Expected relative interstitial volume (eRIV)=12.45+0.11 *age in years

Results

The group under study consisted of 120 cases, of whom 42 were female and 78 male. The mean age was 36.26 years (SE 1.50; range 15–77 years). IgA nephropathy was diagnosed in 35 cases; membranous nephropathy in 24 patients; membranoproliferative glomerulonephritis in 14; minimal change disease in 14, mesangioproliferative glomerulonephritis in 13 cases, focal segmental glomerulosclerosis in 5 individuals, postreptococcal glomerulonephritis in 4 cases and end stage glomerulonephritis in 7 cases. Four cases did not fit into any category. The controls included 11 cases (6 women and 5 men). Their mean age was 55.82 years (range 28–72 years).

The mean number of glomeruli in the biopsy specimen was 14.16 (median 12; range 6–49; SE 0.69). When the completely sclerosed glomeruli were excluded, the mean number of glomeruli was 12.97 (median 11; SE 0.68). In 67 biopsies (55.8%) no completely sclerosed glomeruli were found, while in 46 cases (38.3%) their number was 1–5, in 5 specimens (4.2%) – 6–10, and in 2 cases (1.7%) their number exceeded 10. The mean number of completely sclerosed glomeruli in the entire material was 1.19, while in the group of 52 cases with sclerozed glomeruli, the corresponding value was 2.75 (median value, 2; SE 0.41). In the controls, the mean percentage of completely sclerosed glomeruli (PSG) amounted to 3.3%, whereas in the experimental group the corresponding rate was 7.9% ($p<0.01$). Of cases with particular nephropathy types, the highest PSG was seen in patients with chronic glomerulonephritis, while in minimal change disease no such glomeruli were encountered (Table 1). PSG showed a statistically significant positive correlation with age ($R=0.31$; $p<0,0004$). The values were higher than expected (PSG 7.9% vs. ePSG 0.97%, $p<0.01$).

TABLE 1
The proportion of completely sclerosed glomeruli according to glomerulonephritis type

Diagnosis	Mean percentage of sclerosed glomeruli
IgA nephropathy	9.01
membranous glomerulopathy	3.42
membranoproliferative glomerulonephritis	5.82
minimal change disease	0.00
mesangioproliferative glomerulonephritis	3.86
focal segmental glomerulosclerosis	19.83
poststreptococcal glomerulonephritis	2.50
end stage	40.56
unclassified	6.90
controls	3.33

In the control group, the mean relative interstitial volume (RIV) was 6.7% (range 3.7–10.7, SE 0.63). In the experimental group, mean RIV was 16.4% (range 1.1–43.9, SE 0.77). The difference between the controls and the experimental group was highly significant (p<0.0001). In the experimental group, the interstitial volume was correlated with age (R=0.21; p<0.025), while no such a correlation was seen in the controls. The expected relative interstitial volume (eRIV) significantly differed from the measured values (p<0.001). While the mean values were similar (16.3% in empirical measurements vs. 16.4 as the value calculated based on age), the actual relative interstitial volume range and variability were higher (RIV, 1–44%; SE 0.7 vs. eRIV, 14–21%; SE 0.17). The relative interstitial volume variability was 0.7 (range 0.004–3.6; SE 0.06). The mean percentage of infiltrated renal cortex in the experimental group was 0.8% (range 0–10.7; SE 0.17). In the controls, no significant cellular infiltration was observed.

In the controls, the mean tubular atrophy index (TA) value was 0.18 (range 0–1; SE 0.12), while in the experimental group, the corresponding mean TA amounted to 0.80 (range 0–2; SE 0.06). The mean surface area of the proximal tubule in the controls was 3.47 (range 2.71–4.34; SE 0.13), while in the experimental group the corresponding value was 2.71 (range 0.71–4.96, SE 0.08). The mean surface area of the proximal tubule epithelium in the control group was 3.09 (range 2.30–3.77; SE 0.12) and in the experimental group 2.36 (range 0.68–4.87; SE 0.07). In the controls, the mean surface area of the distal tubule was 2.00 (range 0.62–2.95; SE 0.12), whereas in the experimental group the respective value was 1.17 (range 0.08–3.21; SE 0.06). The mean surface area of the distal tubule epithelium in the control group was 1.44 (range 0.58–2.13; SE 0.15), and in the experimental subjects 0.76 (range 0.05–2.23; SE 0.04).

Data on urea levels were obtained in 77 cases; its mean level was 7.38 mmol/l (range 2.7–33.8; SE 0.65). In 80 cases data on creatinine levels were available, with the mean concentrations value being 136.9 mmol/l (range 35.4–1038 and SE 18.9). Data on proteinuria were reported in 86 cases; the mean value was 4.45g/day (range 0–29.7 and SE 0.58). Blood pressure data were available in 75 cases, with the mean systolic pressure of 141.6 mmHg (range 100–240; SE 3.02). The mean diastolic pressure was 88.8mmHg (range 70–170; SE 1.87). In 61 cases data on further course of disease in the patients were available. The mean follow-up time was 1,097 days. Six patients were placed on chronic dialysis therapy.

The relative interstitial volume of the renal cortex was significantly correlated with the percentage of sclerosed glomeruli (R = 0.33; p < 0.001), degree of tubular atrophy (gamma = 0.57; p<0.00001), degree of interstitial fibrosis (gamma = 0.31; p<0.0002) and the increase of mesangial matrix (gamma = 0.24 p<0.001). The semiquantitative and quantitative evaluation of interstitial infiltration also demonstrated a strong correlation (gamma = 0.8; p < 0.001). In the semiquantitative assessment, the degree of tubular loss showed a strong correlation with the surface area of proximal tubule cross-section (gamma = -0.30; p = 0.004) and of epithelial cells (gamma = -0.31; p = 0.000015).

The percentage of fully sclerosed glomeruli was positively correlated with the creatinine level (R=0.24; p=0.03). On the other hand, the correlation with the urea concentration was weaker (R = 0.09; NS). In semiquantitative analysis, the degree of interstitial fibrosis demonstrated a weak correlation with the creatinine level (gamma = 0.18; NS). No correlation was found between the said parameter and the urea level (gamma = - 0.04; NS). The presence of cellular infiltration in the interstitium was correlated with the creatinine (gamma = 0.34; p = 0.002) and urea levels (gamma = 0.22; p=0.06). Tubular loss was positively correlated with the creatinine (gamma = 0.43; p < 0.001) and urea concentration values (gamma = 0.28; p=0.015). The relative

TABLE 2
Variables that discriminate between patients on renal replacement therapy (RRT) and without it

Variable	RRT	no RRT	U	p
RIV	16%	25%	67	0.012
interstitial infiltration – quantitative	1.5%	0.5%	118.5	0.055
age	34.7	49.8	102	0.097
PSG	7%	18%	114	0.102
TA	0.65	1.33	95.5	0.040
interstitial fibrosis	0.43	1.16	67	0.004
interstitial infiltration	0.45	1.5	63	0.003

interstitial volume showed a strong correlation with the creatinine (R=0.47; p<0.0001) and urea levels (R=0.30; p<0.01). The relative volume of interstitial infiltration was correlated to a degree with the creatinine (R=0.19; p<0.09) and urea values (R=0.16, NS).

Table 2 presents the differences between patients on chronic dialysis and those who did not require renal replacement therapy.

Discussion

The most important of morphometric renal parameters is the relative interstitial volume. In numerous reports, including the present study, RIV shows strong correlation to renal function and risk of renal failure progression. The simplest measurement method is the semiquantitative assessment, which is based on viewing the material under a light microscope. Although this method seems subjective, it is quite useful: in fact allowed Risdon et al. to discover the importance of tubulointerstitial lesions [21]. The most often employed method for determining RIV consists in projecting a grid on the evaluated image. The grid consists of a known number of points and the investigator counts the percentage of points that are contained within the analysed structure – in this case within the interstitium (the point counting method). Its advantage is a satisfactory speed and accuracy, while the calculating complexity is minimal and the method is simple to employ. An important feature of the method lies in the fact that is it an unbiased estimator, thus it is free of systematic errors. The disadvantage is the necessity of employing a human operator and the resultant workload. A negative aspect is its limited sensitivity to local RIV increase. To eliminate this problem, Zachariae et al. proposed a simultaneous use of the semiquantitative assessment [24]. In the present study, the RIV variance between fields of vision was used. In view of the disadvantages associated with the point counting method there have been published reports proposing new methods based on the use of other than routine staining techniques or advanced image analysis. Such automated methods seem to be very promising [14, 16].

Isolated sclerosed glomeruli does not necessarily indicate a kidney disease, and to an even lesser degree allows for establishing a prognosis. Some degree of glomerulosclerosis in persons manifesting no signs of renal diseases, as well as the age-dependent RIV increase, is well documented both in experimental animals and in humans. In healthy human population below 40 years, the percentage of sclerosed glomeruli may approximate 5%, while in older than 70 may be as high as 30% [7]. The age-dependent glomerulosclerosis is sometimes explained by changes in renal blood vessels. Such changes also progress with age, what may suggest their common origin. This was confirmed by Kasiske, who stated that the degree of arteriosclerosis was associated with the percentage of sclerosed glomeruli [8]. However, McLachlan did not find any such an association [15]. In our material, the percentage of sclerosed glomeruli showed some association with renal function, weaker than did RIV. The percentage of sclerosed glomeruli was not significantly correlated with the thickness of the arterial intima and media (data not shown), but it was associated with the small vessel hyaline change index (R=0.20; p=0.02). The percentage of completely sclerosed glomeruli showed a strong correlation with age (R=0.31; p=0.001).

An important element of pathogenesis is the appearance of inflammatory infiltrate within the interstitium. Intense cellular infiltration in biopsy is seen in highly active glomerular disease, such as active forms of lupus nephritis or membranoproliferative glomerulonephritis. It appears that cellular infiltration exerts a negative effect on the prognosis. However the question to what degree such an effect is an independent prognostic factors remains open [5]. Lee observed that in patients with Berger disease the number of inflammatory cells in the interstitium showed a positive correlation with creatinine levels. The number of T lymphocytes was also associated with the mean arterial blood pressure value. Nevertheless, such correlation was not present in membranoproliferative glomerulonephritis [11]. Significant prognostic value of interstitial inflammatory infiltration in IgA nephropathy was also demonstrated by Freese et al.; in addition to tubular atrophy and interstitial fibrosis the above factor had the strongest prognostic importance [6]. According to Naiker et al, in membranoproliferative glomerulonephritis the number of interstitial inflammatory cells significantly affected renal function [18]. T lymphocytes were of the greatest importance for renal function. B lymphocytes and macrophages were less important. In our study, the semiquantitatively assessed intensity of infiltration in membranoproliferative glomerulonephritis yielded the mean value of 0.7, while the mean relative infiltration volume was 1.8%; in other glomerulopathy types the corresponding values were 0.5 and 0.6%. The differences in quantitative measurements were statistically significant (p<0.005), while the differences obtained using semiquantitative measurements were not significant. The method for evaluating interstitial infiltration adopted in the present investigations allows for an easy and fast measurement and does not require immunohistochemical procedures. On the other hand, the method provides only an estimate assessment of interstitial infiltration in the kidney and may seem to be insufficiently precise. Nevertheless, a significant correlation was found between the infiltration and creatinine levels. Yet the power of a statistical correlation is lower than in the case of RIV. The association between interstitial infiltration and renal function was not apparent in the multifactorial analysis (data not shown). Thus, one may infer that inflammatory infiltration in the renal interstitium is associated with impaired renal function, but does not constitute an independent factor.

Impaired tubular function is believed to be co-responsible for the development of chronic renal failure in the course of long-term glomerulonephritis, and according to some authors may be the decisive factor. In biopsy material, tubular atrophy is usually seen in conjunction with interstitial scarring or the presence of inflammatory infiltration. Tubular measurements are rarely performed. A very simple method of measuring the size of renal tubules was used by Mackensen-Haen et al., who performed a linear measurement of the internal and external tubular diameter [12]. The group used point counting method to evaluate the surface area of cytoplasm in the cross-sectioned tubular epithelial cells and in the lumen of the tubules, as well as tracing the tubular cross-sections using a digitiser [13]. Okada et al. performed linear measurements of the internal and external tubular diameter and assessed the length density of the tubules by the stereologic method [19]. Yasuda et al. [23] studied tubular lesions in Itai-Itai disease using the point counting method and calculating the mean fraction of renal cortex occupied by the tubules. Khan and Sinniah used also point counting method in conjunction to immunohistochemistry to mark particular tubular segments [10]. In the present investigation, the method modelled on that developed by Yasuda et al. and Mackensen-Haen et al. was used.

Khan and Sinniah demonstrated the association between functional renal parameters and the degree of tubular damage, both proximal and distal. They observed that the measurement of the cross-section area of distant tubule was a particularly efficient prognostic factor [10]. In the report of Mackensen-Haen et al., the cross-section area of the proximal-tubular epithelium and Henle's loop was associated with urine osmolarity [12]. In another paper, Mackensen-Haen et al. demonstrated an association between the level of creatinine and the proximal tubular cross-section area. Such an association was noticeable only when patients with acute renal failure were excluded [13]. In the report by Freese et al., tubular loss correlated with the progression of IgA nephropathy. The authors studied patients with Berger disease who developed chronic renal failure requiring renal replacement therapy and noted that tubular atrophy, interstitial fibrosis and infiltration were significantly more common in such material than in other cases [6]. In the present material no significant association between renal function and tubular changes was found.

Conclusions

In patients with glomerulonephritis, tubulointerstitial changes significantly correlate with renal function, as well as with the prognosis,

These correlations are noticeable both in semiquantitative and in strictly quantitative assessment,

Quantitative assessment, based on stereologic methodology, is of a greater prognostic value, yet information of practical importance may be also obtained through semiquantitative evaluation.

References

1. *Bajema IM, Hagen EC, Hermans J, Noël L-H et al:* Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizig glomerulonephritis. Kidney Int 1999, 56, 1751–1758.
2. *Bohle A, Bader R, Grund KE, Mackensen S, Neunheffer J:* Serum creatinine concentration and renal interstitial volume. Analysis of correlations in endocapillary (acute) glomerulonephritis and in moderately severe mesangioproliferative glomerulonephritis. Virchows Arch A Path Anat Histol 1977, 375, 87–96.
3. *Bohle A, Mackensen–Haen S, von Gise H, Grund KE et al:* The consequences of tubulo – interstitial changes for renal function in glomerulopathies. A morphometric and cytological analysis. Pathol Res Pract 1990, 186, 135–144.
4. *Churg J, Bernstein J, Glassock RJ:* Renal disease: classification and atlas of glomerular disease, second edition. Igaku - Shoin, New York, Tokyo, 1995.
5. *D'amico G:* Tubulo – interstitial damage in glomerular diseases: its role in the progression of the renal damage. Nephrol Dial Transplant 1998, 13, 80–85.
6. *Freese P, Norden G, Nyberg G:* Morphologic high – risk factors in IgA nephropathy. Nephron 1998, 79, 420–425.
7. *Kappel B, Olsen S:* Cortical interstitial tissue and sclerosed glomeruli in the normal human kidney, related to age and sex. Virchows Archiv A Path Anat Histol 1980, 387, 271–277.
8. *Kasiske BL:* Relationship between vascular disease and age-associated changes in the human kidney. Kidney Int 1987, 31, 1153–1159.
9. *Katafuchi R, Takebayashi S:* Morphometrical and functional correlations in benign nephrosclerosis. Clin Nephrol 1987, 28, 238–243.
10. *Khan TN, Sinniah R:* Morphometric study showing the importance of distal tubular damage in impaired creatinine clearance. Am. J Nephrol 1993, 13, 178–183.
11. *Lee AH, Bass PS, Williams JH, Ewans B et al:* CD45RO and CD45RA positive cell populations in idiopathic membranous and IgA glomerulopathy. J Clin Pathol 1996, 49, 43–47.
12. *Mackensen – Haen S, Bohle A, Christensen J, Wehrmann M et al:* The consequences for renal function of widening of the interstitium and changes in the tubular epithelium of the renal cortex and outer medulla in various renal diseases. Clin Nephrol 1992, 37, 70–77.
13. *Mackensen–Haen S, Eissele R, Bohle A:* Contribution on the correlation between morphometric parameters gained from the renal cortex and renal function in IgA nephritis. Lab Invest 1988, 59, 239–244.
14. *Masseroli M, O`valle F, Andujar M, Ramirez C et al:* Design and validation of a new image analysis method for automatic quantification of interstitial fibrosis and glomerular morphometry. Lab Invest 1998, 78, 511–522.

15. *McLachlan MS, Guthrie JC, Anderson CK, Fulker MJ:* Vascular and glomerular changes in the ageing kidney. J Pathol 1977, 121, 65–78.
16. *Moreso F, Gratin C, Vitria J, Condom E:* Automatic evaluation of renal interstial vulume fraction. Transplant Proc 1995, 27, 2231–2232.
17. *Nath KA:* The tubulointerstitium in progressive renal disease. Kidney Int 1998, 54, 992- 994.
18. *Naiker IP, Ramsaroop R, Somers SR, Randeree IG et al:* Leukocyte analysis of tubulointerstitial nephritis in primary membranoproliferative glomerulonephritis. Am J Kidney Dis 1996, 27, 316–320.
19. *Okada H, Moriwaki K, Konishi K, Kobayashi T et al:* Tubular osteopontin expression in human glomerulonephritis and renal vasculitis. Am J Kidney Dis 2000, 36, 498–506.
20. *Remuzzi G, Bertani T:* Pathophysiology of progressive nephropathies. N Engl J Med 1998, 339, 1448–1456.
21. *Risdon RA, Sloper JC, Wardener HE:* Relationship between renal function and histological changes found in renal – biopsy specimens from patients with persistent glomerular nephritis. Lancet 1968, 7564, 363–370.
22. *Weibel ER:* Stereological methods. Vol. 1. Practical methods for biological morphometry. Academic Press. London, Now York, Toronto, Sydney, San Francisco 1979.
23. *Yasuda M, Miwa A, Kitagawa M:* Morphometric studies of renal lesions in Itai - Itai disease: chronic cadmium nephropathy. Nephron 1995, 69, 14–19.
24. *Zachariae H, Hansen HE, Sogaard H, Olsen S:* Kidney biopsies in methotrexate – treated psoriatics. Dermatologica 1990, 181, 273–276.

Address for correspondence and reprint requests to:
Dr Krzysztof Okoń
Chair of Pathomorphology,
Collegium Medicum, Jagiellonian University
ul. Grzegórzecka 16
31-531 Kraków