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Relative Interstitial Volume is Correlated with Renal Function Even in Non-Representative Biopsy

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An useful renal biopsy should be representative, that is should contain a sufficient number of glomeruli. However, a non-representative biopsy could possibly provide some information. The aim of the study was to evaluate the relationship between interstitial expansion, glomerular sclerosis and renal function in such material. The material consisted of 28 renal biopsies containing less than 5 non-sclerosed glomeruli. For each case the percentage of completely sclerosed glomeruli was recorded. The relative interstitial volume was evaluated by point counting method. Clinical data as sex, age, serum creatinine and urea levels were included into analysis. The mean percentage of completely sclerosed glomeruli was 39.6%; mean relative interstitial volume was 29.6%. Creatinine level was strongly correlated to relative interstitial volume (R=0.70), but the correlation of creatinine level to percentage of sclerosed glomeruli was much weaker (R=0.38). The relationship between interstitial expansion and renal function is seen also in deficient biopsy material. The correlation of renal function with interstitial expansion is stronger the correlation of renal function with glomerular sclerosis. These findings can indicate that the better representation is responsible for stronger prognostic impact of interstitial lesions

Introduction

Glomerulonephritis is an important cause of chronic renal failure requiring renal replacement therapy. Histological assessment of a core biopsy is a standard diagnostic method. Although classification of glomerulopathy is based on glomerular lesions, prognostication based only on them is not reliable. Changes in extraglomerular compartments, especially in the interstitium, are thought to play a major role.

A renal biopsy consists of a small sample, which should bear information about whole kidney. Thus it is very important whether the biopsy is representative. The biopsies that are not representative only rarely allow a definite diagnosis to be given and are usually not regarded useful. However such material can be interesting, as contrast of representation of interstitium and glomeruli is emphasized.

The aim of this study was to investigate whether the relationship of relative interstitial volume to kidney function is maintained in such material.

Material and Methods

For the study, cases meeting following criteria were chosen: 1) clinical diagnosis of primary glomerulonephritis, 2) renal cortex present in biopsy but 3) less than 5 non-sclerosed glomeruli available for light microscopy. The control group consisted of 10 representative biopsies with minimal change disease.

The quantitative measurement of the relative interstitial volume was performed using the AnalySIS image analysis system. Silver methenamine stained, trichrome contrasted preparations were used. This stain allows visualizing both renal interstitium and basement membranes (Fig. 1). Image acquisition was done with Zeiss Axioscop microscope (Zeiss GmbH, Germany) and CCD ZVS-47DE camera (Optronics Inc., USA), connected to GraBIT PCI frame grabber (Soft Imaging System GmbH, Germany) installed on a standard PC. The software for image acquisition, processing and measurements operated under the control of Windows NT 4.0 operating system (Microsoft Inc., USA) and consisted of the AnalySIS 3.0 pro image analysis system (Soft Imaging System GmbH, Germany) and custom made applications developed by one of the authors (K.O.). The measurements of the

relative interstitial volume (RIV) were performed using the point counting method, employing a 72-point grid. The images were taken with a 20x PlanNEOFLUAR lens (Zeiss GmbH, Germany), displayed on the screen of image analysis system, and the grid was superimposed on it. The points hitting renal interstitium were counted by pointing with mouse cursor. Glomeruli, larger extraglomerular vessels and unrecognizable elements were disregarded. The results were saved in a text file, and processed on Excel (Microsoft Inc., USA) spreadsheet, to be imported into statistical analysis program. To estimate RIV variability, standard deviation of local RIV between fields of vision was calculated (RIVSD). The measurements were done in a blinded manner that is without knowledge on clinical data and diagnosis.

The serum creatinine and urea levels were taken from patients' records.

The statistical analysis was performed with Statistica 5.5 PL software (StatSoft Inc., USA). Mann-Whitney U test, Spearman R correlation coefficients were used when appropriate. The significance level was set to p=0.05.

Results

The material consisted of 28 cases. Eight (29%) were female, 20 (71%) were male. Mean age of the patients was 44 years, range 20–72, standard error (SE) 2.41. The control group consisted of 10 cases, 4 (40%) were female, 6 (60%) males.

Mean number of glomeruli was 3, range 0 to 10 (Fig. 1). Mean number of non-sclerosed glomeruli was 1.68, range 0 to 4. Mean percentage of completely sclerosed glomeruli (PSG) was 39.6%, range 0–100%, SE 8.5. PSG was not correlated to patients' age. In the control group the mean number of glomeruli was 15.7, range 7 to 41. In this group no globally sclerosed glomeruli were seen. Electron microscopy (EM) was available in 20 cases. In remaining cases EM material did not contain glomeruli (6 cases) or contained only completely sclerosed glomeruli (2 cases). In all cases immunofluorescence examination was performed using paraffin embedded, pronase treated sections. The histological diagnoses are given in Table 1. Mean creatinine level was 207.7 μmol/l, range 45 to 593, SE 25.8. Mean urea level was 15.4 mmol/l, range 3.8 to 100.0, SE 3.5. In controls mean creatinine level was 71.5 μmol/l, range 57 to 98, SE 4.6. Mean urea level was 5.5 mmol/l, range 3.3–9.4, SE 0.8.

Mean RIV was 29.6%, range 5.1 to 68.9, SE 2.6. Mean RIVSD was 12.7, range 3.1 to 29.9, SE 0.9. Mean number of available fields of vision (VFV) was 8.2, range 2 to 18, SE

TABLE 1
Histological diagnoses

Diagnosis	No. cases (%)
None	4 (14.3)
Unclassified nephropathy	4 (14.3)
Unclassified glomerulopathy	14 (50.0)
End stage kidney	1 (3.6)
Membranoproliferative glomerulonephritis	2 (7.1)
IgA nephropathy	1 (3.6)
Membranous glomerulopathy	1 (3.6)
Postinfectious glomerulonephritis	1 (3.6)

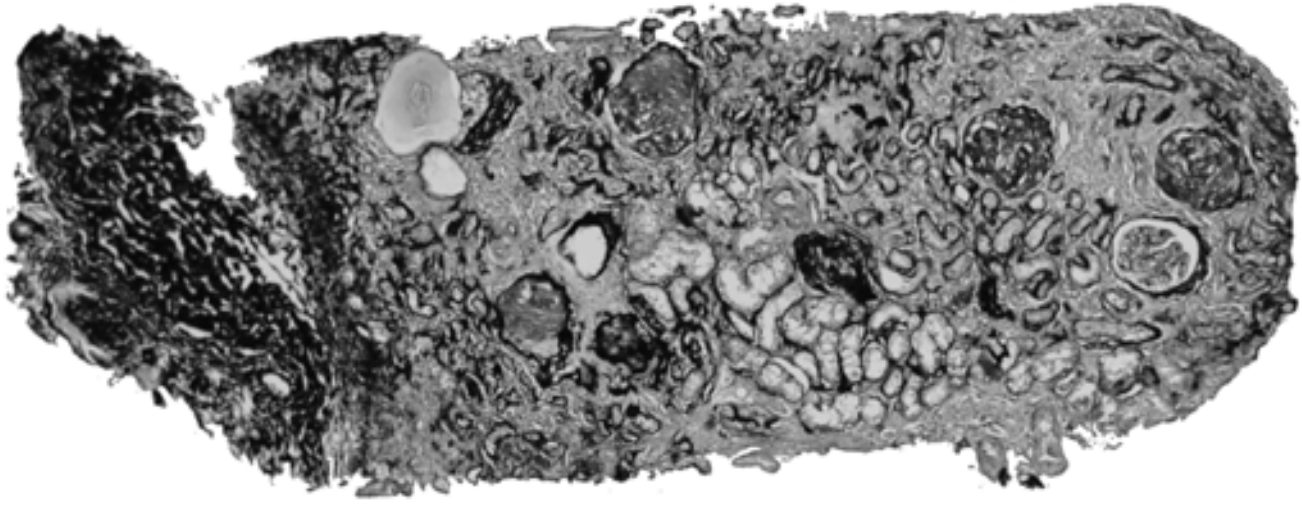

Fig. 1. A typical non-representative renal biopsy. Only one largely intact glomerulus remains. Note sharp contrast of tubules, allowing easy discrimination of the interstitium. Silver methenamine-trichrome stain. Lens magn. 4×.

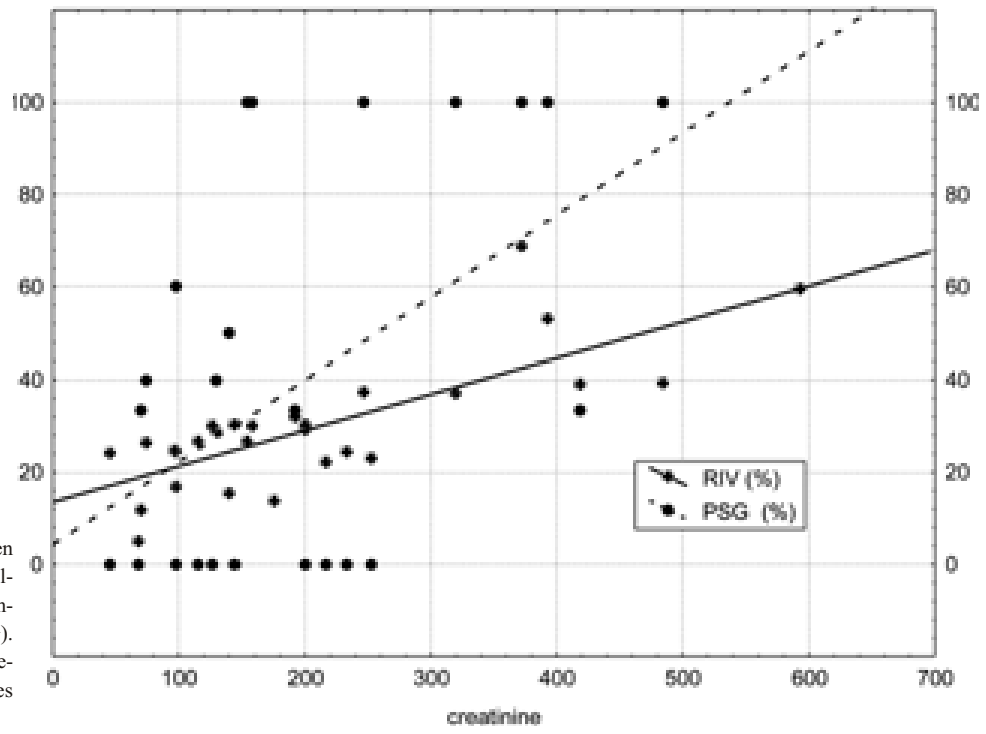

Fig. 2. The relationship between creatinine level, relative interstitial volume (RIV) and percentage of completely sclerosed glomeruli (PSG). Extreme (0 and 100%) PSG values predominate, whereas most of RIV values are near to regression line.

0.8. VFV showed a marginal correlation with RIV (R=0.15, p=0.45). RIV was not correlated with age of the patients, but RIV values were higher in males (32.5% versus 22.5%, p<0.025). PSG was strongly correlated with RIV (R=0.46, p<0.02) and RIVSD (R=0.51, p<0.01). Mean RIV in controls was 8.6%, range 4.8 to 13.8, SE 0.9.

Kidney function parameters were significantly correlated with RIV (creatinine: R=0.70, p<0.0001; urea: R=0.53, p<0.004) and RIVSD (creatinine: R=0.67, p<0.0002; urea R=0.39, p<0.05). Kidney function parameters were also correlated with PSG (creatinine: R=0.38, p<0.06; urea R=0.50, p<0.02). The relationship between RIV, PSG and creatinine level is shown on Figure 2.

Discussion

Glomerulonephrites (GN) are relatively rare. Indeed, diabetes pandemics and increase in vascular nephropathies become the main challenge for nephrologist. GN remain interesting, because of a high progression rate, and difficulty in prognostication [2, 13].

For a kidney biopsy interpretation to be clinically useful, it has to be representative. Representativeness is expressed usually as a number of glomeruli available for examination. According to most authors 5 to 10 glomeruli are sufficient, though detection of a focal process may need twice as much [18, 25]. In some cases the morphological picture of a single glomerulus allows to make diagnosis, especially in the cases when electron microscopy picture is very characteristic. For quantitative pathology, Oberholzer et al. calculate the minimum needed to 6–10 glomeruli [18]. The limit of available glomeruli in our study was set just to the lower limit given by Oberholzer. Amann, analyzing the usefulness of renal morphometry emphasized the necessity of a representative material [1]. However, our own findings show that this requirement is not necessarily strong, and some results can be obtained from seemingly deficient material.

The classification of glomerulonephritis is based on optical microscopy, and seems to be sufficiently reliable [16]; however for optimal performance immunofluorescence and electron microscopy are used. Thus, the obtained material has to be divided into three parts according to processing needed. That routine in a very small biopsy may need to be modified. Immunohistochemistry or immunofluorescence using paraffin embedded tissue can increase the amount of tissue available for optical microscopy. In a case of small specimens we use the later method, though paraffin embedding decreases both sensitivity and specificity of the staining [6, 7]. As shown by Date et al. lack of immunofluorescence has limited influence on diagnosis, at least in a subset of cases [4]. Analogously some authors believe that electron microscopic examination does not need to be performed in all cases. However, its use remains a gold standard [6].

The prognosis in glomerulonephritis based only on glomerular lesions is often unreliable. The main factor which seems to exert influence on the rate of progression to chronic renal failure is interstitial expansion and fibrosis [3, 14, 22,

24]. Since ’90 the strong link of tubulointerstitial lesions and kidney function has become a paradigm. Recently, usefulness of its evaluation for patient management has been challenged, and use of molecular methods is proposed [5]. The mechanisms responsible for interstitial and tubular changes in GN are relatively well known. The initial stimulus appears to be proteinuria. The proteins in tubular lumen induce changes in tubular epithelial cells. These changes consist of: expression of new surface antigens (e.g. MHC-II), exposition of adhesive molecules (e.g. integrins) and production of mediators (e.g. TGF-β). That leads to the recruitment and activation of macrophages and lymphocytes. Signals from both tubular epithelial and inflammatory cells reach myofibroblasts. These cells increase their number and produce extracellular matrix components, seen as increased volume and fibrosis of renal interstitium on microscopic level [19–21]. Why tubulointerstitial lesions are so strongly related to kidney function and prognosis is not completely clear. According to some authors, the main factor is tubular damage and atrophy. In fact, some reports show that tubular lesions are the chief ones for renal function [8]. Another opinion is that renal failure in GN results from reduced number of peritubular capillaries. This phenomenon is indeed observed [14]. Tubulointerstitium is the main mass of the kidney parenchyma and is continuous anatomically. Thus the pathological process can be transmitted all over the kidney [17]. The representation of the glomeruli in the biopsy has been estimated to 0.001% of total, whereas the total volume of an average biopsy would constitute 0.01–0.02% of total renal volume [18]. Thus it can be assumed that average representation of the glomeruli is 10% of the other elements, namely interstitium. The mechanisms responsible for regulation of kidney function in different metabolic and hemodynamic conditions are autoregulation and tubulo-glomerular feedback [12]. The autoregulation can be compromised in experimental models as well in human nephropathy [9]. One of the factors leading to a large discordance between visible glomerular lesions and renal function can be appearance of atubular glomeruli, completely ineffective as sources of ultrafiltrate. That has been shown to be present in animal model as well as human nephropathy, but is difficult to detect by routine methods [15].

The different significance of glomerular and extraglomerular lesions might partially depend on the use of qualitative methods for assessing the former and quantitative methods for the later. Katafuchi et al. [11] showed highly effective prognostication in IgA nephropathy using glomerular factors only. These results were obtained with very multifactorial and controlled assessment of glomerular lesions. Thus, a more effective use of information derived from glomerular lesions could possibly lead to an effective prognostic prediction.

An additional confounder can be the relation of renal sclerosis to age. In fact, in normal population the number of sclerosed glomeruli increases, reaching 30% in the 6th decade. RIV also increases proportionally to age [10]. In this study correlations of RIV and PSG to the age were not present in GN population.

Ting et al. found that in membranous glomerulopathy filtration reduction depends on a fall in filtration coefficient. The increased filtration pressure is not sufficient to keep filtration rate [23]. Such phenomena are not easy to be detected on optical microscopy level, especially not as an increased PSG. As a well known relationship exists between RIV and renal function and prognosis in membranous glomerulopathy [e.g. 24], a doubt on the existence of pathogenic link arises.

The presented results show that the relationship of interstitial expansion with renal function is seen also in deficient biopsy material. The link of renal function with interstitial lesions is stronger than that with global glomerular sclerosis. These findings could support the opinion that better representation is responsible for stronger prognostic impact of interstitial lesions.

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