Is The Histological Scoring System Useful In Assessing Patients With Glomerulonephritis?

Flaviu Raul Bob¹, Diana Herman², Gheorghe Gluhovschi¹, Ligia Petrica¹, Gheorghe Bozdog¹, Silvia Velcio¹, Cristina Gluhovschi¹, Elena Potencz² and Adalbert Schiller¹

¹Department of Nephrology, ²Department of Pathology, University of Medicine and Pharmacy “Victor Babes” Timisoara, Romania

Abstract

Glomerular and tubulo-interstitial lesions are involved in the determination of the degree of renal function impairment and in the outcome of the disease in patients with primary or secondary glomerulonephritis. In order to assess the histological changes we used a scoring system, similar to the one used in lupus nephritis and ANCA associated vasculitis.

The scores obtained from the retrospective evaluation of the renal biopsies of 41 patients with primary and secondary glomerulonephritis were correlated to serum creatinine (SC) and glomerular filtration rate (GFR).

We observed a correlation between interstitial scores for activity with renal function: interstitial edema with SC (R=0.42, P=0.002) and GFR (R=-0.41, P=0.003); interstitial infiltrate with SC (R=0.55, P<0.0001) and GFR (R=-0.62, P<0.0001); total activity index with SC (R=0.54, P=0.0001) and GFR (R=-0.49, P=0.003). We also found significant correlation of renal function tests with tubulo-interstitial scores of chronicity: interstitial fibrosis with SC (R=0.49, P=0.0006) and GFR (R=-0.59, P<0.0001); tubular atrophies with SC (R=0.41, P=0.003) and GFR (R=-0.59, P<0.0001); vascular hyalinosis/fibrosis with SC (R=0.42, P=0.002) and GFR (R=-0.59, P<0.0001); total chronicity index with SC (R=0.50, P=0.0003) and GFR (R=-0.65, P<0.0001). We also observed a correlation between the glomerular segmental sclerosis score and SC (R=0.3, P=0.02) and GFR (R=-0.42, P=0.003).

A scoring system of histological changes is important in the assessment of active and chronic glomerular changes in patients with different primary or secondary glomerulonephritis.

Key words: glomerulonephritis, histology, scoring system

Introduction

Chronic kidney disease progresses towards end stage renal disease, and the final common pathway in this process seems to be fibrosis [1]. Both glomerular and tubulo-interstitial lesions are involved in the determination of the degree of renal function impairment and also in the clinical outcome of the disease in patients with primary or secondary glomerulonephritis. Although the assessment of renal biopsy specimens with immunohistochemistry and immunofluorescence as well as with electron microscopy has improved the diagnostic accuracy, the classical histological investigation through quantification, has not lost its importance in the routine clinical practice, especially when the above mentioned methods are not available [2].

The histological lesions can be estimated using standard staining (hematoxylin and cosin, periodic acid Schiff (PAS), Gomori’s trichrome).

It is well known that differences occur when histological data are assessed by different pathologists. The question is raised if the described lesions can be better assessed by using a scoring system, similar to the one used in lupus nephritis and ANCA associated vasculitis. By adapting such a scoring system we divided the lesions at glomerular or tubulo-interstitial level into active-inflammatory lesions and chronic-sclerotic/fibrotic lesions, and were able to perform some statistical correlations with clinical data.

Patients and methods

Patients

Forty-one patients with chronic glomerulonephritis were studied retrospectively (17 females, 24 males; mean age 45.5±12.9 years, range 18-74). From the kidney biopsies that were performed in the Nephrology Department, Timisoara, cases with fewer than 5 glomeruli were excluded from the study.

The patients had either primary (30 cases) or secondary glomerulonephritis due to systemic vasculitis n=4, infectious diseases n=3, connective tissue diseases n=2 and neoplastic diseases n=2 cases. The histopathological diagnoses were: mesangial proliferative glomerulonephritis (n=12), mesangiocapillary glomerulonephritis (n=1), membranous nephropathy (n=5), minimal change disease (n=5), focal and segmental glomerulosclerosis (n=15) and crescentic glomerulonephritis (n=3 cases).
All biopsies were performed after obtaining informed consent from patients regarding the procedure and the possible use of the obtained material for scientific purposes. The present study has the approval of the local ethical committee.

**Parameters**

Clinical, biological and histological parameters at the time of the biopsy were assessed. In all patients, renal function (serum creatinine and glomerular filtration rate (GFR)), blood pressure and proteinuria were available. GFR was estimated using the MDRD4 formula. In patients that were followed up for long time, delta GFR was calculated using the following formula: delta GFR (ml/min/year) = 12* (GFR1-GFR2)/number of months between the two assessments; where GFR1=GFR at the time of kidney biopsy, GFR2= GFR at follow up.

**Histology**

Routinely fixed and processed sections of kidney were processed for light microscopy and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) and Gomori’s trichrome techniques using routine methods. All stained slides were assessed separately by two pathologists. In order to better quantify the histological lesions, a scoring system adapted by Neumann et al. (2005) for ANCA-associated vasculitis, based on the standardized scoring system for activity and chronicity developed for lupus nephritis, was employed. We extended this scoring system also to other glomerulopathies, because biological processes that take place in vasculitis or lupus nephritis are present also in other types of primary or secondary glomerular diseases.

**Table 1.** Correlations between biological and histological parameters (mes. prolif-mesangial proliferation; interst. edema-Interstitial edema; interst. infiltr-interstitial infiltrate, AI-activity index, glom scler-glomerulosclerosis; mesang. matrix incr-mesangial matrix increase; interst. fibr-interstitial fibrosis; tubular atroph.-tubular atrophy, hyalin.-hyalosis; CI-chronicity index)

<table>
<thead>
<tr>
<th>Pearson correlation</th>
<th>mes. prolif.</th>
<th>interst. edema</th>
<th>interst. infiltr</th>
<th>total AI</th>
<th>glom. scler.</th>
<th>mesang. matrix incr.</th>
<th>interst. fibr.</th>
<th>tubular atroph.</th>
<th>hialin./fibrosis</th>
<th>total CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>proteinuria</td>
<td>-0.150</td>
<td>-0.070</td>
<td>-0.080</td>
<td>-0.038</td>
<td>-0.194</td>
<td>0.095</td>
<td>-0.130</td>
<td>-0.237</td>
<td>-0.440</td>
<td>-0.213</td>
</tr>
<tr>
<td>p</td>
<td>0.177</td>
<td>0.333</td>
<td>0.311</td>
<td>0.408</td>
<td>0.115</td>
<td>0.278</td>
<td>0.210</td>
<td>0.070</td>
<td>0.002</td>
<td>0.093</td>
</tr>
<tr>
<td>serum creatinine</td>
<td>0.097</td>
<td>0.423</td>
<td>0.552</td>
<td>0.539</td>
<td>0.304</td>
<td>0.121</td>
<td>0.489</td>
<td>0.419</td>
<td>0.427</td>
<td>0.508</td>
</tr>
<tr>
<td>p</td>
<td>0.271</td>
<td>0.002</td>
<td>8.9E-05</td>
<td>0.000</td>
<td>0.026</td>
<td>0.224</td>
<td>0.000</td>
<td>0.003</td>
<td>0.002</td>
<td>0.000</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.122</td>
<td>-0.41</td>
<td>-0.629</td>
<td>-0.497</td>
<td>-0.421</td>
<td>-0.139</td>
<td>-0.592</td>
<td>-0.575</td>
<td>-0.597</td>
<td>-0.658</td>
</tr>
<tr>
<td>p</td>
<td>0.222</td>
<td>0.003</td>
<td>5.3E-06</td>
<td>0.000</td>
<td>0.003</td>
<td>0.192</td>
<td>2.00E-05</td>
<td>4.00E-05</td>
<td>1.9E-05</td>
<td>1.00E-06</td>
</tr>
</tbody>
</table>

In order to assess the degree of glomerular injury, glomeruli have been divided into 8 segments, each segment being assessed for the presence of necrosis, hypercellularity, mesangial matrix increase and sclerosis. The number of segments has been used to calculate the percentage of glomeruli affected by each of the above mentioned changes. The score has been established as follows: 1 point for <20% affection, 2 points for 21-40%, 3 points for 41-60%, 4 points for 61-80% and 5 points for >80% affection. At the tubulo-interstitial level, inflammatory lesions (edema, interstitial infiltrate) and sclerotic/ fibrotic lesions (interstitial fibrosis, tubular atrophy, vascular hyalnosis/fibrosis) were assessed semi-quantitatively, as follows: <30% of tubules or interstitial area affected was considered as mild (1 point), 31-60% affected as moderate (2 points) and >60% affected as severe (3 points).

By adding the different scores we obtained the total activity index (mesangial cell proliferation, intracapillary/ extracapillary proliferation, interstitial edema, interstitial infiltrate) and the total chronicity index (mesangial matrix increase, segmental sclerosis, fibrosed crescents, interstitial fibrosis, tubular atrophy, vascular hyalnosis/ fibrosis) [3].

**Table 2.** Correlation between delta eGFR and histological parameters in the patients followed up in time. (mes. prolif-mesangial proliferation; interst. edema-interstitial edema; interst. infiltr-interstitial infiltrate, AI-activity index, glom scler-glomerulosclerosis; mesang. matrix incr-mesangial matrix increase; interst. fibr-interstitial fibrosis; tubular atroph.-tubular atrophy, hyalin.-hyalosis; CI-chronicity index)

<table>
<thead>
<tr>
<th>Pearson correlation</th>
<th>delta eGFR</th>
<th>mes. prolif.</th>
<th>interst. edema</th>
<th>interst. infiltr</th>
<th>total AI</th>
<th>glom. scler.</th>
<th>mesang. matrix incr.</th>
<th>interst. fibr.</th>
<th>tubular atroph.</th>
<th>hialin./fibrosis</th>
<th>total CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.167</td>
<td>0.454</td>
<td>0.508</td>
<td>0.586</td>
<td>0.501</td>
<td>0.376</td>
<td>0.162</td>
<td>0.199</td>
<td>0.093</td>
<td>0.335</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.321</td>
<td>0.093</td>
<td>0.066</td>
<td>0.037</td>
<td>0.069</td>
<td>0.142</td>
<td>0.327</td>
<td>0.290</td>
<td>0.399</td>
<td>0.171</td>
<td></td>
</tr>
</tbody>
</table>
Statistical analysis

Data were recorded on a file created in Microsoft Excel, organized and managed as a database. Correlations between clinical, biological data and histological scores were performed using parametric Pearson’s test. Correlation coefficients of linear regression analysis are presented in relation with p values. The significance of the correlation coefficient (r) is as follows: r=0-0.25 indicates little or no correlation; r=0.25-0.50 indicates a fair degree of relationship; r=0.5-0.75 indicates moderate to good correlation; r>0.75-1 indicates very good to excellent correlation [4]. In order to perform these test we used WinStat for Microsoft Excel and Epi 3.2.2.

Results

We observed a statistically significant correlation between the interstitial scores for activity, such as interstitial edema, interstitial infiltrate, and also of the total activity index with renal function at the moment of the renal biopsy: interstitial edema with serum creatinine (R=0.42, P=0.002) and GFR (R=-0.41, P=0.003); interstitial infiltrate with serum creatinine (R=0.55, P<0.0001) and GFR (R=0.62, P<0.0001); total activity index with serum creatinine (R=0.54, P=0.0001) and GFR (R=-0.49, P=0.003).

We also found a moderate correlation of the renal function with tubulo-interstitial scores for chronicity (interstitial fibrosis, tubular atrophy, hyalnosis/fibrosis of interstitial vessels) and also with the total chronicity score: interstitial fibrosis with serum creatinine (R=0.49, P=0.0006), and GFR (R=-0.59, P<0.0001); tubular atrophy with serum creatinine (R=0.41, P=0.003) and GFR (R=-0.59, P<0.0001); vascular hyalnosis/ fibrosis with SC (R=0.42, P=0.002) with GFR (R=-0.59, P<0.0001); total chronicity index with serum creatinine (R=0.50, P=0.0003) and GFR (R=-0.65, P<0.0001). Hemoglobin showed an indirect correlation with interstitial fibrosis (R=-0.3, p<0.05) and with vascular hyalnosis/fibrosis (R=-0.36, p<0.05).

We also observed a correlation between the glomerular segmental sclerosis score and serum creatinine (R=0.3, P=0.02) and with GFR (R= -0.42, P=0.003). However, we could not find any statistically significant correlation of the renal function tests with mesangial cell proliferation and mesangial matrix increase.

No significant correlations were found between proteinuria or blood pressure and histological scores of glomerular or tubulo-interstitial injury.

For the 10 patients followed up for a mean period of 22.9±11.9 months we observed a mean renal function decline of 5.2±12.11 ml/min/year. We observed that the renal function decline in these patients correlated with active lesions: interstitial edema (R=0.45, P=0.09), interstitial infiltrate (R=0.50, P=0.06), total activity index (R=0.58, P=0.03) as well as with chronic lesions: glomerular segmental sclerosis (R=0.50, P=0.06).

Discussion

Despite the fact that histological lesions were variable in the studied biopsies, the use of the proposed scoring system made it possible to find statistically significant correlations of the renal function at the moment of the biopsy with interstitial lesions (both active and chronic), as well as with glomerulosclerosis.

Glomerulosclerosis and interstitial fibrosis appeared to be the strongest, most reliable predictors of unfavorable prognosis, as it was shown in a meta-analysis published by D’Amico. Despite this fact, in individual cases, the assessment of these prognosis markers has not always been consistent with the evolution of the disease, possibly because of the heterogeneity of the injury [5].

In the group of patients studied, we observed a statistically significant correlation of the renal function tests with tubulo-interstitial scores for chronicity (interstitial fibrosis, tubular atrophies, hyalnosis/fibrosis of interstitial vessels) and also with the total chronicity score. At the interstitial level, renal function at the moment of biopsy, but also renal function decline (in those patients followed up for a period of time) correlated with activity scores, such as inter-
stitial edema, interstitial infiltrate, and also with the total activity index. The importance of the tubulo-interstitial lesions in chronic glomerulonephritis was found in numerous other studies, too.

In a multicentric study performed on 150 patients with chronic glomerulonephritis, Ratner et al. showed that the tubulo-interstitial injury had an important role in the evolution of the disease, together with the histological form of the disease and clinical data (proteinuria and blood pressure) [6]. Similar to these findings, Hrouby et al. showed on 46 patients with chronic primary glomerulonephritis, that interstitial extension and tubular atrophy are important predictors of the renal function decline [7].

Roberts et al. showed, in patients with membranous nephropathy, that the number of interstitial myofibroblasts, as well as the interstitial volume correlated with creatinine clearance at the time of renal biopsy. On the contrary, the percentage of sclerosed glomeruli did not show any correlation with renal function in this study [8]. Similar results were obtained by Rocha et al. in patients with membranous nephropathy, but without any involvement of interstitial myofibroblasts as progression markers [9]. Wu et al. showed that the extension of tubulo-interstitial changes (cellular infiltrate and fibrosis) determines the prognosis of the disease [10]. Other authors showed that besides tubulo-interstitial lesions, an important role in the progression of membranous nephropathy is played by the association of focal segmental glomerulosclerosis lesions [11].

Thus it became clear that not only tubulointerstitial lesions correlate with renal function but also glomerular lesions, and Vleeming et al. proposed more than a decade ago, to abandon the paradigm of the absence of correlation of glomerular pathology with renal function. In his study quantitative estimates of the severity of glomerular and tubulointerstitial extracellular matrix accumulation correlated well with the severity of renal failure [12]. Rauta et al. showed also a correlation between glomerular injury and the progression of the disease in IgA nephropathy [13]. Lee et al. showed the importance of using a grading system of glomerular lesions in IgA nephropathy. In their study the severity of glomerular grading was significantly related to serum creatinine levels at the time of biopsy [14]. In the cases studied by us, we found only a correlation of the glomerulosclerosis score with renal function at the moment of the renal biopsy and with renal function decline. It can be mentioned according to the results of our study that glomerular and tubulointerstitial lesions, either active or chronic correlate with renal function or with the progression of renal disease, and these results are consistent with data found by other authors in different glomerulonephritides. Glomerulosclerosis and interstitial fibrosis were, according to Yang, independent risk factors predicting the renal survival [15]. Shiiki et al. studied the relationship between the prognosis of focal segmental glomerulosclerosis and histological parameters (number of segmental sclerosis, mean glomerular diameter, degree of tubulo-interstitial changes, the presence of vascular lesions), and indicated tubulo-interstitial changes and glomerular diameter as independent risk factors for an unfavourable prognosis [16]. In the same type of glomerulonephritis, Shilov et al. showed that both indices of activity, as well as those for sclerosis are important for the prognosis of the disease [17]. In a study performed on 293 patients it was shown that some semiquantitative histological indices, such as mesangial proliferation index, glomerular chronicity index and tubulointerstitial chronicity index correlated with end stage renal disease (that was chosen as an end point by the authors) [18].

Conclusions

It becomes clear that both, glomerular and interstitial, active and chronic histological lesions are involved in the outcome of the glomerular disease.

A scoring system of histological changes is important in the assessment of active and chronic glomerular changes in patients with different primary or secondary glomerulonephritis. The use of a scoring system in glomerulonephritis could permit a unified approach of renal biopsies by different pathologists or nephrologists, and could facilitate the study of the histological features involved in the progression of renal disease.

Conflict of interest statement. None declared.

References