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Interobserver agreement of scoring of histopathological characteristics and classification of lupus nephritis

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Abstract

Background. Assessing renal biopsies from patients with lupus nephritis (LN) is a difficult task and it is subject to interobserver variability. In this study the interobserver agreement amongst five nephropathologists was analysed.

Methods. Five specialized nephropathologists scored 126 biopsies, comprising 87 first and 39 repeat biopsies from 87 patients with biopsy-proven proliferative LN, included in a randomized controlled trial. The interobserver agreement [expressed as intraclass correlation coefficients (ICC)] of the scored histopathological items was calculated. Also, the WHO1995 and ISN/RPS2003 classification systems for LN were compared, with extra attention being given to the comparison between patients with diffuse proliferative LN with either segmental (IV-S) or global (IV-G) lesions.

Results. There was a wide range of agreement. A good interobserver agreement (ICC > 0.6) was present in 15%, and a moderate interobserver agreement (ICC 0.4–0.6) in 31% of the scored items. The activity index for LN showed a good (ICC 0.716) and the chronicity index a moderate (ICC 0.494) interobserver agreement. Both classification systems showed low agreement, although consensus was easily reached. Patients classified as IV-S (n = 15) had more favorable clinical parameters at study entry than those with class IV-G (n = 57). Although suggested by others, we found no differences in outcome between these two subclasses.

Conclusions. This study shows that, although definitions were agreed upon beforehand, even specialized on nephropathologists have difficulties with scoring histopathological characteristics of LN, particularly with SLE the classification systems.

Keywords: agreement; kidney biopsy; lupus nephritis; observer; systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease in which virtually any organ can be affected [1]. Involvement of the kidney occurs frequently [2] and the morphological changes observed in the kidney show a great diversity [3]. Attempts have been made to categorize the morphological changes in order to recognize certain patterns, with the aim of providing better guidelines for therapy and prognosis. Until the beginning of this century, one of the WHO classification systems for lupus nephritis (LN) was used, the latest being the WHO1995 classification [3]. In 2003, the classification for LN was redefined by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) [4] in order to allow better standardization of renal biopsies in lupus patients than before [4,5]; in particular as it separates segmental and global lesions. The importance of the latter was suggested by Najafi et al. [6] in 2001. Patients with diffuse proliferative LN and segmental lesions (n = 24) were more likely to reach end-stage renal disease than patients with diffuse proliferative LN and global lesions (n = 35), although the number of patients...
entering remission and developing a renal flare was not different.

In a search for a less toxic alternative for cyclophosphamide pulse therapy, the first Dutch LN study started in 1995. In this study, 87 patients with biopsy-proven proliferative LN were randomized to either cyclophosphamide pulse therapy, or to azathioprine combined with methylprednisolone pulses [7]. After 2 years a repeat renal biopsy was performed in 39 participants.

In order to evaluate the histomorphological lesions of these biopsies in detail, a standardized scoring form was developed. Here we report the interobserver agreement among five specialized nephropathologists. We also compare the WHO1995 classification with the ISN/RPS2003 classification for LN.

**Subjects and methods**

**Patient selection**

From September 1995 until September 2001, 87 patients with biopsy-proven proliferative LN were included in the first Dutch LN study [7]. In this randomized controlled trial, patients were treated with either azathioprine combined with methylprednisolone and prednisone, or cyclophosphamide pulse therapy with prednisone for 2 years. After 2 years, this regimen was followed by long-term azathioprine and prednisone. All patients were followed regularly, and clinical and laboratory parameters were collected according to the study protocol [7].

**Renal biopsy**

All biopsies were performed and processed locally. Before randomization, all 87 inclusion biopsies were reviewed and classified by at least one of three experienced nephropathologists according to the WHO1995 criteria for LN [3]. The activity index (AI) and chronicity index (CI) were calculated for each specimen as described previously [8], with maximum scores of 24 for the AI and 12 for the CI. After 2 years, all available patients were asked to have a repeat renal biopsy performed for study purposes only. It was agreed that findings in these biopsies would be anonymized and not used for therapeutic decisions. The ethics committees of all participating hospitals approved this pathology study, and it was conducted according to the Declaration of Helsinki. After informed consent, 41 patients underwent a repeat renal biopsy. For this study silver-stained slides (with HE-background), and for some items PAS slides, were used.

**Scoring procedure**

**Scoring form.** Histological characteristics described in LN, together with its definitions, were gathered from the literature. In three consensus meetings with three specialized nephropathologists, the list of items was reduced and a concept scoring form was made. In a pilot study, 10 biopsies were scored to solve discrepancies and problems. In a consensus meeting, these discrepancies were discussed and the scoring form was adjusted. In another consensus meeting, the scoring form was again discussed and revised with five different, experienced nephropathologists (I.B., S.F., E.S., C.P., R.G.) and a specialized nephropathologist from abroad (Dr G. S. Hill, Hôpital Européen Georges Pompidoa, Paris, France). With the final version and after collecting the slides at a central office, three scoring sessions followed. Each biopsy was scored by three of five nephropathologists.

**Item selection and definitions.** The first pathologist marked the section with the most glomeruli and this slide was subsequently used for the scoring procedure. For almost all assessments, silver-stained slides were used (exceptions were predefined). Scoring the biopsy was divided into several sections: glomerular, tubular, interstitial and vascular. For definitions see our previous publication [9]. For each glomerulus, the presence of a variety of histopathological items was counted. After having scored the section, the nephropathologist was asked to give an opinion on the overall quality of the biopsy: either poor, reasonable or excellent. The quality was evaluated on both the quality of the staining and the quality of the material.

The assessment was completed by filling out the WHO1995 and ISN/RPS2003 classification and activity and chronicity indices for LN [3,4,8]. For these aspects of the assessment, the definitions belonging to the classification systems and the activity and chronicity indices were used. To simplify the comparisons between the two classification methods, membranous features were mentioned as ‘+V’.

After all scoring sessions were finished, the discrepancies in classification were discussed and a final joint judgement was made, during three consensus meetings.

**Statistics**

All data were analysed by using SPSS 12.0.1 software. All continuous glomerular variables were expressed as the percentage of the total number of non-sclerotic and non-ischemic glomeruli scored in the section.

Intraclase correlation coefficients (ICC) were used to evaluate interobserver readings for the entire set of slides [10]. The ICC is an index of concordance that indicates the degree of agreement beyond that expected by chance alone, and is appropriate when assessing agreement between two or more raters. It is expressed in a score ranging between 0 and 1:0 indicating agreement by chance and 1 total agreement. In general, values higher than 0.8 are considered as excellent, values between 0.6 and 0.8 as good, values between 0.4 and 0.6 as moderate, and values below 0.4 as poor concordance. The ICCs of both continuous, binominal and ordinal variables can be computed, which also takes into account the ranking order of the variables. The ICC was favored over the Cohen Kappa value since each of the five observers scored only 3/5 of the biopsies. The scoring of the biopsies was organized in such a way that an overlap of at least one-third of the slides existed between two nephropathologists.

To account for possible systematic differences between nephropathologists, the nephropathologists were added as ‘random effects’. In doing so, the found
Results

Biopsies

In total 128 renal biopsies were performed in 87 patients. All patients underwent a biopsy at study entry. Forty one patients agreed to undergo a repeat biopsy after two years of therapy. Of these 41 repeat biopsies, two did not contain glomeruli and were not included, leaving 126 biopsies for scoring. Overall, the quality of the preparation of the biopsies was good: 55% were judged as excellent and 28% as reasonable. However, in 17% of the biopsies either the staining or cutting quality was judged as poor. A total of nine biopsies could not be classified. Reasons were: six or less glomeruli (n = 7; five repeat biopsies and two taken at study entry), or poor quality (n = 2, both repeat biopsies).

Interobserver agreement

The analyses were performed for all 126 biopsies. A wide range of agreement for the diverse characteristics was found. Good or excellent concordance (ICC > 0.6) was present in 15% of all 54 scored characteristics. Agreement was moderate (ICC 0.4–0.6) in 31% and low (ICC < 0.4) in 54% of all scored characteristics. The ICCs did not improve if the analyses were done after exclusion of biopsies with six or less glomeruli or biopsies of poor quality. The observed ICCs also did not change if only the biopsies taken at study entry were analysed. In Table 1 the variables for which excellent, good or moderate agreement existed are listed.

Glomeruli. Excellent or good intraclass correlation coefficients were found for the following items: total number of glomeruli ICC 0.950, completely sclerosed glomeruli (0.821), endocapillary proliferation (0.646), extracapillary proliferation (0.639) and spikes (0.629). For several items a moderate ICC was found (Table 1).

Tubules and interstitium. Two tubular variables (tubular atrophy and casts) were scored with moderate agreement. Interstitial infiltrate and fibrosis showed acceptable ICCs, while disagreement existed for edema.

Table 1. Interobserver agreement: items for which agreement was excellent (ICC > 0.8), good (ICC 0.6–0.8) or moderate (ICC 0.4–0.6)

<table>
<thead>
<tr>
<th>Item</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular</td>
<td>0.950</td>
</tr>
<tr>
<td>Total glomeruli</td>
<td></td>
</tr>
<tr>
<td>Completely sclerosed glomeruli</td>
<td>0.821</td>
</tr>
<tr>
<td>Endocapillary proliferation</td>
<td>0.646</td>
</tr>
<tr>
<td>Extracapillary proliferation</td>
<td>0.639</td>
</tr>
<tr>
<td>Spikes</td>
<td>0.629</td>
</tr>
<tr>
<td>Synechia</td>
<td>0.522</td>
</tr>
<tr>
<td>Wire loops</td>
<td>0.498</td>
</tr>
<tr>
<td>Karyorrhexis</td>
<td>0.482</td>
</tr>
<tr>
<td>Ischemic glomeruli</td>
<td>0.455</td>
</tr>
<tr>
<td>Loop necrosis</td>
<td>0.439</td>
</tr>
<tr>
<td>Tubulo-interstitial</td>
<td>0.514</td>
</tr>
<tr>
<td>Intervascular infiltrate</td>
<td></td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>0.511</td>
</tr>
<tr>
<td>Casts</td>
<td>0.458</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>0.418</td>
</tr>
<tr>
<td>Indices</td>
<td></td>
</tr>
<tr>
<td>Activity index</td>
<td>0.716</td>
</tr>
<tr>
<td>Chronicity index</td>
<td>0.494</td>
</tr>
</tbody>
</table>

Vascular changes. Vascular changes were not observed very often. Only for arterial fibrous intimal hyperplasia and arterial thrombosis, acceptable agreement was observed (ICC 0.461 and 0.421, respectively).

activity and chronicity index. Good concordance was present for the AI (ICC 0.716) and moderate concordance for the CI (ICC 0.494).

WHO1995 classification and ISN/RPS2003 classification

Remarkably low ICCs (ICC < 0.2) were found for the classification systems, using both the WHO1995 and the ISN/RPS2003 classification. If for the WHO1995 classification the subclasses were excluded, and the classes II, III, IV, V and VI were compared, the level of agreement slightly improved (ICC improved from 0.108 to 0.368). And, if the classes were divided into class II, III, IV-S, IV-G, V and VI (ISN/RPS2003 classification) the ICC increased from 0.182 to 0.414.

Table 2 shows how, after reaching consensus, all biopsies were classified. Membranous features were present in 6 (25%) and in 8 (10%) of the biopsies classified as class III and IV, respectively.
In view of the assumed prognostic importance, patients with ISN/RPS2003 class IV-S and IV-G were assessed separately. At study entry, 15 biopsies were classified as class IV-S and 57 as class IV-G. The clinical characteristics are summarized in Table 3. Patients with segmental lesions had more favorable characteristics: their renal function was better, hypertension was less often present, and complement values and hemoglobin were higher. A number of outcome parameters were not different between the two groups. Actually, more patients had doubling of serum creatinine in the IV-G group (n = 5) than in the IV-S group (n = 0), after a median follow-up of 77 months (interquartile range (IQR) 54–96), but the differences were not statistically significant. Log rank analysis of (time to) partial and complete remission did not show differences between the two groups. Not unexpectedly, more endocapillary proliferation and higher scores for wire loops were present in the biopsies categorized as class IV-G. Also, the total activity index was lower in class IV-S (7.7 vs 9.7), but this difference was not significant (Table 4 gives the histological characteristics).

Table 2. WHO1995 and ISN/RPS2003 classification in 117 biopsies (85 biopsies at study entry and 32 repeat biopsies)

<table>
<thead>
<tr>
<th>WHO1995</th>
<th>n</th>
<th>ISN/RPS2003</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIa</td>
<td>11</td>
<td>II</td>
<td>10</td>
</tr>
<tr>
<td>Ib</td>
<td>3</td>
<td>II</td>
<td>1</td>
</tr>
<tr>
<td>IIIa</td>
<td>5</td>
<td>III-A</td>
<td>5</td>
</tr>
<tr>
<td>IIIb</td>
<td>7</td>
<td>III-A/C</td>
<td>7</td>
</tr>
<tr>
<td>IIc</td>
<td>12</td>
<td>III-C</td>
<td>12</td>
</tr>
<tr>
<td>IVa</td>
<td>4</td>
<td>IV-G-A</td>
<td>4</td>
</tr>
<tr>
<td>IVb</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVc</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVd</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>VI</td>
<td>1</td>
</tr>
<tr>
<td>total</td>
<td>117</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Three biopsies were classified as class II LN in the WHO1995 classification, but due to the presence of membranous features these were classified as class V in the ISN/RPS2003 classification.

Table 3. Clinical characteristics of 72 patients with class IV lupus nephritis at study entry and during follow-up.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>IV-S</th>
<th>IV-G</th>
<th>P</th>
<th>IVa or IVb</th>
<th>IVc or IVd</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY/AZA (n)</td>
<td>9/6</td>
<td>35/22</td>
<td>ns</td>
<td>7/10</td>
<td>37/18</td>
<td>ns</td>
</tr>
<tr>
<td>Age (year)</td>
<td>37 (28–47)</td>
<td>31 (24–41)</td>
<td>ns</td>
<td>26 (21–34)</td>
<td>33 (24–44)</td>
<td>0.037</td>
</tr>
<tr>
<td>Female (n) (%)</td>
<td>12 (80)</td>
<td>50 (88)</td>
<td>ns</td>
<td>12 (71)</td>
<td>50 (91)</td>
<td>0.034</td>
</tr>
<tr>
<td>Caucasian (n) (%)</td>
<td>12 (87)</td>
<td>40 (70)</td>
<td>ns</td>
<td>12 (71)</td>
<td>41 (75)</td>
<td>ns</td>
</tr>
<tr>
<td>Hypertension (n) (%)</td>
<td>3 (20)</td>
<td>12 (21)</td>
<td>ns</td>
<td>1 (6)</td>
<td>14 (26)</td>
<td>ns</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>140 (120–145)</td>
<td>140 (120–150)</td>
<td>ns</td>
<td>140 (120–153)</td>
<td>140 (120–150)</td>
<td>ns</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 (70–90)</td>
<td>80 (80–90)</td>
<td>ns</td>
<td>80 (78–98)</td>
<td>80 (80–90)</td>
<td>ns</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>2.5 (0.4–109)</td>
<td>22 (1–82)</td>
<td>ns</td>
<td>2 (1–27)</td>
<td>22 (1–102)</td>
<td>ns</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>20 (14–26)</td>
<td>21 (16–24)</td>
<td>ns</td>
<td>22 (17–27)</td>
<td>20 (14–24)</td>
<td>ns</td>
</tr>
<tr>
<td>Laboratory parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scrat (μmol/l)</td>
<td>95 (72–121)</td>
<td>112 (87–154)</td>
<td>0.039</td>
<td>98 (84–139)</td>
<td>115 (85–152)</td>
<td>ns</td>
</tr>
<tr>
<td>Proteinuria (g/24h)</td>
<td>3.5 (1.8–6.5)</td>
<td>4.2 (2.7–6.6)</td>
<td>ns</td>
<td>3.9 (2.8–6.2)</td>
<td>4.1 (2.2–6.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Hemoglobin (g/l)</td>
<td>7.6 (7.0–8.3)</td>
<td>6.2 (5.6–7.2)</td>
<td>0.000</td>
<td>6.9 (5.6–7.6)</td>
<td>6.3 (5.6–7.5)</td>
<td>ns</td>
</tr>
<tr>
<td>C3 (g/l)</td>
<td>0.54 (0.47–0.77)</td>
<td>0.43 (0.32–0.60)</td>
<td>0.012</td>
<td>0.45 (0.24–0.62)</td>
<td>0.49 (0.38–0.63)</td>
<td>ns</td>
</tr>
<tr>
<td>C4 (g/l)</td>
<td>0.12 (0.01–0.22)</td>
<td>0.10 (0.07–0.12)</td>
<td>0.008</td>
<td>0.08 (0.07–0.14)</td>
<td>0.10 (0.08–0.14)</td>
<td>ns</td>
</tr>
<tr>
<td>Anti-dsDNA (IU/ml)</td>
<td>141 (37–558)</td>
<td>166 (18–553)</td>
<td>ns</td>
<td>342 (93–907)</td>
<td>124 (22–502)</td>
<td>ns</td>
</tr>
<tr>
<td>Outcome parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>78 (63–95)</td>
<td>76 (54–95)</td>
<td>ns</td>
<td>93 (54–107)</td>
<td>75 (54–88)</td>
<td>ns</td>
</tr>
<tr>
<td>Scrat x 2 (n) (%)</td>
<td>0 (0)</td>
<td>5 (9)</td>
<td>ns</td>
<td>1 (6)</td>
<td>4 (7)</td>
<td>ns</td>
</tr>
<tr>
<td>Failure/relapse (n) (%)</td>
<td>2 (13)</td>
<td>10 (18)</td>
<td>ns</td>
<td>2 (12)</td>
<td>10 (18)</td>
<td>ns</td>
</tr>
<tr>
<td>Complete remission (n) (%)</td>
<td>7 (53)</td>
<td>32 (56)</td>
<td>ns</td>
<td>12 (71)</td>
<td>28 (51)</td>
<td>ns</td>
</tr>
<tr>
<td>Total activity index (n) (%)</td>
<td>13 (87)</td>
<td>54 (94)</td>
<td>ns</td>
<td>16 (94)</td>
<td>51 (93)</td>
<td>ns</td>
</tr>
<tr>
<td>Scrat at last follow-up (μmol/l)</td>
<td>86 (71–94)</td>
<td>79 (73–108)</td>
<td>ns</td>
<td>81 (63–111)</td>
<td>79 (72–104)</td>
<td>ns</td>
</tr>
<tr>
<td>Proteinuria at last follow-up (g/24h)</td>
<td>0.5 (0.1–1.4)</td>
<td>0.2 (0.1–0.8)</td>
<td>ns</td>
<td>0.1 (0.1–0.5)</td>
<td>0.3 (0.1–1.1)</td>
<td>ns</td>
</tr>
</tbody>
</table>

IV-S, class IV-segmental; IV-G, class IV-global; CY, patients treated with cyclophosphamide pulses; AZA, patients treated with azathioprine and methylprednisolone pulses; SLEDAI, SLE disease activity index; Scrat, serum creatinine; C3, complement C3; C4, complement C4; Scrat x 2, doubling of serum creatinine. Comparison of ISN/RPS2003 class IV-S and IV-G, and of WHO1995 class IVa/IVb and IVc/IVd. Number and percentage, or median with interquartile range is given.

aTo convert serum creatinine to mg/dl divide by 88.4.
bPrimary study end point.
Table 4. Histopathological characteristics of 72 patients with class IV lupus nephritis at study entry

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IV-S n = 15 (range)</th>
<th>IV-G n = 57 (range)</th>
<th>P</th>
<th>IVa or IVb n = 17 (range)</th>
<th>P</th>
<th>IVc or IVd n = 55 (range)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocapillary proliferation</td>
<td>50 (37–77)</td>
<td>80 (67–96)</td>
<td>0.002</td>
<td>86 (68–93)</td>
<td>0.020</td>
<td>74 (52–92)</td>
<td>ns</td>
</tr>
<tr>
<td>Extracapillary proliferation</td>
<td>21 (5–34)</td>
<td>12 (2–31)</td>
<td>ns</td>
<td>33 (13–41)</td>
<td>0.020</td>
<td>10 (2–28)</td>
<td>ns</td>
</tr>
<tr>
<td>Wire loop score ≥25% (n) (%)</td>
<td>1 (7)</td>
<td>27 (47)</td>
<td>0.004</td>
<td>8 (47)</td>
<td>0.004</td>
<td>20 (36)</td>
<td>ns</td>
</tr>
<tr>
<td>Activity index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>7.7 (5.3–11.7)</td>
<td>9.7 (7.3–12.5)</td>
<td>ns</td>
<td>10.7 (9.2–13.0)</td>
<td>0.036</td>
<td>9.3 (7.0–11.0)</td>
<td>ns</td>
</tr>
<tr>
<td>Score ≥10 (n) (%)</td>
<td>5 (33)</td>
<td>28 (49)</td>
<td>ns</td>
<td>12 (71)</td>
<td>0.019</td>
<td>21 (38)</td>
<td>ns</td>
</tr>
<tr>
<td>Chronicity index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completely sclerosed glomeruli</td>
<td>0 (0–5)</td>
<td>5 (0–9)</td>
<td>ns</td>
<td>0 (0–1)</td>
<td>0.002</td>
<td>5 (0–11)</td>
<td>ns</td>
</tr>
<tr>
<td>Synechiae</td>
<td>27 (7–38)</td>
<td>16 (6–28)</td>
<td>ns</td>
<td>10 (0–14)</td>
<td>0.01</td>
<td>23 (9–38)</td>
<td>ns</td>
</tr>
<tr>
<td>Tubular atrophy score ≥25% (n) (%)</td>
<td>1 (7)</td>
<td>6 (11)</td>
<td>ns</td>
<td>0 (0)</td>
<td>7 (13)</td>
<td>12 (10)</td>
<td>ns</td>
</tr>
<tr>
<td>Interstitial fibrosis score ≥25% (n) (%)</td>
<td>1 (7)</td>
<td>3 (5)</td>
<td>ns</td>
<td>0 (0)</td>
<td>4 (7)</td>
<td>7 (13)</td>
<td>ns</td>
</tr>
<tr>
<td>Chronicity index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>2.3 (1.7–3.3)</td>
<td>2.7 (20–3.7)</td>
<td>ns</td>
<td>2.0 (1.3–2.7)</td>
<td>0.000</td>
<td>3.0 (2.0–3.7)</td>
<td>0.000</td>
</tr>
<tr>
<td>Score ≥4 (n) (%)</td>
<td>1 (7)</td>
<td>8 (14)</td>
<td>ns</td>
<td>0 (0)</td>
<td>9 (16)</td>
<td>7 (14)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Comparison of ISN/RPS2003 class IV-S and IV-G, and comparison of WHO1995 class IVa/IVb and IVc/IVd. Median with interquartile range, or number and percentage are given.

*Fibrous crescents are chronic lesions, while cellular crescents are active lesions. Fibrous crescents were scored rarely.

histopathological characteristics were also evaluated for patients with WHO1995 class IVa or IVb vs those classified as class IVc or IVd (data are added to Tables 3 and 4). We argued that class IVa and IVb represent the biopsies with more acute lesions, while IVc and IVd are more chronic forms of LN. Of the 15 patients classified as ISN/RPS2003 class IV-S, only 2 were categorized as WHO1995 class IVa or IVb and 13 as IVc or IVd. For the 57 patients classified as ISN/RPS2003 class IV-G this was 15 and 42, respectively.

Discussion

This is the first time that the interobserver agreement for many predefined histopathological characteristics of LN was studied thoroughly, along with both the WHO1995 and the ISN/RPS2003 classification for LN. Previous studies have hinted on this subject, but either did not incorporate classification data or used the histological characteristics for (prognostic) regression analyses only. The present study was based on 126 renal biopsies of 87 patients with proliferative LN in their initial biopsy, who were included in a randomized controlled trial. We found a wide range of agreement, not only for the diverse histological characteristics but also for the WHO1995 and ISN/RPS2003 classification, of which the latter was assumed to be easily reproducible [5]. A good interobserver agreement was present in only 15% of the 54 scored characteristics, and moderate in 31%. The observation that if more pathologists examine a biopsy, the disagreement will increase, was described in many other pathology studies [11,12], and is therefore not unique for LN. However, since in lupus the variety of histological abnormalities is huge as compared to other diseases, the problem of variable interobserver agreement in lupus will probably be larger. We can only speculate about the reasons for the lack of agreement.

The agreement of the total score of AI and CI appeared to be good to moderate (0.716 and 0.494, respectively). This is in line with observations by others [13].

During the scoring sessions and the consensus meetings, we encountered several difficulties in interpreting the definitions and slides. Although definitions for each item were agreed upon beforehand, the nephropathologists may have interpreted some of these definitions differently during the scoring rounds. Most importantly, however, some of the lesions seem to defy definition, of which we give a number of examples in Figure 1.We were somewhat hampered by the variable quality of the slides, caused by the fact that the biopsies were taken, cut and stained locally. Unfortunately, immunofluorescence slides were not available for this study.

The disagreement on classification was striking: both for the WHO1995 and ISN/RPS2003 classification low ICCs were found (<0.2). Potential causes for these low levels of agreement are: poor quality of the slides, smaller biopsies, biopsies in which around half of the glomeruli were affected, affected glomeruli with both segmental and global lesions, and the presence of the most prominent proliferative lesions in the glomeruli at the edge of the slide. According to the definition of total number of glomeruli, the latter had to be disregarded, thereby hampering a proper classification. No clinical data were given to the pathologists. One could argue that disagreement over a class III or IV LN is less cumbersome than disagreement over class II or IV. Therefore, we categorized the biopsies in proliferative vs non-proliferative LN. This did not reveal better ICCs (ICC for WHO1995 was 0.312 and for ISN/RPS2003 was 0.251). So the discrepancies in classification cannot be explained by disagreement...
Fig. 1. Several examples of histomorphological characteristics, which might explain why a low interobserver agreement was found. Furthermore, since the ISN/RPS 2003 classification makes an explicit distinction between segmental and global lesions, we would like to show you how difficult it can sometimes be to make this distinction. Considerations/answers are given in parentheses following the questions. (A) Is this a normal glomerulus? (This is clearly not a normal glomerulus because it shows signs of ischemia, namely wrinkling of GBM and irregular Bowman’s capsule. However, it does not contain lupus-related lesions. In counting glomeruli with and without lupus-related lesions, for instance, to make a decision on a class III or class IV, this glomerulus would be on the ‘normal’ side, and was categorized as such on the scoring form.) (B) Glomerulus with mesangial changes, but is there endocapillary proliferation as well? (Next to mesangial changes this glomerulus shows chronic lesions, namely doubling of GBM and ischemia. However, in a number of capillary loops, endothelial cells are prominently present. However, leucocytes are absent and obliteration of capillary loops due to proliferation hardly occurs. Three out of five pathologists scored this as segmental endocapillary proliferation. However, the other two scored it as global endocapillary proliferation. It is certainly a case of A/C.) (C) Glomerulus with extensive mesangial proliferation, but is there a component of endocapillary proliferation
regarding focal or diffuse LN. Finally, for a correct
classification, a minimum number of ten glomeruli is
required [4]. In several cases, our classification was
made on less than ten glomeruli. However, this did not
explain the low interobserver agreement, since ICCs
did not change if the data were analysed for biopsies
with ten or more glomeruli only. Strikingly, we found
that during the consensus meetings, consensus was
often reached without difficulties.

Reports on the interobserver agreement of the
WHO1995 or ISN/RPS2003 classification are scarce
[14,15]. In most studies on LN only one nephropathologist
classified the biopsies [16,17], or two nephro-
pathologists classified the biopsies in consensus
[6,18,19]. An exception is a report on the ISN/
RPS2003 classification which described 60 Japanese
patients with LN [14]. The consensus between two
pathologists for the ISN/RPS2003 and WHO1995
classification was 98 and 83%, respectively. Another
report showed that the consensus between two
pathologists who studied 420 biopsies, was better for
the ISN/RPS2003 classification than for the WHO1995
classification (96% vs 87%) [20]. Recently, Furness
et al. reported a study on interobserver variability
throughout the UK [15]. Twenty renal biopsies,
accompanied with clinical information and immuno-
fluorescence results, were sent to (untrained) patholo-
gists twice: the first time the pathologist was asked to
classify the biopsies according to the WHO1995
classification, the second according to the ISN/
RPS2003 classification. Calculated kappa values were
0.44 and 0.53 for the WHO1995 and ISN/RPS2003
classification, respectively. Disease activity (acute vs
chronic vs acute and chronic) was poorly agreed upon
(κ 0.33). They concluded that the ISN/RPS2003
classification is better reproducible. In our study, the
pathologists were unaware of clinical data.

The differences between class IV-S and IV-G may
become clinically important and lead to different
therapeutical guidelines. Our patients with IV-G had
more active disease and worse clinical parameters at
study entry. The outcome however was not different,
and none of the patients who were classified as IV-S
doubled their serum creatinine. One of the reasons that
we did not find differences in outcome could be due to
the fact that doubling of serum creatinine did not occur
often in our study (n = 5), after a reasonable follow-up.
Like others [14,21,22], we could not confirm the
findings by Najafi et al. [6] that IV-S had a worse
prognosis than IV-G. All these studies [14,21,22] also
found worse clinical and histopathological character-
istics in the IV-G patients. The chronicity index in our
population was lower than that in the original study
[6], and higher than that found in the French study
[22]. It is important to realize that the initial report
described an American population, while most of the
recent studies, including ours, were carried out on
either European or Asian patients. The comparison of
patients with WHO1995 class IVa or IVb with those
classified as class IVc or IVd revealed that IV-S and
IV-G present as different clinical entities, while IVa/b
and IVc/d are histopathologically different. Since for
the patients with class III LN an impaired renal
function was obligatory for inclusion in our trial, a
comparison between the patients with class IV-S and
IV-G with those with class III (n = 11) is not
meaningful.

In summary, although standardizing the assess-
ment of renal biopsies in LN is needed both for clinical and
research purposes, and although the new ISN/
RPS2003 classification seems to be of help, our study shows
that the detailed evaluation by specialized
nephropathologists shows variable degrees of agree-
ment with regard to various histopathological char-
acteristics. However, the detailed scoring system we
used is too laborious and unpractical for daily clinical
use.

Our results do not weaken the importance of the
classification for LN, but they raise the question
whether a further subclassification is meaningful.
Indeed, we did not find differences in the outcome
between the patients with class IV-S or IV-G lesions.
We believe that, until proven clinically useful, sub-
classifying diffuse proliferative LN into segmental or
global should be used for research purposes only.
Additional research has to reveal whether or not these
groups are (clinically) different and should be treated
differently.

Acknowledgements. We would like to thank all patients and
the members of the Dutch Working Party on SLE who were willing to
participate in the first Dutch Lupus Nephritis Study. This study was
possible because of the skilful coordination by Dr G. Ligtenberg
(Department of Nephrology, and Department of Rheumatology
& Clinical Immunology, University Medical Center Utrecht,
Utrecht, The Netherlands). We thank the pathologists who supplied
the renal biopsies. Furthermore, we have appreciated the help of
Drs J. J. Weening (Department of Pathology, Academic Medical
Center, Amsterdam, The Netherlands), J. A. Bruijn (Department of

as well? (The mesangial proliferation is dominant and most of the capillary loops surround the mesangial areas like a corona, without apparent endocapillary proliferation. However, there seems to be endocapillary proliferation at 7 o’clock, and also at 9 o’clock, to be scored as segmental.) (D) Glomerulus with a segmental proliferative lesion, but is it active or chronic? (The mesangial changes in the background of the proliferative lesions are disregarded in making this decision. The proliferative lesion next to the proximal tube has an element of chronicity, namely its adhesion to the capsule, and of activity, namely its active endocapillary proliferation. Two pathologists scored it as a chronic lesion, letting the adhesion prevail on the decision. The others chose for A.) (E) Glomerulus with mesangial sclerosis, but is it global or segmental? According to the definition, global mesangial sclerosis involves more than 50% of the glomerular tuft, segmental mesangial sclerosis less than 50%. (Mesangial sclerosis is most prominently present in the center and on the right side of the glomerulus. To a lesser extent, it is present on the left side and upper side as well. Does it reach an area of more than 50%? All pathologists decided it did.) (F) Glomerulus with minor inflammation, enough for a positive score? According to the definition, at least five inflammatory cells should be present. (There seem to be five inflammatory cells present in capillary loops, so the answer is yes, and all pathologists agreed on this. However, some inflammatory cells are difficult to recognize, and could easily be mistaken for endothelial cells. There are also cells with apoptotic changes, of which it is uncertain whether they are inflammatory cells.)
Conflict of interest statement. None declared.

References


Received for publication: 24.6.07
Accepted in revised form: 20.7.07