Scoring system for renal pathology in Fabry disease: report of the International Study Group of Fabry Nephropathy (ISGFN)

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Abstract

Background. In Fabry nephropathy, alpha-galactosidase deficiency leads to accumulation of glycosphingolipids in all kidney cell types, proteinuria and progressive loss of kidney function.

Methods. An international working group of nephrologists from 11 Fabry centres identified adult Fabry patients, and pathologists scored histologic changes on renal biopsies. A standardized scoring system was developed with a modified Delphi technique assessing 59 Fabry nephropathy cases. Each case was scored independently of clinical information by at least three pathologists with an average final score reported.

Results. We assessed 35 males (mean age 36.4 years) and 24 females (43.9 years) who mostly had clinically mild Fabry nephropathy. The average serum creatinine was 1.3 mg/dl (114.9 μmol/l); estimated glomerular filtration rate was 81.7 ml/min/1.73 m² and urine protein to creatinine ratio was 1.08 g/g (122.0 mg/mmol). Males had greater podocyte vacuolization on light microscopy (mean score) and glycosphingolipid inclusions on semi-thin sections than females. Males also had significantly more proximal tubule, peritubular capillary and vascular intimal inclusions. Arteriolar hyalinosis was similar, but females had significantly more arterial hyalinosis. Chronic kidney disease stage correlated with arteriolar and glomerular sclerosis scores. Significant changes, including segmental and global sclerosis, and interstitial fibrosis were seen even in patients with stage 1–2 chronic kidney disease with minimal proteinuria.

Conclusions. The development of a standardized scoring system of both disease-specific lesions, i.e. lipid deposition related, and general lesions of progression, i.e. fibrosis and sclerosis, showed a spectrum of histologic appearances even in early clinical stage of Fabry nephropathy. These findings support the role of kidney biopsy in the baseline evaluation of Fabry nephropathy, even with mild clinical disease. The scoring system will be useful for longitudinal assessment of prognosis and responses to therapy for Fabry nephropathy.

Keywords: chronic kidney disease; Fabry disease; pathology; sclerosis; scoring

Introduction

Fabry disease is an X-linked genetic disorder with cellular accumulation of globotriosylceramides (GL-3) due to lysosomal alpha-galactosidase enzyme activity deficiency [1,2]. Males classically develop chronic kidney disease...
(CKD) and progress to end-stage renal disease (ESRD) be-


ter than the males (28.1 ± 3.3 years, range 18.0–45.2) and
diabetic nephropathy (21.4 ± 1.9 years, range 15.3–


tors in the dataset are described in the late-onset cardiac
variants of Fabry disease [1]. The mean age was 39.4 ± 13.2 years (range 16–70), and the serum creatinine was 1.3 ± 1.2 mg/dl (range 0.6–7.1) [114.9 ± 106.1 μmol/l (range 53.0–627)]. UPCR was 1.08 ± 1.34 g/g (range 0.01–5.62) [122.0 ± 151.4 mg/mmol (range 1.1–635.1)] (Table 1). Forty-six patients (78.0%, 25 males) had stage 1 or 2 CKD, 8 (13.6%, 7 males) had stage 3 CKD and 5 males (8.5%) had stage 4 or 5 CKD (Table 2). Males with CKD stage 1 were significantly younger than the females (28.1 ± 10.3 versus 42.4 ± 10.6 years, P < 0.003).

Average systolic and diastolic blood pressures were

125.7 ± 13.0 (range 100–167) and 75.6 ± 10.5 mmHg
(range 54–105; Table 1), respectively. Systolic blood
pressure was >135 mmHg in 13 (22%) patients, and
diastolic blood pressure was >85 mmHg in 9 patients
(15.3%). Angiotensin-converting enzyme inhibitors or
angiotensin receptor blockers were given to 24 patients
(40.7%).

Nine patients started ERT 1.5 ± 1.0 months (range 0.6–
3.9, median 1.4) before biopsy at a dose of 0.2 (n = 3) or
1.0 mg/kg every 2 weeks (n = 6). Forty-six patients started
ERT 8.2 ± 13.1 months (range 0.03–76.2, median 4.0) after
biopsy at a dose of 0.2 (n = 13) or 1.0 mg/kg every 2 weeks
(n = 33).

Results

Summary of clinical assessments

All patients had defined mutations, and five male pa-
tients had the p.N215S missense mutation, previously de-
scribed in the late-onset cardiac variants of Fabry disease
[1]. The mean age was 39.4 ± 13.2 years (range 16–70), and
the serum creatinine was 1.3 ± 1.2 mg/dl (range 0.6–7.1) [114.9 ± 106.1 μmol/l (range 53.0–627)]. UPCR was 1.08 ± 1.34 g/g (range 0.01–5.62) [122.0 ± 151.4 mg/mmol (range 1.1–635.1)] (Table 1). Forty-six patients (78.0%, 25 males) had stage 1 or 2 CKD, 8 (13.6%, 7 males) had stage 3 CKD and 5 males (8.5%) had stage 4 or 5 CKD (Table 2). Males with CKD stage 1 were significantly younger than the females (28.1 ± 10.3 versus 42.4 ± 10.6 years, P < 0.003).

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biopsy at a dose of 0.2 (n = 13) or 1.0 mg/kg every 2 weeks
(n = 33).
### Table 1. Summary of patient characteristics by CKD stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>Males (n = 35)</th>
<th>Female (n = 24)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Age (years) 39.4 ± 13.2 (40.0)</td>
<td>Age (years) 42.4 ± 10.6</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td>Serum creatinine (mg/dL) 1.3 ± 1.2 (0.9)</td>
<td>Serum creatinine (mg/dL) 1.6 ± 1.4 (1.2)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>eGFR (ml/min/1.73 m²) 81.7 ± 34.8 (81.9)</td>
<td>eGFR (ml/min/1.73 m²) 79.0 ± 42.3 (80.0)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>UPCR (g/g) 1.08 ± 1.34</td>
<td>UPCR (g/g) 0.73 ± 0.83</td>
<td>0.007*</td>
</tr>
<tr>
<td></td>
<td>Systolic BP (mmHg) 125.7 ± 13.0 (126.0)</td>
<td>Systolic BP (mmHg) 126.1 ± 13.1 (125.0)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Diastolic BP (mmHg) 75.6 ± 10.5 (75.0)</td>
<td>Diastolic BP (mmHg) 74.3 ± 11.5 (73.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Age (years) 39.9 ± 14.1</td>
<td>Age (years) 43.5 ± 9.9</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Serum creatinine (mg/dL) 73.4 ± 7.8</td>
<td>Serum creatinine (mg/dL) 83.5 ± 9.0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>eGFR (ml/min/1.73 m²) 79.0 ± 42.3 (80.0)</td>
<td>eGFR (ml/min/1.73 m²) 63.0</td>
<td>NS</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Age (years) 46.4 ± 11.3</td>
<td>Age (years) 1.3 ± 0.8</td>
<td>0.007*</td>
</tr>
<tr>
<td></td>
<td>Serum creatinine (mg/dL) 43.9 ± 8.9</td>
<td>Serum creatinine (mg/dL) 46.0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>eGFR (ml/min/1.73 m²) 79.0 ± 42.3 (80.0)</td>
<td>eGFR (ml/min/1.73 m²)</td>
<td>NS</td>
</tr>
<tr>
<td>Stage 4, 5</td>
<td>Age (years) 41.5 ± 16.6</td>
<td>Age (years) 0.003; males compared to females, two-tailed t-test.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Serum creatinine (mg/dL) 16.3 ± 10.3</td>
<td>Serum creatinine (mg/dL)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>eGFR (ml/min/1.73 m²) 79.0 ± 42.3 (80.0)</td>
<td>eGFR (ml/min/1.73 m²)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>UPCR (g/g) 0.80 ± 1.09</td>
<td>UPCR (g/g)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD and (range; median) for UPCR. To convert UPCR to mg/mmol, multiply by 113.12.

### Biopsy findings

The histologic evaluations are summarized in Tables 3–6. On average, 16.1 ± 11.9 glomeruli were present for light microscopic assessment (range 1–67), and 2.6 ± 1.9 (range 0–8) glomeruli were examined on toluidine blue-stained semi-thin sections. Two cases were not scored on the semi-thin sections because of inadequate staining.

**Glomerular sclerosis.** There was a wide range of glomerular sclerosis scores (Table 3). Mild or severe segmental sclerosis was observed in 19 of 35 males (9 stage 1 CKD, 3 stage 2 CKD, remainder with more advanced CKD), and 11 of 24 females (2 stage 1 CKD, 8 stage 2 CKD, 1 patient with more advanced CKD). The average extent of segmental sclerosis was 4.7 ± 7.8% of glomeruli, with mild and severe lesions seen in the same biopsy. Global sclerosis was present in 14.8 ± 22.7% of glomeruli. Ten of the 15 males (66.7%) with stage 1 CKD had segmental or global sclerosis in 6.2 ± 8.7% (median 2.9) of their glomeruli, and 7 of the 10 females with stage 1 CKD had segmental or global sclerosis in 5.7 ± 9.0% (median 3.0) of their glomeruli. On average, 43.5 ± 23.0% of the glomeruli had global sclerosis for the 13 patients with eGFR <60 ml/min/1.73 m², with only 1 without global sclerosis.

**Interstitial fibrosis and chronic damage index.** The semi-quantitative and morphometric scores for fibrosis were closely correlated (top section, Table 4; Spearman’s coefficient = 0.871, P < 0.001). Bland–Altman analysis (Figure 1) [28] showed that the semi-quantitative assessment had no significant bias compared to the morphometry (mean difference of average score minus index of chronic damage = +0.79%; 95% Confidence Interval: −1.74 to +3.32%). The limits of agreement (mean difference ± 2 SD) ranged between −18.6% and 20.2%.

The reliability of scoring among the pathologists was assessed with ICC analysis [25,26]. The variance in scoring attributable to the cases and the residual variance were used to estimate the ICC (0.6539). The variance within cases was 52.9% of the variance between cases (Table 4), indicating reasonably good agreement between the different...
Pathologists with respect to the semi-quantitative scoring of interstitial fibrosis.

**Podocytes vacuoles and inclusions.** Vacuoles were frequently observed in podocytes (Figure 2A), corresponding to extracted GL-3 deposits. Vacuole average severity (0–3 scale) was $2.3 \pm 0.7$ (median 2.6) for males and $1.9 \pm 0.8$ (median 1.9) for females ($P < 0.03$, Table 3). GL-3 inclusions were scored for the semi-thin sections stained with toluidine blue (0–4 scale). The distribution of GL-3 inclusions was quite variable (Figure 2B and C). Women had significantly fewer deposits than men ($2.4 \pm 1.2$ versus $3.3 \pm 1.1$; $P < 0.03$; Table 3). The most common pattern in male patients was a mixture of large expanded deposits with concurrent small deposits.

Of patients with glomeruli available on semi-thin sections, one 46-year-old woman did not have any GL-3 deposits, and seven additional female patients and one male patient had small deposits (i.e. inclusion score <1.5); none had received ERT. The female patient without podocyte deposits had distal tubular and arterial medial deposits and was known to have mutation p.R363P. Parietal epithelial inclusions were present (Figure 2B), but were not reliably scored.

The podocyte vacuole scores and GL-3 inclusion scores agreed reasonably well (Table 4). The reliability of the scoring among the pathologists was assessed with ICC analysis [25,26]. The variance attributable to the cases, and the residual variance were used to estimate the ICC. The variance component within cases was 42.2% and between cases was 25.7%, respectively, for the scorings of podocyte vacuoles on light microscopy and GL-3 inclusions on semi-thin sections. Of the three ICC comparisons, the reliability of the scoring of the podocyte GL-3 inclusions appeared to be superior to the semi-quantitative scoring of interstitial fibrosis or podocyte vacuoles.

**Tubular inclusions.** Distal tubule inclusions were present in 42 of the 56 cases (75.0%) (Table 5, Figure 2D–F). Proximal tubule inclusions were more prevalent in males than females (47.1% versus 19.0%, $P = 0.046$). Inclusions in both proximal and distal tubules were present in 14 males and 4 females, and 14 males and 9 females only had distal tubule inclusions.

**Capillary and arterial inclusions.** Peritubular capillary inclusions were identified in 26 males and 7 females ($P = 0.002$). Intimal vascular inclusions were more prevalent in males than females. Vascular medial inclusions were present in 25 out of 38 patients (Figure 3). Lesions included typical arteriosclerotic lesions and severe medial injury and necrosis with inclusions, often associated with luminal dilatation. Arterial sclerosis was mild (Table 3). Hyalinosis was present in arterioles of 34 patients and in arteries of 22 patients out of 55 evaluated (Table 5). Arteriolar hyalinosis was scored in 22 of 34 males (64.7%) and 12 of 21 females

### Table 3. Kidney morphologic findings in Fabry patients

<table>
<thead>
<tr>
<th>Score</th>
<th>All (n = 59)</th>
<th>Male (n = 35)</th>
<th>Female (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomeruli, number in biopsy (light microscopy sections)</td>
<td>16.1 ± 11.9 (13.6)</td>
<td>17.3 ± 12.8 (14.3)</td>
<td>14.3 ± 10.3 (13.6)</td>
</tr>
<tr>
<td>Segmental sclerosis; (mild + severe) (%)</td>
<td>4.7 ± 7.8 (0.9)</td>
<td>5.1 ± 8.5 (1.9)</td>
<td>4.0 ± 6.7 (0.0)</td>
</tr>
<tr>
<td>Global sclerosis (%)</td>
<td>14.8 ± 22.7 (2.9)</td>
<td>18.3 ± 24.3 (5.9)</td>
<td>9.8 ± 19.5 (0.5)</td>
</tr>
<tr>
<td>Glomeruli without sclerosis (%)</td>
<td>69.9 ± 27.3 (75.2)</td>
<td>66.3 ± 30.4 (75.2)</td>
<td>75.2 ± 21.6 (77.7)</td>
</tr>
<tr>
<td>Interstitial fibrosis (%)</td>
<td>16.2 ± 22.9 (6.0)</td>
<td>20.4 ± 25.0 (10.0)</td>
<td>10.1 ± 18.3 (2.5)</td>
</tr>
<tr>
<td>Arterial sclerosis (0–3)</td>
<td>0.71 ± 0.72 (0.43)</td>
<td>0.75 ± 0.77 (0.33)</td>
<td>0.64 ± 0.66 (0.47)</td>
</tr>
<tr>
<td>Podocyte vacuoles (light microscopy; 0–3)*</td>
<td>2.2 ± 0.8 (2.5)</td>
<td>2.3 ± 0.7 (2.6)</td>
<td>1.9 ± 0.8 (1.9)</td>
</tr>
<tr>
<td>Podocyte inclusions (semi-thin sections; 0–4)*</td>
<td>3.0 ± 1.2 (3.5)</td>
<td>3.3 ± 1.1 (3.9)</td>
<td>2.4 ± 1.2 (2.8)</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD and (median). Assessment from standard light microscopy sections unless otherwise indicated. Percentage represents proportion of glomeruli with lesion. Of note, glomeruli with periglomerular fibrosis, extensive corrugation, ischaemia or adhesion were not scored as ‘non-sclerotic’, and thus the sum of global or segmental sclerosis and ‘non-sclerotic’ glomeruli does not necessarily equal 100%. *$P < 0.03$, two-tailed t-test, females compared to males.

### Table 4. Interclass and intraclass correlation coefficients for interstitial fibrosis, podocyte vacuoles and podocyte GL-3 inclusions

<table>
<thead>
<tr>
<th>Scoring (n)</th>
<th>Mean (%)</th>
<th>SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphometric score (59)</td>
<td>16.22</td>
<td>22.94</td>
<td>6.00</td>
<td>0</td>
<td>89.0</td>
</tr>
<tr>
<td>Average semi-quantitative score (59)</td>
<td>17.01</td>
<td>10.56</td>
<td>8.33</td>
<td>0</td>
<td>75.0</td>
</tr>
<tr>
<td>Spearmann’s interclass correlation coefficient = 0.871, P &lt; 0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scoring (scale)</td>
<td>Mean (%)</td>
<td>SD</td>
<td>Median</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Podocyte vacuoles (1–3)</td>
<td>2.19</td>
<td>0.80</td>
<td>2.5</td>
<td>0</td>
<td>3.00</td>
</tr>
<tr>
<td>Podocyte inclusions (1–4)</td>
<td>2.95</td>
<td>1.21</td>
<td>3.4</td>
<td>0</td>
<td>4.00</td>
</tr>
<tr>
<td>Spearmann’s interclass correlation coefficient = 0.5774, P &lt; 0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scoring (n)</td>
<td>Variance of cases (V) (SE)</td>
<td>Residual variance (R) (SE)</td>
<td>ICC (V/V+R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intersitial fibrosis score (59)</td>
<td>340.3 (71.8)*</td>
<td>180.1 (18.9)*</td>
<td>0.6539</td>
<td>52.9%</td>
<td></td>
</tr>
<tr>
<td>Podocyte vacuoles (59)</td>
<td>0.557 (0.119)*</td>
<td>0.235 (0.0310)*</td>
<td>0.7030</td>
<td>42.2%</td>
<td></td>
</tr>
<tr>
<td>Podocyte inclusions (57)</td>
<td>1.344 (0.278)*</td>
<td>0.3443 (0.047)*</td>
<td>0.7961</td>
<td>25.7%</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.0001.
ICC, intraclass correlation coefficient; R, residual variance; V, variance of cases.
Fig. 2. Vacuolization and glycosphingolipid deposits in Fabry nephropathy. (A) The glomerulus shows vacuolization of podocytes (arrowheads), and mild corrugation of glomerular basement membranes, with periglomerular and interstitial fibrosis (periodic acid-Schiff, ×400). (B) The glomerulus shows massive expanded deposits in most podocytes (arrowheads) and also in mesangial and endothelial cells and parietal epithelial cells in this male Fabry patient (toluidine blue, ×400). (C) The glomerulus shows occasional small deposits in podocytes (arrowheads) and rare deposits in mesangial areas in this female patient (toluidine blue, ×400). (D) There are prominent deposits in some proximal tubular cells and peritubular capillary endothelium (toluidine blue, ×1000). (E) Numerous deposits in distal tubules and very rare deposits in proximal tubules. The vascular smooth muscle of a large artery (top left corner) also shows deposits, as do parietal epithelial cells lining the Bowman's capsule (bottom) (toluidine blue, ×1000). (F) Numerous deposits in distal tubules with rare deposits in proximal tubules and frequent deposits in peritubular capillaries and interstitium (toluidine blue, ×200).

Table 5. Kidney morphologic findings in Fabry patients

<table>
<thead>
<tr>
<th></th>
<th>All (n = 59)</th>
<th>Male (n = 35)</th>
<th>Female (n = 24)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal tubule inclusions</td>
<td>42/14 (75.0%)</td>
<td>28/6 (82.4%)</td>
<td>14/8 (63.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Proximal tubule inclusions</td>
<td>20/35 (36.4%)</td>
<td>16/18 (47.1%)</td>
<td>4/17 (19.0%)</td>
<td>0.046</td>
</tr>
<tr>
<td>Peritubular capillary inclusions</td>
<td>33/23 (58.9%)</td>
<td>26/8 (76.5%)</td>
<td>7/15 (31.8%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Vascular intimal inclusions</td>
<td>19/19 (50.0%)</td>
<td>16/9 (64.0%)</td>
<td>3/10 (23.1%)</td>
<td>0.038</td>
</tr>
<tr>
<td>Vascular medial inclusions</td>
<td>25/13 (65.8%)</td>
<td>17/8 (68.0%)</td>
<td>8/5 (61.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>Arteriolar hyalinosis (light microscopy sections)</td>
<td>34/21 (61.8%)</td>
<td>22/12 (64.7%)</td>
<td>12/9 (57.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial hyalinosis (light microscopy sections)</td>
<td>22/33 (40.0%)</td>
<td>9/25 (26.5%)</td>
<td>13/8 (61.9%)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Data are presented as present/absent and percentage present/(present + absent); Excluded cases are those that could not be scored due to insufficient sampling for that variable. Lesions scored on semi-thin sections except as noted.

*Fisher exact two-tailed test statistic, females compared to males; NS, non-significant.

Arterial hyalinosis was present in 9 of 34 males (26.5%) and 13 of 21 females (61.9%; P = 0.012).

Trend analysis. Arterial sclerosis was more severe with advanced CKD (P = 0.0003), but blood pressure was not correlated (not shown). Glomerular sclerosis was more severe with more advanced CKD. In stage 1 CKD, 7.8 ± 17.6% (median 0.85) of the glomeruli had segmental or global sclerosis despite minimal proteinuria.

Discussion

We have described the renal biopsies of mild Fabry nephropathy in adult patients. Histologic evidence of kidney involvement precedes clinical signs in early Fabry nephropathy, confirming previous reports [16,17,19,29]. Glomerulosclerosis and interstitial fibrosis were observed in both genders, even in patients with minimal or no proteinuria in our series. In the evaluation of early Fabry nephropathy, clinical assessments lack sensitivity, potentially delaying initiation of ERT [30], highlighting the utility of kidney biopsies as part of the baseline assessment.

A similar scoring strategy was used for lupus nephritis [31] and focal segmental glomerulosclerosis [32]: inclusion of pathologists and clinicians; multiple iterations; validation and adjudication of difficult lesions; and refinement of definitions using a modified Delphi technique [23]. We evaluated disease-specific GL-3 lesions and secondary
alterations that occur in Fabry nephropathy [33], as well as any other form of CKD (e.g. arterial and glomerular sclerosis, and interstitial fibrosis). Interstitial fibrosis, global and segmental sclerosis and podocyte inclusions were robustly scored. The latter parameter showed the highest degree of inter-individual agreement. The semi-quantitative scoring of interstitial fibrosis showed similar reliability when compared to the morphometric assessment. Other lesions (e.g. parietal epithelial cell inclusions, interstitial inflammation) could not be reliably scored with the current approaches.

Glomerulosclerosis occurs early in Fabry nephropathy and is more severe with more advanced CKD. Only 37% of our patients with eGFR >60 ml/min/1.73 m² did not have any glomerular sclerotic lesions, and ~13% had sclerosis in >20% of glomeruli. Although proportions of segmental and global sclerosis varied among cases, no significant histologic differences between genders could be identified in early CKD. This finding may have important prognostic implications; Germain et al. [6] have described a marked loss of GFR in male Fabry patients with segmental and global glomerular sclerosis in >50% of their glomeruli.

GL-3 inclusions in podocytes were larger in males than in females, despite the 7.5-year higher mean age of males. Even in early CKD, half of the males had large podocyte inclusions involving >50% of the glomerular tuft. The podocyte scores for vacuolization and inclusions were significantly correlated, with only two patients having discordant findings.

Most of our patients (78%) had mild nephropathy (stage 1–2 CKD). Nearly half of the stage 1–2 CKD patients were females. Some conspicuous gender differences were observed. Clinical disease was milder in the female cohort, with corresponding lesser degree of global sclerosis and less podocyte, peritubular, vascular and proximal tubule inclusions than in the males. However, segmental and global glomerulosclerosis, interstitial fibrosis, distal tubular inclusions, arteriolar hyalinosis and vascular medial inclusions were of similar degrees in females as in males. Arterial hyalinosis was the only lesion more prevalent in females than males, which may be related to their higher mean age. These gender differences indicate that prognostic scoring elements may be assessed differently in male and female Fabry patients. Arterial sclerosis increased with CKD stage, and there appears to be a non-linear relationship between interstitial fibrosis and CKD stage, with minimal fibrosis scores in the stage 1–2 biopsies (Table 6).

Attempts to develop prognostic markers and/or pathogenic insights from renal biopsies have been undertaken in other forms of CKD. Glomerulosclerosis, interstitial fibrosis, endocapillary proliferation and surprisingly, mesangial proliferation were signs of poor prognosis in IgA nephropathy [34]. In the African-American Study of Kidney Diseases, global sclerosis was minimally related to mean arterial pressure, but was predicted by systolic blood pressure, serum cholesterol and reciprocal of serum creatinine [35]. With longitudinal studies of Fabry patients, a similar strategy may support the development of a chronicity index that reflects the long-term outcome. The variables summarized in Tables 3–5 may serve as the basis for such studies. The amount of chronic renal damage at the time of biopsy is likely to be related to the rate of loss of kidney function [23]. In addition, some lesions may influence the rate of decline, as well as baseline GFR. There may also be an indication as to response to therapy for some of the scored variables.

Several limitations of our study are evident. (1) We have assigned CKD stages to the patients based on baseline serum creatinine values and calculated eGFR.
majority of patients had GFR >60 ml/min/1.73 m², beyond the current validation range of the MDRD. UPCR was used as an index of proteinuria. KDOQI [24] defined CKD as either kidney damage or eGFR <60 ml/min/1.73 m² for ≥3 months, with kidney damage defined as pathologic abnormality or a marker of damage. Since all of our patients had biopsy-proven Fabry nephropathy, we feel justified in using CKD staging to describe the severity of their kidney damage. Furthermore, the significant increases in the various sclerosis scores with CKD stage, with more severe scores in stage 2 than stage 1 (Table 6), lend further credence to the use of CKD staging, bearing in mind the reservations about using estimating equations for GFR. (2) Nine patients received ERT before biopsy, but the median time before biopsy was only 1.4 months, with none more than 4 months. It is not likely that short-term ERT would influence sclerotic lesions. (3) Biopsies were performed according to the local standard of care, and the criteria for submitting cases for this study were not restrictive. This retrospective, cross-sectional design raises a possible selection bias for inclusion in the study. (4) Few patients with advanced CKD were included, limiting the trend analysis as well as comparisons between genders. Nevertheless, the pathology was similar in both genders, although developing at a later age in females.

The present study focused on histologic sections for developing the scoring system. For this purpose, standard histologic sections provided sufficient glomeruli and more extensive vascular and interstitial sampling, overcoming the limited tissue sampling with semi-thin sections used in previous studies [5]. On the other hand, histological findings suggestive of Fabry nephropathy (e.g. vacuolization) may be missed on routine histologic sections; semi-thin sections are best for characterizing podocyte GL-3 inclusions. This conclusion is strengthened by the ICC analysis in Table 4.

In conclusion, our description and validation of a scoring system of histologic involvement in Fabry nephropathy are notable because of the inclusion of a large number of adult females. Chronic glomerular and interstitial damage develop early in the course of Fabry disease, and the absence of typical clinical signs of CKD does not rule out Fabry nephropathy. Overall, our results are in agreement with previous, smaller series of Fabry patients, showing significant histologic changes before renal function is decreased [12,16,19]. Access to detailed illustrations and definitions of lesions (see Appendix) should enhance the utility and application of the scoring system for future studies of Fabry nephropathy. Future development of a chronicity index based on longitudinal data may be useful for describing severity of pathologic changes and prognostic implications for progressive Fabry nephropathy. For now, we conclude that important information is provided by kidney biopsy in Fabry nephropathy that is not available from routine assessment of kidney function and proteinuria. Our results support the role of kidney biopsy in the baseline evaluation of all Fabry patients, even with mild clinical disease.

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Appendix. Histologic scoring

General approach

The initial effort involved six cases coded and blinded as to origin of slides and was intended to identify common features in the biopsies (fibrosis, sclerosis, vascular changes, GL-3 deposits, etc.) that would be included in the final scoring sheet. Round-robin slide reviews and two face-to-face scoring sessions with a modified Delphi technique [23] were used to refine the scoring system. Then, slides from 20 cases were circulated, with each group scoring each element specified in the score sheet (Figure A1). In a face-to-face meeting, cases with difficult lesions were discussed for further refinement and clarification of definitions. This sequence of scoring, Delphi consensus process and
rescoring, was adopted to validate the scoring system. The final 59 cases were then circulated with each biopsy scored by three different centres. Final scores of all 59 cases represent the average in each case from all validated scorings from these centres. An average for each parameter was calculated, with a range. For lesions scored as present or absent, cases with divergent scoring were reviewed for a final score by A.B.F. Electron micrographs were not examined in the current project. Morphometry was done on all slides by one centre (see below).

Lesions were scored on the same sections on the circulated slide, so that all observers assessed the exact same

**Fig. A1.** Scoring sheet for Fabry nephropathy by light microscopy.
tissue. For most cases, tissue was assessed on periodic acid-Schiff (PAS)-stained slides. In a few cases, when PAS-stained slides were not available, Jones’ stained slides were scored instead. Of note, more than one lesion could be present on light microscopy in any one glomerulus, e.g. segmental sclerosis and ischaemia/collapse could coexist in the same glomerulus, and both features were then scored as ‘present’ for that glomerulus. From our experience, optimal slides for scoring should ideally include >10 glomeruli for light microscopic assessment, and at least 3 glomeruli for semi-thin section scoring.

Light microscopic slide scoring

Glomeruli that could not be scored due to, for instance, incomplete sections or global sclerosis were indicated in the box as ‘indeterminate’ for scoring. Glomeruli with <25% of the tuft represented in the section were also marked as ‘indeterminate’ for vacuolization scoring. The total was calculated as a sum of all scored and non-scored glomeruli.

Podocyte vacuolization was scored for each individual glomerulus (‘0’ none, and ‘1’, ‘2’, or ‘3’, respectively, when <25%, 25–50%, and >50% of podocytes showed cytoplasmic vacuoles). A final average score was calculated for each biopsy.

The total number of glomeruli without any degree of sclerosis, as well as with no adhesions, evidence of ischaemia or periglomerular fibrosis, was counted and reported as non-sclerotic glomeruli. Segmental sclerosis was defined as obliteration of capillary lumen with a increased matrix involving a portion of the glomerulus (<50%, ‘mild’; or ≥50% of the tuft affected, ‘severe’). Global sclerosis was scored when the entire glomerular tuft was sclerosed. Adhesions were scored when there was continuity of connective tissue between the glomerular tuft and Bowman’s capsule without concomitant well-defined sclerosis. Ischaemia/collapse was scored when >50% of the glomerular tuft showed corrugation of the glomerular basement membrane and retraction of the tuft. Periglomerular fibrosis and/or Bowman’s capsule reduplication were defined as present when >25% of the circumference of Bowman’s capsule was affected by these processes, in the absence of globally sclerosed glomeruli.

Of note, as a consequence of our definition of non-sclerotic glomeruli, the sum of the percentages of globally and segmentally sclerotic glomeruli and non-sclerotic glomeruli is not mathematically 100%. Arterial sclerosis was scored based on lesions in vessels as an average and also as a separate score for the most severely affected as described by Remuzzi et al. [36]: ‘0’, vascular lesions absent; ‘1+’, wall thickness increased but to a degree less than the diameter of the lumen; ‘2+’, wall thickness equal or slightly greater than the diameter of the lumen; and ‘3+’, wall thickness far exceeding the diameter of the lumen with extreme luminal narrowing or occlusion. Arteriolar or arterial hyalinosis was scored as ‘present’ or ‘absent’ and specified as involving the media or subendothelial location.

Interstitial fibrosis was semi-quantitatively estimated on cortical tissue. Interstitial inflammation was noted as ‘present’ or ‘absent’. If present, its location in scarred areas or non-scarred areas or both was noted. However, this parameter was variably scored. Any area involved with either tubular atrophy and/or interstitial fibrosis was scored as ‘involved with interstitial fibrosis’. Each ×20 field was assessed, and degree of involvement with interstitial fibrosis estimated. An average of all cortical fields was then calculated, and scored as the nearest 10%, except for minimal fibrosis of ≤5%, which was scored as 5%. The results were compared with morphometric assessment on the same slides by the method published by Howie et al. [21].

Additional scoring was done on the epoxy-embedded toluidine blue-stained semi-thin section slides. The number of glomeruli available on the single section was designated. The extent of segmental and global sclerosis, defined as above, was noted. A podocyte inclusion score (see below) was given separately for each glomerulus, and an average score was thereby calculated for each biopsy. Podocyte inclusions were scored as follows: ‘0’, no deposits; ‘1+’, rare small inconspicuous deposits; ‘2+’, more frequent small deposits; ‘3+’, <50% of the tuft involved with large expanded deposits in the presence or absence of concurrent small deposits; and ‘4+’, >50% of the tuft involved with large expanded deposits. Inclusions in parietal epithelial, proximal and distal tubular, peritubular capillary, vascular intimal and vascular medial cells were scored as ‘present’, ‘absent’ or ‘not sampled’ in the specimen.

Morphometric assessment of chronic renal damage

Each biopsy specimen was examined to give a measure of the amount of chronic damage called ‘the index of chronic damage’ [21]. Briefly, images of the cortex were captured on a computer, and areas with chronic damage, defined as globally sclerosed glomeruli, atrophic tubules and interstitial fibrosis, were outlined using an interactive image analysis program. The index of chronic damage in a specimen was the total cross-sectional area with chronic damage expressed as a percentage of the cortical cross-sectional area.

The mean estimates of the extent of interstitial fibrosis were compared with the index of chronic damage in two ways, firstly by calculation of the Spearman’s correlation coefficient, and secondly by assessment of the agreement between them using the Bland–Altman method [28]. This method gave the mean difference, or bias, with 95% confidence interval, and the limits of agreement, which were two standard deviations of the difference on each side of the mean difference. Preliminary analysis of 20 specimens showed that exclusion of the area of globally sclerosed glomeruli, which was not included in semi-quantitative estimation of the extent of interstitial fibrosis, made little difference to the index of chronic damage, because inclusion of globally sclerosed glomeruli added only 1% to the index. Subjective scoring of interstitial fibrosis was generally recorded to the nearest 10%, or to 5% for minimal fibrosis, and so 1% on the index was considered insignificant. The values of the morphometric index used were those including the area of globally sclerosed glomeruli.

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