Living donor kidney transplants: a biopsy study 1 year after transplantation, compared with baseline changes and correlation to kidney function at 1 and 3 years

Ståle Sund¹, Anna V. Reisæter², Per Fauchald², Øystein Bentdal³, Kirsten Sundby Hall⁴ and Torstein Hovig¹

¹Institute of Pathology, ²Department of Medicine, ³Department of Surgery and ⁴Department of Clinical Chemistry, The National Hospital, Rikshospitalet, University of Oslo, Oslo, Norway

Abstract

Introduction. Chronic changes in biopsies from long-term stable kidney allografts have been reported to correlate with graft prognosis. Morphological changes in baseline ('zero-hour') biopsies have been described as well, but their importance for long-term prognosis have been less clear. The aim of the present study was to evaluate biopsy changes from baseline to 1 year after transplantation in patients receiving kidneys from living donors, and to assess the possible prognostic implications of these findings.

Methods. Light microscopical changes in 18 gauge full-core biopsies were scored semi-quantitatively in 33 patients 1 year after transplantation, and compared to baseline changes previously reported [1]. All cases were also examined with transmission electron microscopy. The semi-quantitative data from baseline and at 1 year were correlated with kidney function 1 and 3 years after transplantation. The reproducibility of baseline findings regarding arteriosclerosis and arteriolar hyalinosis was tested by comparison with biopsies 1 week after transplantation (n = 43).

Results. We found a significant increase in mesangial glomerular sclerosis (P < 0.001), interstitial fibrosis/tubular atrophy (if/ta) (P = 0.002), and mononuclear cell interstitial infiltration (P = 0.003) after 1 year, compared to baseline changes. There was an increase of arteriosclerosis (P = 0.028) and arteriolar hyalinosis (P = 0.006) when compared to biopsies taken 1 week after transplantation, but not when compared to the 'zero-hour' findings. Electron microscopy revealed one case of recurrent immune-complex glomerulonephritis and another case of recurrent light chain deposition kidney disease. Comparing 1-week vascular findings with baseline gave a low level of reproducibility, probably due to sampling error. Baseline biopsy findings could not predict long-term kidney function. In the 1-year biopsy, if/ta was significantly correlated with serum creatinine (P = 0.007) and glomerular filtration rate (GFR) (P < 0.001) at 1 year, with serum creatinine at 3 years (P = 0.011), and with the first-year cumulative dose of methylprednisolone (P = 0.004). Serum creatinine at 1 year, however, was found to be the most accurate predictor of 3-year kidney function (P < 0.001). Donor age was correlated to kidney function at 3 years (P = 0.013) but not at 1 year after transplantation.

Conclusion. Morphological changes in baseline biopsies of living donor kidneys tend to become more pronounced in well-functioning allografts during the first year after transplantation. In the 1 year biopsy, if/ta seems to be the most reliable variate for grading of chronic changes. However, 1-year serum creatinine predicted long-term kidney function more precisely than did the biopsy scores. Based on the results of the present study, a protocol 1-year biopsy does not seem warranted in the management of the graft recipient with a stable kidney function.

Key words: kidney transplant biopsy; baseline; prognosis; transplant outcome; reproducibility

Introduction

In a previous study, we described the morphological changes in kidney baseline ('zero-hour') biopsies from living donors [1], and found that significant alterations, especially arteriosclerosis and arteriolar hyalinosis, were prevalent even in normally functioning kidneys. The possible impact of such baseline changes on kidney graft function have been reported in several studies, but the results have been somewhat conflicting [2–7]. Morphological changes in arterial vessels are part of the chronic transplant nephropathy, together with interstitial fibrosis, tubular atrophy and glomerulopathy [8,9]. Similar changes in kidney transplants with a
stable function have been shown to predict long-term renal function [10].

The impact of morphological changes in cadaveric donor kidneys may be difficult to evaluate because of the confounding effects of donor factors, such as pre-existing disease, and primary renal dysfunction related to pre-transplantation shock-induced kidney damage and prolonged ‘cold’ ischaemia [11,12]. We therefore wanted to study changes in grafts from living donors, in whom a pre-operative screening has confirmed the absence of kidney disease or hypertension, and in whom the pre-operative regimen as well as the surgical procedure are optimally standardized. Furthermore, our material offered the possibility to compare the developing changes with those of the baseline biopsies.

In the current study, our main purpose was to answer the following questions: (i) what is the evolution of the histopathological changes in follow-up full core biopsies up to 1-year after transplantation; in particular, what happens to the arterial and arteriolar changes? (ii) Do the graft biopsy findings predict graft outcome? (iii) What is the effect of sampling error in studying these questions? (iv) Should a protocol 1-year biopsy be recommended in the management of the kidney graft recipient?

Subjects and methods

Patients

Thirty-three patients who were transplanted with a kidney from a living donor from March 1992 to January 1994 at our centre were included in this study. Patients were included consecutively and exclusion criteria were as follows: recipient aged less than 18 years, HLA-A, -B and -DR identity between donor and recipient, anticoagulation treatment with warfarin, or known bleeding diathesis, and intra-abdominal placement of the kidney graft.

A baseline biopsy was obtained from 57 patients [1], and 33 of these were available for a biopsy 1 year after transplantation. Twenty-four patients withdrew from the longitudinal study for the following reasons: 14 patients declined further protocol biopsies, three patients were withdrawn for medical reasons, six patients lost their grafts within the first 3 months because of rejection, and one patient died after 3 months from a cerebrovascular insult. From 1 to 3 years after transplantation, three patients lost their grafts because of chronic rejection and two patients died with functioning grafts (one with pneumonia and one with diabetes mellitus suffering sudden death).

There were 20 males and 13 females among the 33 kidney graft recipients and the mean age was 41.9 years (range 20.4–74.4). Thirty-two patients were transplanted for the first time and one patient received a second graft. Among the donors there were 14 males and 19 females, and the mean age was 51.3 years (range 20.7–71.5). The donors were screened pre-operatively according to standard procedure, and were confirmed to have normal kidney function, normal urine and normal peripheral blood pressure [1].

The recipients suffered from chronic glomerulonephritis (n = 14), focal segmental glomerulosclerosis (n = 3), chronic pyelonephritis (n = 4), diabetic nephropathy (n = 3), plasma cell dyscrasia (n = 3), autosomal dominant polycystic kidney disease (n = 2), nephrosclerosis (n = 2), interstitial nephritis (n = 1) and nephropathia (n = 1).

Immunosuppressive treatment

The post-transplant immunosuppression consisted of a standard triple therapy regimen: cyclosporin A, started at 10 mg/kg/day tapered to 2–5 mg/kg/day, aiming at a trough value of 75–175 μg/l at 3 months and 75–125 μg/l 1 year after transplantation; prednisolone 80 mg/day tapered to 10 mg/day after 8 weeks, and azathioprine 2.0 mg/kg/day tapered to 1.0 mg/kg/day after 7 days.

Patients with an increase of serum creatinine of 20% or more, when other causes of renal dysfunction were excluded, were treated for acute rejection. Pulses of methylprednisolone were given for 4 days, to a total of 1.25 g. Steroid resistant rejections were verified by biopsy and treated with antithymocyte globulin (ATG Fresenius, Bad Homburg, Germany) a total of 1–2 g in doses of 200 mg or OKT3 (Ortho Pharmaceutical Corp, New Jersey, USA) 2.5 or 5 mg/day for 10 days [14]. Repeated episodes of acute rejection were primarily treated with methylprednisolone and if steroid resistant, with ATG or OKT3. Twenty-seven out of 33 patients (82%) experienced one or more episodes of acute rejection during the first 3 months. Cumulative i.v. doses of methylprednisolone were 1.0–3.875 g during the first 3 months, and 1.25–4.125 g during the first year after transplantation. Fourteen patients needed additional treatment with ATG and/or OKT3.

HLA typing, antibody screening and cross-match tests

Prospective serological HLA typing of recipients and donors was performed twice on peripheral blood mononuclear cells, isolated by the immunomagnetic method [13]. Patient sera were screened for lymphocytotoxic panel reactive antibodies (PRA) by the immunomagnetic method [13], each time using 15 random blood donors. Patients whose sera reacted against one or more of the panel cell donors on more than one occasion during the last year, in the absence of autoreactive antibodies, were considered PRA positive. Immunomagnetic lymphocytotoxic B and T cell cross-matches [13] were negative in all cases. One patient had PRA against 5–20% of the panel of T lymphocytes and was treated with prednisolone and cyclophosphamide for 4 weeks prior to transplantation.

Thirty donors were first degree relatives and three were spouses. Twenty-eight recipient–donor pairs were one haplotype mismatched and two pairs were two haplo-types mismatched. Between the spouses the number of HLA-A and -B mismatches were three and of HLA-DR mismatches zero, one and two, respectively.
Kidney biopsies

Full-core biopsies were obtained with a biopticut biopsy gun (BiopTiCut, Radioplast, Bromma, Sweden) with a needle size of 18 gauge. The baseline biopsies were taken during donor nephrectomy, shortly before the kidneys were removed from the donors [1]. Follow-up biopsies were taken at 1 week, 8–12 weeks (not reported here), and 1 year, guided by ultrasonography. Specimens for light and electron microscopy were processed in the same manner as previously described [1]. Most biopsies were representative for renal cortical tissue according to the Banff-93 criteria [15], containing at least seven glomeruli and one artery (Table 1).

Scoring of biopsies

Light microscopical changes were estimated and scored semi-quantitatively (0–3) according to a scoring system partly based on the Banff schema—93/95 [15,16], as previously described [1]. The following variables were graded: arteriosclerosis (as), arteriolar hyalinosis (ah), glomerular mesangial sclerosis (gs), interstitial fibrosis and tubular atrophy (if/ta), and monocellular cell interstitial infiltration (mi). The evaluation of glomerular changes included estimation of the fraction of globally sclerosed glomeruli (gsg) and a ‘combined’ glomerulosclerosis score (gsc), combining the gs score and the gsg as described in a preceding publication [1]. The sum of scores in each biopsy was recorded as the chronicity index (CI) (CI = as + ah + gsc + if/ta + mi) [1]. Lastly, the differences between the 1-year and baseline biopsy scores were recorded in each case. Biopsies obtained 1 week after transplantation were graded for as and ah, in order to analyse the reproducibility of the vascular scores at baseline (n=43).

Kidney function

Renal function was measured as serum creatinine at 1 and 3 years after transplantation. In addition, GFR was measured at 1 year as 99mTc-DPTA clearance [17].

Statistical methods

The data were entered on SPSS for Windows Release 7.5.1 [18] and partly on S-PLUS version 3.4 Release 1 [19]. For analyses of changes in morphology scores we used the Wilcoxon one-sample test. Relationships between morphological changes and renal function were calculated with Spearman’s rank correlation, supplemented by testing of group differences with the ANOVA F-test. Relationships between data of renal function were analysed with Pearson’s correlation and linear regression. Group differences with respect to rejection data (rejection vs non-rejection, different subsets of rejection) were analysed with the Mann–Whitney U test. The predictive power of 1-year functional and histopathological variates with respect to 3-year kidney function (serum creatinine more or less than 130 μmol/l) was assessed by cross validation. A logistic model was used for the prediction. For each of the patients a logistic model was fitted using the remaining variates. All tests were two-tailed. Cohen’s kappa analysis was used for testing reproducibility of early vascular scores in kidney biopsies.

Results

Vascular changes in 1-week biopsies compared to baseline: as and ah

There was no significant difference between the mean arteriosclerosis scores at baseline (1.40) and at 1 week (1.28) (Table 2a). When comparing these two scores in each patient, however, they often differed from each other. A Cohen’s kappa [18] of 0.046 for arteriosclerosis grading confirmed a poor reproducibility of the baseline score.

The baseline ah could often not be reproduced in the 1-week sample. Thus, the mean ah score at baseline was significantly higher (1.19) than the 1-week score (mean 0.56) (P<0.001) (Table 2a). When comparing the ah scores in each individual, the reproducibility of the baseline grading was again found to be low (Cohen’s kappa 0.122). When recording only absence or presence of hyalinosis, however, the discrepancy was less: out of 33 ah positive cases at baseline, 22 (67%) remained positive at 1 week. And among 10/43 cases without hyalinosis at baseline, all but one showed absence of ah at 1 week as well. The intra-observer reliability of the scoring both with respect to arteriosclerosis and arteriolar hyalinosis was previously found to be satisfactory [1].

The number of glomeruli, arteries and arterioles in general did not differ in baseline [1] vs the 1 week biopsies (Table 1). The fraction of biopsies containing renal capsular tissue was 21/43 (48.8%) in baseline, vs 27/43 (62.8%) at 1 week (NS). Medullary tissue or tissue from the corticomedullary junction was found in 33/43 (76.7%) and 20/43 (46.5%) at baseline and at 1 week, respectively (P=0.002).

One-year biopsy findings compared to baseline

Arteriosclerosis (as)

The arterial changes in the 1-year biopsies consisted mainly of fibrointimal thickening of the vessel wall. In three biopsies, a small number of intimal lymphocytes were demonstrated (Fig. 1). No case was interpreted as acute vascular rejection. Foam cells were not observed.

The as scores are given in Table 2b, compared with baseline data. In the 1-year biopsies, as well as in baseline, more than 50% of the specimen showed as grade 2–3, and the as scores in the two groups were
### Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Mean score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(a) Vascular changes: baseline vs 1-week biopsy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteriosclerosis ((n = 40))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>9</td>
<td>13</td>
<td>11</td>
<td>7</td>
<td>1.40</td>
</tr>
<tr>
<td>1 week</td>
<td>10</td>
<td>15</td>
<td>9</td>
<td>6</td>
<td>1.28 (NS)</td>
</tr>
<tr>
<td>Arteriolar hyalinosis ((n = 43))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>10</td>
<td>19</td>
<td>10</td>
<td>4</td>
<td>1.19</td>
</tr>
<tr>
<td>1 week</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>1</td>
<td>0.56 (P &lt; 0.001)</td>
</tr>
<tr>
<td><strong>(b) Vascular changes: baseline vs 1-year biopsy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteriosclerosis ((n = 31))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>1.61</td>
</tr>
<tr>
<td>1 year</td>
<td>2</td>
<td>12</td>
<td>8</td>
<td>9</td>
<td>1.77 (NS)</td>
</tr>
<tr>
<td>Arteriolar hyalinosis ((n = 32))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>6</td>
<td>16</td>
<td>6</td>
<td>4</td>
<td>1.25</td>
</tr>
<tr>
<td>1 year</td>
<td>11</td>
<td>12</td>
<td>3</td>
<td>6</td>
<td>1.13 (NS)</td>
</tr>
<tr>
<td><strong>(c) Vascular changes: 1-week vs 1-year biopsy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteriosclerosis ((n = 28))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>6</td>
<td>11</td>
<td>8</td>
<td>3</td>
<td>1.29</td>
</tr>
<tr>
<td>1 year</td>
<td>2</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>1.89 (P = 0.028)</td>
</tr>
<tr>
<td>Arteriolar hyalinosis ((n = 30))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>16</td>
<td>13</td>
<td>0</td>
<td>1</td>
<td>0.53</td>
</tr>
<tr>
<td>1 year</td>
<td>11</td>
<td>11</td>
<td>2</td>
<td>6</td>
<td>1.10 (P = 0.006)</td>
</tr>
</tbody>
</table>

**Fig. 1.** Graft biopsy 1 year after transplantation: Arcuate artery with intimal lymphocytes (arrow). Serum creatinine at 1 year, 96; at 3 years 89 \(\mu\)mol/l. (PAS stain, original magnification \(\times 400\).)

not significantly different. Although 10 patients showed an increase in as score during the first year, 14 patients demonstrated the same score and seven cases even a lower score in the 1-year biopsy. When comparing the 1-year changes with the biopsies taken 1 week after transplantation, we found an increase of arteriosclerosis (Table 2c). Comparing the two biopsies from each patient, 11 cases showed an increase and seven a decrease of as score, while 10 patients showed identical scores at 1 week and 1 year. The 1-year arteriosclerosis was also compared with the mean score of baseline and 1-week as. The difference between the two sets of scores was then not significant (\(P = 0.12\)).

**Arteriolar hyalinosis (ah)**

Approximately 2/3 of the 1-year biopsies showed ah (Table 2b). Compared to baseline changes, 9/31 (29%) patients showed an increase of hyalinosis, 10 patients remained unchanged, and 13 biopsies showed a reduced score.

There was, on the other hand, a significant increase in ah from 1 week to 1 year (Table 2c). Thus, 40% of the patients (12/30) showed an increase of ah, while most of the remaining biopsies showed the same score and only two patients a reduced score at 1 year. An increase was seen both with respect to high-grade (2–3) changes, and when recording all cases including mild (grade 1) changes.

**Glomerulosclerosis (gs)**

The glomerular changes are summarized in Table 3. The mean score of mesangial sclerosis increased from baseline to 1 year. There was a similar increase of the glomerulosclerosis score gsc, combining mesangial sclerosis with the fraction of globally sclerosed glomeruli [1]. Thus, an increase of mesangial sclerosis by one grade in preserved glomeruli was present in 19 cases; no change was found in 12 biopsies, while one single case showed reduction of mesangial sclerosis by one grade. An increase of the combined score gsc was found at 1 year in 14/32 biopsies, whereas no change was found in the remaining samples.
Table 3. Glomerular and tubulointerstitial changes: baseline vs 1-year biopsy (n=32)

<table>
<thead>
<tr>
<th>(a)</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Mean score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gs1</td>
<td>Baseline</td>
<td>9</td>
<td>22</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 year</td>
<td>0</td>
<td>22</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>(b)</td>
<td>Gsc2</td>
<td>Baseline</td>
<td>6</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 year</td>
<td>0</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>(c)</td>
<td>If/ta3</td>
<td>Baseline</td>
<td>5</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 year</td>
<td>3</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>(d)</td>
<td>Mi4</td>
<td>Baseline</td>
<td>31</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 year</td>
<td>20</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

1 Gs: glomerular mesangial sclerosis (in preserved glomeruli). 2 Gsc: Glomerulosclerosis score, combining Gs with fraction of globally sclerosed glomeruli (see text). 3 If/ta: interstitial fibrosis/tubular atrophy. 4 Mi: mononuclear cell interstitial infiltration.

Thickening of capillary walls was not observed, except for occasionally short capillary segments in continuity with areas of mesangial sclerosis. No case with double contours of basement membranes was found.

The fraction of globally sclerosed glomeruli was found to increase from a mean value of 5.25% in baseline to 8.59% in the corresponding 1-year biopsies (NS). In 22/32 (69%) of the 1-year biopsies, 10% or less of the glomeruli were sclerosed, while two cases showed more than 25% sclerosed glomeruli.

Electron microscopical examination confirmed mesangial sclerosis to be the most consistent morphological feature. One case revealed recurrence of immune-complex glomerulonephritis, and one showed recurrence of light chain deposition in the graft. Another case with dense deposits and glomerular staining of IgA in the baseline biopsy [1], did not show deposits at 1 year. No case of typical chronic transplant glomerulopathy [9] was found.

**Interstitial fibrosis/tubular atrophy (If/ta)**

There was a significant increase compared to baseline (Table 3). Thus, grade 3 if/ta was found in nine cases vs none at baseline. Altogether 18 patients (56%) showed an increased score, whereas if/ta grade remained unchanged in nine biopsies and reduced by one grade in five cases.

**Mononuclear cell interstitial infiltration (mi)**

No case of unequivocal acute cellular rejection was found. Two cases were, however, consistent with Banff borderline and interpreted as probable acute-on-chronic rejection. One of these biopsies revealed a cortical stenosing arteriolopathy as well as signs of ‘arteriolitis’ in the vasa recta. A few mononuclear cells lying within tubular epithelium was a common finding in atrophic tubuli.

A mononuclear cell infiltration grade 1–2 was found in approximately 40% of the biopsies (Table 3). In 12/32 cases, there was an increase in mi score compared to baseline; 19 patients showed an unchanged grade 0 score (not reaching the 5% limit for a grade 1 score [1]), and one single case a reduction of infiltrating cells by one grade.

**Chronicity index (CI)**

CI was significantly increased compared to baseline (Table 4). While two biopsies had the same CI at baseline and at 1 year, 17 biopsies showed an increase of CI by 1–3 grades and six biopsies by 4–6 grades. On the other hand, six biopsies showed a reduction of CI by 1–5 grades compared to baseline.

**Correlation of biopsy findings to renal function**

**Functional significance of 1-year biopsy findings**

As shown in Table 5, serum creatinine showed a very slight increasing tendency only from 1 to 3 years after transplantation (P=0.06). Thirteen out of 33 patients (39%) at 1 year, and 10 out of 28 patients (36%) at 3 years had excellent kidney function with serum creatinine of 130 μmol/l or less. Four out of 28 patients (14%) showed an increase of more than 20% in serum creatinine between 1 and 3 years; one of these suffered from recurrence of light chain deposition in the graft, and another had a recurrence of immune-complex glomerulonephritis.

The correlations of the various histopathological parameters with kidney function at 1 and 3 years are shown in Table 6 and Figure 2. If/ta correlated well with kidney function at 1 and 3 years. The extent of mi also correlated with renal function. One of the two mi-grade 2 biopsies showed recurrence of light chain deposition, and the other (patient no. 2) had a failing graft function; when excluding these from the analysis, no significant correlation between the mi score and serum creatinine at 1 or 3 years was found.

The occurrence of a few arterial intimal lymphocytes (Figure 1) did not seem to influence kidney function. In three patients with this finding, serum creatinine at 1 and 3 years was 96–89, 122–116, and 126–147 μmol/l, respectively.

Kidney function at 1 and 3 years did not correlate with the 1-year biopsy scores of as, ah, or gs. The fraction of globally sclerosed glomeruli correlated with kidney function at 3 years (P=0.009), but not at 1 year. Estimates of the respective differences between the 1-year and baseline scores did not improve the correlation between morphological and functional parameters (data not shown).

The CI tended to be higher in those patients with less well preserved kidney function; the correlation was significant, however, only to GFR at 1 year (Table 6). Subtracting the ah score from the CI improved the correlation to kidney function both at 1 and 3 years (Figure 2b). By combining the chronicity scores in
Table 4. Chronicity index (CI): baseline vs 1-year biopsy (n=31)

<table>
<thead>
<tr>
<th>Score 0–3</th>
<th>Score 4–6</th>
<th>Score 7–9</th>
<th>Score &gt;9</th>
<th>Mean score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline¹</td>
<td>8</td>
<td>16</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>1 year²</td>
<td>4</td>
<td>12</td>
<td>13</td>
<td>2</td>
</tr>
</tbody>
</table>

¹Range 0–9, ²Range 2–11.

Table 5. Renal function data 1 and 3 years after transplantation

<table>
<thead>
<tr>
<th>Serum creatinineᵃ</th>
<th>GFRᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>(median; range)</td>
<td>(median; range)</td>
</tr>
<tr>
<td>1 year (n=32)</td>
<td>141.5, 96–230</td>
</tr>
<tr>
<td>3 years (n=28)</td>
<td>145.0, 76–277</td>
</tr>
</tbody>
</table>

ᵃμmol/l;ᵇml/min · 1.73 m²³; ³Patient no. 2 not included.

Impact of donor age

Donor age correlated significantly with kidney function at 3 years (P=0.013), but not with 1-year kidney function. The fraction of globally sclerosed glomeruli at 1 year correlated with donor age (P=0.011) but not the 1-year if/ta.

Impact of rejection

In patients with one or more episodes of acute rejection (n=27) the mean score of if/ta was 1.9 vs 1.0 in patients with no rejection episodes (n=6; P=0.052). There was no difference in the score for if/ta in patients with steroid sensitive rejections and patients receiving additional T cell antibody treatment. There was, however, a correlation between the first year cumulative doses of methylprednisolone in the individual patient and the 1-year if/ta (P=0.004).

Functional significance of baseline biopsy findings

We found no significant correlation between kidney function at 1 or 3 years with the baseline scores of as, ah, gs, if/ta, CI, or fraction of globally sclerosed glomeruli.

Furthermore, there was no correlation between as/ah in the 1-week biopsies and renal function at 1 and 3 years.

Table 6. One-year variates: correlation with kidney function 1 and 3 years after transplantation

<table>
<thead>
<tr>
<th>GFR 1 year (n=31)</th>
<th>Se-creatinine 1 year (n=32)</th>
<th>Se-creatinine 3 years (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>If/ta</td>
<td>P&lt;0.001</td>
<td>P=0.007</td>
</tr>
<tr>
<td>Mi</td>
<td>P=0.003</td>
<td>P=0.017</td>
</tr>
<tr>
<td>If/ta + Mi</td>
<td>P&lt;0.001</td>
<td>P=0.008</td>
</tr>
<tr>
<td>Asᵃ + Gs + If/ta + Mi</td>
<td>P&lt;0.001</td>
<td>P=0.03</td>
</tr>
<tr>
<td>CI</td>
<td>P=0.012</td>
<td>NS</td>
</tr>
<tr>
<td>Se-creatinine</td>
<td>P&lt;0.001</td>
<td>—</td>
</tr>
<tr>
<td>GFR</td>
<td>—</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

ᵃAs, Arteriosclerosis. For abbreviations see otherwise Tables 3 and 4.
Living donor kidney transplants

...if/ta score from baseline to 1 year. CI was unchanged or in fact reduced from baseline to 1 year in 8/31 cases.

These data may seem paradoxical, but are most likely explained by sampling error. Mild-to-moderate morphological changes may be seen at baseline as an expression of benign (partly age-related) nephrosclerosis [1]. These changes may be essentially similar to those of chronic transplant nephropathy [8,9]. A difference in morphological score between baseline and a follow-up graft biopsy may therefore merely reflect the focal nature of the processes involved. We have shown that even high-grade vascular changes may be seen in ‘zero-hour’ biopsies from living donors [1], adding to the uncertainty in comparing with follow-up samples.

We assume this point has been generally underestimated in studying chronic allograft changes. In our opinion, the value of the allograft biopsy in diagnosing low-grade chronic transplant nephropathy may be questioned. On the other hand, severe (grade 3) interstitial fibrosis/tubular atrophy is restricted to the 1-year biopsies in our study and seems a reliable marker of chronic post-transplantation parenchymal damage.

Sampling error may be especially relevant in the study of early vascular changes, as shown by our comparison between baseline and biopsies taken 1 week after transplantation. Thus, we found the reproducibility of baseline as to be only slightly better than random. This may be explained by the sample size: the single 18-gauge biopsy, with an average of four arterial profiles, is too small for a reproducible scoring of this lesion.

The scoring of baseline ah seemed even more unreliable compared to the 1-week biopsy. The difference in the biopsy procedures may contribute to this result: at our centre, the baseline biopsies were obtained with the biopsy gun positioned directly onto the surface of the kidney. These biopsies would therefore be expected to contain tissue from a scarcely deeper level of the renal parenchyma. This is supported by our finding that the baseline samples more often included tissue from the corticomedullary junction, and may indicate that the baseline ah is more pronounced in a juxtamedullary location. Reporting only presence or absence of hyalinosis improved the reliability of the baseline finding: we then found a 67% reproducibility of baseline Arteriosclerosis (as) + Glomerulosclerosis (gsc) + Interstitial fibrosis/tubular atrophy (if/ta) + Mononuclear cell interstitial infiltration (mi). Spearman’s rank correlation 0.401, P = 0.037. (c) Serum creatinine. Pearson’s correlation 0.762, P < 0.001.

Fig. 2. One-year variates correlated with serum creatinine (μmol/l) 3 years after transplantation (n = 28). (a) Interstitial fibrosis/tubular atrophy (if/ta) of graft biopsy. Spearman’s rank correlation 0.472, P = 0.011. (b) Combination of the following variates in graft biopsy: Arteriosclerosis (as) + Glomerulosclerosis (gsc) + Interstitial fibrosis/tubular atrophy (if/ta) + Mononuclear cell interstitial infiltration (mi). Spearman’s rank correlation 0.401, P = 0.037. (c) Serum creatinine. Pearson’s correlation 0.762, P < 0.001.

pronounced during the first year after transplantation. This could be shown for if/ta, gs, and mi but not for the vascular changes. When comparing the 1-year samples with biopsies taken 1 week after transplantation, however, an increase in ah and as was found.

In the follow-up of the individual patient, however, the 1-year chronicity scores were less predictable. Thus, even though if/ta was increased in the 1-year biopsy group, 14/32 biopsies showed unchanged or decreased...
cellular infiltrates, and glomerular hypercellularity (data not shown).

The clinical significance of the ah at 1 year is uncertain. We found no correlation between the hyalinosis and kidney function at 1 or 3 years. Nor was there any correlation to acute rejection, as measured by cumulative 1-year methylprednisolone dose. On the other hand, long-term cyclosporin treatment—even in low doses—has been shown to induce ah in native kidneys as well as in allografts [20–22]. Our patients were treated with a standard triple regimen, aiming at low trough values of CsA; consequently our study was not designed to reveal possible morphological effects of differences in CsA doses. Because of individual differences in susceptibility to CsA nephrotoxicity, such morphological effects are, anyway, unpredictable [20].

Gs was described by Isoniemi et al. [10,23] as part of their CADI (Chronic Allograft Index) score. Our 1-year findings confirm the evolvement of such mesangial alterations in the graft with stable function. This mesangial sclerosis may reflect a subclinical or ‘silent’ transplant glomerulopathy [23], and seems much more common than the more specific ‘chronic transplant glomerulopathy’ [15]. Quantitative analyses of mesangial sclerosis of various aetiologies have recently been shown to correlate with renal function [24]. We did not find any significant impact on renal function after 1 or 3 years, possibly because the changes tended to be mild or mild-to-moderate only. In our study, electron microscopy was implemented mainly to find or exclude specific causes of glomerular changes, such as recurrence of kidney disease or de novo glomerulonephritis. The findings of recurrent disease in two patients (vide supra) underlines the importance of ultrastructural examination.

Allograft inflammatory infiltrates are known to occur—apart from in acute rejection—in ‘well-to-do’ grafts. Our finding of leukocyte infiltration in many biopsies, as well as frequently occurring mild tubulitis in atrophic tubuli [25] is in conjunction with other studies [10,26,27]. The pathologological significance of such infiltrates in the single case is often difficult to assess. Croker et al. [28] studied the effect of macrophage infiltration in graft biopsies and found correlation between their Macrophage index (MI) and graft outcome. We found an uncertain correlation to long-term renal function, but the majority of biopsies showed mild (grade 1) changes only.

A few of our biopsies showed a scarce arterial lymphocytic infiltration within a fibrous intima, that might be interpreted as ‘chronic vascular rejection’. This finding did not, however, have any obvious impact on kidney function, and its relevance seems therefore doubtful.

If/ta was the histopathological variate that most closely correlated to long-term kidney function. This finding confirms studies previously presented by several investigators [10,23,29,30]. Nicholson et al. [31] studied the development of allograft fibrosis by histomorphometric assessment and found that changes at 6 months after transplantation can be used as a surrogate endpoint marker of chronic graft damage. Furthermore, our data support the proposal by the Banff Working Classification, that chronic allograft changes be graded essentially by the extent of if/ta [15,16].

Serum creatinine 1 year after transplantation correlated highly significantly with creatinine at 3 years, and proved to be the single most reliable predictor of long-term kidney function. Adding any of the histopathological scores, or any combinations thereof, did not at all, or only marginally, increase the predictive value of 1-year creatinine in cross validation analysis. The reason for this result is probably a lack of precision in the semi-quantitative scoring system, together with the effect of sampling error already discussed.

Serum creatinine is not, either, an ideal measure of kidney function. It cannot be ruled out that the biopsy findings might have shown a better correlation to a more precise measure of kidney function like GFR at 3 years. The 1-year data might indicate such a closer correlation between the biopsy findings and GFR (Table 6). Recording of GFR 3 years after transplantation was not, however, part of our protocol.

The frequency of cases with one or more acute rejection episodes in this study was 82%. This figure corresponds to that reported in another study from our centre including a higher number of patients [32] and to data published by Olbricht [33]. The frequency with which acute rejection occurs depends on several factors, such as HLA mismatch [32,34], immunosuppressive protocol [35,36], and the current definition of acute rejection at the actual centre. In the present study, HLA identical siblings were excluded, and the majority of transplantations were one HLA haplo-type mismatched. All patients were treated with cyclosporin A, prednisolone, and azathioprine, with no induction therapy. At the time of patient inclusion in the study, the protocol CsA dosage was, in fact, lower than what is currently recommended (vide supra). In the majority of the patients, acute rejection was verified by graft biopsy. Our graft survival is, in general, at the same level as that of other centres [32,37]. As reported by Opelz [38], successfully treated episodes of acute rejection had a negligible influence on long-term graft function.

None of our baseline biopsy variates correlated significantly with graft outcome. This is in agreement with the reports by Nyberg et al. [2] and Curschellas et al. [3], who studied ‘zero-hour’ biopsies from living and cadaveric donors, respectively. Minakawa et al. [4], on the other hand, observed their baseline ‘vasculopathy’ to correlate with short-term but not with long-term prognosis; similar observations were made by Taub et al. [5], who found reduced graft survival in patients with donor baseline as at 2 years, relating however mainly to increased early graft loss during the first 3 months after transplantation. Wang et al. [6] found that absence of ah was correlated with a better graft prognosis, and have recently reported [39] that fibrous intimal thickening in arteries in cadaveric donor
baseline biopsies was correlated with occurrence of graft loss and delayed graft function.

We could not show any prognostic significance of our vascular baseline changes. Our analysis is, however, based mainly on patients with long-functioning grafts. The number of early graft losses were too small for a meaningful correlation with ‘zero-hour’ changes. One might speculate, furthermore, that the significance of vascular changes is less in living than in cadaveric donors; in the latter, a potentially reduced renal reserve capacity might be challenged by additional stress such as prolonged ischaemia.

In conclusion, we have found that if/ta in the well-functioning allograft 1-year after transplantation is the most reliable histopathological parameter in predicting kidney function at 3 years. Serum creatinine at 1 year seems, however, an even better predictor of long-term prognosis. In fact, none of the chronic biopsy scores in our study added substantially to the predictive value of the 1-year serum creatinine. It might be that increasing the amount of tissue, by taking larger biopsies or more than one biopsy at the time, could improve the reliability of the histopathological findings [29,40].

According to our experience, the value of the morphological examination of the long-term allograft is first and foremost in the case of declining kidney function; the graft biopsy is the superior tool in diagnosing recurrent disease vs chronic transplant glomerulopathy or de novo nephritis. Recent data [41] suggest that protocol biopsies may be helpful to optimize the level of immunosuppression in patients with ‘subclinical’ rejection, perhaps as a step towards a more individualized approach in patient follow-up. We do not at our centre, however, recommend a routine 1-year biopsy in the management of the patient with a well-functioning transplant, at least not in the case of the living donor and with the current biopsy procedures.

Acknowledgements. The authors want to thank Odd Kolbjørnsen, Institute of Mathematical Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, for his performance of the kidney biopsies. Dr Jari Jakobsen for performance of the kidney biopsies.

References

12. Rokasni P, Cecchi JM, Takemoto S. More than one biopsy at the time, could improve the 1986; 28: 301–312
14. Midvetti K, Tafjord AB, Hartmann A. Long-Term Results


Received for publication: 2.12.98
Accepted in revised form: 8.6.99