Original Article

Computerized image analysis vs semiquantitative scoring in evaluation of kidney allograft fibrosis and prognosis

Ståle Sund1, Paul Grimm2, Anna Varberg Reisæter3 and Torstein Hovig1

1Department/Institute of Pathology and 3Department of Internal Medicine, Rikshospitalet University Hospital, Oslo, Norway and 2Division of Pediatric Nephrology, University of California at San Diego, CA, USA

Abstract

Background. Chronic morphological changes in the kidney allograft predict long-term graft function, but there are few studies comparing different methods in assessing chronic lesions. In the present study, we evaluated allograft cortical interstitial fibrosis, and compared semiquantitative assessment with computerized image analysis of Sirius red-stained collagen in prediction of graft prognosis.

Methods. Sections were obtained from a series of 1-year protocol living donor kidney graft biopsies (n = 33) and their corresponding baseline specimens (n = 32). At light microscopy, the biopsies were scored for interstitial fibrosis as a percentage of involved tubulointerstitium according to the Banff schema. Quantitation of cortical fractional interstitial fibrosis volume (Vint) was performed with computerized image analysis on coded sections stained with Sirius red. The results were correlated with kidney function at 8–10 years after transplantation, and with late graft loss.

Results. There was a significant correlation between the semiquantitative and quantitative methods for measuring cortical interstitial fibrosis in all the biopsies (n = 65, percentage area vs Vint: R = 0.439, P = 0.0003). The correlation further improved when analysing the baseline specimens separately (n = 32, R = 0.704, P < 0.0001) and was still significant, but less precise for the 1-year biopsies (n = 33, R = 0.384, P = 0.0274). One-year semiquantitative fibrosis (percentage area) was correlated to serum creatinine at 8–10 years (P = 0.010) and to late graft loss (P = 0.0445). The 1-year Vint values for interstitial fibrosis showed a similar trend but did not reach statistical significance in prediction of long-term graft function.

Conclusions. Image analysis quantitation of interstitial collagen with Sirius red corresponded well to light microscopic semiquantitative assessment of interstitial fibrosis. In prediction of long-term graft function, the semiquantitative method was superior, indicating that accumulation of matrix molecules other than fibrillary collagens, oedema and inflammation are also important in graft prognosis.

Keywords: collagen; image analysis; interstitial fibrosis; kidney allograft prognosis; protocol biopsies; Sirius red

Introduction

Kidney transplant function and prognosis are correlated with chronic morphological changes in the allograft, which have been demonstrated both with semiquantitative lesion scoring and with morphometric techniques [1–4]. Because traditional morphometric methods are considered time consuming, computerized systems for automatic quantitation of chronic interstitial lesions have been developed [5,6]. Recently, image analysis quantitation of allograft interstitial fibrosis has been performed on sections stained with Sirius red [7,8], which when observed under polarized light can be considered specific for collagen types I and III [7,9]. There are, however, few reports systematically comparing semiquantitative methods with quantitative assessment of fibrosis in the kidney graft [7].

In a previous study, we reported that interstitial fibrosis and tubular atrophy in 1-year protocol kidney allograft biopsies predicted long-term kidney function, when using semiquantitative lesion scoring [4]. In the present study, we extended the lesion scoring with semiquantitative assessment of the percentage of tubulointerstitial area affected by fibrosis, and with measuring interstitial fibrosis by computerized image analysis.
analysis. The latter was performed on Sirius redstained coded sections from the same kidney allograft biopsies as previously reported. The 1-year biopsy findings were correlated with graft prognosis, with a follow-up of 8–10 years after transplantation.

**Subjects and methods**

**Patients and biopsies**

Protocol allograft biopsies were taken at baseline (n = 32) and 1 year after transplantation (n = 33) in a series of patients consecutively receiving a graft from a living donor from March 1992 to January 1994 at the Rikshospitalet University Hospital in Oslo, Norway, as previously described [4]. Among the exclusion criteria were recipient age < 18 years, and HLA-A, -B and -DR identity between donor and recipient. Of the 33 recipients, there were 20 males and 13 females, aged 20.4–74.4 years (mean age 41.9) at the time of transplantation. Among the donors, there were 14 males and 19 females, 20.7–71.5 years of age (mean age 51.3). Thirty-two patients were transplanted for the first time and one patient received a second graft. Thirty donors were first-degree relatives and three were spouses. Twenty-eight recipient–donor pairs were one haplotype mismatched and two pairs were two haplotypes mismatched. Between the spouses, the number of HLA-A and -B mismatches were three and of HLA-DR mismatches zero, one and two, respectively. 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), nephroclerosis (n = 2), interstitial nephritis (n = 1) or nephronophthisis (n = 1). All patients received a standard triple immunosuppressive regimen, consisting of prednisolone, azathioprine and cyclosporin A. The project was approved by the regional ethical committee, and all patients gave their written consent to participate. Further patient data are detailed in our previous publication [4].

In the previous study, the light microscopic changes were assessed semiquantitatively in 18 gauge needle biopsies according to a grading system partly based on the Banff schema [4,10]. The scoring of interstitial fibrosis and tubular atrophy (if/ta) was identical to the Banff grading for these changes [11] except that one single score was given for these variables together. The area of tubulointerstitial involvement by fibrosis, as expressed by the ci score of the Banff schema, equals the area affected by our if/ta change, since we did not report any cases with tubular atrophy without fibrosis.

In the present study, our reporting of interstitial fibrosis was extended to an assessment of the percentage of cortical tubulointerstitial involvement. By estimating this ‘percentage area’ for each biopsy, consecutive visual fields were assessed for interstitial fibrosis, generally applying one of the intermediate objectives of the microscope to include the entire width of the biopsy, and covering all areas of the biopsy. In each visual field, the approximate fraction of affected tubulointerstitium was estimated. The final score for interstitial fibrosis was then given as the average for all visual fields, in percentage area. Medullary tissue, glomeruli and vessels were excluded from the calculated areas of fibrotic involvement. One of the sections stained with haemat-oxylin–erythrocิน–saffron (HES) was routinely chosen for this part of the study. The other sections including the trichrome stain of Masson–Goldner [10] were always checked not to overlook areas of more pronounced fibrosis in deeper levels of the biopsy. The difference in interstitial fibrosis between the 1-year and baseline biopsy was, for each patient, recorded as the Delta value.

For the Sirius red staining, further sections were obtained from the same paraffin blocks at ~4 µm. If inadequate tissue was available in the block, trichrome or HES sections from the original studies were de-stained and then re-stained with Sirius red.

**Image analysis**

Sirius red staining and image analysis were performed at the Division of Pediatric Nephrology, UCSD, as previously described [7]. In short, unstained paraffin-embedded sections fixed in formalin were used for staining with Sirius red F3BA (Aldrich Chemicals). The slides, after heating at 60°C for 1 h and taken through xylene, graded ethanol and distilled water, were stained overnight in saturated picric acid with 0.1% Sirius red. Slides that had been stained previously were de-stained with acid alcohol and washed in distilled water before staining with Sirius red.

The total of 65 specimens were coded, so that the observer had no knowledge of the source of the slide (baseline or 1-year biopsy), of the clinical record or the result from the semi-quantitative study.

The slides were observed under polarized light in a Nikon E600 microscope. A Hitachi analogue 3 CCD camera was used to capture grey scale 256 bit images, and background correction was performed in real time while the images were being acquired using the 40× objective. Images of the entire cortex were obtained, avoiding glomeruli, large vessels and tissue from the renal medulla, and stored in a sequential fashion. Image analysis was performed using an automated macro (Rasbind W. NIH image 1.61, 1997). Separate data files with the cortical fractional interstitial fibrosis volume were obtained. These files were transferred to the statistical analysis package Statview 5.0 for subsequent analysis on a Macintosh G3 personal computer.

After breaking the specimen coding, a Delta value was estimated in each case, as the difference between the Vint value in the 1-year and baseline biopsy.

**Kidney function**

The morphological changes were correlated with serum creatinine and glomerular filtration rate (GFR) measured by 99mTc-DPTA clearance at 1 year after transplantation, by serum creatinine at 1 year and at 8–10 years, and by late graft loss. The data of kidney function at 1 year were taken from our previous study [4] and the long-term follow-up data were obtained from The Norwegian Renal Registry. In one of the 33 patients, 1-year GFR was calculated according to the Cockcroft–Gault formula [12]. Depending on the year of transplantation, the latest creatinine value was available 8, 9 or 10 years later; in the text, they are all referred to as 10 years serum creatinine.
Statistics

Statistics were analysed using Microsoft Excel and with the statistical analysis package Statview 5.0 on a Macintosh G3 personal computer. Data files from image analysis were transferred to the computer as previously described [7]. Correlations were analysed using the simple linear regression option of the software. Group differences were analysed with the Mann–Whitney U-test, and comparisons of morphological changes in the 1-year and baseline biopsies were performed with the Wilcoxon one-sample test; all tests were two-tailed. In correlation analyses of 1-year variates with 10-year serum creatinine, the cases with late graft loss (between 1 and 10 years) were given a creatinine at 10 years as end-point. Multiple logistic regression was performed for the same covariates vs graft loss.

Results

Semiquantitative assessment

Baseline biopsies. In the baseline biopsies, the tubulointerstitial area involved by fibrosis was found to be within the range of 1–36%, with a mean of 12% (SD 8.9) and a median of 9.5%. Five cases (16%) corresponded to the Banff ci grade 0 (range 1–4% of tubulointerstitium involved), 23 biopsies (72%) showed fibrosis consistent with Banff ci grade 1 (6–21%), and four biopsies (13%) showed Banff ci grade 2 (29–36%).

One-year biopsies. The tubulointerstitial area involved by fibrosis was within the range of 2–94%, with a mean of 30% (SD 24) and a median of 22%. Four biopsies (12%) showed Banff ci grade 0 (2–5%), 13 (40%) ci grade 1 (7–22%), eight (24%) ci grade 2 (31–43%), and eight biopsies (24%) ci grade 3 (53–94%).

Baseline vs 1-year biopsies: Delta values. Interstitial fibrosis at 1 year was significantly increased compared with baseline (median 22 vs 9.5%, P < 0.0001). The difference of estimates in each pair of baseline and 1-year biopsies was recorded as the Delta percentage area of tubulointerstitium involved. The mean Delta value was 19% (SD 26), median 16.5%. No change or minimal change of fibrosis was found in 10 cases (31%) (Delta value −4 to +9%). Increased fibrosis was found in 18 cases (56%), with mild to moderate change (16–27%) in six cases and severely increased fibrosis in 12 (30–88%) compared with baseline. In four cases (13%), reduced interstitial fibrosis was found in the second biopsy (−14 to −25%).

Computerized image analysis

Baseline biopsies. The Vint was found to be within the range of 0.037–0.475 with a mean of 0.159 (SD 0.090) and a median of 0.150. While 19 out of 32 biopsies (59%) showed Vint values of 0.105–0.184, seven cases (22%) showed minimal involvement with a Vint ≤ 0.074, and six cases (19%) a substantial interstitial fibrosis with a Vint ≥ 0.241 including one extreme value of 0.475.

One-year biopsies. The Vint values were within the range of 0.076–0.374, with a mean of 0.183 (SD 0.076) and a median of 0.166. Twenty of the 33 biopsies (61%) showed values of 0.110–0.199, four (12%) showed minimal fibrosis with a Vint ≤ 0.096, and in nine cases (27%) the fibrosis was substantial with a Vint ≥ 0.207, including three cases with high-grade Vint values of ≥ 0.323.

Baseline vs 1-year biopsies: Delta Vint. Although slightly more pronounced, interstitial fibrosis at 1 year was not significantly increased compared with baseline (median Vint 0.166 vs 0.150). The mean Delta Vint was 0.024 (SD 0.131), median 0.013. In three cases (9%), Delta Vint was close to 0 (−0.017 to +0.003). Sixteen cases (50%) showed an increase of fibrosis up to 1 year, with a Delta Vint in the range of 0.337–0.020. In six of these cases, the increase of fibrosis was minimal (Delta Vint ≤ 0.084), in seven cases mild to moderate (Delta Vint 0.107–0.167), and in three cases pronounced (Delta Vint ≥ 0.230).

Thirteen cases (41%) showed less fibrosis at 1 year than at baseline, with a Delta Vint of −0.022 to −0.309. In nine cases, the reduction in fibrosis was minimal (Delta Vint ≥ −0.098), in three cases mild to moderate (Delta Vint −0.167 to −0.110), and in one case pronounced.

Correlation of image analysis and semiquantitative assessment

Comparison of the quantitative estimates with the semiquantitative assessment showed a significant correlation between the two methods.

The correlation improved and was highly significant when comparing the baseline biopsies separately. When comparing the 1-year estimates, the correlation was still acceptable and significant, although there were individual cases with a considerable discrepancy between them (Figure 1a–c).

The findings at baseline were not, for either method, predictive for the extent of interstitial fibrosis 1 year after transplantation.
Graft function

Median GFR at 1 year was 46 ml/min/1.73 m² (8–77) and median 1-year serum creatinine was 142 μmol/l (96–556). Among the 33 patients undergoing the 1-year project biopsy, eight lost their graft between 15 months and 8 years 8 months, due to chronic allograft nephropathy (CAN) (n = 4) or recurrent renal disease (n = 4). Five patients died with a functioning graft between 16 months and 10 years 2 months after transplantation. In the 21 patients with a functioning graft at 10 years, median serum creatinine was 148 μmol/l (81–351).

Correlation of interstitial fibrosis with graft function

**Baseline biopsies.** There was no correlation between baseline interstitial fibrosis and kidney function at 1 and 10 years after transplantation, using either the semiquantitative or the computerized quantitative analysis.

**One-year biopsies.** Interstitial fibrosis in the 1-year graft biopsy correlated with 1-year GFR, both with the semiquantitative method (R = 0.515, P = 0.0022), and with Sirius red (R = 0.361, P = 0.039).

The correlations of 1-year biopsy findings with kidney function at 10 years and with late graft losses are given in Table 1. Percentage area of interstitial fibrosis correlated significantly both with 10-year serum creatinine and with late graft loss. By Sirius red quantitation of collagen at 1 year, correlation with long-term kidney function did not reach statistical significance, and did not improve by replacing the 1-year measurements with the Delta values (Table 1).

**Baseline vs 1-year biopsies: Delta values.** The Delta values for percentage area of interstitial fibrosis but not the Delta Vint correlated significantly with late graft loss and serum creatinine at 10 years (Table 1). The small group of patients with an

### Table 1. Protocol biopsy findings and kidney function 1 year after transplantation: correlation with graft prognosis

<table>
<thead>
<tr>
<th>Variate 1 year post-tx</th>
<th>Serum creatinine 10 years</th>
<th>Graft loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial fibrosis, if/ta</td>
<td>R = 0.404, P = 0.0242</td>
<td>P = 0.0449</td>
</tr>
<tr>
<td>Interstitial fibrosis, percentage area</td>
<td>R = 0.455, P = 0.010⁴</td>
<td>P = 0.0445</td>
</tr>
<tr>
<td>Interstitial fibrosis, Vint</td>
<td>R = 0.314, P = 0.085⁴</td>
<td>NS</td>
</tr>
<tr>
<td>Delta percentage area</td>
<td>R = 0.469, P = 0.009⁴</td>
<td>P = 0.0225</td>
</tr>
<tr>
<td>Delta Vint</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>GFR</td>
<td>R = 0.458, P = 0.0096</td>
<td>P = 0.0876</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>R = 0.465, P = 0.0085</td>
<td>P = 0.1501</td>
</tr>
</tbody>
</table>

Interstitial fibrosis was estimated by a semiquantitative method by lesion scoring (if/ta) and as a percentage of involved tubulointerstitium, and by image analysis with Sirius red staining as cortical fractional interstitial fibrosis volume (Vint). Delta values. Differences in interstitial fibrosis are between 1-year and baseline biopsies. Number of graft loss in the analysis: n = 8, see text.

⁴Results of re-analysis after exclusion of four patients with diagnosis of recurrent disease in the graft were essentially unchanged: R = 0.440, P = 0.0217 for percentage area and R = 0.320, P = 0.1039 for Vint vs 10 year serum creatinine; R = 0.489 and P = 0.0112 for Delta percentage area. Graft losses with recurrent disease occurred at 3.7–8.7 years because of plasma cell dyscrasia (n = 2), immune complex glomerulonephritis (n = 1) and IgA nephropathy (n = 1).

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Table 2. Cases with regression of graft biopsy interstitial fibrosis from baseline to 1 year after transplantation (n = 4)

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Delta Delta</th>
<th>Delta</th>
<th>Donor/recipient</th>
<th>Donor/recipient</th>
<th>No. AR</th>
<th>Serum creatinine at 10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>-14</td>
<td>-0.06</td>
<td>35/42 Brother/brother</td>
<td>1</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>-25</td>
<td>-0.167</td>
<td>65/65 Wife/husband</td>
<td>0</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>-14</td>
<td>-0.026</td>
<td>56/31 Mother/daughter</td>
<td>1</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>-15</td>
<td>-0.309</td>
<td>67/37 Father/daughter</td>
<td>1</td>
<td>228</td>
<td></td>
</tr>
</tbody>
</table>

Differences in interstitial fibrosis between 1 year and baseline reported as Delta values, see text. AR=treated acute rejections for each of the patients. Serum creatinine values are given as \( \mu \text{mol/l} \).

apparent regression of fibrosis up to 1 year (n = 4) showed stable graft function (Table 2).

Correlation of 1-year kidney function and long-term graft prognosis

The 1-year variates for kidney function—measured GFR and serum creatinine—correlated with long-term graft prognosis, at a level comparable with the 1-year interstitial fibrosis in the semiquantitative analysis (Table 1).

Multiple regression analyses: interstitial fibrosis and kidney function at 1 year vs long-term graft prognosis

Semi-quantitative interstitial fibrosis, expressed as percentage area, was analysed with GFR and serum creatinine—correlated with long-term graft prognosis, at a level comparable with the 1-year interstitial fibrosis in the semiquantitative analysis (Table 1).

Donor age

Donor age correlated significantly with semi-quantitative interstitial fibrosis in the baseline biopsies, both for lesion scoring \( (P = 0.0009) \) and for estimation of percentage parenchymal area involved \( (P = 0.0039) \). By using the Sirius red data for interstitial fibrosis, a similar trend was found \( (P = 0.140) \).

There was no correlation between donor age and 1-year interstitial fibrosis for either of the methods.

Acute rejection

The 1-year interstitial fibrosis correlated significantly with the cumulative dose of bolus methylprednisolone given with acute rejection during the first year with the semiquantitative approach (percentage area, \( R = 0.394, P = 0.024 \)), but not with image analysis \( (R = 0.226, P = 0.20) \).

Discussion

In the present kidney allograft protocol biopsy study, we have compared image analysis quantitation of renal cortical interstitial fibrosis, performed on coded specimens, with semiquantitative assessment according to the Banff working classification. The 1-year morphological data were correlated with graft function up to 10 years after transplantation.

To our knowledge, this protocol biopsy study is the first to predict graft prognosis with a 10 year follow-up, and the first such long-term study to apply a chronic lesion assessment as defined in the Banff classification [11].

In the Banff schema, continuous data are transformed into numerical categories, and the 0–3 scale is not linear. Biopsies may be placed in different categories for interstitial fibrosis, even if the ‘true’ difference between them is small. In particular, this effect may be misleading in assessing sequential biopsies. In protocols for studying progression, or possibly regression, of graft fibrosis, the percentage of parenchymal area involved should therefore be included as well as the simpler 0–3 lesion scoring.

Interstitial fibrosis in the Banff schema is estimated as the combined area of interstitium and tubuli—the tubulointerstitium—involved by the process. In Sirius red image analysis, on the other hand, fibrosis is reported as the fraction of cortical tubulointerstitial tissue occupied with birefringent collagen fibres [7]. Thus, the numerical values obtained from the two methods are different even if the same areas are recognized as fibrotic.

Even so, an approximately linear correlation between the two sets of measurements could be expected in mild fibrosis. In high-grade fibrosis, however, the correlation seems less predictable. Thus, ‘100%’ involvement of the tubulointerstitium corresponds to a continuum of different Vint values, depending on the amount of fibrous tissue between the tubuli. The maximum Vint value of 1 corresponds to the extreme that all tubuli are replaced by fibrous tissue, which is hardly ever seen in the graft biopsy and certainly not in our series.

Expansion of the interstitial area in the graft may be due to several factors other than fibrillar collagen. Accumulation of non-fibrillar collagen, especially collagen IV, and non-collagen extracellular matrix protein [13–15] cannot with certainty be distinguished from collagen I and III by routine light microscopy, and even tubular basement membrane thickening may be interpreted as interstitial fibrosis in H&E and trichrome sections. Another possible confounding variable is expansion of the intertubular space due to oedema and inflammation, in which the extent of interstitial fibrosis can be very difficult to assess by light microscopy. With Sirius red, on the other hand (Figure 2), inflammation...
may cause understaining of collagen. This is probably due to the action of metalloproteases elaborated by mononuclear cells such as macrophages and lymphocytes, that may degrade collagen, as recently discussed by Grimm et al. [7].

An important source of error is intra-specimen variation, especially the disappearance of fibrous areas when obtaining sections from different levels of the paraffin block for the two studies. Ideally, future comparative studies should use the same slide if possible.

In spite of these methodological problems, the present series showed a significant correlation between the Vint values and the semiquantitative assessments; it was excellent for the overall low-grade fibrosis of the baseline biopsies, and less consistent but still significant for the group of 1-year protocol biopsies. This finding is at variance with a recent protocol biopsy study [7], in which a high number of grade 0 cases were reported by the semiquantitative method, and the light microscopic scoring did not correlate with the Sirius red quantitation of fibrosis. Comparison between studies is complicated by inter-observer and inter-centre variation [16]. There is, furthermore, no consensus as to the threshold of ‘very mild’ fibrosis vs ‘normal’ amounts of connective tissue in the kidney. In our series, we made the effort to report even areas of minimal interstitial fibrosis at light microscopy, regardless of donor age or considerations of whether the fibrosis was ‘pathological’.

Even if there was a significant correlation between the semiquantitative and quantitative measurements of interstitial fibrosis, the two methods differed in their ability to predict long-term graft prognosis. Thus, the extent of tubulointerstitium affected by fibrosis was a better discriminator with respect to long-term graft dysfunction and loss than the quantitatively assessed cross-sectional area of interstitial collagen only. This result indicates that factors other than fibrillary collagen expanding the interstitial area, as discussed above, contribute to the long-term prognosis of the stable allograft.

Sirius red estimated fibrosis alone did not significantly predict long-term graft prognosis, as opposed to previous reports [7,8], or to similar studies using immunohistochemical staining for collagen III [6]. We assume this discrepancy can be explained by the nature of our material, comprising biopsies from stable living donor transplants, most of which showed only mild to moderate fibrosis. In contrast, comparable series [6–8] comprised mostly or exclusively cadaveric donor grafts. In the study by Nicholson et al. [6], the cut-off point for comparison of groups was at 40% area fraction of immunostained collagen III, which indicates a more advanced overall fibrosis than in our biopsies. In the recent study by Pape et al. [8], the analysed specimens were clinical biopsies taken because of declining graft function, at larger median time intervals after transplantation than in our series.

Our baseline biopsies revealed a substantial amount of Sirius red-stained interstitial collagen fibres, and, notably, the amount of fibrillary collagen did not increase up to 1 year after transplantation. It seems, therefore, that the impetus for increase of collagen I and III has not been strong in these allografts or, alternatively, collagen synthesis has been counter-balanced by degradation in tissue remodelling. Our results may be comparable with the series published by Vleming et al. [14], who studied biopsies in several chronic native kidney diseases, and found...
that substances generally considered basement membrane constituents contributed more to interstitial fibrosis than collagen I/III that were already expressed in controls.

The peritubular capillary (PTC) bed is an important target for interstitial tissue injury in CAN, as shown by ultrastructural studies [17] and more recently by the demonstration of complement factor C4d deposition in PTC [18] in long-term allografts. In various chronic native kidney diseases, renal function correlates closely with interstitial fibrosis but, according to Bohle [19], there is also a highly significant inverse correlation between serum creatinine and the relative interstitial capillary surface of the renal cortex. The prognostic impact by graft fibrosis, expressed as involved tubulointerstitium, might therefore be explained by the specific effect on PTC, rather than by the amount of interstitial fibrotic tissue per se.

Whatever the pathogenetic mechanisms are, our data indicate that a developing fibrosis in the graft may be detectable in terms of area of involved tubulointerstitium, even if the amount of fibrous tissue is only slightly increased.

The assumption of an early low-degree fibrosis in the graft predictive of long-term graft function suggests the development of CAN as a diffuse or multifocal process, which is supported by a recent report by Ellingsen et al. [20]. The authors studied the extent of interstitial tissue in kidney grafts, and found that biopsies from one region of the kidney were representative for all of the explanted graft in chronic rejection. These results indicate that the impact of sampling error might be less than previously assumed in the estimation of CAN-related fibrosis [4,21].

The question of sampling error is especially important in the assessment of possible regression of fibrosis, as four of our patients showed reduced interstitial fibrosis between peri-transplant and 1-year post-transplant biopsies using both methods (Table 2). One of these cases showed a focal scar only in the baseline biopsy and can therefore be explained by sampling variation. Although sampling error is impossible to rule out in the remaining three cases, an alternative explanation could actually be a ‘true’ reduction of interstitial fibrosis from baseline to 1 year after transplantation. Regression of fibrosis has been demonstrated after cisplatin-induced renal injury in rats [22]. We are not aware of any previous documentation of this phenomenon in human kidney transplants, although the possibility of regression of fibrosis in CAN is suggested recently by Seron et al. [21].

The computerized image analysis confirmed our previous observation [4,10] that a certain amount of interstitial fibrosis is a common finding even in baseline biopsies from well-functioning living donor kidneys. Thus, a median cortical interstitial fraction value of 0.15 is indicated for ‘normal’ kidney fibrosis, and a median value of 10% for affected tubulointerstitial area by the semiquantitative assessment. Larger series will be needed for more precise estimates of normal values and for normal ranges related to age.

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Conflict of interest statement. A.V.R. is currently involved in a study conducted by Novartis.

References

10. Sund S, Reiseter AV, Scott H et al. Morphological studies of baseline needle biopsies from living donor kidneys: light microscopic, immunohistochemical and ultrastructural findings. APAMIS 1998; 106: 1017–1034


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