Chronic allograft nephropathy—biopsy findings and outcome

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Abstract

Background. Chronic allograft nephropathy (CAN) is a composite term for various types of damage to a kidney transplant. We wanted to analyse its components in relation to baseline biopsy findings, transplant function, and outcome.

Methods. Among renal transplantations performed from 1985 to 1997, 156 were identified where allograft biopsies had been obtained on clinical indication 6 months after transplantation or later, baseline biopsies were available in each case and the patient’s original disease was known. Time after transplantation was median 2.2 years (range 0.5–13). The biopsies were reviewed and the Banff 1997 CAN score obtained.

Results. All but one late biopsy showed some CAN grade, 48% grade II, and 7.5% grade III. Acute tubulointerstitial rejection was seen in 9% but vascular rejection in only 3%. Arterial wall thickening was present in 66% of the late biopsies, correlated with donor age and its presence at baseline but also with time after transplantation. The Banff CAN score and serum creatinine level were both independent predictors of further graft survival, relative risk 0.35 (confidence interval 0.15–0.82, P = 0.015) for CAN grade I vs III and 0.30 (0.14–0.67, P = 0.003) for serum creatinine < 170 vs > 250 μmol/l. Presence of arterial wall thickening had no prognostic impact.

Conclusion. The CAN grade is predictive of further graft survival independently of the serum creatinine level. Interstitial fibrosis and tubular atrophy are more prominent features of chronic graft damage than vascular rejection. Unspecific arterial wall thickening is partly dependent on baseline conditions and lacks prognostic impact in this late stage.

Keywords: Banff criteria; chronic rejection; graft survival; renal allograft biopsy

Introduction

The majority of kidney allografts deteriorate in the long-term and are eventually lost. The process has often been termed chronic rejection, although it is widely recognized that many non-immunological factors may contribute [1,2]. Nephrotoxicity, ischaemia, hypertension, scarring as a consequence of previous acute rejections, and hyperfiltration due to low nephron mass are among those discussed [1,2]. Pre-existing age-related damage of the donated kidney is another relevant factor [3–7]. Inflammatory or proliferative processes in the arterial walls attract much interest, and are sometimes described as key events [8–10]. Interstitial fibrosis and tubular atrophy are other prominent features [11,12]. The Banff 1997 classification uses the term chronic allograft nephropathy (CAN) to describe and measure the extent of all types of changes [12]. The term includes cases with typical chronic vascular rejection but does not require such changes. In the present study of late allograft biopsies, we used the Banff 1997 CAN classification and analysed the various components of tissue damage. Their significance for the further graft survival was determined. Donor-related pathology and clinical data were also included in the analysis.

Patients and methods

Patients

The total number of kidneys transplanted in the Sahlgrenska University Hospital between 1985 and 1997 was 1692. Among these, 156 fulfilled the following inclusion criteria: the original disease was known (i.e. chronic glomerulonephritis was accepted as a diagnosis only if biopsy-verified, and allograft biopsies had been obtained at baseline as well as 6 months post-transplant or later), the median time from transplantation until biopsy was 2.2 years (range 0.5–13) and the further follow-up time 2.3 years (0–13).

Table 1 shows background data for the study group and the remainder of the contemporary transplants. The patients who underwent late graft biopsies were younger than the main group and more often had a diagnosis of...
Table 1. Comparison between the group of transplant recipients studied by late allograft biopsies and the remainder of the contemporary renal transplant population. Values are median (range)

<table>
<thead>
<tr>
<th></th>
<th>Biopsy series (n = 156)</th>
<th>Main population (n = 1536)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at transplantation (years)</td>
<td>37 (10–70)</td>
<td>46 (1–73)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender, female (%)</td>
<td>38</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Original disease (%)</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glomerulonephritis/vasculitis</td>
<td>42</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>23</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Pyelo-interstitial nephritis</td>
<td>17</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Hereditary</td>
<td>15</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Other specified</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>First transplant (%)</td>
<td>77</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Living donor (%)</td>
<td>35</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>48 (11–74)</td>
<td>46 (3–82)</td>
<td>0.003</td>
</tr>
<tr>
<td>DR-mismatches, 0/1/2 (%)</td>
<td>20; 56; 24</td>
<td>20; 50; 29</td>
<td></td>
</tr>
<tr>
<td>Treated for rejection first year (%)</td>
<td>60</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Number of days treated</td>
<td>4 (0–22)</td>
<td>4 (0–33)</td>
<td></td>
</tr>
</tbody>
</table>

glomerulonephritis, SLE, or vasculitis. The proportion with living donors was somewhat higher. There was no difference with respect to donor age, the number of DR mismatches, the proportion treated for rejection in the first year after transplantation, or the number of days within the first year post-transplant when any kind of antirejection therapy was given.

Allograft biopsies

The late biopsies were obtained by core needles, G16 or G18, while the baseline biopsies were surgically excised. For light microscopy, specimens were processed from formaldehyde-fixed, paraffin-embedded tissue, sectioned at 4 µ and sequentially distributed with three to four sections on each of six slides. The sections were stained with haematoxylin and eosin, van Gieson, periodic acid-Schiff (PAS), orcein and van Gieson, trichrome according to Ladewig, and silvermethane amin (PASM) with light green, respectively.

A preliminary chart of biopsy findings was made based on the original protocols. All biopsies—except 10 that could not be retrieved—were then reviewed by two of the investigators (J.M. and C.T.S.) without knowledge of the clinical data. The median number of glomeruli was 15; 20 biopsies had only three to nine. Arteries were present in all but seven biopsies.

For the grading of chronic allograft lesions, the Banff 1997 classification was followed as regards interstitial fibrosis and inflammation, tubular atrophy, tubulitis, and arteriolar hyalinosis [12]. They were all graded 0–3. Similarly, the degree of mesangial matrix increase (sclerosis) was graded as none, mild, moderate, or severe. The total number of glomeruli and the fraction of glomeruli with global sclerosis were calculated. The presence or absence was noted for glomerular cell increase (proliferation), basement membrane thickening, segmental sclerosis, and tubular hypertrophy. The term glomerulopathy will only be used for membranous or proliferative changes. In the interstitium, the presence or absence of lymphocytes, plasma cells, and granulocytes was noted. In the arteries, the occurrence of fibrous intimal thickening, inflammation, presence of foam cells, and duplication/break of the elastic lamina was noted but not graded. For a diagnosis of chronic vascular rejection, infiltration of the thickened intima by inflammatory cells or a break of the internal elastic lamina was required. Finally, the severity of CAN was graded as 0–3, with focus on interstitial fibrosis and tubular atrophy [12].

Whenever technically possible (129 cases), immunofluorescence or immunoperoxidase stainings were performed. These investigations were used to identify recurrent glomerulopathy.

The baseline biopsies were also retrieved, except four, and reviewed by the same pathologists evaluating interstitial inflammation and fibrosis, glomerulosclerosis, arteriosclerosis, and arteriolar hyalinosis according to the same principles as for the late biopsies. Nephrosclerosis was then graded as none, mild, moderate, or severe.

Clinical data

The following clinical data were retrieved from the registry or from the medical record: the patients’ immunosuppressive protocol initially and at the time of biopsy as well as serum creatinine, cyclosporin A (CsA) trough level and dose at the time of biopsy. Triple therapy, i.e. a combination of CsA, prednisolon and azathioprine, was taken by 87% initially, while 10% had CsA and prednisolon, 2% only azathioprine and prednisolon, and 1% combinations based on tacrolimus. At the time of biopsy, these proportions were 76%, 14%, 6%, and 4%, respectively. The median CsA trough level value at the time of biopsy was 93 ng·ml⁻¹. Serum creatinine levels were classified as ≤170, 171–250, or >250 µmol·l⁻¹. The indication for obtaining a biopsy was a rise in serum creatinine in <93% of the cases, combined with gross proteinuria in 8%. Isolated gross proteinuria was the indication in 7%. Hypertension was present in almost all cases. Due to the anti-hypertensive therapy given, we did not attempt evaluation of the significance of this factor.

Statistics

The database was kept as a StatView 5.0.1. document (SAS Institute Inc., Cary, NC, USA) and all calculations were performed using this software. Unless otherwise stated, values are median and range. Differences between groups in continuous variables were calculated with the Mann–Whitney U rank test. The χ² test was used to test differences
in frequencies. Cumulative survival was calculated according to Kaplan–Meier and multiple regression with the Cox proportional hazards test. Graft loss due to death of the patient was counted as lost to follow-up. Significance testing in the Kaplan–Meier analyses was made using the Mantel–Cox log rank test.

Results

Baseline biopsies

Table 2 shows the prevalence of certain structural findings in baseline and late biopsies. Arteriosclerosis, arteriolohyalinosis, interstitial fibrosis, mesangial sclerosis, and other features of nephrosclerosis were frequently seen in the baseline biopsies, however, in lower numbers than at the time of follow-up, and less pronounced. The fraction of globally sclerosed glomeruli was 4% (0–52) vs 14% (0–92). With increasing degree of nephrosclerosis in the baseline biopsies; the age of the donor was significantly higher \( P < 0.0001 \) between biopsies with no and slight nephrosclerosis and \( P = 0.03 \) between those with slight and moderate nephrosclerosis. Few of the youngest donors had nephrosclerosis, whereas the degree ranged from none to moderate among elderly donors.

Correlations between baseline and late biopsies

The only isolated factor where a correlation was observed between findings in the baseline and the late biopsy was arteriosclerosis \( (P = 0.001) \). Grafts with arteriosclerosis at baseline were from significantly older donors than those with normal arteries \( (P < 0.0001) \); median 37 (8–65) vs 53 (23–71) years. An impact of donor age on arteriosclerosis remained in the late biopsies. Table 3 shows the donor age for grafts with and without arteriosclerosis in late biopsies. As shown in Table 3, there was also an effect of time after the transplantation on this variable. Donor age was not different between biopsies with different CAN grades.

Late biopsies

The conclusive late biopsy histopathologic diagnoses are shown in Table 4. All biopsies but one showed some degree of CAN, and CAN was the dominant or only finding in half of the cases. Vascular rejection was seen in only 3% and acute tubulointerstitial rejection in another 9%. However, the cellular infiltrates observed in 87% of the CAN biopsies were sometimes difficult to interpret due to tubulitis in atrophic tubuli, therefore, the possibility of a borderline acute rejection could not always be ruled out. Twelve of 83 (14%) had borderline grade tubulitis. Glomerulopathy was observed in almost one-third of the cases. It was always accompanied by some degree of CAN.

Clinical data

Clinical data were tested against late biopsy findings. Serum creatinine was significantly higher in patients

Table 2. Prevalence of structural histopathologic findings in biopsies obtained from 156 renal allografts at baseline and, on clinical indication, later than 6 months after transplantation

<table>
<thead>
<tr>
<th>Findings</th>
<th>Baseline</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriosclerosis</td>
<td>50</td>
<td>66</td>
</tr>
<tr>
<td>Arteriolohyalinosis</td>
<td>50</td>
<td>30/18/1</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>Tubulitis</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Tubular hypertrophy</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>54</td>
<td>49/5/0</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>41</td>
<td>87</td>
</tr>
<tr>
<td>Mesangial sclerosis</td>
<td>64</td>
<td>55/9/0</td>
</tr>
<tr>
<td>Glomerulopathy (proliferative/membranous)</td>
<td>2</td>
<td>33</td>
</tr>
</tbody>
</table>

All values are percentage of 156.

Table 3. Correlations between the presence or absence of arterial wall thickening in late renal allograft biopsies and time variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal artery ((n = 46))</th>
<th>Arterial wall thickening ((n = 98))</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor age (years)</td>
<td>40.5 (8–71)</td>
<td>46.5 (14–69)</td>
<td>0.021</td>
</tr>
<tr>
<td>Time since transplantation (months)</td>
<td>17 (6–76)</td>
<td>42 (6–166)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Graft age (sum of the above) (years)</td>
<td>43 (11–73)</td>
<td>52 (19–77)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

The five cases with active vascular rejection have been excluded. Seven biopsies lacking interlobular size arteries could not be evaluated in this respect.
with graft biopsies showing increasing grades of interstitial fibrosis (median values 200–234–264 μmol/l, \( P = 0.007 \)). There was also a weak correlation between serum creatinine and glomerular sclerosis (\( P = 0.04 \)). In addition, the total Banff CAN score showed a strong correlation to serum creatinine (\( P = 0.004 \)). There was no significant difference in time since transplantation between biopsies with different CAN grades, e.g. CAN I was found after median 2 years (range 0.5–13), CAN II after median 3 years (range 0.5–13), and CAN III after median 4 years (range 1–8). No correlation was seen between the CsA trough level at the time of biopsy and the Banff CAN score for those 147 that could be reviewed after transplantation and the Banff CAN score for those 147 that could be reviewed.

Outcome

A low Banff CAN score conferred a better graft survival after the biopsy (\( P = 0.002 \); Figure 1). The single most important histopathologic component was interstitial fibrosis which had a significant impact on further graft survival (\( P = 0.02 \)) whereas tubular atrophy and arteriolohyalinosis were not significant and arterial wall thickening did not seem to have any influence (Figure 2). Among the clinical variables, the serum creatinine value at the time of biopsy was highly significant (\( P < 0.0001 \); Figure 3), but a history of treated rejection during the first year after transplantation was insignificant. Furthermore, there was no correlation between the duration of antirejection therapy (0–22 days) and the CAN grade.

In a Cox proportional hazards analysis, the serum creatinine level was entered in the first step and the CAN grade in the second. Compared with serum creatinine > 250 μmol/l, the relative risk for graft loss with a value < 170 μmol/l was 0.30 (confidence interval 0.14–0.67, \( P = 0.003 \)) and with 171–250 μmol/l 0.55–1.0.

Table 4. Main histopathologic diagnosis in biopsies obtained on clinical indication from 156 renal allografts later than 6 months after transplantation and the Banff CAN score for those 147 that could be reviewed

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number (%)</th>
<th>CAN grade</th>
<th>CAN grade 0/1/II/III numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular rejection</td>
<td>5 (3)</td>
<td>0.1/2/2</td>
<td></td>
</tr>
<tr>
<td>Acute tubulointerstitial rejection</td>
<td>14 (9)</td>
<td>0.9/5.0</td>
<td></td>
</tr>
<tr>
<td>Chronic allograft nephropathy</td>
<td>83 (53)</td>
<td>0.31/43/7</td>
<td></td>
</tr>
<tr>
<td>Glomerulopathy, other</td>
<td>25 (16)</td>
<td>0.8/12.2</td>
<td></td>
</tr>
<tr>
<td>Recurrent glomerulonephritis</td>
<td>22 (14)</td>
<td>0.12/6.0</td>
<td></td>
</tr>
<tr>
<td>'Normal'</td>
<td>1 (1)</td>
<td>1/0/0/0</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>6 (4)</td>
<td>0.4/2.0</td>
<td></td>
</tr>
</tbody>
</table>

According to written protocols, the CAN grade was not zero in any of the missing nine.

Fig. 2. Cumulative survival according to Kaplan–Meier for renal allografts subjected to graft biopsy later than 6 months after transplantation. Grafts with unspecified arterial wall thickening (\( n = 98 \)) indicated by dotted line, not significantly different from those with normal arteries (\( n = 46 \)), full line. Five biopsies with vascular rejection not included. The numbers at risk for 1, 2, and 5 years are 70/33, 54/28, and 22/12, respectively.

Fig. 3. Cumulative survival according to Kaplan–Meier for renal allografts subjected to graft biopsy later than 6 months after transplantation. Grafts with three different classes of serum creatinine values at the time of biopsy have significantly different survival (\( P < 0.0001 \)). The numbers at risk are 30, 62, and 53 at the time of biopsy, 28/51/26 after 1 year, 23/42/18 after 2 years and 13/15/6 after 5 years.

Fig. 1. Cumulative survival according to Kaplan–Meier for renal allografts subjected to graft biopsy later than 6 months after transplantation, grouped according to the Banff CAN score. The numbers at risk in each group (I/II/III) was 65/70/11 at the time of biopsy, 50/49/6 after 1 year, 43/34/6 after 2 years, and 18/12/2 after 5 years. There is a significant difference in survival between grafts with different scores (\( P = 0.002 \)).
(confidence interval 0.33–0.91, \( P = 0.02 \)). The relative risk with a Banff CAN score of I compared with III was 0.35 (confidence interval 0.15–0.82, \( P = 0.015 \)) whereas grade II was not significantly different from grade III.

**Discussion**

To our knowledge, this is the first study in which the Banff 1997 working classification of chronic changes has been evaluated in a biopsy material obtained on clinical indication. However, valuable information was provided by the investigators behind its predecessors, the CADI and CGD scores [4,5]. In particular, these scores were found to be predictive of the further transplant function. We confirm that this is also true for the Banff score of chronic allograft nephropathy.

The main purpose of the study was to investigate the significance of singular components of the score, such as the arterial changes. Considering the fact that arterial vascular rejection is often described as the hallmark of chronic rejection, it is remarkable that only five cases were observed [8,10,11]. Some under-estimation of the true prevalence in our series may be caused by irregular distribution of the process within the vascular tree. However, chronic vascular rejection has a more generalized distribution than arteriosclerosis and should, therefore, be more correctly sampled [2,8].

Yet, in a late fibrotic stage it may be difficult to differentiate chronic vascular rejection from arteriosclerosis. The overall damage score systems CADI and CGD do not distinguish between various forms of arterial wall thickening [4,5]. The Banff revised classification stresses the value of a closer characterization of the arterial wall pathology, but takes such differences into account only to a limited extent [12]. The frequently used term ‘transplant arteriosclerosis’, includes inflammatory and proliferative changes, but does not seem to require this strict definition [2]. Mihatsch et al. [10] describe the evolution of transplant vasculopathy over time, from inflammatory, proliferative phases to a sclerotic stage. They do not, however, differentiate this condition from ‘normal’ arteriosclerosis or the consequences of uncontrolled hypertension. The concept of ‘accelerated senescence’, coined by Halloran, meaning that the allograft is damaged by a more rapid but otherwise normal ageing process, fits our observations of a rapid evolution of non-specific arteriosclerosis [1].

‘Chronic vascular rejection’ is sometimes used as a synonym to chronic graft dysfunction [4,8,9,13]. In the study of pathogenetic mechanisms, a useful step might be to apply a more precise terminology. Criteria for ‘transplant arteriosclerosis’ should also be clearly defined, otherwise differences in its prevalence between various studies cannot be judged. If, in fact, the arterial wall thickening observed in many of our graft biopsies was a form of vascular rejection, this should be expected to influence further graft survival. Yet, no such effect was observed. Clearly, so-called chronic rejection sometimes does not involve the arteries but causes damage to the parenchyma in other ways.

The two clinical variables in this study were episodes of acute rejection and the level of serum creatinine at the time of biopsy. There was no effect of the prevalence or severity of acute rejection in the first year on any of the morphologic parameters. The graft outcome following biopsy was unrelated to a history of acute rejection. Partly, this may be explained by the fact that only grafts functioning for at least 6 months were evaluated. Efficacy of the early anti-rejection therapy may explain a low degree of resulting graft damage. Isoniemi et al. [5] did corresponding observations in protocol biopsies of well-functioning grafts. Solez et al. [7] found a small difference in the CAN score in protocol biopsies obtained after 2 years depending on whether or not biopsy-proven acute rejection had occurred in the first year. Our data, which are based on biopsies obtained on clinical indication, suggest that if the progressive long-term damage is a consequence of on-going chronic rejection it is not closely related to the early acute forms.

Arteriolohyalinosis was present in half of the baseline biopsies, but the severity increased at follow-up, certainly to some extent related to the CsA treatment. However, as the CsA dosage had changed during follow-up and we could not evaluate the dynamics in each case, the role of CsA toxicity on the established graft damage could not be determined. The low doses used at the time of biopsy and the absence of an impact of arteriolohyalinosis on graft survival in the univariate analysis may suggest that the contribution was small.

The CADI and CGD scores have been found to correlate to the patients’ serum creatinine values [4,5]. In the Helsinki study, and in a recent study from Norway, the extent of interstitial fibrosis, as a single factor, showed a strong correlation to serum creatinine [5,14]. We could confirm this connection, using both the CAN score and the degree of interstitial fibrosis alone. The serum creatinine level and the CAN score both have a prognostic impact [4,14–16]. As the two are correlated, the question is whether the CAN score is a better predictor of graft survival than the more readily available serum creatinine value. To our knowledge an advantage of the damage score has never been demonstrated. In our analysis, serum creatinine was somewhat superior, but the CAN grade added prognostic information.

Our study is an evaluation of biopsies obtained on clinical indication. The results are not valid for all renal transplants, as when protocol biopsies are obtained. However, they are of higher clinical relevance, particularly because the series was selected to include only cases with a certified renal diagnosis and a maximum of other baseline data.

Following an analysis of decisions taken based on late allograft biopsies Kon et al. [17] drew the rather desolate conclusion that the guidance given by the biopsies is so little that they need not be carried out.
Our findings contradict this conclusion. The CAN grade gives prognostic information and ongoing rejection and active glomerulonephritis may be treatable. The study of the latter calls for more advanced analyses than those necessary for the definition of a CAN score. A closer study of the glomerulopathies in our material is in preparation.

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References


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