Glomerular and tubulointerstitial infiltrates in chronic allograft dysfunction

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Immunohistochemical characterization of glomerular and tubulointerstitial infiltrates in renal transplant patients with chronic allograft dysfunction

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Abstract

Background. The term chronic allograft nephropathy (CAN) was deleted in the Eighth Banff Classification and two new categories were introduced: chronic T-cell-mediated rejection (CTMR) and chronic active humoral rejection (CAHR). The aim of this study was to revise our CAN cases diagnosed in the last 4 years, analyse allograft survival rates and identify types of infiltrating cells in the different settings.

Methods. Seventy-nine patients with biopsy-proven CAN were examined and classified into four groups according to the Banff 2005 criteria: CTMR, CAHR, chronic calcineurin inhibitor toxicity (CNITOX) and interstitial fibrosis and tubular atrophy not otherwise specified (NOS). CD4, CD8, CD20, CD68, CD103, Foxp3 and IL-17 protein expression and C4d deposits were investigated.

Results. We diagnosed 20 CTMR, 13 CAHR, 28 CNITOX, and 18 NOS. Death-censored graft survival at 4 years from renal biopsy was worse in CAHR compared with the other types of chronic injury. Glomerular CD8+ cells were increased in CTMR vs CNITOX and NOS. Interstitial CD4+ and CD8+ cells were increased in CTMR vs CNITOX. CD68+ cells in glomerular and peritubular capillaries were higher in CAHR vs CNITOX, CTMR and NOS. CD103+ cells were higher in cases with tubulitis than in those without. T regulatory and T helper 17 cells were rarely observed in the different settings.

Conclusions. Graft survival was worse in patients with CAHR. The presence of any grade transplant glomerulopathy and chronic allograft vasculopathy are poorer prognostic factors. Infiltrating CD8+, CD103+ and CD4+ cells may help to differentiate CTMR from other types of chronic injury, thus improving diagnostic/prognostic features of biopsy in patients with chronic allograft dysfunction.

Keywords: Banff schema; chronic active antibody-mediated rejection; chronic T-cell-mediated rejection; immunohistochemical markers; renal transplantation

Introduction

‘Chronic allograft nephropathy’ (CAN) represents the leading cause of late allograft loss in kidney transplant patients. However, the term ‘CAN’ does not indicate a specif-
ic disease occurring in the graft, since different aetielogic factors may result in chronic (sclerosing) changes in the allograft, alone or in combination [1–3]. Therefore, CAN is a complex nosographic entity including both immune-mediated and non-immune-mediated lesions [4,5].

The new updated Banff classification [6] deleted the term CAN, encouraging pathologists to search for histological findings specific of defined aetiopathogenetic mechanisms of chronic graft injury. In this perspective, this classification provides two new diagnostic categories: chronic active humoral rejection (CAHR; histologic evidence of chronic structural changes with C4d-positive and with anti-HLA antibodies detected in serum samples) and chronic T-cell-mediated rejection (CTMR; histologic evidence of chronic structural changes and an active T-cell component in sites of chronic damage: arterial intima, tubules, interstitium and glomeruli). The diagnosis of ‘interstitial fibrosis and tubular atrophy, no evidence of any specific aetiology’ is to be made only when the histological findings do not allow any conclusions on the aetiopathogenetic mechanism of the chronic lesions.

The identification of the main aetiologic factors responsible for the chronic lesions occurring in the graft is of great importance since it may suggest changes in the immunosuppressive regimen and can recognize those types of allograft injury that are at higher risk of progression.

However, the routine evaluation of renal biopsies not always provides clues on aetiologic factors of chronic damage. A specific tissue marker of humoral rejection has been identified in C4d deposition at the peritubular capillary (PTC) level [8]. C4d is a split product of C4, derived from the activation of the classical complement pathway, which covalently binds to PTCs of renal allograft during humoral rejection [9]. Humoral rejection is often associated with glomerular and interstitial accumulation of monocytes [10,11]. Although C4d is a reliable marker of humoral rejection, specific markers of CTMR in the routine evaluation of renal allograft biopsies are lacking. The presence of asymptomatic tubulointerstitial cellular infiltrates in renal allograft biopsy in a chronic setting remains poorly understood since we lack reliable biomarkers to discriminate whether these cellular infiltrates represent formal injuries rejection or, conversely, are merely protective T-cell infiltrates necessary for graft acceptance [12]. Mengel et al. recently showed that inflammatory infiltrates in protocol biopsies predict allograft function outcome independently of their localization [13].

T regulatory cells (Tregs), expressing the transcription factor Foxp3, play a crucial role in the overall immune response and are also present within transplanted tissue, so the identification of Tregs in recipients of an allograft may help to identify infiltrates that are playing a role in the graft acceptance [12]. Foxp3+ Treg cells were found to be able to produce IL-17 [14]; furthermore, the IL-17-producing CD4+ T helper 17 (Th17) cells have been associated with inflammatory disease and allograft rejection [15,16]. Conversely, the integrin heterodimer αv (CD103)β7 (herein referred to as CD103) defines a subset of CD8 effectors elicited in response to renal allograft whose recognition within infiltrate should be regarded as a potential marker of aggressiveness [17].

The aim of our study was, therefore, to revise all cases of ‘CAN’ diagnosed at our renal unit in the last 4 years and to analyse renal survival rates for the different types of chronic damage. We further studied the composition of glomerular and interstitial infiltrates in order to identify possible expression patterns exploitable for the diagnosis of specific types of graft injury.

Materials and methods

Patients

From May 2003 to December 2007, 79 patients with a biopsy-proven CAN were included in this retrospective study. Renal specimens taken for clinical indication were obtained by needle core biopsies (16-gauge) performed under ultrasonographic guidance.

Table 1. Main clinical and histological features of the patients included in the study

<table>
<thead>
<tr>
<th></th>
<th>CTMR</th>
<th>CAHR</th>
<th>CNITOX</th>
<th>NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (N)</td>
<td>20</td>
<td>13</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>Recipient age (years)</td>
<td>40±13</td>
<td>44±11</td>
<td>47±12</td>
<td>45±14</td>
</tr>
<tr>
<td>Recipient gender (M/F)</td>
<td>11/9</td>
<td>8/5</td>
<td>21/7</td>
<td>12/6</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>42±14*</td>
<td>41±16*</td>
<td>52±15</td>
<td>54±13</td>
</tr>
<tr>
<td>HLA mismatches</td>
<td>3.2±0.8</td>
<td>2.8±0.6</td>
<td>3.0±1.2</td>
<td>3.1±0.9</td>
</tr>
<tr>
<td>Interstitial fibrosis (%)</td>
<td>36.5±20.8</td>
<td>39.3±15.9</td>
<td>31.7±14.1</td>
<td>29.4±24.7</td>
</tr>
<tr>
<td>Segmental sclerosis (%)</td>
<td>5.8±6.0</td>
<td>17.4±18.2***</td>
<td>2.0±4.6</td>
<td>4.2±9.3</td>
</tr>
<tr>
<td>Global sclerosis (%)</td>
<td>15.0±19.3</td>
<td>24.3±11.0</td>
<td>24.5±22.2</td>
<td>23.2±25.5</td>
</tr>
<tr>
<td>TGP (g0/g1/g2–3)</td>
<td>11/5/4</td>
<td>5/2/6</td>
<td>26/2/0</td>
<td>18/0/0</td>
</tr>
<tr>
<td>CAV (yes/no)</td>
<td>10/10</td>
<td>8/5</td>
<td>0/28</td>
<td>0/18</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>3.1±3.1</td>
<td>2.6±1.0</td>
<td>2.3±1.1</td>
<td>2.4±0.9</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>1.2±1.2</td>
<td>2.6±3.5***</td>
<td>0.6±0.8</td>
<td>1.4±1.4</td>
</tr>
<tr>
<td>Time post transplant (months)</td>
<td>52.7±32.3</td>
<td>58.1±47.4</td>
<td>48.6±45.5</td>
<td>51.5±38.4</td>
</tr>
</tbody>
</table>

CTMR, chronic T-cell-mediated rejection; CAHR, chronic active humoral rejection; CNITOX, chronic CNI toxicity; NOS, interstitial fibrosis and tubular atrophy not otherwise specified.

*P<0.05 vs CNITOX and NOS.

**P<0.05 vs CNITOX, CTMR and NOS.

***P<0.05 vs CTMR and CNITOX.
Mean time of biopsy from transplant was 53±47 months. At the time of biopsy, all patients were treated with a conventional immunosuppressive regimen using prednisone and calcineurin inhibitors (CsA or tacrolimus) and mycophenolate mofetil or azathioprine. The main clinical and histological features of the patients included in the study are summarized in Table 1. The study was approved by the local ethics committee.

Histological diagnosis

Tissue samples were fixed in 4% formaldehyde, paraffin-embedded and then processed for routine histological staining (hematoxylin–eosin, periodic acid–Schiff, silver methenamine and Masson’s trichrome). Snap-frozen tissue sections were used for routine immunofluorescence study (IgG, IgA, IgM, C3, C1q and fibrinogen). Cases with recurrent de novo glomerular diseases were excluded from the study. Only biopsies with at least seven glomeruli and two medium-sized arteries available for light microscopy examination were considered for the study. Semi-quantitative scoring for acute and chronic tubular, interstitial, vascular and glomerular changes was performed by two independent pathologists (M.R. and C.D.). Transplant glomerulopathy (TGP) was scored according to Banff criteria from g0 to g3. The percentage of globally and segmentally sclerotic glomeruli was evaluated. Interstitial fibrosis was assessed in consecutive fields of the biopsies avoiding sub-capsular and perivascular areas on trichrome-stained slides using Adobe Photoshop 8.0.1 (Adobe Systems Corporation, San Jose, CA) [18,19]. The percentage of blue (fibrous tissue) stained area/total area was measured. Values from all consecutive images for each biopsy were averaged. Diagnosis was made according to the Banff 2005 Meeting Report [6]. Cases were assigned to four groups based on the main histological lesions: CTMR in the presence of chronic allograft vasculopathy (CAV: mononuclear cells infiltrating thickened intima of medium-sized arteries in the absence of internal elastic lamina duplication assessed on silver stain) and/or evidence of ongoing T-cell activity at the tubulointerstitial level including tubulitis in non-atrophic tubules and interstitial infiltrate in non-completely scarred areas (below the threshold of acute T-cell-mediated rejection), CAHR when C4d was diffusely positive in PTCs in the presence of chronic lesions (i.e. glomerular double contours and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries), chronic calcineurin inhibitor toxicity (CNITOX; in the presence of both medial arterial hyalinosis and striped interstitial fibrosis in the absence of signs of CAHR and/or CTMR) or not otherwise specified (NOS). Cases with more than one lesion were classified according to the prevalent histological feature.

Antibodies

The primary antibodies used in this study recognized the following markers: CD20 (Dako Cytomation, Carpinteria, CA), a cell surface marker specific for B lymphocytes; CD8 (Dako Cytomation, Carpinteria, CA), a marker of cytotoxic/suppressor T lymphocytes [20]; CD103/Integrin αE (Abcam, Cambridge, UK), a specific marker for a subset of CD8+ effector T cells [21,22]; CD4 (Santa Cruz Biotechnology, Inc.) a specific marker for T helper cells; Foxp3 (e-Bioscience, San Diego, CA), a marker for a subset of CD4+ effector T cells (Th17) [23]. Moreover, we used CD68 (Santa Cruz Biotechnology, Inc.), a specific marker for monocytes/macrophages, and C4d (Biomedica Group, Vienna, Austria), an immunohistochemical marker for the humoral alloresponse.

Immunohistochemical analysis

Immunohistochemistry evaluation of CD20, CD8, CD103, CD4, Foxp3, IL-17 and CD68 protein expression and C4d deposits in renal biopsies was carried out on paraffin tissue sections. Thin (2 μm) sections of paraffin-embedded tissue were deparaffinized and rehydrated through xylenes and graded alcohol series. The slides were subjected to specific epitope denoising: by microwave in citrate buffer (0.01 M, pH 6.0) for CD4 and IL-17, by pressure cooking in EDTA buffer (0.01 M, pH 8.0) for CD20, CD8, CD68, CD103 and Foxp3 and in citrate buffer (0.01 M, pH 6.0) for C4d. After antigen retrieval, the sections were blocked with protein block serum-free (Dako, Glostrup, Denmark) at room temperature (RT) for 10 min. Only for CD103 and Foxp3 BSA 2% for 1 h at RT was also used.

Then, the sections were incubated with the following primary antibodies: CD20 antibody (ready to use, 10 min incubation at RT), CD8 antibody (ready to use, 10 min incubation at RT), CD103 antibody (1:20, 1 h incubation at RT), CD4 antibody (1:400, 1 h incubation at RT), Foxp3 antibody (1:100, 1 h incubation at RT), IL-17 antibody (1:50, 1 h incubation at RT), CD68 antibody (1:100, overnight incubation at +4°C) and C4d antibody (1:10, 1 h incubation at RT).

For CD20, CD8, CD4, CD68 and C4d, the binding of the secondary biotinylated antibody was detected by the Dako EnVision G/2 System/AP kit (Dako Cytomation, Glostrup, Denmark), according to the manufacturer’s instructions and the visualization of alkaline phosphatase was achieved by incubation in Ryno Blue Phosphate Solution, resulting in a red precipitate. For CD103, Foxp3 and IL-17, the binding of the secondary biotinylated antibody was detected by the Dako Real EnVision Detection System, Peroxidase/DAB kit (Dako Cytomation, Glostrup, Denmark), according to the manufacturer’s instructions. Visualization of peroxidase was achieved by incubation in DAB Chromogen Solution, giving a brown precipitate. The sections were counterstained with Mayer haematoxylin (blue) and mounted with glycerol (Dako Cytomation, Glostrup, Denmark). Negative controls were obtained by omitting the primary antibody. Paraffin-embedded tissue sections of lymph nodes were used as positive controls for CD20, CD8, CD4, CD68 and Foxp3. Sections from acute graft rejection were used as positive controls for IL-17 and CD103 antibodies.

Positive staining was evaluated by optical light microscopy using a Leica microscope fitted with a Coolpix 990 digital camera (Nikon, Calenzano, Italy).

The number of CD20+, CD8+, CD4+, CD68+, CD103+, Foxp3+ and IL-17+ intracellular cells was measured at high power (>400) field on the entire cortical region of biopsies by two independent observers blinded to the origin of the slides. The number of CD20+, CD8+, CD4+, CD68+, CD103+, Foxp3+ and IL-17+ glomerular cells was counted in all glomeruli/sections.

Positive C4d staining was defined as the presence of C4d deposition in more than 50% of PTC cross-sections [24].

Statistical analysis

Data are expressed as mean values ± standard deviation. Differences between groups were tested using ANOVA. Dichotomous variables were analyzed with the chi-square test. A P-value <0.05 was considered to be statistically significant. Graft survival curves, starting at the time of diagnosis of CAN, were censored by death with a functioning graft and analysed by the Kaplan–Meier actuarial method and log-rank test. Statistical analysis was performed using the Statview Software package (version 5.0, SAS Inc. Cary, NC).

Results

Renal function and histology

In 33 patients, the renal biopsy showed prevalent histologic signs of chronic rejection, of which 13 (39.4%) were CAHR and 20 (60.6%) were CTMR. Twenty-eight patients showed signs of CNITOX and 18 cases were classified as NOS, based on the presence of interstitial fibrosis and tubular atrophy in the absence of specific lesions (Table 1). Serum creatinine at the time of renal biopsy, extent of interstitial fibrosis and global glomerulosclerosis were not significantly different among groups. The percentage of segmentally sclerosed glomeruli was significantly higher in the CAHR group compared with the CTMR (P=0.0008), CNITOX (P<0.0001) and NOS (P=0.0002) groups. Twenty-four hour proteinuria at the time of renal biopsy was significantly increased in the CAHR group compared with the CTMR (P=0.03) and CNITOX (P=0.003) groups (Table 1).
Death-censored graft survival at 4 years from the renal biopsy-proven diagnosis was worse in CAHR (44%) compared with the other types of chronic injury (CTMR 66%, CNITOX 89% and NOS 81%) (log-rank test P=0.04; Figure 1). The presence of TGP was associated with lower graft survival at 4 years from renal biopsy even in cases with mild glomerular lesions (g1) (g0, 92%; g1, 37%; g2–3, 20%) (log-rank test P<0.0001; Figure 2). CAV was present in 10 cases of CTMR and in 8 cases of CAHR. The presence of CAV significantly affected graft survival at 4 years post-renal biopsy (with CAV, 40%; without CAV, 87%) (log-rank test P=0.001; Figure 3).

Only CD8\(^+\) (R\(^2\)=0.06, P=0.03) and CD103\(^+\) (R\(^2\)=0.21, P=0.02) cells at the tubulointerstitial level correlated with graft function, expressed as serum creatinine at the time of renal biopsy at single-regression analysis. The number of tubulointerstitial CD8\(^+\), CD4\(^+\) and CD68\(^+\) cells directly correlated with interstitial fibrosis (CD4: R\(^2\)=0.12, P=0.001, CD8: R\(^2\)=0.06, P=0.02, CD68: R\(^2\)=0.08, P=0.01) at single-regression analysis. No correlation was found for CD20\(^+\), CD103\(^+\) and Foxp3\(^+\) cells. Only tubulointerstitial CD4\(^+\) cells independently correlated with fibrosis at multiple-regression analysis (R\(^2\)=0.19, P=0.006).

The number of glomerular CD68\(^+\) cells correlated with the percentage of segmental (R\(^2\)=0.16, P=0.0002) glomerulosclerosis.

Infiltrate features and Banff classification

CTMR was characterized by an increased number of CD4\(^+\) and CD8\(^+\) cells at the tubulointerstitial level compared with the CNITOX group and by an increased number of glomerular-infiltrating CD8\(^+\) cells compared with the CNITOX and NOS groups (Table 2). CAHR was characterized by an increased number of CD68\(^+\) infiltrating cells at the glomerular level compared with all the other groups and at the PTC level compared with the CTMR and NOS groups (CD68\(^+\) infiltrating cells in PTCs where numerically higher in the CAHR group compared with the CNITOX group, although the difference did not reach statistical significance) (Table 2).

The number of CD103\(^+\) cells at the tubulointerstitial level was significantly increased (P=0.005) in cases with tubulitis compared with cases without tubulitis (2.69±2.75 vs 0.9±1.0, respectively). Treg (Foxp3\(^+\)) and Th17 (IL-17\(^+\)) cells were rarely observed at the glomerular and tubulointerstitial levels and no difference was observed among all groups.

Fig. 1. Death-censored graft survival after the diagnosis of CAN according to the Banff 2005 classification, showed that CAHR has a poorer prognosis compared with other groups (log-rank test, P=0.04).

Fig. 2. Death-censored graft survival after the diagnosis of CAN according to the presence of TGP (g0, g1, g2–3) pointed out that TGP has a poorer prognosis regardless of grading (log-rank test, P<0.0001).

Fig. 3. Death-censored graft survival after the diagnosis of CAN according to the presence of CAV, proved that CAV significantly affected graft survival at 4 years (log-rank test, P=0.001).
Glomerular and tubulointerstitial infiltrates in chronic allograft dysfunction

Table 2. Number of infiltrating cells at tubulointerstitial and glomerular levels

<table>
<thead>
<tr>
<th></th>
<th>CTMR</th>
<th>CAHR</th>
<th>CTMR</th>
<th>NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>G CD20 (+cells/hpf)</td>
<td>12.54±12.44</td>
<td>8.15±8.12</td>
<td>8.21±8.62</td>
<td>7.23±9.15</td>
</tr>
<tr>
<td>G CD20 (+cells/glom)</td>
<td>0.15±0.46</td>
<td>0.09±0.19</td>
<td>0.10±0.25</td>
<td>0.04±0.07</td>
</tr>
<tr>
<td>PTC CD20 (+cells/PTC)</td>
<td>0.25±0.44</td>
<td>0.64±1.01</td>
<td>0.24±0.51</td>
<td>0.25±0.44</td>
</tr>
<tr>
<td>G CD8 (+cells/hpf)</td>
<td>32.02±16.12</td>
<td>29.31±19.91</td>
<td>22.29±16.12</td>
<td>23.58±18.26</td>
</tr>
<tr>
<td>G CD8 (+cells/glom)</td>
<td>1.45±1.77**</td>
<td>0.90±1.27</td>
<td>0.26±0.32</td>
<td>0.53±1.01</td>
</tr>
<tr>
<td>PTC CD8 (+cells/PTC)</td>
<td>1.75±1.21</td>
<td>1.29±1.07</td>
<td>1.72±1.22</td>
<td>1.50±1.19</td>
</tr>
<tr>
<td>G CD4 (+cells/glom)</td>
<td>0.64±1.71</td>
<td>0.41±0.89</td>
<td>0.32±0.54</td>
<td>0.38±0.92</td>
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<tr>
<td>PTC CD4 (+cells/PTC)</td>
<td>1.05±1.23</td>
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<tr>
<td>G CD8 (+cells/glom)</td>
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<td>0.90±1.27</td>
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<td>0.53±1.01</td>
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<tr>
<td>TI CD8 (+cells/hpf)</td>
<td>32.02±16.12</td>
<td>29.31±19.91</td>
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<td>23.58±18.26</td>
</tr>
<tr>
<td>G CD20 (+cells/hpf)</td>
<td>1.75±1.21</td>
<td>1.29±1.07</td>
<td>1.72±1.22</td>
<td>1.50±1.19</td>
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<tr>
<td>G CD20 (+cells/glom)</td>
<td>0.15±0.46</td>
<td>0.09±0.19</td>
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<td>0.04±0.07</td>
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<td>PTC CD20 (+cells/PTC)</td>
<td>0.25±0.44</td>
<td>0.64±1.01</td>
<td>0.24±0.51</td>
<td>0.25±0.44</td>
</tr>
<tr>
<td>TI CD68 (+cells/hpf)</td>
<td>16.81±14.40*</td>
<td>13.64±10.67</td>
<td>10.46±6.59</td>
<td>11.87±12.93</td>
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<td>G CD68 (+cells/glom)</td>
<td>1.45±1.77**</td>
<td>0.90±1.27</td>
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<td>0.53±1.01</td>
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<td>G CD8 (+cells/glom)</td>
<td>1.45±1.77**</td>
<td>0.90±1.27</td>
<td>0.26±0.32</td>
<td>0.53±1.01</td>
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<tr>
<td>PTC CD68 (+cells/PTC)</td>
<td>0.80±1.06</td>
<td>1.79±1.19***</td>
<td>1.14±1.12</td>
<td>0.70±1.03</td>
</tr>
</tbody>
</table>

CTMR, chronic T-cell-mediated rejection; CAHR, chronic active humoral rejection; CNITOX, chronic CNI toxicity; NOS, interstitial fibrosis and tubular atrophy not otherwise specified; TI, tubulointerstitial level; G, glomerular level; PTC, peritubular capillaries.

TGP was found in 19 cases (8 in CAHR, 9 in CTMR and 2 in CNITOX). It was associated with positive C4d staining at the PTC level in 42% of cases. Of the remaining 11 cases of TGP that were not C4d PTC-positive, 6 were associated with positive C4d staining at the glomerular level. We found a significant increase of glomerular CD68+ cells (TGP 1.86±2.21 vs no TGP 0.87±1.14, P=0.02) in cases with g1 TGP compared with cases without TGP. CD68+ cells in g2–3 TGP were numerically higher compared with cases without TGP, although the difference was not statistically significant. No significant difference was found for glomerular CD4+, CD8+ and CD20+ cells.

Discussion

In the present study, we revised all cases of CAN diagnosed at our renal unit in the last 4 years, assigning a new histological diagnosis according to the Banff 2005 classification, and evaluated the renal survival rate of the different types of chronic allograft injury. We observed that CAHR had a poorer prognosis compared with other groups including CTMR. This is, to our knowledge, the first report on allograft survival rates using the new Banff classification. It seems that CTMR behaves ‘more benignly’ compared with chronic injury related to alloantibodies. This observation, if confirmed, may have important clinical implications. Indeed, if an increase in immunosuppression may be justified in cases of CAHR, in ways that remain to be elucidated possibly through large multicentre clinical trials, the poor prognosis of this type of chronic injury, a similar approach may be hazardous in patients with T-cell-mediated chronic injury where the long-term side effects related to increasing immunosuppression may not be justified. We also observed that the presence of TGP and/or CAV was associated with a worse prognosis.

TGP results from a persistent immune-mediated injury (both humoral and cellular) against the glomerular endothelium [8,25–28]. In a few case series of sequential biopsies, glomerular infiltrates (i.e. glomerulitis) seem to precede the occurrence of chronic lesions [8,29]. In our cohort of TGP patients, we found that the number of CD68+ cells was increased in g1 cases in both C4d+ and C4d− cases compared with g0, remarking the role of the cellular component in the development of TGP lesion. TGP has long been recognized as a poor prognostic feature in allograft pathology. In particular, in our cohort of patients, we found that the presence of even the mild form of TGP (Banff g1) was associated with a significantly worse prognosis compared with cases without.

Another observation that should be made is that CAHR was significantly associated with segmental glomerulosclerosis. This should suggest that all cases displaying such a lesion on light microscopy must receive careful examination in order to rule out CAHR before considering it as the result of ‘non-specific’ injury.

In this study, we also characterized glomerular, tubulointerstitial and PTC infiltrating cells in different types of chronic graft injury in the attempt to identify possible expression patterns exploitable for the diagnosis of specific chronic graft lesions. The number of CD8+ cells was significantly higher in the CTMR group at the glomerular level compared with the CNITOX and NOS groups and at tubulointerstitial level, along with CD4+ cells, only when compared with the CNITOX group. The number of CD103+ cells at the tubulointerstitial level was significantly increased in cases with tubulitis compared with cases without tubulitis. On the other hand, CD68+ cells were increased in glomerular and peritubular capillaries in CAHR when compared with other groups.

So far, the diagnosis of CTMR is based on the presence of a specific vascular lesion (so-called ‘CAV’) consisting of intimal thickening with inflammatory cells within the intimal layer and without duplication of internal elastic lamina (this last change being observed in hypertension-associated changes). Sometimes the recognition of such a lesion may be difficult for several reasons (e.g. borderline changes, inadequate sampling, overlapping aetiologic factors that may contribute to the vascular lesions). On the other hand, interstitial infiltrates and tubulitis, when present in an
In conclusion, our data show that the recognition of CAHR in kidney allograft biopsy and the presence of any grade TGP and CAV are poorer prognostic factors. Furthermore, characterization of inflammatory infiltrates in renal tissue may improve the routine evaluation of biopsies in patients with chronic allograft dysfunction and different renal outcomes.

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Conflict of interest statement. None declared.

References

Low-density array PCR analysis of reperfusion biopsies: an adjunct to histological analysis

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Abstract

Background. Histologic evaluation of baseline kidney biopsies is an inconsistent tool to predict graft outcomes, which might be assisted by gene expression analysis.

Methods. We evaluated 49 consecutive kidney graft biopsies obtained post-reperfusion in 18 deceased donors (DD) and 31 living donors (LD) at our center. Biopsies were evaluated and scored using Banff criteria. Low-density real-time polymerase chain reaction arrays were used to measure intragraft expression of 95 genes associated with programmed cell death, fibrosis, innate and adaptive immunity and oxidative stress signaling. A pool of 25 normal kidney biopsies was used as control. We applied a stepwise forward selection procedure to build a multiple regression model predicting estimated glomerular filtration rate (eGFR) at 1 year after transplant using baseline clinical characteristics and gene expression levels.

Results. DD grafts displayed a pattern of gene expression remarkably different from LD, including an increased expression of complement protein C3, and chemokines, CXCL1 and CXCL2, consistent with the proinflammatory setting of ischaemia–reperfusion injury. There was no association between any of the reperfusion biopsy histological features and either renal function at 1 year post-transplant or risk of acute rejection. Conversely, older donor age ($R^2 = 0.17$, $P < 0.001$) and higher integrin $\beta_2$ gene expression levels ($\text{incremental } R^2$ versus Donor Age-only model = 0.23, $P < 0.001$) jointly predicted lower eGFR at 1 year after transplant (multiple regression $R^2 = 0.40$). Patients with higher ITG$\beta_2$ expression levels in baseline biopsies showed lower eGFR, higher levels of proteinuria and more transplant glomerulopathy on the 1-year per-protocol biopsies.

Conclusion. ITG$\beta_2$ gene expression in reperfusion biopsies may represent a prognostic marker for kidney transplant

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