C4d Deposition in Acute Rejection: An Independent Long-Term Prognostic Factor

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Abstract. Peritubular capillary deposition of C4d has been demonstrated to be associated with both acute humoral and vascular rejection and increased graft loss. Whether it is an independent predictor of long-term graft survival rates is uncertain. The biopsies (n = 126) from all patients (n = 93) with a tissue diagnosis of acute rejection that were performed between July 1, 1995, and December 31, 1997, were classified according to Cooperative Clinical Trials in Transplantation (CCTT) criteria. Fresh frozen tissue was immunostained for C4d. There were 58 patients with CCTT type I (interstitial) rejection and 35 with CCTT type II (vascular) rejection. For 34 patients, at least one biopsy exhibited peritubular C4d deposition (C4d+ group). The C4d+ group had proportionately more female patients (P = 0.003), more patients with high (>30%) panel-reactive antibody levels (P = 0.024), more patients with resistance to conventional antirejection therapy (P = 0.010), and fewer patients with postrejection hypertension (P = 0.021) and exhibited a greater rate of graft loss (38 versus 7%, P = 0.001). Peritubular C4d deposition was associated with significantly lower graft survival rates in the CCTT type I rejection group (P = 0.003) and the CCTT type II rejection group (P = 0.003). Multivariate analyses demonstrated that peritubular C4d deposition (P = 0.0002), donor age (P = 0.0002), cold ischemic time (P = 0.0211), and HLA matches (P = 0.0460) were significant independent determinants of graft survival rates. Peritubular C4d deposition is a significant predictor of graft survival rates and is independent of histologic rejection type and a variety of clinical prognostic factors.

Acute allograft rejection, both cellular and humoral, is associated with worsened graft survival rates (1–4). Peritubular capillary deposition of the complement fragment C4d can identify patients who develop acute humoral rejection (3) and those at high immunologic risk, who are more likely to develop graft failure (5), and deposition has been demonstrated to be associated with increased graft loss among patients with acute rejection (6,7). In addition, there is a close correlation between peritubular C4d deposition and vascular rejection (6,7), which itself is a significant risk factor for graft failure (8). Other potential risk factors for poor graft survival that have been observed among patients with acute rejection include postrejection hypertension (2), histologic type (8) and grade (9) of acute rejection, late-occurring acute rejection (2,10), young recipient age (2), old donor age (11), high serum creatinine (Cr) levels before, during, and after acute rejection (2), and increased number of rejection episodes (12). Whether peritubular C4d deposition is a significant predictor of graft failure independent of these clinical and pathologic factors is uncertain.

The primary purpose of this study was to determine the effects of peritubular C4d deposition on long-term renal allograft survival rates and to test its independence as a prognostic factor. The results indicate that peritubular C4d deposition is a significant predictor of long-term graft survival rates and that, as a prognostic factor, it is independent of histologic rejection type and clinical factors demonstrated to be of prognostic significance.

Materials and Methods

Patients

All renal transplant patients in British Columbia with biopsy-proven acute rejection, according to the Cooperative Clinical Trials in Transplantation (CCTT) criteria (8), between July 1, 1995, and December 31, 1997, were selected for the study. The latter date was chosen as an ending date because it would provide a potential follow-up period of at least 24 mo for study patients. Patient and donor data were compiled primarily from the British Columbia Transplant Society renal transplant database. Chart reviews were performed when specific information was not available from the database.

Histologic Analyses

Renal biopsies were divided into three portions, for light-microscopic, electron-microscopic, and immunohistochemical analyses. For light-microscopic analyses, tissue was either fixed in 3% paraformaldehyde or Karnovsky’s fixative and embedded in polyglycol methacrylate (116 biopsies) or fixed in 10% buffered formalin (six biopsies)
or B5 fixative (four biopsies) and embedded in paraffin. Sections embedded in polyglycol methacrylate were cut at 1 μm and stained with hematoxylin-eosin and periodic acid-silver methenamine. Sections embedded in paraffin were cut at 2 μm and stained with hematoxylin-eosin, periodic acid-Schiff stain, and periodic acid-silver methenamine.

The biopsies were examined by two of the authors (Drs. Herzenberg and Magil), without knowledge of the immunohistochemical results or the clinical outcomes. The CCTT criteria for acute interstitial (CCTT type I) and vascular (CCTT type II) rejection were used (8). The Banff 97 classification (13) for the diagnosis and grading of acute rejection was not used because most of the biopsies were embedded in polyglycol methacrylate.

**Immunohistochemical Analyses**

The avidin-biotin-peroxidase complex procedure for antibody localization was used to detect C4d in acetone-fixed sections of snap-frozen renal tissue stained with a commercially available mouse monoclonal antibody specific for complement fragment C4d (Quidel, San Diego, CA). Snap-frozen sections from membranous glomerulonephritis biopsies, which demonstrated strong glomerular staining for C4d, served as positive control samples. Negative control samples consisted of thin basement membrane disease specimens. Additional control studies were performed by omitting the primary monoclonal antibody in the staining procedure and by using an irrelevant mouse monoclonal antibody as the primary antibody.

Biopsies were assessed for C4d immunostaining by two of the authors (Drs. Herzenberg and Magil), without knowledge of the histologic rejection types or the clinical outcomes. Patients were considered positive for C4d if at least one of their biopsies exhibited circumferential staining of at least 25% of the peritubular capillaries. In the few cases in which there were discrepancies regarding C4d positivity, agreement was reached by consensus.

**Clinical Follow-up Monitoring**

The primary end point was graft failure, and times were measured from the date of renal transplantation to the date of permanent dialysis initiation. The following clinical parameters were assessed: (1) patient and donor ages at the time of transplantation; (2) patient gender; (3) graft type, classified as either living related donor, living unrelated donor, or cadaveric; (4) cold and warm ischemic times; (5) postrejection hypertension, defined as elevated average systolic (>140 mmHg) or diastolic (>90 mmHg) BP from 2 mo after acute rejection to the end of the follow-up period (2); (6) serum panel-reactive antibody (PRA) levels of the patients, as determined in standard lymphocytotoxic assays before transplantation; (7) number of previous transplants (the cause of previous graft losses was not assessed); (8) number of HLA matches (zero to six) between donor and recipient HLA types A, B, C, and DR (genotype data were not included); (9) delayed graft function, defined as anuria or dialysis dependence at 1 wk after transplantation; (10) times to first and last rejections, defined as the time from the transplant date to the date of the first and last biopsies demonstrating acute rejection; and (11) baseline and follow-up serum Cr levels. Neither PRA nor anti-HLA antibody levels were measured after transplantation.

Maintenance immunosuppression was achieved with prednisone (0.3 mg/kg per d, tapered in 6 mo to 0.15 mg/kg per d) (93 patients), cyclosporine (9.0 mg/kg per d, adjusted to achieve trough blood levels of 350 to 450 μg/ml for the first 30 to 60 d and gradually tapered to 150 to 250 mg/d for long-term maintenance) (75 patients), or tacrolimus (0.15 mg/kg per d, adjusted to achieve trough blood levels of 8 to 12 μg/ml and gradually tapered to 5 to 10 mg/d for long-term maintenance) (18 patients), with azathioprine (1.5 mg/kg per d) (43 patients) or mycophenolate mofetil (MMF) (1.0 g twice daily) (50 patients). Induction therapy was used for four patients at high risk (PRA levels of >30%) and consisted of a 10-d course of treatment with monoclonal anti-lymphocyte antibodies (5.0 mg/d). CCTT type I rejection was treated with three pulses of Solu-Medrol (500 mg/d for 3 d; Pharmacia & Upjohn, Don Mills, Ontario). Patients with CCTT type II rejection received a 10-d course of treatment with monoclonal anti-lymphocyte antibodies (OKT3, 5.0 mg/d; Ortho Biotech, Raritan, NJ). A full response to therapy was defined as a return of renal function to prerejection baseline Cr levels. A partial response was defined as a decrease in Cr levels of at least 20% from the peak levels but not to prerejection baseline levels.

**Statistical Analyses**

Descriptive statistical values are presented as means ± SD or as medians with 25th and 75th percentile values, depending on the underlying distribution. Continuous variables were compared by using the t test or the Wilcoxon rank-sum test where appropriate, and categorical variables were compared by using the χ² test. A P value of <0.05 for two-sided univariate tests was considered significant.

We examined the association between C4d positivity and CCTT type at both the patient and biopsy levels. Because patients can undergo multiple biopsies during each rejection episode, we used generalized estimating equations to analyze biopsy-level data. This technique is similar to standard regression modeling but is also able to account for repeated measurements (e.g., biopsies) for a patient.

Graft survival rates were estimated by using the Kaplan-Meier method, and survival curves according to C4d positivity and CCTT type were compared by using the log-rank test. The Cox proportional-hazards model was used to identify important predictors of the time to graft loss. The variables included in multivariate analyses were patient age, patient gender, previous transplants, pretransplant serum PRA levels, graft type (cadaveric versus living donor), donor age, number of HLA matches, cold ischemic time, delayed graft function, time to the first episode of acute rejection, number of rejection episodes, postrejection hypertension, C4d deposition, and CCTT rejection type. C4d deposition and CCTT type were modeled as time-dependent covariates.

**Results**

**Renal Biopsy Findings**

The 93 patients in the study underwent a total of 126 biopsies, which indicated 109 acute rejection episodes. Sixty-seven patients underwent one biopsy, 20 patients underwent two biopsies, five patients underwent three biopsies, and one patient underwent four biopsies. For 58 patients, biopsies demonstrated only CCTT type I rejection. For 35 patients, at least one biopsy demonstrated CCTT type II rejection. None of the biopsies demonstrated significant chronic transplant nephropathy or severe acute vascular rejection (CCTT type III rejection). Peritubular capillary staining for C4d (Figure 1) was observed in at least one biopsy for 34 patients. Staining was diffuse (involving >50% of peritubular capillaries) for 28 patients, whereas it was focal (involving 25 to 40% of peritubular capillaries) for six patients. In specimens in which both the cortex and medulla were present, the positive reaction was present in both areas. Fifty-nine patients did not demonstrate any peritubular C4d staining in any of their biopsies. All
biopsies exhibited varying degrees of glomerular, arterial, and arteriolar staining for C4d.

For 43 patients, at least one biopsy demonstrated at least one of the histologic features associated with acute humoral rejection (14). These features include glomerulitis, neutrophils in more than two peritubular capillaries, thrombosis, and infarction. Severe vascular lesions were not observed in any of the biopsies. Peritubular capillary staining for C4d was observed for 27 of those patients (63%).

Focal margination of mononuclear cells in peritubular capillaries was observed in the majority of biopsies. There was no correlation of this finding with positive peritubular capillary C4d staining.

**Patient Characteristics**

**All Patients.** The detailed general characteristics of the entire cohort are presented in Table 1. The age range of the recipients was 7 to 74 yr, with a mean age of 45.8 ± 13.4 yr, whereas that of the donors was 3 to 72 yr. The median follow-up time was 37 mo. Seventeen patients (18%) developed graft failure during the study period.

**C4d+ and C4d− Patients.** The general characteristics and statistical comparisons of the C4d+ and C4d− groups are presented in Table 1. There were proportionately more female patients in the C4d+ group (59%) than in the C4d− group (27%), with the difference being significant ($\chi^2 = 9.14, P = 0.003$). Of the 14 female patients for whom parity information was available, eight were multiparous. Seven of the eight multiparous women exhibited C4d positivity, whereas only one of the six other women exhibited C4d positivity; this difference was significant ($\chi^2 = 4.43, P = 0.0353$). Four C4d+ patients (two female and two male patients) exhibited PRA levels of $>30\%$, whereas none of the C4d− patients did ($\chi^2 = 7.44, P = 0.024$). A significantly higher proportion of C4d+ patients (79%) had at least one biopsy with histologic evidence of acute humoral rejection, compared with the C4d− patients (27%) ($\chi^2 = 23.73, P = 0.001$). A higher percentage of C4d− patients (71%) developed postrejection hypertension, compared with the C4d+ patients (47%) ($\chi^2 = 5.35, P = 0.021$). There was no significant difference between the proportion of C4d+ patients receiving MMF (50%) and the proportion of C4d− patients receiving MMF (56%). Eight of the C4d+ patients (24%) demonstrated no response to treatment, compared with two patients in the C4d− group (3%) ($\chi^2 = 9.16, P = 0.010$).

The times from transplantation to the finding of peritubular capillary C4d deposition ranged from 4 to 61 d. For two C4d+ patients, repeat biopsies were negative for C4d. Those negative biopsies were performed 47 and 67 d after the preceding C4d+ biopsies.

Twenty-four patients exhibited elevated blood cyclosporine levels just before at least one of their biopsies. For three of those patients, focal isometric vacuolation of tubular epithelium was observed in the biopsies. For six of the patients, at least one biopsy was positive for C4d. There was no significant association between elevated blood cyclosporine levels and positive peritubular capillary C4d staining.

Fourteen C4d+ patients were treated with OKT3. Seven exhibited no response to this therapy.

![Figure 1. Immunostaining of a renal biopsy for C4d. There is positive staining of the cortical peritubular capillaries for C4d. A glomerulus at the lower right demonstrates strong staining for C4d. Magnification, ×200.](image-url)


Patients with CCTT Type I and Type II Rejection. There were 58 patients with CCTT type I rejection, 18 of whom were C4d⁺, and 35 patients with CCTT type II rejection, 16 of whom were C4d⁻. There were proportionately more female patients in the CCTT type I rejection/C4d⁺ subgroup (72%) than in the CCTT type I rejection/C4d⁻ subgroup (25%) (χ² = 11.57, P = 0.001). In the group with CCTT type II rejection, there were no significant differences in the male/female ratios between the C4d⁺ and C4d⁻ subgroups. A higher proportion of C4d⁺ patients with CCTT type I rejection (11%) exhibited PRA levels of 30%, compared with the CCTT type I rejection/C4d⁻ subgroup (0%) (χ² = 4.60, P = 0.032). Similarly, more C4d⁺ patients with CCTT type II rejection (13%) exhibited PRA levels of >30%, compared with the CCTT type II rejection/C4d⁻ subgroup, but the difference was not significant, because of the smaller number of patients with CCTT type II rejection. There were no significant differences between the C4d⁺ and C4d⁻ subgroups, for each CCTT rejection type, with respect to the mean recipient and donor ages, graft types, mean cold and warm ischemic times, mean number of donor-recipient HLA matches, proportion of patients with delayed graft function, median time to first rejection, proportion of patients with postrejection hypertension, and proportion of patients with a second transplant.

Four of the patients with CCTT type I rejection would have been classified as exhibiting suspicious findings with the Banff 97 criteria (13). One of those patients was C4d⁺ and experienced graft loss. The others were C4d⁻ and all had functioning grafts at the end of the study period.

Association of C4d Deposition with Rejection Type

There was an association between the type of rejection and C4d staining, as assessed at the biopsy level. Biopsies demonstrating CCTT type I rejection were C4d⁺ in 24% of cases; biopsies demonstrating CCTT type II rejection were C4d⁺ in 46% of cases (χ² = 6.73, P = 0.009). When we used generalized estimating equation modeling, the CCTT rejection type was a significant explanatory variable for C4d positivity (β = 1.03, P = 0.010) (after inclusion of the

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Table 1. Patient population characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Patients (n = 93)</th>
<th>C4d⁺ Patients (n = 34)</th>
<th>C4d⁻ Patients (n = 59)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient gender (n female)</td>
<td>36 (39%)</td>
<td>20 (59%)</td>
<td>16 (27%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Recipient age (yr)</td>
<td>45.8 ± 13.4</td>
<td>45.8 ± 10.7</td>
<td>45.7 ± 14.9</td>
<td>0.98</td>
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<tr>
<td>Previous renal transplant (n)</td>
<td>14 (15%)</td>
<td>5 (15%)</td>
<td>9 (15%)</td>
<td>0.94</td>
</tr>
<tr>
<td>PRA level before transplantation (n)</td>
<td></td>
<td></td>
<td></td>
<td>0.024</td>
</tr>
<tr>
<td>0%</td>
<td>79 (85%)</td>
<td>26 (76%)</td>
<td>53 (90%)</td>
<td></td>
</tr>
<tr>
<td>1 to 30%</td>
<td>10 (11%)</td>
<td>4 (12%)</td>
<td>6 (10%)</td>
<td></td>
</tr>
<tr>
<td>&gt;30%</td>
<td>4 (4%)</td>
<td>4 (12%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Graft type (n)</td>
<td></td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>cadaveric</td>
<td>74 (80%)</td>
<td>29 (85%)</td>
<td>4 (76%)</td>
<td></td>
</tr>
<tr>
<td>LUD</td>
<td>8 (9%)</td>
<td>3 (9%)</td>
<td>5 (8%)</td>
<td></td>
</tr>
<tr>
<td>LRD</td>
<td>11 (12%)</td>
<td>2 (6%)</td>
<td>9 (15%)</td>
<td></td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>36.7 ± 17.0</td>
<td>36.7 ± 17.8</td>
<td>36.7 ± 16.7</td>
<td>0.99</td>
</tr>
<tr>
<td>Cold ischemic time (min)</td>
<td>742 ± 440</td>
<td>795 ± 432</td>
<td>711 ± 445</td>
<td>0.38</td>
</tr>
<tr>
<td>Warm ischemic time (min)</td>
<td>32 ± 6</td>
<td>31 ± 7</td>
<td>32 ± 6</td>
<td>0.49</td>
</tr>
<tr>
<td>Number of donor-recipient HLA matches</td>
<td>3.7 ± 1.8</td>
<td>3.4 ± 2.1</td>
<td>3.9 ± 1.6</td>
<td>0.13</td>
</tr>
<tr>
<td>Delayed graft function (n)</td>
<td>15 (16%)</td>
<td>5 (15%)</td>
<td>10 (17%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Baseline serum creatinine level (µM)</td>
<td>244 ± 187</td>
<td>283 ± 196</td>
<td>221 ± 178</td>
<td>0.12</td>
</tr>
<tr>
<td>Time to first acute rejection (d)</td>
<td>12 (7 to 30)</td>
<td>10 (7 to 30)</td>
<td>15 (7 to 32)</td>
<td>0.85</td>
</tr>
<tr>
<td>Acute humoral rejection (n)</td>
<td>43 (46%)</td>
<td>27 (79%)</td>
<td>16 (27%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Response to treatment of acute rejection (n)</td>
<td></td>
<td></td>
<td></td>
<td>0.010</td>
</tr>
<tr>
<td>no response</td>
<td>10 (11%)</td>
<td>8 (24%)</td>
<td>2 (3%)</td>
<td></td>
</tr>
<tr>
<td>partial</td>
<td>18 (19%)</td>
<td>6 (18%)</td>
<td>12 (22%)</td>
<td></td>
</tr>
<tr>
<td>full</td>
<td>65 (70%)</td>
<td>20 (59%)</td>
<td>45 (76%)</td>
<td></td>
</tr>
<tr>
<td>Postrejection hypertension (n)</td>
<td>58 (62%)</td>
<td>16 (47%)</td>
<td>42 (71%)</td>
<td>0.021</td>
</tr>
<tr>
<td>Number of rejections</td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>one</td>
<td>78 (84%)</td>
<td>29 (85%)</td>
<td>49 (83%)</td>
<td></td>
</tr>
<tr>
<td>two</td>
<td>11 (12%)</td>
<td>2 (6%)</td>
<td>9 (15%)</td>
<td></td>
</tr>
<tr>
<td>three</td>
<td>4 (4%)</td>
<td>3 (9%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Time to last acute rejection (d)</td>
<td>17 (8 to 45)</td>
<td>13 (8 to 45)</td>
<td>18 (9 to 52)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

*PRA, panel-reactive antibody; LUD, living unrelated donor; LRD, living related donor.
number of biopsies each patient had undergone), confirming the association between CCTT rejection type and C4d positivity.

**C4d Positivity and Graft Survival Rates**

Significantly more C4d\(^+\) patients (13 of 34 patients, 38\%) lost their grafts during the study period, compared with the C4d\(^-\) group (4 of 59 patients, 7\%) ($\chi^2 = 14.29$, $P = 0.001$). Although there were significantly more female patients than male patients in the C4d\(^+\) group, a greater proportion of male patients (8 of 14 male patients, 57\%) lost their grafts than did female patients (5 of 20 female patients, 25\%); the difference was not significant. Similarly, the risk of graft loss during the study period was much higher within the subgroup of patients with type I rejection who were C4d\(^+\). Six of 18 C4d\(^+\) patients (33\%) with CCTT type I rejection experienced graft loss, whereas only two of 40 C4d\(^-\) patients (5\%) with CCTT type I rejection lost their grafts ($\chi^2 = 8.38$, $P = 0.004$). Among patients with type II rejection, seven of the 16 C4d\(^-\) patients (44\%) experienced graft loss, compared with two of the 19 C4d\(^-\) patients (11\%) ($\chi^2 = 5.02$, $P = 0.025$). Of the six patients (all with CCTT type I rejection) whose biopsies were focally positive for C4d, two (33\%) lost their grafts, a result identical to that for patients with CCTT type I rejection and diffuse C4d\(^+\) staining. Nine of 35 patients (26\%) with CCTT type II rejection and eight of 58 patients (14\%) with CCTT type I rejection lost their grafts during the study period ($\chi^2 = 2.08$, $P = 0.150$).

Follow-up Cr levels were significantly higher for the C4d\(^+\) patients (382 ± 282 \(\mu\)M), compared with the C4d\(^-\) patients (197 ± 149 \(\mu\)M) ($P = 0.004$). This difference became insignificant when the patients with graft loss were excluded (C4d\(^+\), 131 ± 50 \(\mu\)M; C4d\(^-\), 165 ± 85 \(\mu\)M).

Twelve (44\%) of the 27 patients whose biopsies were C4d\(^+\) and exhibited at least one of the histologic changes associated with acute humoral rejection lost their grafts, whereas only 2 of the 16 patients (13\%) whose biopsies were C4d\(^-\) but exhibited histologic evidence of acute humoral rejection experienced graft loss; this difference was significant ($\chi^2 = 4.67$, $P = 0.031$).

Estimated 6-mo, 1-yr, and 2-yr cumulative renal allograft survival rates were 76, 71, and 68\%, respectively, for C4d\(^+\) patients and 98, 95, and 93\%, respectively, for C4d\(^-\) patients ($P = 0.0002$, log-rank test) (Figure 2). Among patients with either CCTT type I or type II rejection, the cumulative graft survival rates were significantly lower for those who were C4d\(^+\) ($P = 0.003$ for CCTT type I rejection, $P = 0.033$ for CCTT type II rejection, log-rank test) (Figure 3).

Four patients underwent repeat biopsies after biopsies that were positive for C4d. In two cases, the positive reaction persisted in two or three repeat biopsies. One of those patients experienced graft loss. In one case, the C4d staining was observed in two consecutive biopsies but disappeared in the third. In another case, the repeat biopsy was negative for C4d. Graft loss occurred for both of those patients.

We tested the prognostic value of CCTT rejection type among patients, acknowledging that patients with acute vascular rejection were treated more aggressively (usually with monoclonal anti-lymphocyte antibody). Similarly, patients with high pretransplant serum PRA levels were treated with more aggressive immunosuppression (with OKT3) from the time of transplantation. Two of four patients with PRA levels

![Figure 2. Kaplan-Meier curves of graft survival rates for C4d\(^+\) and C4d\(^-\) patients. The C4d\(^+\) group exhibited a significantly lower graft survival rate ($P = 0.0002$, log-rank test).](image-url)
of >30% exhibited CCTT type II rejection, whereas two exhibited CCTT type I rejection. Patients with CCTT type I rejection exhibited higher cumulative graft survival rates (86%) than did patients with CCTT type II rejection (74%), but the difference did not reach statistical significance (P = 0.13).

C4d deposition remained a significant independent predictor of graft failure in Cox proportional-hazards multivariate analyses (Table 2). The adjusted risk ratio of graft failure for C4d+ patients was 8.7 (95% confidence interval, 2.8 to 27.3; P = 0.0002). Other independent predictors of graft failure were donor age, number of HLA matches, and cold ischemic time (Table 2).

Discussion

The results of this retrospective study establish for the first time that peritubular capillary C4d deposition in acute allograft rejection is independent of histologic rejection type (interstitial or vascular), and they confirm the significance of C4d deposition as a predictor of long-term graft failure. Peritubular C4d deposition was also demonstrated to be independent of a number of clinical variables that are considered to be prognostically significant. These factors include postrejection hypertension (2), delayed graft function (15,16), late occurrence of acute rejection (10), donor age (11), and HLA matches (17,18).

A limitation of this study was that alloantibody assessment of patient serum samples obtained at the time of biopsy could not be performed. Therefore, we were unable to determine whether an association between peritubular C4d deposition and circulating alloantibodies existed for our patients. Previous studies demonstrated an association between these two factors (3,5,7), suggesting that peritubular C4d deposition might serve as a marker for humoral rejection (and thus might possibly not be an independent prognostic factor). However, in one large series, circulating alloantibodies were not demonstrated for 44% of patients with peritubular C4d deposition in their grafts (19), raising some uncertainty regarding the nature of the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Risk Ratio (95% Confidence Interval)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritubular C4d deposition</td>
<td>8.72 (2.24 to 19.03)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Donor age</td>
<td>1.06 (1.03 to 1.10)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Cold ischemic time</td>
<td>1.10 (1.01 to 1.19)</td>
<td>0.0211</td>
</tr>
<tr>
<td>Number of donor-recipient HLA matches</td>
<td>0.73 (0.54 to 0.99)</td>
<td>0.0460</td>
</tr>
</tbody>
</table>
relationship between C4d deposition and alloantibodies, especially with respect to the independence of C4d as a prognostic factor.

Within the CCTT type I rejection subgroup with positive C4d staining, one third of the patients lost their grafts. Evaluation of the histologic parameters failed to reveal any significant differences between patients with graft failure and those with graft survival that would allow more accurate prediction of the course for individual recipients. In addition, the C4d+ patients with CCTT type I rejection whose grafts survived did not exhibit significantly worse renal function, compared with the C4d− patients with CCTT type I rejection and surviving grafts.

C4d is a complement fragment that is generated through classic pathway activation and is covalently bound to antigen. The results of several studies suggest that peritubular capillary C4d deposition is a marker for acute humoral rejection (3,5,7). In this study, peritubular C4d deposition was observed significantly more frequently in biopsies demonstrating at least one of the histologic markers of acute humoral rejection (14), which is consistent with the findings of two of the aforementioned investigations (3,7). In addition, in this investigation, peritubular C4d deposition was observed in the biopsies of all patients with high (>30%) PRA levels.

In this study, peritubular C4d deposition was associated with the female gender. The association of multiparity with C4d positivity in this study suggests that prior pregnancies in which there was exposure to foreign HLA antigens increase the risk of development of donor-specific antibodies and acute humoral rejection, through an anamnestic response that is not detected by conventional pretransplant PRA testing. This finding was reported for one multiparous woman who received a renal allograft from her spouse (20).

The deleterious effect of the male gender on renal function was previously reported for renal transplant recipients (20,21) and patients with a variety of diseases in their native kidneys (22–25). Sex hormones have been postulated to be one of the main causes of the gender difference in disease progression (25).

There were four patients whose biopsies met the CCTT criteria for acute rejection but would have been placed in the suspicious or borderline category on the basis of Banff 97 standards. The significance of borderline lesions is controversial. The results of some studies have suggested that they represent acute rejection reactions (9,26,27), whereas another study reported a high rate of remission without specific anti-rejection therapy (28). As in the former studies (8,26,27), the four patients referred to above exhibited no other cause for the acute increases in serum Cr levels and all responded fully or partially to pulse Solu-Medrol therapy, suggesting that they did experience episodes of acute rejection.

Cosio et al. (2) have drawn attention to the correlation of postrejection hypertension with reduced graft survival rates. An unexpected finding in this study was the inverse association of C4d positivity with postrejection hypertension. A possible explanation for this result may involve the observation of mildly reduced BP in association with elevated serum C4d levels among a group of cardiopulmonary bypass patients after protamine administration (29).

Because conventional antirejection therapy seems to be inadequate for many patients with peritubular C4d deposition (3,30), an alternate therapeutic approach, using plasma exchange with tacrolimus-MMF rescue, has been proposed (30). Whether this form of therapy will modify the long-term outcomes for patients with C4d+ acute rejection warrants further investigation.

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References