Banff criteria as predictors of outcome following acute renal allograft rejection


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Abstract

Background. The Banff classification of renal allograft rejection grades acute tubulointerstitial rejection (AIR) by severity of tubulitis and acute vascular rejection (AVR) by severity of arteritis. The intensity of tubulitis has not, however, been demonstrated to be of prognostic value and other features such as glomerulitis and eosinophil infiltration are of uncertain significance. This study was performed in order to determine the clinical value of this pathological classification.

Methods. Banff criteria were correlated with outcome in 134 consecutive graft recipients transplanted in our unit over a 3-year period (1994–1996) who experienced at least one biopsy-confirmed acute rejection episode. Of 197 biopsies performed for the diagnosis of rejection, 177 contained at least one artery and were suitable for Banff grading. Tissue eosinophil counts were available for 101 biopsies. Clinical severity of rejection was classified as mild (fully responsive to pulse steroid therapy), moderate (partially steroid responsive) and severe (steroid unresponsive/requiring ATG therapy).

Results. Graft failure ensued in 18 of 58 patients with AVR compared with 10 of 65 patients with AIR (P = <0.05). Clinical severity of rejection correlated with the presence of arteritis, but not severity of tubulitis; rejections graded I, IIA and IIB according to the Banff’93 classification were clinically severe in 3/68 (4%), 2/28 (7%) and 15/67 (22%) respectively (P = <0.05). The presence of glomerulitis showed no correlation with clinical severity or graft loss. Tissue eosinophilia (>10 eosinophils/mm²) was present in 18 of 33 patients who had at least one episode of AVR (v1/2), compared with 11 of 45 patients who suffered only AIR (P = <0.02).

Conclusions. We conclude that: arteritis, but not severe tubulitis or glomerulitis, is an adverse prognostic factor in acute rejection and that tissue eosinophilia is associated with vascular rejection. Our findings support the 1997 revision of the Banff classification, replacing grades with types of acute rejection.

Key words: acute rejection; Banff classification; eosinophilia; renal transplant

Introduction

The renal allograft biopsy plays an important role in the management of graft dysfunction; it is essential for an accurate diagnosis of rejection and may also demonstrate features of rejection which have prognostic and therapeutic implications. Identification of such features has been hindered in the past by the subjectivity of histological assessment. The Banff classification of renal allograft rejection [1] was introduced in an attempt to achieve greater accuracy and uniformity in the histological definition of rejection and in addition to produce a clinically meaningful grading system. In this classification acute tubulointerstitial rejection (AIR) is defined by lymphohistiocytic infiltration in the interstitium (i score) with a lymphocytic tubulitis (t score). Acute vascular rejection (AVR) is defined by an intimal or transmural arteritis (v score). Each of these criteria is graded variably 0–3 and in the original Banff schema (Banff’93) acute rejection is then graded according to severity of both tubulitis and arteritis. Grade II acute rejection encompasses severe tubulitis without arteritis and mild/moderate arteritis. Glomerulitis (g score) and the presence of other inflammatory cells, such as neutrophils and eosinophils, are also noted but their presence is of uncertain significance and is not used in the grading of rejection. While several studies have provided objective evidence that the presence of acute vascular rejection is an adverse prognostic factor, both in the short- and long-term [2–8], the importance of severity of tubulitis is less certain. In recognition of this uncertainty it was proposed to replace grades with types of rejection at the 1997 Banff conference of renal allograft pathology (Banff’97).

In this study, Banff criteria are correlated with outcome in order to determine the clinical value of this classification system. In particular, we aim to determine the clinical significance of severity of tubulitis, arteritis and glomerulitis and of eosinophilia within the graft.
Methods

Renal biopsies

All graft recipients who were transplanted on our unit over a 3-year period (1994–1996) and who suffered at least one biopsy-confirmed acute rejection (n=134) were included in the study. Biopsies were assessed using the Banff criteria by a single renal pathologist (ISDR) who was blinded to the clinical data. Of 197 biopsies performed to confirm or refute a diagnosis of acute rejection in these patients, 177 were suitable for grading (contained at least eight glomeruli and one arterial cross-section). Biopsies which showed chronic transplant nephropathy (CTN) in addition to acute rejection were excluded from analysis. All biopsies were performed for graft dysfunction; there were no protocol biopsies from normally functioning grafts. Tissue eosinophil counts were available for 101 biopsies (1994–1995). The numbers of eosinophils were counted in haematoxylin and eosin (H&E)-stained sections at high power (40 × obj) over the entire renal cortex in each biopsy, minimum 10 high power fields (hpf). Tissue eosinophilia was defined as >4 eosinophils/hpf (≥ >10 eosinophils/mm²).

Clinical data

The following data were obtained from review of case notes: recipient age and sex, history of allergy, CMV status, transplant number, HLA mismatch, donor status (cadaveric vs living related/unrelated), cold ischaemic time, presence of delayed graft function (DGF), initial immunosuppression, number and timing of acute rejection episodes (all were biopsy confirmed) and presence and duration of blood eosinophilia (>0.4 × 10⁹/l). Follow-up was for a minimum of 1 year. The outcome measures considered were clinical severity (reversibility) of rejections, graft failure and serum creatinine at 1 and 2 years; rejection episodes were classified as mild (return of serum creatinine to within 10% of pre-rejection value following pulse steroid therapy), moderate (serum creatinine remains >10% above the pre-rejection low following pulse steroid therapy) and severe (rejections that were steroid unresponsive and require the use of ATG or OKT3 therapy). Graft failure was defined by a permanent return to dialysis dependence.

Statistics

All data were analysed using SPSS for Windows software. Chi-squared was used to compare the different categories of clinical and rejection variables, and outcome measures.

Results

Clinical variables

The mean age was 40 years (range: 18–52 years) and 91 patients (68%) were male. One hundred and eighteen grafts (88%) were from cadaveric donors and 16 from living related donors. The mean cold ischaemic time was 23 h (range: 15 min to 44 h) and incidence of delayed graft function was 22.4%. The mean number of mismatches at the HLA A/B loci was 1.6. Seventy-one patients (53%) had a single DR mismatch, no patient had two mismatches at the DR locus. Initial immunosuppression was triple therapy (cyclosporin, azathioprine and prednisolone) in the 21 patients with DGF secondary to acute tubular necrosis (ATN), cyclosporin monotherapy in 84 patients and tacrolimus monotherapy in 29 patients.

Eighty-three patients suffered a single rejection episode, 39 suffered two, nine suffered three and three suffered four rejection episodes. The median time of acute rejection was 20 days post-transplant (range 3–346 days). ‘Late’ acute rejections were associated with a poor prognosis; 11 of 27 patients experiencing acute rejection after 2 months post-transplant suffered graft loss, compared with 21 of 107 patients who only experienced acute rejection within the first 2 months (P = <0.05). Five of 12 patients (42%) with more than two rejection episodes suffered graft loss, compared with 26 of 122 (21%) of patients with one or two rejection episodes. There was no correlation between histological type of rejection, serum creatinine at 1 and 2 years post-transplant, graft loss or number of rejections and cold ischaemic time, delayed graft function, primary immunosuppression, type of donor (cadaveric vs living related) or CMV status of donor and recipient.

Histological variables

Type of rejection vs graft loss. Banff grades were recorded for 123 patients; biopsies in 11 patients did not contain an artery and were, therefore, unsuitable for full Banff grading. Taking the highest grade for patients who experienced multiple rejection episodes, 65 suffered AIR (42 t2; 19 t3) and 58 AVR. Eighteen of 58 patients with AVR suffered graft loss, compared with 10 of 65 patients with AIR (P = <0.05). In patients with AIR there was no correlation between graft loss and severity of tubulitis, presence of glomerulitis or extent of interstitial inflammation (Table 1). There was no association between type or grade of worst acute rejection, serum creatinine at 1 or 2 years or biopsy-proven CTN.

Banff score vs clinical severity of rejection. As defined by reversibility, 122 (59.2%) rejections were mild, 54 (26.2%) moderate and 23 (11.2%) were severe. Of the 177 biopsies suitable for Banff grading, 96 showed AIR, 73 AVR and eight borderline changes. The latter group was excluded from further analysis. The presence of a vascular component, but not severe tubulitis, was associated clinically with more severe rejection (Table 2). There was no association between severity of tubulitis and presence or severity of arteritis. Fifty-two of 71 biopsies with AVR showed a glomerulitis, compared with 27 of 102 with AIR/borderline change (P = <0.0001). Neither the extent of interstitial mononuclear cell infiltrate nor presence of glomerulitis correlated with clinical severity (Table 2).
Table 1. Banff criteria in the highest grade of rejection experienced by each patient vs graft loss

<table>
<thead>
<tr>
<th>Graft loss</th>
<th>v0</th>
<th>v1–3</th>
<th>t2</th>
<th>t3</th>
<th>g0</th>
<th>g1–3</th>
<th>i1</th>
<th>i2</th>
<th>i3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>10</td>
<td>18</td>
<td>18</td>
<td>10</td>
<td>13</td>
<td>16</td>
<td>1</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>No</td>
<td>55</td>
<td>40</td>
<td>57</td>
<td>30</td>
<td>49</td>
<td>45</td>
<td>5</td>
<td>33</td>
<td>59</td>
</tr>
</tbody>
</table>

v0 vs v1–3 $P < 0.05$; t, g, i scores n.s.

Table 2. Banff criteria vs clinical severity of rejection

<table>
<thead>
<tr>
<th>Clinical severity</th>
<th>t2,v0 (I)</th>
<th>t3,v0 (IIA)</th>
<th>v1/2 (IIB)</th>
<th>v3 (III)</th>
<th>i1</th>
<th>i2</th>
<th>i3</th>
<th>g0</th>
<th>g1–3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>47</td>
<td>18</td>
<td>35</td>
<td>2</td>
<td>5</td>
<td>42</td>
<td>58</td>
<td>58</td>
<td>46</td>
</tr>
<tr>
<td>Moderate</td>
<td>18</td>
<td>8</td>
<td>17</td>
<td>3</td>
<td>5</td>
<td>20</td>
<td>25</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
<td>2</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>14</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

t2 vs t3 n.s.; v0 vs v1/2/3 $P < 0.01$.

developed AVR. This latter group was analysed separately as it is possible that the diagnosis of AVR was missed in the initial biopsy due to the absence of an affected vessel. Of the biopsies showing AVR 39.5% revealed eosinophilia, compared with 19.6% of those showing AIR ($P < 0.05$; Table 3). Eighteen of 33 patients who had at least one episode of AVR revealed tissue eosinophilia, compared with 11 of 45 patients who suffered only AIR ($P < 0.02$). Eosinophil infiltration was predominantly within the interstitium but there was focal infiltration of tubules and arterial intima. Typically, it was patchy and often most marked at the corticomедulillary junction. Tissue eosinophilia did not correlate with the extent of interstitial mononuclear cell infiltrate or presence of glomerulitis (Table 3). Twelve biopsies showing AIR were from patients who subsequently suffered AVR; nine of these showed tissue eosinophilia, compared with 10 of 51 biopsies showing AIR from patients who never showed AVR ($P < 0.001$).

Tissue eosinophilia was present in 31 of 88 (35%) early (<60 day) rejections and one of 13 (8%) late (>60 day) rejections ($P < 0.05$). Similarly blood eosinophilia was present in 59% of early acute rejections, compared with only 22% of late acute rejections ($P < 0.001$). This difference may be due to a greater proportion of patients receiving maintenance corticosteroids after 60 days as tissue eosinophilia was associated most frequently with cyclosporin monotherapy; 42% of biopsies showing rejection from patients receiving cyclosporin monotherapy showed eosinophilia, compared with only 8% of biopsies from patients on triple therapy ($P < 0.05$; Table 4). Blood eosinophilia was significantly associated with both tissue eosinophilia and with cyclosporin and tacrolimus monotherapy (Table 5). Ten of 30 patients (33%) whose rejections were associated with tissue eosinophilia suffered graft loss, compared with 10 of 52 patients (19%) who never showed tissue eosinophilia. This difference did not reach statistical significance. Typically, blood eosinophil levels rose from 1 to several days before the diagnosis of rejection and fell rapidly (within 1 day), to almost zero, with pulse steroid therapy. Neither blood nor tissue eosinophilia were associated with a history of vasculitis, allergy or drug-related hypersensitivity.

Discussion

The Banff classification has proved to be a workable and clinically relevant scoring system for histological

Table 3. Banff grade vs tissue eosinophilia

<table>
<thead>
<tr>
<th>Banff criteria (Banff '93 grade)</th>
<th>t2,v0 (I)</th>
<th>t3,v0 (IIA)</th>
<th>v1/2 (IIB)</th>
<th>i1</th>
<th>i2</th>
<th>i3</th>
<th>g0</th>
<th>g1–3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils $&gt;10/\text{mm}^2$</td>
<td>6</td>
<td>4</td>
<td>15</td>
<td>2</td>
<td>5</td>
<td>21</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Eosinophils $&lt;10/\text{mm}^2$</td>
<td>29</td>
<td>12</td>
<td>23</td>
<td>0</td>
<td>30</td>
<td>40</td>
<td>41</td>
<td>29</td>
</tr>
</tbody>
</table>

t2 vs t3 n.s.; v0 vs v1/2 $P < 0.05$; i and g scores n.s.
Table 4. Immunosuppressive therapy vs tissue eosinophilia

<table>
<thead>
<tr>
<th>Initial immunosuppressive therapy</th>
<th>Cyclosporin monotherapy</th>
<th>Triple therapy</th>
<th>Tacrolimus monotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils &gt; 10/mm²</td>
<td>25</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Eosinophils &lt; 10/mm²</td>
<td>34</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

Cyclosporin vs triple therapy \(P = < 0.05\); tacrolimus vs triple therapy n.s.

Table 5. Blood eosinophilia vs tissue eosinophilia and initial immunosuppression

<table>
<thead>
<tr>
<th>Blood eosinophils</th>
<th>Blood eosinophils</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&gt; 0.4 \times 10^9/l)</td>
<td>(&lt; 0.4 \times 10^9/l)</td>
<td></td>
</tr>
<tr>
<td>Tissue eosinophils &gt; 10/mm²</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>Tissue eosinophils &lt; 10/mm²</td>
<td>30</td>
<td>58</td>
</tr>
<tr>
<td>Cyclosporin monotherapy</td>
<td>47</td>
<td>37</td>
</tr>
<tr>
<td>Triple therapy</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Tacrolimus monotherapy</td>
<td>17</td>
<td>12</td>
</tr>
</tbody>
</table>

classification of renal allograft rejection [9]. Interobserver variation is generally low [10] and there is evidence that the classification has increased the consistency of diagnosis of rejection, although possibly not the accuracy [11]. In the present study we aimed to determine which of the Banff criteria have clinical relevance in terms of predicting outcome following acute rejection. The Banff criteria are under continual review and at the 1997 Banff conference a modified schema was proposed in the light of data from clinical trials and from the use of the NIH CCTT classification [12]. It was acknowledged that the clinical relevance of severity of tubulitis is unknown and, as a consequence, in the Banff’97 classification (13) grades are replaced with types of rejection. Our findings would support such a modification. In agreement with previous studies in the pre- and post-cyclosporin era [2–7] we find that the presence of acute vascular rejection, as defined by an intimal or transmural arteritis, is predictive of steroid-resistant rejection and an increased likelihood of graft loss. The relevance of severity of tubulitis is less certain. Gaber et al. [5], in a study of 56 episodes of acute rejection, reported that mean tubulitis score was significantly higher for irreversible rejection. While they did not determine whether patients whose biopsies showed severe tubulitis had a poorer outcome than those with moderate tubulitis, they did find that severity of tubulitis was not predictive of steroid sensitivity. In this study we found no difference in steroid sensitivity or graft loss for up to 2 years following rejection episodes with moderate or severe tubulitis. Similarly, we did not find the presence of glomerulitis to be of prognostic value. Previous studies reporting the significance of glomerulitis have provided conflicting findings; two earlier studies showed acute glomerulitis [14,15] to be associated with a poor outcome, as did the evaluation of the CCTT criteria [12], while a recent study by Olsen et al. [16] did not. Furthermore, we did not find the extent of interstitial inflammation to be related to outcome, which is in agreement with previous reports [12,17,18]. One possible explanation for these apparently conflicting published results is that the significance of the Banff criteria may depend, in part, on the clinical situation in which the biopsies were performed. This certainly applies to biopsies which show a borderline infiltrate; in the context of graft dysfunction ‘borderline changes’ often represent acute rejection, as indicated by response to anti-rejection therapy [19,20], while a mild or moderate tubulitis has also been found in a high proportion of protocol biopsies from normally functioning grafts [21]. The baseline immunosuppressive therapy and timing of biopsy during an acute rejection episode may have a similar impact. There is considerable variation between centres in the readiness to perform a transplant biopsy; in some biopsies are performed at the earliest indication of graft dysfunction; in others biopsy is delayed until there is a high clinical suspicion of rejection, while in some centres biopsy may not be undertaken until after initiation of anti-rejection therapy.

In the Banff classification the presence of large numbers of eosinophils, neutrophils or plasma cells within the interstitial infiltrate is recorded by appending an asterisk to the ‘i’ score but it is not included in the grading system. An infiltrate of neutrophils is often related to damaged tubules or incipient cortical necrosis. The former may reflect either a reaction to severe tubular injury due to rejection or concurrent bacterial infection. The presence of large numbers of eosinophils within the leucocytic infiltrate of rejection is one feature which is easily identified in routine transplant biopsies but is of unknown clinical significance. Two fine-needle aspiration and three histology-based studies of eosino-
philia following renal transplantation have demonstrated an association between tissue and/or blood eosinophilia and acute rejection [22–26]. Four of these studies also reported that the presence of increased numbers of eosinophils within the transplant kidney is associated with irreversible rejection and increased graft loss [23–26]. These findings may reflect an association between eosinophilia and severe acute rejection as defined by the other generally accepted histological criteria, such as arteritis. Only one published study has investigated the association between eosinophilia and grade of rejection. This did not use the Banff criteria but found no correlation between tissue eosinophilia and presence of eosinophilia; the immunosuppressive protocol may have altered these apparently contradictory results. First, as we have stated, the immunosuppressive protocol may have a major impact on the presence of eosinophilia; the inclusion of steroids in maintenance immunosuppression is associated with greatly reduced numbers of eosinophils within the both the graft and blood. This may also be true for other histological variables and highlights the dangers of interpreting clinicopathological data independent of immunosuppressive protocol. Most of our patients received cyclosporin or tacrolimus monotherapy and none had ATG induction; in the study by Hongwei et al. [26] all patients received ATG induction followed by triple therapy (cyclosporin, azathioprine, prednisolone). A second confounding factor is that there is no conformity to the definition of tissue eosinophilia; of all previous histology-based reports only that of Hongwei et al. quantifies numbers of eosinophils in a manner which enables comparison (number of cells/section area). They define significant eosinophilia as >1/mm², whereas in our study, in which eosinophil counts are generally much higher, we define eosinophilia as >10/mm². We find that within the group of patients showing acute vascular rejection, blood or tissue eosinophilia is not an adverse prognostic factor. This suggests that the significance of eosinophilia demonstrated by others may reflect an association with a more severe histological grade of rejection. The association between AVR and eosinophilia may be of diagnostic value in identifying cases of possible vascular rejection, which may otherwise have been missed; histological features of rejection are not uniform throughout the transplant kidney and may not be represented in a small needle core biopsy. This is particularly true for vascular rejection because appropriately sized vessels are commonly not present to enable the diagnosis to be made. We have found that eosinophilia in biopsies showing pure AIR is highly predictive of recurrent acute rejection with a vascular component.

In conclusion, we have demonstrated that the presence of an intimal arteritis, but not severity of glomerulitis and tubulitis or extent of interstitial inflammation, is associated with steroid-resistant rejection and increased graft loss in the short-term (up to 2 years post-transplant). Eosinophilia within the graft is associated with vascular rejection and steroid-free immunosuppressive therapy, but is not of independent prognostic significance. This is the first large historical study in which the majority of patients received either CsA or tacrolimus monotherapy and our findings highlight the potential impact of the immunosuppressive protocol on the histology of graft rejection. The prognostic significance of the histological criteria may alter as new immunosuppressive agents are introduced. We would, therefore, advocate that recording the severity of tubulitis, glomerulitis and interstitial inflammation be continued. Furthermore, while acute vascular rejection appears to correlate with chronic graft loss [27], the relevance of the other Banff criteria to the long term outcome of the graft has yet to be determined.

References

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