Identification of lesions indicating rejection in kidney transplant biopsies: tubulitis is severely under-detected by conventional microscopy

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Abstract

Background. In the current international Banff classification of kidney transplant rejection, tubulitis and intimal arteritis are regarded as the key histological features of acute rejection. Grade 1 tubulitis can sometimes be seen in biopsies that do not represent acute rejection; but in the case of intimal arteritis, just one lymphocyte can justify anti-rejection treatment. Our aim was to audit reliability and accuracy of recognizing tubulitis and intimal arteritis using the approach recommended by the Banff classification and correlate any discrepancies with subsequent graft function.

Methods. This is a retrospective review of all kidney transplant biopsies reported as negative for rejection from 1 January 2009 to 31 December 2009 to assess the presence or absence of occult tubulitis and arteritis. Lymphocytes were immunostained with CD3, using Periodic Acid Schiff as a counterstain. Sections were reviewed to detect missed intimal arteritis and tubulitis. Discrepancies between the report and the immunostain results were analysed by biopsy type and broken down by the reporting pathologist. The graft function of any patient with missed lesions was checked to test for adverse impact on the patient.

Results. ‘Missed’ tubulitis was found in 68% of biopsies, but only two such cases subsequently developed biopsy-proven acute rejection. Only one case of missed intimal arteritis was found (1%) and the subsequent clinical course suggested that this was probably early rejection. There was no significant difference between the reporting pathologists.

Conclusions. We conclude that tubulitis is missed very frequently, but the Banff classification seems to be ‘calibrated’ to allow for this and it does not seriously affect the identification of clinically significant acute rejection. Immunostaining is therefore not indicated in routine practice because (by Banff criteria) it would result in over-diagnosis of rejection. Intimal arteritis can indicate acute rejection even if extremely mild.

Keywords: Banff; kidney transplant biopsies; rejection; tubulitis; arteritis

Introduction

Kidney transplantation is the best available treatment modality for end-stage kidney disease. When graft dysfunction occurs, the renal biopsy plays an important role in defining the underlying cause and in assisting the clinician in determining the most suitable therapeutic intervention [1–3]. The Banff schema was introduced in 1993 to unify nomenclature and classification of changes in renal allograft biopsies, to guide therapy in transplant patients and to establish an objective rejection end point in clinical trials [4]. The schema is regularly updated [5–7] and is universally accepted in clinical trials and research publications where the interpretation of renal transplant biopsies is required. However, its reproducibility between centres is suboptimal [8] and it has always been accepted that its use in guiding the treatment of individual patients demands interpretation in the light of each patient’s clinical condition.

Using the Banff classification scheme for acute rejection, pathologists score the intensity and distribution of infiltrating cells to assess the type and severity of a rejection episode, specifically noting the extent of interstitial mononuclear cell infiltration, the translocation of lymphocytes across the tubular basement membrane (tubulitis) and the invasion of arterial walls by lymphocytes (intimal arteritis) [6, 9]. Interstitial infiltration by mononuclear inflammatory cells in isolation is not sufficient to justify a diagnosis of acute rejection. It is only when interstitial inflammation is accompanied by other changes, notably tubulitis and intimal arteritis, that classification as acute rejection is considered [9]. The accurate and consistent identification of these changes is therefore central to transplant biopsy interpretation.

It has long been recognized that tubulitis can be seen in transplants showing stable graft function [10]. Grade 1 tubulitis (up to four lymphocytes per tubular cross section) is described as ‘borderline’ or ‘suspicious for acute rejection’, while more severe tubulitis in a stable graft is classified as ‘subclinical acute rejection’ and may imply
Missed tubulitis in kidney transplant biopsies

Results

Using immunohistochemistry, all the biopsies reported as t1 had this confirmed. However, of the biopsies reported as t0, ‘missed’ tubulitis was found in 68% (Figure 1). Twenty-six per cent of these were protocol biopsies from stable grafts and 74% were negative biopsies taken to investigate graft dysfunction. However, this difference between stable grafts and grafts showing dysfunction was not statistically significant using Fisher’s exact test (P < 0.23).

Discussion

Recognizing early tubulitis and intimal arteritis in conventionally stained sections in the analysis of kidney transplant

Materials and methods

We reviewed the histopathology reports of 144 sequential renal transplant biopsies between 1 January till 31 December 2009 at the histopathology department, University Hospitals of Leicester. Forty-four cases were excluded because they were reported to show evidence of rejection in the form of intimal arteritis or tubulitis (t2 and t3) by conventional assessment. The original reports had been based on examination of sections stained with H&E, PAS and an elastin stain (Elastic Van Gieson), each using sections cut at three different levels—i.e. a minimum of nine sections.

One paraffin section (3–5 μm) from each of the biopsies (protocol and for graft dysfunction) that were reported as not showing rejection (i.e. neither intimal arteritis nor tubulitis t2 or t3) was immunostained for CD3 using the avidin–biotin–peroxidase immunolabelling method. The resultant high contrast of T lymphocyte cytoplasm facilitates their rapid identification. A conventional PAS counter stain was applied to highlight the basement membrane of the renal tubules and arteries, thereby facilitating accurate localization of the lymphocytes highlighted by the brown immunostain. The PAS counterstain also facilitated the identification of atrophic tubules by virtue of their thickened and ‘wrinkled’ basement membranes. Tubulitis in atrophic tubules was ignored, in accordance with the recommendations of the Banff schema.

One immunostained section from each biopsy was examined carefully to identify and grade intimal arteritis and tubulitis. Discrepancies between the original report and observations from the immunostained specimens were then analysed by the type of biopsy (protocol biopsy of a stable graft or biopsy to investigate graft dysfunction) and broken down by the reporting consultant. Finally, the history of serum creatinine levels of any patient with missed intimal arteritis or missed tubulitis (defined as tubulitis of any severity in a biopsy originally reported as t0) was checked to test whether or not an undiagnosed episode of graft dysfunction developed soon after the ‘negative’ biopsy.

Statistical analyses of the data were performed using GraphPad software (GraphPad Software, Inc. San Diego). Responses were compared using the two-tailed Fisher’s exact test and P < 0.05 was considered significant.

Discussion

Recognizing early tubulitis and intimal arteritis in conventionally stained sections in the analysis of kidney transplant

Fig. 1. A single T lymphocyte in the epithelial compartment of a tubule, representing a case of ‘missed tubulitis’. Note the case with which one cannot only identify lymphocytes but also their location in relation to the tubular basement membrane. Immunostain for CD3, PAS counterstain, ×400.

In most cases of missed tubulitis, the tubulitis was Banff Grade t1 but in 7% of negative biopsies, it was t2 (i.e. more than four lymphocytes in one tubular cross section). In all seven cases where t2 was detected, mononuclear cell infiltration was seen in <25% of the cortex so reclassification as acute rejection under the Banff schema was not justified. In 32%, there was no discrepancy between the report and the immunostain. Of these, 12% showed t1 on both the report and the immunostain and 20% showed no evidence of tubulitis.

The cases which were originally found to show t1 were reported as ‘suspicious for rejection’ and in 83% of them, this was accompanied by an increase in serum creatinine level.

In the 2 weeks following biopsy, a diagnosis of acute rejection was made in none of the grafts found to have missed mild tubulitis (t1) by immunohistochemistry. Five of the seven cases with missed moderate tubulitis (t2) showed an increased serum creatinine level post-biopsy, indicating unstable kidney function. Two of these required a further biopsy; in both cases, the second biopsy showed features reported as acute rejection.

However, only one case of missed intimal arteritis was detected (1%) (Figure 2). This patient showed an increased creatinine serum level after the biopsy and a subsequent biopsy was found to show changes suspicious for acute rejection, justifying treatment, although the second biopsy did not show intimal arteritis.

Immunostaining did not detect any case where the grade of tubulitis had originally been over-called.

Table 1 shows the discrepancies between the original report and the results of immuno-histochemistry for T lymphocytes. Table 2 details the rate of missed tubulitis and missed intimal arteritis of different consultant pathologists. There was no significant difference between them using Fisher’s exact test.
biopsies can be difficult because it demands the accurate identification and localization of single lymphocytes. Furthermore, it has been reported that it can be very difficult to distinguish between apoptotic tubular epithelial cells and infiltrating lymphocytes [12]—although it is notable that this study did not detect any cases where apoptosis had led to an over-diagnosis of tubulitis. To avoid under-diagnosis, the Banff 97 schema recommended the preparation of multiple slides, of which three should be stained with H&E and three with PAS [13] but immunostaining to highlight lymphocytes is not recommended for routine diagnosis.

When interpreting biopsies for graft dysfunction, previous studies have provided conflicting results as to the proportion of cases showing borderline changes that actually prove to represent acute rejection and therefore justify treatment. A review of this literature suggested that ‘about one-third of patients with borderline infiltrates and clinical evidence of graft dysfunction do indeed have acute rejection’ [14]. We are not aware of any previous study where borderline changes had as little clinical impact as in the cases identified by immunohistochemistry in this study. The possibility is raised that our immunohistochemistry for CD3-positive cells is identifying a different subset of T lymphocytes perhaps ones that are morphologically less obvious and functionally less damaging; although our impression was that the CD3-positive cells were morphologically no different and had simply been missed on initial examination.

With the advent of protocol biopsies, it is now well recognized that in some centres, there is a high incidence of unexpected tubulitis, described as subclinical acute rejection, in biopsies from renal allografts with stable graft function [15]. Borderline changes within the Banff schema can behave as either mild acute rejection or can be insignificant [16, 17]. However, the distinction between borderline changes and Grade 1 rejection depends largely on the grade of tubulitis. We have previously reported that there is a large variation in grading of tubulitis in conventionally stained kidney transplant biopsies among expert pathologists in 22 major transplant units [8].

The use of immunohistochemistry stain for T cells with PAS counter stain in this study allowed the rapid and reliable identification and grading of tubulitis as well as the identification of mild intimal arteritis, even if represented by only one lymphocyte. Using this approach, we found that mild tubulitis is identifiable in a majority of negative biopsies, whether from stable grafts or to investigate graft dysfunction. This is a far higher proportion than has ever previously been reported in biopsies from stable grafts, when examined by conventional histology. Moderate tubulitis was missed in 7% of the original negative reports and two of these seven cases showed features of acute rejection in further biopsies.

The data hint that missed tubulitis might be more common in biopsies taken to investigate graft dysfunction than it was in protocol biopsies taken from stable grafts, but this was not statistically significant and it would be difficult to explain. In many negative biopsies taken to investigate graft dysfunction, the cause of graft dysfunction was identified clinically, after the biopsy had been reported, as probable calcineurin inhibitor toxicity. In some, it was attributed to dehydration. In such cases, tubulitis would not be expected.

This remarkably high rate of ‘missed tubulitis’ cannot be attributed to a lack of care in the initial examination of the biopsies for three reasons:

- First, almost all the missed tubulitis cases behaved as though they did not have acute rejection, which is not the published experience of biopsies reported as suspicious for acute rejection in the Banff classification [17].
- Second, all three transplant pathologists showed similar rates of missed tubulitis.
- Third, one of the pathologists (P.N.F.) had previously participated in a pan-European study of transplant biopsy consistency [8], which had demonstrated his sensitivity for the detection of tubulitis to be close to the European average.

Table 1. Discrepancies between the original report and the results of immunohistochemistry for T lymphocytes (n = 100)

<table>
<thead>
<tr>
<th>Tubulitis</th>
<th>Number of biopsies reported</th>
<th>% of missed tubulitis</th>
<th>% of missed arteritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tubulitis on the report and immunostain</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t1 on the report and immunostain</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t1 on the report and no tubulitis on immunostain</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No tubulitis on report but t1 on immunostain</td>
<td>61</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>No tubulitis on report but t2 on immunostain</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intimal arteritis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No intimal arteritis in report or immunostain</td>
<td>99</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Intimal arteritis in immunostain but not reported</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The percentage of missed tubulitis or arteritis among different consultant histopathologists (n = 100)

<table>
<thead>
<tr>
<th>Consultant</th>
<th>Number of biopsies reported</th>
<th>% of missed tubulitis</th>
<th>% of missed arteritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>64</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>17</td>
<td>65</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>19</td>
<td>89</td>
<td>0</td>
</tr>
</tbody>
</table>
It is therefore reasonable to suggest that this rate of missed tubulitis is likely to be seen in the practice of any renal transplant pathologist.

Our results suggest that, whether by serendipity or intent, the Banff criteria for the diagnosis of acute rejection have been appropriately designed to allow for this high rate of missed tubulitis. In our hands, routine use of immunostaining for T cells is not justified because it would result in the over-diagnosis of acute rejection. Nonetheless, some centres routinely use, and even recommend, immunohistochemistry for T cells with a PAS counter stain [18]. Our results suggest that this approach would demand the use of radically different criteria for the diagnosis and classification of acute rejection.

In summary, this study showed that tubulitis is missed frequently, but the Banff classification seems to have been ‘calibrated’ to allow for this and it does not adversely affect the identification of clinically significant acute rejection. Immunostaining, to detect missed tubulitis, is therefore not indicated in routine practice. Intimal arteritis is rare, but when present, it is indicative of acute rejection, even if it involves only one intimal lymphocyte.

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Conflict of interest statement. None declared.

References


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