Transplant capillaropathy and transplant glomerulopathy: ultrastructural markers of chronic renal allograft rejection

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Introduction

A late dysfunction of a renal allograft refers to a progressive decline in renal function manifested >3 months after transplantation. A late dysfunction may have several causes, such as chronic rejection, chronic allograft nephropathy, chronic calcineurin inhibitor toxicity, de novo or recurrent renal disease and acute rejection. An allograft biopsy is necessary to establish a definitive diagnosis. The standard interpretation of alterations is widely carried out on the basis of the ‘Banff 97 classification’, which relies on the evaluation of light microscopic stains [1]. The assessment of allograft biopsies by light microscopy per se, however, is hampered by the fact that the histological examination of the specimen is not sufficient to identify all types of rejection. Whereas acute cellular rejection can be appropriately diagnosed, the recognition of chronic rejection seems impossible in a certain number of cases involving chronic rejection, and the verification of an alloantibody-mediated graft injury requires the application of immunohistochemistry [2,3]. Renal capillary lesions attributed to chronic rejection have recently been described ultrastructurally. The present communication reviews these markers, and demonstrates how the incorporation of electron microscopy into the evaluation of allograft biopsies with a late dysfunction can help the pathologist establish the proper diagnosis.

Chronic rejection, chronic allograft nephropathy and allograft sclerosis

Chronic rejection is a clinicopathological entity characterized clinically by an insidious, progressive increase in the serum creatinine level, frequently in association with proteinuria (often in the nephrotic range) as well as arterial hypertension, and histologically by the changes listed in Table 1. Pathogenetically, there is ongoing, smouldering damage to the allograft, mediated by cellular and/or humoral alloimmune mechanisms. The clinical risk factors include an HLA mismatch, panel-reactive antibodies, acute rejection episodes,

Table 1. Morphologic features of chronic rejection and chronic allograft nephropathy (based on 1, 4, 16, 18)

<table>
<thead>
<tr>
<th>CR</th>
<th>CAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteries</td>
<td>Intimal fibroelastosis or arteries without any change</td>
</tr>
<tr>
<td>Transplant arteriopathy</td>
<td>(intimal fibrosis in the absence of elastosis ± foam cells/mononuclears in the intima ± breaks in the internal elastic lamina ± formation of neo-media)</td>
</tr>
<tr>
<td>Arterioles</td>
<td>No change</td>
</tr>
<tr>
<td>No or excentric subendothelial hyalinosis</td>
<td></td>
</tr>
<tr>
<td>Glomeruli</td>
<td>Non-specific segmental or global glomerular sclerosis</td>
</tr>
<tr>
<td>Transplant glomerulopathy</td>
<td>(double contours in &lt;10% of the capillaries or formation of a new layer(s) of basal lamina in at least three capillary loops by EM*)</td>
</tr>
<tr>
<td>Peritubular capillaries</td>
<td>No or mild basement membrane changes by EM</td>
</tr>
<tr>
<td>Transplant capillaropathy</td>
<td>(at least three capillary profiles with five or more circumferential basement membrane layers by EM)</td>
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<tr>
<td>Interstitium</td>
<td>Fibrosis</td>
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<tr>
<td>Mononuclear infiltrates</td>
<td></td>
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<tr>
<td>Fibrosis</td>
<td></td>
</tr>
<tr>
<td>Tubules</td>
<td>Atrophy</td>
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<td>Atrophy</td>
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*Should be assessed in glomeruli not affected by segmental scarring or hyperfiltration. EM, electron microscopy; ±, and/or; CR, chronic rejection; CAN, chronic allograft nephropathy. Chronic rejection is diagnosed in the presence of transplant arteriopathy and/or transplant glomerulopathy and/or transplant capillaropathy.
inadequate immunosuppression and drug compliance. The histological markers of chronic rejection are transplant arteriopathy and transplant glomerulopathy. Transplant arteriopathy (Figure 1) leads to progressive obliteration of the vessels, and in turn to ischaemic glomerulopathy, interstitial fibrosis and tubular atrophy. The lesion is focal, and is usually more pronounced in the interlobar and arcuate arteries (Figure 2) than in the small arteries of the superficial cortex. The morphology of transplant glomerulopathy resembles that seen in renal thrombotic microangiopathy, suggesting that humoral immunity and nephron depleting factors can produce chronic allograft nephropathy, such as injury to the graft during the implantation period (brain death, ischaemia/reperfusion injury or a delayed graft function), advanced donor age, ischaemia, hypertension, drug toxicity, infection, increased ureteral pressure, a size mismatch and hyperfiltrating glomeruli [5–8].

The alloimmune and nephron depleting pathways of renal fibrogenesis usually act in parallel and ultimately lead to the end-stage of allograft sclerosis.

Limitations in the histological diagnosis of chronic rejection

The traditional markers of chronic rejection are relatively infrequently encountered in biopsy series. The low incidence of transplant arteriopathy (25–40%) may have at least two possible explanations. First, since allograft biopsies usually miss large arteries, and the arteriopathy is focal, the lesion may not be present in the biopsy specimen (sampling error). Secondly, the fibrous phase of arteriopathy is not or cannot be distinguished from intimal fibroelastosis of small cortical arteries induced by nephron depleting factors. Intimal fibroelastosis may have been present in the donor organ at the time of transplantation or may have occurred de novo in the graft. Unfortunately, the Banff 97 classification does not recommend use of elastin stain [1], which is an unfavourable compromise between diagnostic accuracy and cost, because the stain is indispensable to verify rejection-induced intimal fibrosis. For no clear reasons, the incidence of transplant glomerulopathy is fairly rare (15–30%), and the light microscopic distinction between the presence or absence of transplant glomerulopathy may occasionally be difficult. It is plausible, therefore, to assume that cases that are in fact chronic rejection, but lack the histological indicators of chronic rejection, are diagnosed as chronic allograft nephropathy. The limitations in the histological diagnosis of chronic rejection have had unfavourable consequences: the
true incidence of chronic rejection is not known, there is a frustrating debate on the relative contributions of alloantigen-dependent factors and nephron depleting factors in the evolution of allograft sclerosis [9], and the efficacy of immunosuppressive drugs on long-term graft survival cannot be properly assessed.

**Ultrastructural markers of chronic rejection**

The routine application of electron microscopy seems to overcome the limitations in the histological diagnosis of chronic rejection. Renal capillary pathology can be better recognized ultrastructurally than by light microscopy. The renal capillary endothelial cells express HLA class I and class II antigens and are therefore targets of the rejection responses [10,11]. Two lesions develop in response to alloantigen-induced, smouldering damage to the renal capillaries: transplant capillaropathy and transplant glomerulopathy.

**Transplant capillaropathy**

[Synonyms: duplication of lamina densa in peritubular capillaries; typical circumferential multisplitting of capillary basement membrane; splitting and reduplication of basal lamina in intertubular capillaries; ‘cpc lesion’ (chronic peritubular capillary lesion).]

(i) In 1990, the Italian pathologist G. Monga, first reported the circumferential multilayering and splitting of the peritubular capillary basement membranes in allograft biopsies from patients with transplant glomerulopathy, and suggested to consider this lesion as an ultrastructural marker of chronic rejection [12]. For a decade the specificity of the basement membrane changes remained unproven because sufficient knowledge on the reaction patterns of peritubular capillaries in response to injury was not available. During the past few years, we have learned that the peritubular capillary basement membrane undergoes multiplication in pathogenetically different conditions. The multiplication can be focal or circumferential, and the number of layers is usually higher along the wide interstitial aspect than along the narrow interstitial aspect of the capillary profile [13–17].

(ii) We earlier investigated the specificity of basement membrane changes in rigorously defined biopsy cases of chronic rejection and selected series of controls [16]. The number of circumferential basement membrane layers was arbitrarily graded as mild (two to four layers), moderate (five or six layers) or severe (seven or more layers). The mean number of circumferential layers was calculated and the incidence of the grades was determined. The site of reading was the narrow peritubular interstitium. The circumferentially multilayered peritubular capillaries were significantly correlated to the presence of chronic rejection. A capillary profile with seven or more layers or at least three profiles with five or six layers was found exclusively in patients with chronic rejection. A mild lesion was not suggestive of chronic rejection at all. The severe or moderate lesions had tightly packed basement membrane layers, which were frequently split. The endothelial layer was thickened and non-fenestrated, and had a serrated contour along the interstitial aspect of the cell body (Figure 4). It was concluded that the reliable indicators of chronic rejection were a capillary profile with the severe lesion or at least three profiles with the moderate lesion [16]. When the capillaries exhibited such features, the situation was termed transplant capillaropathy [18]. This capillaropathy appeared as a serrated profile with a thick, ribbon-like basement membrane layer in semithin plastic sections, promoting better sampling of the lesion. The incidence of transplant capillaropathy increased as the rejection process progressed.

(iii) Four other groups have also dealt with the specificity of circumferential multilayering of the basement membrane of peritubular capillaries in chronic rejection [15,17,19,20]. Two groups have used well-defined criteria on the extent of multiplication [15,17], which allows comparison of the data obtained. Accordingly, capillary profiles with more than six layers can be safely used as a marker of chronic rejection. It should be recalled that the grades of multilayering have been arbitrarily defined. In the study by Drachenberg et al. [15] and Gough et al. [17], the range of the moderate grade was wider than our own (four to six layers vs five or six layers). Consequently, the association between the grade and chronic rejection was not as striking as in our study. Our experience and a review of the literature lead us to suggest that in the event of one profile or two profiles with five or six layers, a few conditions
should be borne in mind before the change is definitely regarded as an indicator of chronic rejection. These conditions include obstructive uropathy, chronic tubulointerstitial nephritis, thrombotic microangiopathy, radiation nephritis, analgesic nephropathy and Balkan nephropathy. In daily practice, only the first three of these merit deep thought. The clinical data on the existence of urinary tract obstruction and/or reflux nephropathy, an earlier history of the occurrence of thrombotic microangiopathy in the allograft, and a search for transplant arteriopathy and/or transplant glomerulopathy help the pathologist reach a conclusive decision.

Transplant glomerulopathy (Figure 5)

In our and others’ experience, electron microscopy verifies transplant glomerulopathy more precisely than light microscopy does [13–16,18]. There is, however, a lack of an agreed ultrastructural cut-off of transplant glomerulopathy. We defined transplant glomerulopathy as the thickening of the capillary wall in at least three loops as a result of the widening of the subendothelial space by abnormal basement membrane material, and the formation of a new layer(s) of basal lamina [16].

Electron microscopy in the diagnosis of chronic rejection: a biopsy review

In our department, biopsies of patients with late graft dysfunction are routinely investigated by the combined use of light microscopy and immunofluorescence (IgG, IgA, IgM, C3, C1q, fibrinogen and also C4d since 1999). In addition, pieces of renal tissue are embedded for electron microscopy in each case. This fortunate situation allowed us to carry out a systemic retrospective ultrastructural search for transplant capillaropathy and transplant glomerulopathy in a consecutive series of 117 biopsies performed ≥6 months after implantation (median: 25 months, range 6–186; median serum creatinine concentration at biopsy: 380 μmol/l, range 104–1100). The results of the study of 91 biopsies have been published [18]. All but one patient had received a cadaveric kidney. The biopsies

![Fig. 4. Transplant capillaropathy. The peritubular capillary endothelium (E) is thickened, non-fenestrated and displays a serrated contour along the interstitial aspect of the cell body. The basement membrane has 5 or 6 layers (arrows). The lymphocyte (Ly) in the capillary lumen adheres to the endothelium. IS, interstitium.](image)

![Fig. 5. Transplant glomerulopathy. Subendothelial layer of basal lamina (arrows) between the lamina densa of glomerular basement membrane (GBM) and the glomerular capillary endothelium (E).](image)
were carried out between 1993 and 2001. They met the criteria of specimen adequacy [1]. The diagnoses were reclassified on the basis of the ultrastructural findings (Table 2).

The application of electron microscopy increased the diagnosis of chronic rejection to 68%, and decreased chronic allograft nephropathy to 17%. In the group of chronic rejection, the individual incidence of transplant capillaropathy and of transplant glomerulopathy was 82% and 57%, respectively, and their cumulative incidence was 92%. Electron microscopy verified transplant glomerulopathy on 10 occasions. The ultrastructural examination disclosed the coexistence of chronic rejection in several cases of cyclosporine toxicity, acute rejection, and glomerulonephritis (not detailed here). The late dysfunction was usually bi- or multifatorial, and the most frequent combination was chronic rejection and acute borderline acute rejection. Chronic rejection was also observed in patients treated with the new generation of immunosuppressive drugs (basiliximab in four patients; mycophenolate mofetil in 31 patients; tacrolimus in four patients).

Comment

The revised diagnoses showed that in a non-selected, consecutive series of allograft biopsies of patients with late graft dysfunction, 68% of the cases displayed features of chronic rejection. To the best of our knowledge, the data are the first in the literature that reflect the true occurrence of chronic rejection. The results confirm the assumption that if light microscopy alone is used to explore the cause of a late dysfunction, chronic rejection is markedly underdiagnosed and chronic allograft nephropathy is overdiagnosed. The application of electron microscopy more than doubled the frequency of the diagnosis of chronic rejection. The ultrastructural evaluation of the renal allograft tissue offered further advantages. Late dysfunction of the allograft may be related to de novo or recurrent glomerular disease, or a combination of different diseases, which are difficult to explore adequately by means of standard light microscopy. We experienced that electron microscopy was a relevant diagnostic tool in about half of our cases. Herrera et al. [21] foresaw already in 1997 that electron microscopy will have a significant diagnostic value in the evaluation of late dysfunction biopsies.

In conclusion, chronic rejection can be adequately diagnosed by electron microscopy. Once the existence of chronic rejection has been confirmed, important topics, such as the pathogenetic aspects of chronic rejection, the efficacy of newer immunosuppressive regimens in preventing long-term alloimmune damage to the allograft, etc., can be analysed. Our preliminary data indicate that ongoing, smouldering rejection continues to be a major problem in the era of new immunosuppressive drugs. The descriptive histologic term 'chronic allograft nephropathy' should be reduced as much as possible because it provides uncertainty rather than precision.

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References