Prospects and limitations of post-transplant alloantibody detection in renal transplantation

Georg A. Böhmig, Gregor Bartel, Heinz Regele, Markus Wahrmann

PII: S0198-8859(09)00103-7
DOI: 10.1016/j.humimm.2009.04.014
Reference: HIM 8231

To appear in: Human Immunology

Received date: 2 April 2009
Accepted date: 9 April 2009


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Prospects and limitations of post-transplant alloantibody detection in renal transplantation

Georg A. Böhmig, Gregor Bartel, Heinz Regele, and Markus Wahrmann

Department of Medicine III, Medical University of Vienna, Vienna, Austria
Institute of Clinical Pathology, Medical University of Vienna, Vienna, Austria

Address reprint requests to Dr. Georg A. Böhmig, Division of Nephrology and Dialysis, Department of Medicine III, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria. Tel.: +43-1-40400-4363; Fax: +43-1-40400-2194; e-mail: georg.boehmig@meduniwien.ac.at

Key words: alloantibody; C4d; HLA; monitoring; renal transplantation

Abbreviated title: post-transplant alloantibody detection

Abbreviations
AMR antibody-mediated rejection
CDC complement-dependent cytotoxicity
DSA donor-specific antigens
HLA human leukocyte antigen
FCXM flow cytometry crossmatch
Abstract

Antibody-mediated immunity is generally accepted to be a major cause of kidney allograft injury and loss. Post-transplant monitoring for circulating alloantibodies was proposed to represent a useful non-invasive diagnostic approach to assess individual immunological risks and to guide the implementation of specific therapeutic measures. This was supported by the recent establishment of highly sensitive and specific solid-phase immunoassays for detailed characterisation of reactivity patterns. Nevertheless, imperfect associations of serological results with biopsy-based criteria of antibody-mediated rejection as well as the disputed long-term significance of circulating alloantibody detected in recipients with normal graft function necessitate a careful interpretation of monitoring results.
Introduction and general remarks

It is well established that antibodies directed against polymorphic alloantigens, primarily HLA antigens, can cause kidney allograft injury and loss (1, 2). The spectrum of antibody-mediated injury ranges from hyperacute rejection triggered by preformed complement-activating donor-specific antibodies (DSA) to chronic antibody-mediated rejection (AMR) culminating in graft loss many years after transplantation. The implementation of C4d as a biopsy-based rejection marker has provided a valuable basis for the diagnosis of AMR (3). Capillary C4d staining has now been incorporated into the Banff scheme to define both acute and chronic AMR (4-6). A critical finding was its tight association with the detection of circulating alloantibodies in sera taken at the time of indication biopsy (7-10). These data and recent evidence derived from animal models of AMR (11-13) strongly support the initial assumption that C4d deposition is primarily triggered upon classical complement activation by DSA bound to the graft endothelium (for unknown reasons, however, binding of reactive IgG itself along the graft endothelium usually remains undetectable). In addition, associations of C4d and circulating alloantibody with the occurrence of typical morphological features, such as capillary monocyte/granulocyte accumulation or transplant glomerulopathy helped to define AMR as a separate entity (4-6).

In clinical routine, a renal allograft biopsy represents the indispensable diagnostic gold standard. It is well accepted that morphological and immunohistochemical biopsy results are critical to guide targeted treatment. For acute AMR various specific anti-humoral strategies including antibody depletion by plasmapheresis or immunoadsorption were effectively used to reverse dysfunction and prevent graft loss (14-16). For chronic AMR associated with advanced tissue
injury and impaired graft function, however, the efficiency of such measures has not been determined conclusively (17-19). A critical advantage would be a preferentially non-invasive diagnostic tool uncovering deleterious humoral reactivity at a very early stage to give a reliable and timely warning in advance of injury. Early detection of subclinical rejection can be speculated to provide a useful basis for (presently not defined) potential therapeutic strategies that are able to prevent deterioration of graft function at a later time. It is obvious, that, especially in the face of current sophisticated technical developments, the serological detection of alloantibodies following transplantation could be a useful strategy for non-invasive immunological monitoring.

The concept of post-transplant antibody monitoring is based on several considerations: (a) anti-HLA alloantibodies, primarily of the IgG class, play a causative role in humoral rejection; (b) clinically deleterious anti-HLA antibodies causing overt humoral rejection are reliably detectable by sensitive diagnostic assays, (c) circulating anti-HLA antibodies in stable recipients reflect subclinical AMR, which culminates in progressive tissue injury and loss of graft function at a later time; and (d) early anti-humoral treatment abrogates subclinical rejection and thereby prevents subsequent development of chronic rejection.

The repertoire of alloantibody detection tests comprises a wide array of method approaches, including a variety of techniques for antigen-specific solid phase-based testing (20-28). An increasing number of laboratories have now included Luminex-based antibody detection using HLA antigen-coated microspheres for sophisticated risk stratification in advance of transplantation (28-30). This highly sensitive approach of solid phase-based alloantibody detection provides the opportunity to characterize individual patterns of HLA specificities. In addition, Luminex testing has recently been extended to polymorphic non-HLA antigens (31).
Using the Luminex platform, however, a major challenge is its extensive level of sensitivity and possible interference by false positive reactions (32). Future studies will have to clarify how to actually define thresholds in a clinical context (e.g. according to the variance of negative control serum binding to each defined single bead or according to a more generalized and simplified arbitrary level of mean fluorescence intensity). A standardization of test performance and interpretation would be highly desirable to minimize day-to-day and inter-laboratory variations. Applying super-sensitive solid phase immunoassays, a major challenge, however, will be to distinguish reliably between clinically relevant and irrelevant alloreactivities, either by using qualitative (e.g. complement- versus non-complement-fixing reactivity (33-35)) or quantitative parameters (e.g. documentation of the binding strength of DSA (36)).

In this review, we provide a brief discussion of the clinical relevance of post-transplant serology with special focus on the utility of antibody detection as an independent diagnostic tool to identify clinical and subclinical AMR.

**Antibody detection in patients with graft dysfunction**

Following the Banff scheme, the diagnosis of both acute and chronic AMR relies on the presence of three different criteria, that are, two biopsy-based criteria (capillary C4d, typical morphology), and, to provide definitive proof for AMR, the detection of circulating DSA (4-6).

Serological studies have demonstrated tight associations of antibody detection applying flow cytometry and complement-dependent cytotoxicity (CDC) testing with biopsy-based features of AMR (7-10, 37). However, such associations may be far
from being perfect. Potential discrepancies between antibody detection and biopsy results are illustrated in Figure 1.

In an earlier study of indication biopsies, we observed that sensitive antibody tests [flow cytometry crossmatch (FCXM), FlowPRA screening and single antigen testing] frequently yielded a positive result also in recipients with C4d negative graft dysfunction (8). Compared to a group of C4d-positive recipients, C4d-negative but antibody-positive patients had superior graft performance, which was suggested to be a result of the inability of detected reactivities to activate complement in vivo (8). The high sensitivity but modest specificity of FCXM and FlowPRA strongly reinforced the superior diagnostic accuracy of C4d biopsy staining as a marker of clinically relevant humoral injury. A major challenge will be to delineate distinct qualitative properties of detected alloantibodies which could help stratify the clinical impact of a given alloreactivity pattern. One attractive approach to increase the predictive value of antibody testing could be to determine the ability of a given pattern to fix complement (e.g. C4d) in vitro (38, 39). The test principle of in vitro C4d detection using HLA-coated microparticles (FlowPRA or Luminex beads) is illustrated in Figure 2.

In several studies, it was noted that some recipients with C4d deposits in biopsy were negative by anti-HLA IgG testing (7-10). This may be a result of changing patterns of C4d deposition over time which may not always reflect the time course of circulating antibody. Other explanations could be subthreshold levels of alloreactive IgG, the presence of IgM type alloantibodies, or, alternatively, antibodies against ill-defined non-HLA antigens. Most importantly, recent studies suggest absorption of donor-specific antibodies by the graft itself (40, 41). Moreover, efficient antibody clearance by the transplanted tissue is reinforced by studies demonstrating
a substantial increase of circulating HLA antibodies following transplant nephrectomy (42-44).

The above mentioned discrepancies between biopsy results and serology suggest a careful interpretation of negative as well as positive test results in the context of graft dysfunction. In our opinion, the apparent limitations of post-transplant antibody monitoring may preclude its use as a sole diagnostic tool. However, in line with the recommendations of the Banff classification, alloantibody detection may be useful to narrow the diagnostic alternatives and should therefore have its permanent place as an important adjunct in a multi-faceted diagnostic algorithm.

**Antibody monitoring in stable recipients**

Analysing large patient cohorts it was shown that post-transplant detection of circulating alloantibodies is associated with worse graft outcomes (1, 45-49). Viewed superficially, observed associations may suggest that early detection of circulating alloantibodies could reliably predict graft injury at a later time. Reinforcing a causative role of antibody, it was even suggested that chronic allograft losses are always preceded by detectable HLA alloreactivity (47). Such tight interrelationships between early alloantibody formation, subsequent complement activation, chronic graft injury and finally progressive graft dysfunction were also demonstrated in a large animal model (50).

Nevertheless, a thorough analysis of available data clearly demonstrates that antibody/complement does apparently not inevitably cause graft injury and loss. The following important observations have to be taken into account when interpreting the results of HLA antibody monitoring in a clinical and therapeutic context:
In a recent multicenter analysis, a cohort of 2231 patients having functioning kidney allografts for at least 6 months were tested for the presence HLA antibodies, and followed for 2 years (51). In accordance with numerous previous studies, antibody-positive patients had a higher graft loss rate than antibody-negative patients (15.1% versus 6.8%). However, stratifying patients according to baseline serum creatinine levels, they found that among subjects with a baseline creatinine between 0.5 and 1.9 mg/dl, a single snap shot of HLA antibody detection was not predictive of subsequent graft failure. These data suggested that the value of antibody monitoring may depend on serum creatinine levels at the time of testing. Discussing their data, the authors argued that the follow up in their study was too short to uncover potential deleterious effects far in advance of failure (51). However, a very recent study suggested that despite detectable circulating alloreactivity patients with normal allograft function at the time of testing may maintain stable function over a long period of time (38). Bartel et al. (38) evaluated the time course of HLA reactivity patterns in a cohort of 164 deceased donor kidney allograft recipients having a functioning graft for at least one year. In their analysis, the authors focused on a group of 34 patients defined according to stringent clinical criteria, i.e. normal graft function, no proteinuria, and no documented episode of graft dysfunction within the first year. Nine of these patients had detectable anti-HLA alloreactivity. Interestingly, binding intensities, C4d-fixing abilities, and proportions of donor reactivities were similar to those reported for patients with dysfunctioning grafts. The most surprising finding, however, was that, also in the long term, antibody-positive patients with normal 1-year graft function maintained stable graft function without development of proteinuria for up to 80 months (38).

Similarly, also other features of humoral immunity, such as capillary C4d deposition, were reported to occur in stable transplants without apparent adverse
effects on subsequent graft performance. Fiebeler et al. (52) reported a patient with C4d staining in a protocol biopsy, who maintained long-term allograft function. This anecdotal case suggested that some patients could maintain stable graft function despite local complement activation and inflammation. Mengel et al. (53) found that 4.4% of 551 protocol biopsies showed focal or diffuse C4d staining. In many cases, C4d staining was found to be associated with morphological features of AMR. Interestingly, in this large analysis, C4d staining was not associated with worse graft function at one year or inferior graft survival after 12 to 143 months of follow-up. Moreover, in a recent smaller study, Yoon et al. (54), observed C4d staining in 4 of 79 protocol biopsies. The authors observed that C4d staining was not associated with inferior graft function or the development of proteinuria over an extended follow-up period.

These data suggest that features of AMR in the absence of dysfunction may not necessarily implicate graft injury at a later time. In contrast, if associated with impaired graft function at the time of testing, such features may predict adverse outcomes. Accordingly, for post-transplant antibody monitoring, the absence of graft dysfunction at the time of antibody testing may result in a low pretest probability of harmful humoral reactivity preceding allograft loss. This and some other potential drawbacks mentioned above, most importantly the loss of sensitivity by DSA absorption, may impede the clinical value of serial antibody monitoring.

First, a varying clinical relevance of detected reactivity patterns could result from differences in antibody binding strength, antigen specificity, and distinct functional properties (36, 55). Considering the important role of complement as a trigger of graft injury, another important determining factor could be the actual ability of a given alloreactivity pattern to efficiently trigger complement activation (39).
However, in the study by Bartel et al (38), stable antibody-positive recipients did not significantly differ from those with graft dysfunction with respect to binding intensities and complement-fixing capabilities. Even though limited by small group sizes, this study may be in support of the suggestion that the fate of an allograft may not be solely determined by the quality of a given alloreactivity pattern but also by its individual vulnerability to humoral injury. This leads to the speculation that transplant accommodation could play a major role, an ill-defined antibody-mediated state of resistance of an allograft to injury.

According to the scheme outlined in Figure 3, it can be speculated that, in stable recipients, circulating alloreactivity or other features of AMR could reflect an established state of accommodation associated with stable function over a long period of time. In contrast, if associated with graft dysfunction, such features may reflect ongoing rejection culminating in subsequent chronic injury and progressive deterioration of graft function.

Transplant accommodation has been earlier postulated to play a pivotal role in xenotransplant models or ABO-incompatible transplantation (56, 57). In the particular setting of blood group-incompatible transplantation, high proportions of protocol biopsies were found to be C4d positive and blood group antibody recurrence has been frequently described despite stable long-term function (56, 57). In a recent study, C4d-positivity in early protocol biopsies was even suggested to be associated with less scarring in follow-up biopsies (58). Primarily based on experimental (xenotransplant) models, various mechanisms have been proposed, including altered complement regulation, affected signal transduction, disruption of cellular adhesion or other mechanisms promoting cytoprotection (59-63).
Concluding remarks

Whereas alloantibody detection in advance of kidney transplantation represents an indispensable diagnostic measure for risk stratification and recipient allocation, the actual clinical value of post-transplant alloantibody monitoring remains to be established. While antibody detection in the setting of acute graft dysfunction, as a part of a multi-faceted diagnostic procedure, may help to identify a causative role of humoral immunity, despite impressive technical advancements, the value of antibody detection in recipients with normal functioning grafts is far less clear. Indeed, in some patients, just in those with good graft function at the time of testing, such reactivities may not necessarily predict impending failure.

References


**Figure legends**

**Figure 1.** Possible discrepancies between DSA detection and renal biopsy results. 
*C4d* staining in the absence of circulating DSA may represent ongoing AMR and could be explained by: (a) DSA absorption to the allograft, (b) presence of non-HLA antibodies, (c) IgM alloreactivity, (d) subthreshold levels of circulating alloantibodies, or (e) false positive C4d staining (alloantibody-independent complement deposition).

**Figure 2.** Solid phase detection of C4d deposition in vitro as a possible surrogate of alloantibody-triggered capillary C4d deposition in vivo.

**Figure 3.** The impact of serological/biopsy-based AMR features on long-term outcomes – a function of graft performance at the time of testing?
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>N</th>
<th>Serology</th>
<th>Protocol Bx</th>
<th>Baseline results</th>
<th>Follow-up</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terasaki &amp; Ozawa (2005)</td>
<td>302</td>
<td>CDC-PRA, ELISA, or flow methods (single snapshot at &gt;6 months)</td>
<td></td>
<td>SrCr 0.5-1.9 mg/dl</td>
<td>2 years</td>
<td>4.4% graft loss rate, (no difference to Ab-negative patients)</td>
</tr>
<tr>
<td>Bartel et al. (2008)</td>
<td>34</td>
<td>[IgG]FlowPRA, [C4d]FlowPRA, and FCXM (serial detection at 2, 6, and 12 months)</td>
<td></td>
<td>eGFR ≥60 ml/min/1.73 m², 24-h urinary protein excretion ≤0.5 g, and no rejection at 1 year</td>
<td>40 to 80 months</td>
<td>eGFR and protein excretion comparable to Ab-negative patients</td>
</tr>
<tr>
<td>Mengel et al. (2005)</td>
<td>24</td>
<td>C4d in PTC (median time to Bx: 77, 52, 12 weeks*)</td>
<td></td>
<td>no significant rise in SrCr (90% of Bx) (remaining Bx: incidental impairment of allograft function)</td>
<td>12-143 months</td>
<td>Delta SrCr at 1 year and graft survival at a median of 43 months comparable to C4d-negative patients</td>
</tr>
<tr>
<td>Yoon et al. (2008)</td>
<td>4</td>
<td>C4d in PTC (time to Bx: 13-15 days)</td>
<td></td>
<td>SrCr 1.0±0.2 mg/dL, no proteinuria</td>
<td>24-45 months</td>
<td>SrCr 1.2±0.3 mg/dL, no proteinuria, (no difference to C4d-negative patients)</td>
</tr>
</tbody>
</table>

*three centers participated in the study and differed with respect to the timing of biopsy.

Ab, antibody; Bx, biopsy; CDC, complement-dependent cytotoxicity; ELISA, enzyme-linked immunosorbent assay; FCXM, flow cytometry crossmatch; PTC, peritubular capillaries; SrCr, serum creatinine; eGFR, estimated glomerular filtration rate.
Figure 1

Circulating HLA-DSA

- no
- yes
- no*

Indication biopsy

- no AMR features
- C4d in PTC ± AMR morphology

- no AMR
- AMR?
- AMR
- Suspected AMR
Solid-phase C4d staining in vitro

Surrogate of AMR?

Flow cytometry
Luminex

Capillary C4d deposition in vivo

Immunohistology

Figure 2
Figure 3

Circulating alloantibodies ± C4d in PTC ± AMR morphology

Graft dysfunction?

yes no

Accommodation

Rejection

Chronic injury Stable function