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INVITED COMMENTARY

# Detection of C4d-fixing HLA antibodies in serum – a glass half full and half empty*

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**Conflicts of Interest**
None.

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Research efforts dedicated to provide new diagnostic and therapeutic modalities via better understanding of antibody-mediated (humoral) immune responses dominate the current era of transplantation medicine. The implementation of sensitive solid-phase assays for detection of donor specific anti-HLA antibodies (DSA) in serum together with immunohistochemical detection of complement degradation product C4d in transplant biopsies were most remarkable developments [1,2]. Assays based on single-antigen beads (SAB) or the so called Luminex technology also became broadly accepted because of their high sensitivity [3]. However, the specificity issue of DSAs detected by SAB and their clinical relevance are a matter of continuous debate in the transplant community [4]. Complement-mediated mechanisms are considered as important effectors of HLA-DSA-related pathology in the allograft [5]. Complement binding $IgG_1$ and $IgG_3$ HLA-DSA isotypes initiate the classic complement pathway via binding to C1q. Activated C1q subsequently activates its natural substrate C4 and other down-stream components ultimately leading to target cell lysis and death [5]. Complement fixation in the tissue as detected by C4d, a split product of C4 became a routine diagnostic modality in transplant pathology worldwide [2]. Considering the important role of complement activation, a logical next step is to refine assays for detection of HLA antibodies in serum to better define which circulating HLA-DSA are capable to bind complement and are potentially detrimental. The development of assays for detection of C1q or C4d HLA-DSA binding capability in serum in combination with Luminex technology could enhance immunologic risk stratification [6,7]. Böhmig and associates were first to establish a flow-cytometry-based assay which allows for solid-phase detection of HLA antibody-triggered complement (split) product deposition [8]. The assay was further refined by means of Luminex-based SAB technology for identification of complement-fixing DSA [9]. Similar developments were attempted for C1q and results of first studies were reported [10].

The current study focused on the potential of DSA-triggered C4d deposition (C4d/DSA) detected pretransplant and 6-months post-transplantation to predict outcomes in a cohort of 68 highly sensitized renal transplant recipients who underwent an immunoadsorption-based desensitization procedure. Conventional antibody detection techniques have failed to predict immunological complications in these patients [11]. Median complement-dependent cytotoxicity (CDC) reactivity in these subjects was 73% before desensitization. A single pretransplant immunoadsorption was sufficient to render a positive CDC cross match in 21 recipients negative. HLA single-antigen reactivities were assessed on a Luminex SAB platform. Pretransplant [C4d]DSA positivity in the serum was detected in 44 of 68 patients. Acute AMR with C4d-positive biopsies occurred more frequently in pretransplant [C4d]DSA-positive patients (13/15 detected cases). Interestingly, four recipients who later developed chronic active AMR were also pretransplant [C4d]DSA-positive. Multivariate analysis confirmed [C4d]DSA as independent risk factor for C4d-positive AMR. [C4d]DSA-positive patients displayed a trend toward inferior transplant survival. In contrast, [C4d]DSA detected 6 months after transplantation had no influence on clinical outcomes.

Obviously, detection of [C4d]DSA in serum offers a valuable diagnostic modality for the detection of detrimental complement-fixing antibodies in serum and holds potential for pretransplant risk stratification. However, similar to results obtained by other available SAB-based assays, the problem of detection of [C4d]DSA in serum is that many patients who were detected positive ($n = 28$) did not develop significant allograft pathology. Subsequent analysis focusing on the group of [C4d]DSA-positive recipients found no difference regarding [IgG]DSA number or binding strength, virtual [C4d] panel reactivity, number and HLA class specificity of [C4d]DSA, C4d binding intensity, or the extent of [C4d]DSA depletion upon preoperative immunoadsorption, respectively [11]. Thus, the burning question, as to why more than a half of [C4d]DSA-positive patients showed no clinical deterioration, remains unanswered. As protocol biopsies were not performed it is plausible that detrimental effects of [C4d]DSA positivity were subclinical at least in some of the patients. Conversely, accommodation processes could have also been operative in some allografts rendering them resistant to actions of [C4d]DSA.

Future studies are needed to investigate whether or not a combined assessment of C4d- and C1q-fixing abilities of DSA may provide an additional diagnostic benefit. Another important issue is whether or not further technical refinements would translate into higher specificity and further clinical relevance of serological findings. Meanwhile, the glass remains half full and half empty. The next step would be therapeutic proof of concept to investigate whether or not pre-emptive targeting of complement activation in [C4d]DSA-positive patients may translate into improved outcomes. The availability of eculizumab, a humanized monoclonal antibody against C5a [5], provides an opportunity to test this hypothesis. As we are slowly moving towards better identification of acceptable and non-acceptable alloantibody profiles, we should also not forget that the allograft itself may be an important modifier of alloantibody-mediated response. Some transplants can acquire resistance to AMR via a biologic process called "accommodation" [12]. Availability of more and more refined assays implicate that the future of clinical monitoring of highly sensitized transplant patients will probably not become less demanding. Experienced transplant clinicians are increasingly becoming aware that comprehensive risk assessment including both, factors related to the recipient and the allograft could mostly help the patients. [C4d]DSA in serum and C4d in biopsies will certainly remain a significant part of that concept.

## Funding

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## References

1. Colvin RB. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J Am Soc Nephrol* 2007; **18**: 1046.
2. Cohen D, Colvin RB, Daha MR, *et al.* Pros and cons for C4d as a biomarker. *Kidney Int* 2012; **81**: 628.
3. Gebel HM, Bray RA. The evolution and clinical impact of human leukocyte antigen technology. *Curr Opin Nephrol Hypertens* 2010; **19**: 598.
4. Roelen DL, Doxiadis II, Claas FH. Detection and clinical relevance of donor specific HLA antibodies: a matter of debate. *Transpl Int* 2012; **25**: 604.
5. Sacks SH, Zhou W. The role of complement in the early immune response to transplantation. *Nat Rev Immunol* 2012; **12**: 431.
6. Bartel G, Wahrmann M, Exner M, *et al.* Determinants of the complementfixing ability of recipient presensitization against HLA antigens. *Transplantation* 2007; **83**: 727.
7. Chen G, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. *Hum Immunol* 2011; **72**: 849.
8. Wahrmann M, Exner M, Regele H, *et al.* Flow cytometry based detection of HLA alloantibody mediated classical complement activation. *J Immunol Methods* 2003; **275**: 149.
9. Bartel G, Regele H, Wahrmann M, *et al.* Posttransplant HLA alloreactivity in stable kidney transplant recipients-incidences and impact on long-term allograft outcomes. *Am J Transplant* 2008; **8**: 2652.
10. Yabu JM, Higgins JP, Chen G, Sequeira F, Busque S, Tyan DB. C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. *Transplantation* 2011; **91**: 342.
11. Bartel G, Wahrmann M, Schwaiger E, *et al.* Solid phase detection of C4d-fixing HLA antibodies to predict rejection in high ummunological risk kidney transplant recipients. *Transpl Int* 2013; **26**: 121.
12. Dorling A. Transplant accommodation – are the lessons learned from xenotransplantation pertinent for clinical allotransplantation? *Am J Transplant* 2012; **12**: 545.