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INVITED COMMENTARY

Balancing sensitivity and specificity – unfolding crossmatch biology in renal transplantation*

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Clinical renal transplant programmes have been struggling to understand and to interpret the impact of the solid phase human leukocyte antigen (HLA) antibody assays, especially the microsphere-based assays offering antibody definitions at a level of selectivity and sensitivity previously unseen. We have all started to use the language of the laboratory and to ask each other what do you do about a 'DSA with an MFI of 1840 to a possible DP mm, in a negative Xm recipient?' The answer is of course: the acronyms and abbreviations of the laboratory have infected the clinic and no one knows what the true impact of such a test result is. So what does this string of letters mean, what are the real problems and how can we work out the solutions?

The technology for detecting HLA antibodies has advanced substantially over the past decade. The flow-based microspheres with particular HLA molecules bound to their surface, have become the most widespread solid phase assay [1]. Binding of antibody to the surface-bound HLA molecule is detected by the addition of a fluorochrome attached to an anti-IgG antibody, thus measuring IgG anti-HLA antibodies. The molecules bound to the microspheres may be a mixture of HLA molecules or a single HLA antigen thus giving screening results similar to measurement of panel reactive antibodies in the former, or specific detection of antibody to one HLA molecule in the latter. The single antigen bead (SAB) assay thus gives a clear measure of the HLA antibody specificities present in a particular serum sample. Can we use this technology for better matching and allocation of kidneys to avoid graft loss in presensitized recipients? A consensus meeting on these issues will soon publish the results of their eagerly anticipated work.

The technique gives the possibility of undertaking a 'virtual crossmatch' test by combining the patient's known specificities and matching them against the poten-

tial donor's known antigens to identify donor specific antibodies (DSA) thus reducing unwarranted shipping of kidneys and to speed allocation [2]. One of the catches to this approach is that the antibody specificity is at a level that routine deceased-donor typing does not provide in the middle of the night for donor matching. HLA-DP and HLA-Cw are, for example, seldom typed in deceased-donor renal allocation programmes. Hence, dissecting the question above: are antibodies to HLA-DP directed at the recipient DP if the DP typing has not been performed? One cannot be sure, although linkage disequilibrium may allow a Tissue Typing Laboratory to estimate the chance that the donor has the particular molecule. The next issue is whether or not the mean fluorescence intensity (MFI) measured at 1840 – even if it is directed at the HLA-DP molecule on the donor – will predict any impact on the short-, medium- or long-term outcomes of the transplant? At this strength of antibody the B-cell crossmatch will likely be negative by all methods of cytotoxicity and even flow cytometry, but does it predict outcomes?

The use of microsphere assays in patients with a negative conventional crossmatch is thus under much scrutiny, with results that at first glance might seem contradictory [3,4]. While there remains some disparity in the data, there is a view that pretransplant antibodies to both class I and II, detectable only by solid phase assays, portend a poor outcome in the medium-term.

Huh *et al.* [5] in this issue have taken a different approach to the use of the microsphere technology. By using donor cell lysates and binding actual donor HLA molecules to the surface of microspheres, they have obviated the need for virtuality – the molecules on the beads come from the donor. The capture immunoglobulins are directed at framework components of HLA class I or HLA class II, thus allowing identification of donor-specific HLA antibodies to this level – the Luminex crossmatch (LumXm). Huh *et al.* have selected relatively unsensitized patients with a PRA of <20% who have been transplanted across negative anti-human globulin enhanced T cell and conventional complement dependent cytotoxicity B cell crossmatches. They have identified that 18 of 55 such patients have a positive LumXm (six to class I, five to class II and seven to both I and II), and detecting 10 of the 16 patients known to have class I DSA as identified by the SAB test and nine of nine known to have class II DSA, but also finding three patients with a class I and three with a class II not detected by the SAB test. The real outcome is tested by renal function and by rejection rates. There were no graft or patient losses in the study, but there were more rejection episodes in the LumXm positive grafts than LumXm negative (clinical rejection 12 of 18 vs. 8 of 37, Biopsy proven rejection 9 of 18 vs. 3 of 37). Eight of the nine biopsy proven rejection episodes in the LumXM positive group were Banff grade IIA, and were negative for C4d. Renal function was also worse in the first year after transplantation in the LumXm positive group by about 10–11 ml/min using the MDRD estimating equation.

Do we have the new gold standard crossmatch test described in this study? No, for two reasons: first, the technique has to be validated in much more depth. Are all HLA molecules captured equally and presented on the microspheres such that all relevant epitopes are available for recipient antibody binding? Are any antigens denatured in the process such that the molecular binding occurring on the microspheres reflects binding in-vivo? Is antibody avidity – the strength of binding sufficient to avoid being washed off the microspheres – relevant to both the test result and clinical outcomes? Are all the HLA antibodies detected relevant to graft outcomes? Second, will the test remain predictive in highly sensitized individuals? Is it possible that high titre, high avidity antibodies present in such individuals will cross react and give positive results that falsely predict bad graft outcomes?

Do we have a useful assay about which much more must be known? Yes.

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