Improving Crossmatch Techniques and Graft Outcomes

Sir,

The authors Vimal *et al*.[1] in the last issue of the IJN (IJN 2017; 27: 347-52.) and others[2] recommend the Luminex crossmatch (LMC), a solid-phase immunoassay (SPI), for pretransplant crossmatch, demonstrating good short-term graft outcomes and costs, suiting local reality. LMC can detect the important class II anti-human leukocyte antigen (HLA) antibodies, at lower levels than detected by CDC crossmatch or flow cytometric crossmatch (FCXM), a necessity of the hour.

Technological advances of SPIs have caused new paradigms, but the awareness of new problems to be solved is identified with time, a part of the scientific process. Therefore, an awareness of the drawbacks of the LMC and SPI is important for informed decision-making. First, LMC only detects anti-HLA donor-specific antibody (DSA) and not the non-anti-HLA DSA. Next, LMC may have a "gray zone" for mean fluorescence index (MFI) up to 4000 with a sensitivity of 54% and a specificity of 100%. Further, LMC may fail to detect anti-Cw and anti-DP/DQ antibodies and have a lower sensitivity for anti-A and B Class-1 antibodies.[3] Also, as mentioned by the authors, the much larger issue today is the development of *de nova* DSA (dnDSA) and its detrimental long-term effects. Especially, if C1q-binding anti-HLA DSA, which causes increased rates of severe phenotypic antibody-mediated graft injury, microvascular inflammation and C4d deposition within graft capillaries are undetected.[4] Unfortunately, data on dnDSA in the 1st year of follow-up, C4d staining, and protocol biopsies in very high-risk individuals for subclinical inflammation or rejection are understandably not reported, being out of the study's scope and outcomes. However, the correlation of historical immunization and outcomes may be of explicit local clinical interest.

The MFI, the basis of LMC, is at best a semiquantitative number. This single metric, the "MFI", does not outline in black and white the antibody effects. Nor do the MFI "thresholds" always translate between transplant centers, graft pathology or its dysfunction. Sometimes, the MFI may not tell us happenings within a patient. High MFI thresholds may not always be causative of graft dysfunction. Importantly, the MFI is no absolute quantification of the DSA titers. However, it is only a determination of the reactivity of DSA and the strength of reactivity, which are expressed as a mean of the fluorescence intensity. The quantification of an antibody status is best achieved by titration.

Often overlooked is the fact that no vendor kits of Luminex technology are available with representation for all common HLA alleles, especially in the local immune context. SPI panels have variable results, on testing the same recipient sera, with different vendor protocols or kits. Suboptimal assay reproducibility and variability in test interpretation, from center to center, occurs in the absence of assay standardization. HLA antigen purification may cause denatured epitopes that could significantly affect detection of clinically significant antibodies. Not all positive reactions of preformed antibodies detected by LMC are due to clinically relevant antibodies, as reported by the authors in the recent past. Despite the use of controls, complement-mediated interference may confound clinically relevant anti-HLA DSA, adding complexity with false negatives (prozone effect). LMC does not always prevent false positives (denatured epitopes). The above at best, outline the need for awareness of a few prevalent issues with LMC and SPI. But, absolutely by no means, call for negation of the very important advance of "the SPI" over the last decade in antibody detection and its potential to improve long-term graft outcomes.

The Halifax FCXM protocol, adopted nationally in Canada and used world over at numerous laboratories, is an alternative to emulate. It is done in 30–40 min and found superior to the standard FCXM assay.[5] Such approaches to evolve better interpretation of antibody results today include pretreatment of sera, protocol modifications, method standardization, and the evolving use of HLA epitope-based analyses. Potential long-term immune risks of graft function are best identified early if possible. Indigenous, economically developed local solutions and protocols, with a national consensus and standardization, could improve the long-term post transplant outcomes of our patients.

Finally, the authors are congratulated for their work in unfolding the many unknowns in our patients.

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Conflicts of interest

There are no conflicts of interest.

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