3- Reliability of Virtual Crossmatch: Reality of perception

Abstract

Background: The increased sensitivity of the bead assay has permitted the concept of virtual crossmatch (VXM), whereby the actual crossmatch (AXM) using flowcytometry (FCXM) is predicted to a high degree of accuracy based on single antigen bead (SAB) assay. Nevertheless there is still concerns about the reliability of VXM to replace AXM. Ideally each laboratory should validate the reliability of VXM before the omission of AXM. Objective: In this study, we aimed to evaluate the concordance between VXM and AXM using SAB and to determine a cut-off point for VXM in our laboratory. Method: AXM: 773 crossmatches performed by FCXM were retrospectively analyzed. The cut off for T and B are 64, 100 respectively. This includes 573 crossmatches performed using peripheral blood- or spleen-derived lymphocytes deceased donor cells with potential recipients & 200 consecutive final crossmatches performed for living transplantation. VXM: Donor HLA- antigens were compared against the potential recipient’s antibody specificities. Prediction of vFXM was based on antibody identification using SAB. Normalized MFI values of 3000 were used to identify antibodies predicted to cause a positive FCXM for A, B, DR &5000 for HLA-C and DQ specificities when present as single antibody. A sum of multiple weak antibodies (MFI≤2000) that yield MFI of ≥3000 was used to predict positivity. Result: Only positive FCXM positive for B or B &T simultaneously are considered. The vast majority of aFXM were in concordance with vFXM with an overall concordance of 97%. Twenty-three predicted to be negative showed positive aFXM, 12 of them had 0% cPRA and 11 were found in patients with multiple non DSA- HLA- antibodies, dilution of these sera revealed weak DSA. Three predicted positive vFXM yield negative aFXM, two of them had allele specific antibodies. Because sera of highly sensitized patients are diluted we rarely deceived by prozone phenomenon. Therefore the only cause of false negative VXM is auto or non HLA- antibodies. Due to the extreme sensitivity of SAB, reporting false positive VXM remains a possibility and will continue to be a limitation for VXM. Conclusion: vFXM based on precise characterization of antibody specificities detected by SAB using our cutoff point accurately predicted FXM in the majority of patients and can be used safely to allocate kidney offers without performing physical crossmatches in selected patients.