Surrogate Crossmatches As a Tool to Define the True Incidence of HLA Class II de novo DSA

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AIM

De novo donor-specific HLA antibodies (dnDSA) targeting HLA-DQ and –DR antigens is a leading cause of graft loss. Misfolded HLA on SABs leads to artifactual reactivities that are clinically irrelevant and reporting such reactivities as DSA can change a patient's clinical management. Physical crossmatches (XM) do not display misfolded HLA and can serve as a useful tool to verify potential DSAs. We confirmed possible dnDSAs against HLA-DR or HLA-DQ identified by SAB using flow cytometry surrogate B cell XM (surrBXM).

METHODS

New reactivities against donor DR or DQ antigens that developed post-transplant were confirmed by performing surrBXM comparing previous to current sera. Leveraging our frozen cell stocks from nearly 6,000 donors, we identified cells homozygous for the specific HLA in question. dnDSA were defined as Negative in pre-transplant samples but increased reactivity (MFI≥1000) on SAB post-transplant. Conservative surrBXM reactivity categories, beyond the common binary XM output, were used to assess DSA reactivity: Negative - MCS ≤1 SD of control sera used to establish XM thresholds (≤33 MCS), Possible positive (1-2 SD; 34-66MCS), or Positive if >2 SD (≥67 MCS).

RESULTS

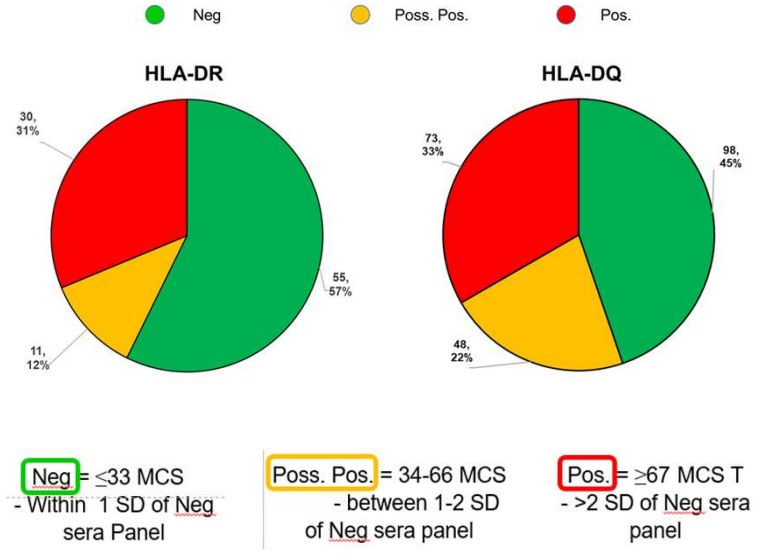


Figure 1. Confirmation of SAB reactivities using surrogate B cell crossmatches. Potential DSA reactivities to HLA-DR or -DQ were grouped as either negative (Neg), possible positive (Poss. Pos.), or positive (Pos.) according to the degree of reactivity against surrogate B cells as described on the LH side.

RESULTS (continued)

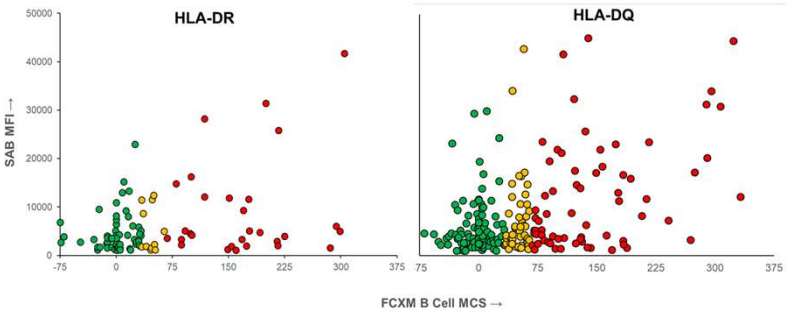
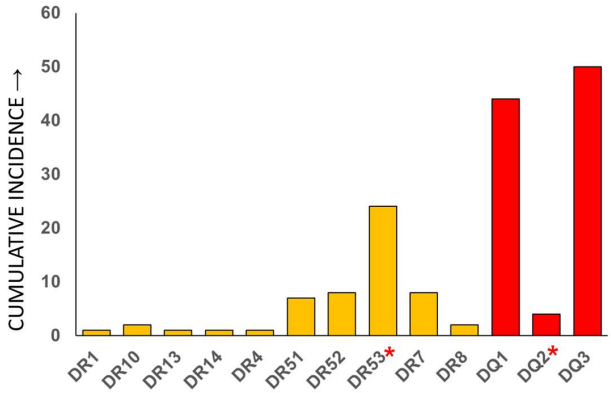


Figure 2. Correlation of SAB reactivities and corresponding surrogate B cell crossmatches. Correlations between MFIs of the possible DSAs targeting HLA-DR or –DQ antigens and the resulting median channel shifts (MCS) on the respective surrogate B cell crossmatches.



* *Common false positive patterns (DR53/DP1/DP5)(DQ2/DQA05 only) were triaged and did not undergo XM verification*

Figure 3. Cumulative Indicence of Potential *de novo* DSAs with Negative Surrogate B Cell Crossmatches. List of potential DSAs and specific HLA targets that showed negative surrBXMs.

CONCLUSION

Our results suggest that the reporting of de novo DSA based solely on SAB MFI thresholds is a dangerous practice and argues that validation using more stable HLA platforms is necessary (Many, but not all, of our confirmatory surrogate crossmatch results were also validated with phenotype beads – data not shown). The use of surrogate crossmatches is a viable option for validation of SAB results as it offers a low cost option to the laboratory but requires the compilation of an expansive frozen cell repository.

Importantly, our study is not an argument against the use of virtual crossmatches but serves as a warning about the importance of SAB interpretation and recognition of false positive SAB reactivities.