Prozone Phenomenon Complicates Detection of Donor Specific Antibodies in Haploidentical Hematopoietic Cell Transplant Patients

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Abbreviations

HLA: Human leukocyte antigen; HCT: Hematopoietic cell transplant; DSA: Donor specific anti-HLA antibody; SAB: Single-antigen bead; MFI: Mean fluorescent intensity; PRA: Panel Reactive antibody

The use of human leukocyte antigen (HLA)-haploidentical related donors has become increasingly common among patients requiring hematopoietic cell transplant (HCT) [1]. Recent studies have shown that the presence of donor specific anti-HLA antibodies (DSA) in the recipient's serum prior to transplant is associated with poor engraftment [2]. Due to the high number of unshared HLA loci in haploidential HCT (haplo-HCT) setting, the odds of the recipients carrying pre-existing DSA are significant. Ciurea et al. [3] found 18% of 122 patients screened prior to haploidential transplant had at least one DSA. As in previous studies, the presence of DSAs was found to be a risk factor for graft failure despite the use of desensitization regimens in 54.3% (12/22) of patients. Consequently, detection of these antibodies is critical in donor selection. Additionally, accuracy of DSA detection is critical for monitoring these antibody levels during the desensitization process. At present, there is no standard screening procedure for DSAs. Herein, we present evidence that undiluted Single-antigen bead (SAB) testing is insufficient for proper detection of DSAs likely due to the complement interference phenomenon.

In the past six years, we performed anti-HLA antibody screening in 102 haplo-HCT patients using a transplantation protocol described by Bhambidipati et al. [4]. We screened the serum of these patients for DSAs using a Single Antigen Kit from One Lambda. In our institution, mean fluorescent intensity (MFI) of 2000 was used as the positive cutoff for clinically relevant antibody titer based on our correlation study between fluorescent intensity (MFI) of 2000 was used as the positive cutoff for clinically relevant antibody titer based on our correlation study between solid phase immunoassay and cytotoxic crossmatch in solid organ transplant settings [5]. Panel reactive antibodies (PRA) were calculated with the online tool provided by the U.S. Department of Health and Human Services.

Fifty-nine (59%) of these patients were found to have anti-HLA antibodies. Sixteen (16%) patients had a total of 37 antibodies which were classified as DSAs based on their donor’s HLA typing. Patient demographics are summarized in Table 1. The majority of patients were women (88%) and diagnosed with AML (69%). Median follow-up was 140 days (range: 6-455) in all patients and 438 days (range: 225-455) in surviving patients. In patients who engrafted, median time to neutrophil recovery was 16.5 days (range: 14-78). PRA scores were uniformly high (median: 97.5, range: 32-100).

We subsequently performed serial dilutions (1:25 and 1:50) and C1q testing on the patients with DSAs. We discovered that, in a subset of antibodies, undiluted (or “neat”) MFI as measured by SAB did not accurately represent antibody strength. The neat MFI was found to be significantly lower than the MFI on 1:25 dilution of serum in 27% (10/35) of DSAs detected (Figure 1A). The inhibition of an antibody-based assay in the setting of high antibody titers is a classic presentation of the prozone effect. Loiseau and colleagues recently raised the possibility of a complement-mediated prozone effect interfering with detection of DSAs in the HCT setting but this has not, to our knowledge, been previously reported in the literature [6].

In our population, we found no difference in frequency of the prozone effect between antibodies to class I and class II antigens (95% CI: 0.83-13.9). The presence of multiple DSAs was not associated with a higher likelihood of any particular antibody exhibiting the prozone effect (RR: 2.1, 95% CI: 0.32-14.0). Patients with DSAs exhibiting the prozone effect had significantly PRA scores (Wilcoxon Sum-Rank: p<0.04). None of these patients had PRA scores less than 90%. All antibodies demonstrating the prozone effect were complement fixing, as measured by the C1q test. Consequently, we propose that complement interference is responsible for this phenomenon in our cohort. As reported in kidney transplantation, it is likely caused by the impairment of the detection of the anti-HLA IgG antibody with the anti-IgG secondary antibody by complement activation products [7].

Figure 1B-C demonstrates that undiluted SAB testing correlates poorly with both 1:25 dilution (R2<0.001) and C1q testing (R2=0.019). On the other hand, a high degree of association between C1q and 1:25 dilution SAB testing is observed (R2=0.69) (Figure 1D). Similarly, the SAB test on 1:50 dilution is highly correlated with 1:25 dilution (R2=0.97, p<0.001) and C1q testing (R2=0.58, p<0.001) (Data not shown). All of these secondary tests provide similar complementary information to

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Table 1: Donor specific antibodies in patients undergoing haploidentical hematopoietic cell transplant. Antibodies exhibiting the prozone effect are highlighted in red. Abbreviations: Diagnosis (Dx) Neutrophil engraftment (NE), overall survival (OS), panel reactive antibodies (PRA), mean fluorescence intensity (MFI).

Based on the evidence presented here, we believe that undiluted SAB testing is not sufficient to characterize clinically relevant DSAs in the haplo-HCT setting. Furthermore, we have shown in our cohort antibodies demonstrating the prozone effect were all complement fixing. This complement-mediated prozone effect could be simply fixed by routinely performing serial dilutions on all DSA testing. Given the importance of DSA detection and the high prevalence of the prozone effect, we assert that undiluted SAB testing alone is not sufficient for pre-transplant screening in Haplo-HCT. As an example of this phenomenon, we would like to highlight the case of a patient #3 in whom we found DSA to HLA B*57:01 which, because of the prozone effect, had initially not met our threshold for reporting as a positive (MFI>2000). The patient was included in the group undergoing undiluted SAB testing. Given the evidence presented here, we believe that undiluted SAB testing is not sufficient to characterize clinically relevant DSAs in the haplo-HCT setting. Furthermore, we have shown in our cohort antibodies demonstrating the prozone effect were all complement fixing. This complement-mediated prozone effect could be simply fixed by routinely treatment of EDTA without significant increasing the cost or additional screens to trend antibody strength. Other studies have explored the use of ethylenediaminetetraacetic acid (EDTA), dithiotreitol (DDT) or heat to eliminate the prozone effect in SAB testing. Tambour et al. [9] performed serial dilution on serum pre-treated with DTT which abrogates the complement interfering and then tested the serum in SAB assays. They revealed a few of antibodies that still demonstrated prozone effect with the DTT treatment, suggesting other non-complement mediated may play a role in causing the falsely decreasing readings. On the other hand, compared to noncomplement binding antibodies, the antibodies demonstrating complement mediated prozone effect shown in our study might confer greater risk of graft failure [3].

In other settings, several different approaches have been recommended to eliminate the prozone effect in SAB testing. Tambour et al. [9] recommended performing at least two serial dilutions on all DSA screens to trend antibody strength. Other studies have explored the use of ethylenediaminetetraacetic acid (EDTA), dithiotreitol (DDT) or heat inactivation [10,11]. Of these, EDTA is the most promising, but lack of standard concentration makes the literature difficult to interpret. At present, sufficient evidence is not available in the HCT setting to evaluate these methods of secondary testing.
Screening with either C1q testing or a single dilution provides complementary information when used along with undiluted SAB testing and we recommend this approach for all sensitized patients undergoing haplo-HCT, especially with PRA scores ≥ 90%. Further studies examining the relative efficacy and cost effectiveness of the individual approaches in this setting are still needed.

Conflict of interest

All authors have no financial disclosure related to the study described in the submitted manuscript. The authors declare no conflict of interest.

References
