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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 haploidentical 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 haploidentical 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 54.5% (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 Bhamidipati 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 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 majorities 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 detecting 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% C.I. 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% C.I. 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<.001) and C1q testing (R2=0.58, p<0.001) (Data not shown). All of these secondary tests provide similar complementary information to

PID	Age	Sex	Dx	NE	OS	Status	PRA	DSA HLA Loci	Undiluted (MFI)	1:25 Dilution (MFI)	1:50 Dilution (MFI)	C1q (MFI)	Prozone Effect
1	42	F	AML	17	441	Alive	100	C*03:04	3,621	9,559	6,505	26,097	Yes
2	65	M	AML	20	176	Dead	76	B*35:01	2,415	759	407	70	No
3	26	F	AML	-	6	Dead	91	A*02:01	2,963	826	356	44	No
								B*57:01	1,479	23,022	20,930	25,572	Yes
								C*06:02	2,819	228	57	159	No
4	54	F	AML	31	341	Dead	89	A*25:01	9,341	3,235	2,062	18,996	No
5	69	F	AML	-	35	Dead	100	B*57:01	2,109	21,643	19,412	25,932	Yes
								DRB1*07:01	2,517	22,970	23,506	25,204	Yes
6	48	F	CML	57	126	Dead	100	DRB1*08:04	17,061	4,807	2,807	11,782	No
7	61	F	AML	-	67	Dead	99	A*02:01	5,828	22,373	19,286	26,244	Yes
								B*07:02	8,104	23,120	20,378	26,645	Yes
								DRB1*15:01	17,042	9,804	6,306	24,886	No
								DRB5*01	19,097	10,129	6,274	2,489	No
								DQB1*06:02	17,527	6,080	3,668	18,764	No
8	53	F	AML	17	120	Dead	100	A*68:01	2,943	223	115	0	No
								B*27:05	3,466	14,236	9,473	26,780	Yes
								DRB1*14:01	2,000	89	16	0	No
9	44	F	AML	14	235	Dead	99	B*07:02	8,225	12,338	7,583	26,645	Yes
								DRB1*15:01	11,519	9,371	5,067	24,886	No
								DRB5*01	13,088	4,352	2,298	2,489	No
								DQB1*06:02	10,521	2,165	962	959	No
10	41	F	ALL	15	35	Dead	32	B*40:01	2,182	180	82	40	No
11	53	F	AML	14	455	Alive	96	DRB1*11:04	2,096	283	79	0	No
								DQB1*03:01	7,248	2,880	1,283	6,966	No
12	53	F	AML	78	434	Alive	96	A*02:01	3,184	95	53	55	No
								DRB1*15:01	10,446	6,839	3,836	25,886	No
								DRB5*01	6,963	400	0	148	No
								DQB1*0602	8,547	260	78	110	No
13	57	F	NHL	16	148	Dead	48	A*02:01	2,053	126	49	0	No
14	49	M	MDS	-	11	Dead	100	A*03:01	4,705	11,761	8,650	12,703	Yes
								DRB1*15:01	6,387	10,995	8,207	13,428	Yes
								DRB5*01	7,474	2,934	1,959	2,002	No
15	37	F	AML	14	225	Alive	95	B*07:02	11,796	1,588	880	2	No
16	55	F	ALL	15	140	Dead	100	A*03:01	7,285	660	294	95	No
								B*07:02	9,302	4,132	1,869	10,807	No

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).

undiluted SAB 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 serial dilution and C1q testing due to other DSAs. This case demonstrates the possibility that relying on undiluted SAB testing alone can not only underestimate antibody strength, but may miss relevant DSAs entirely.

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 treatment of EDTA without significant increasing the cost or additional tests. It is worth noting that a traditional noncomplement mediated prozone effect caused by excessed antibody(ies) is not ruled out by our current study. In kidney transplant settings, Konvalinka and colleague [8] 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.

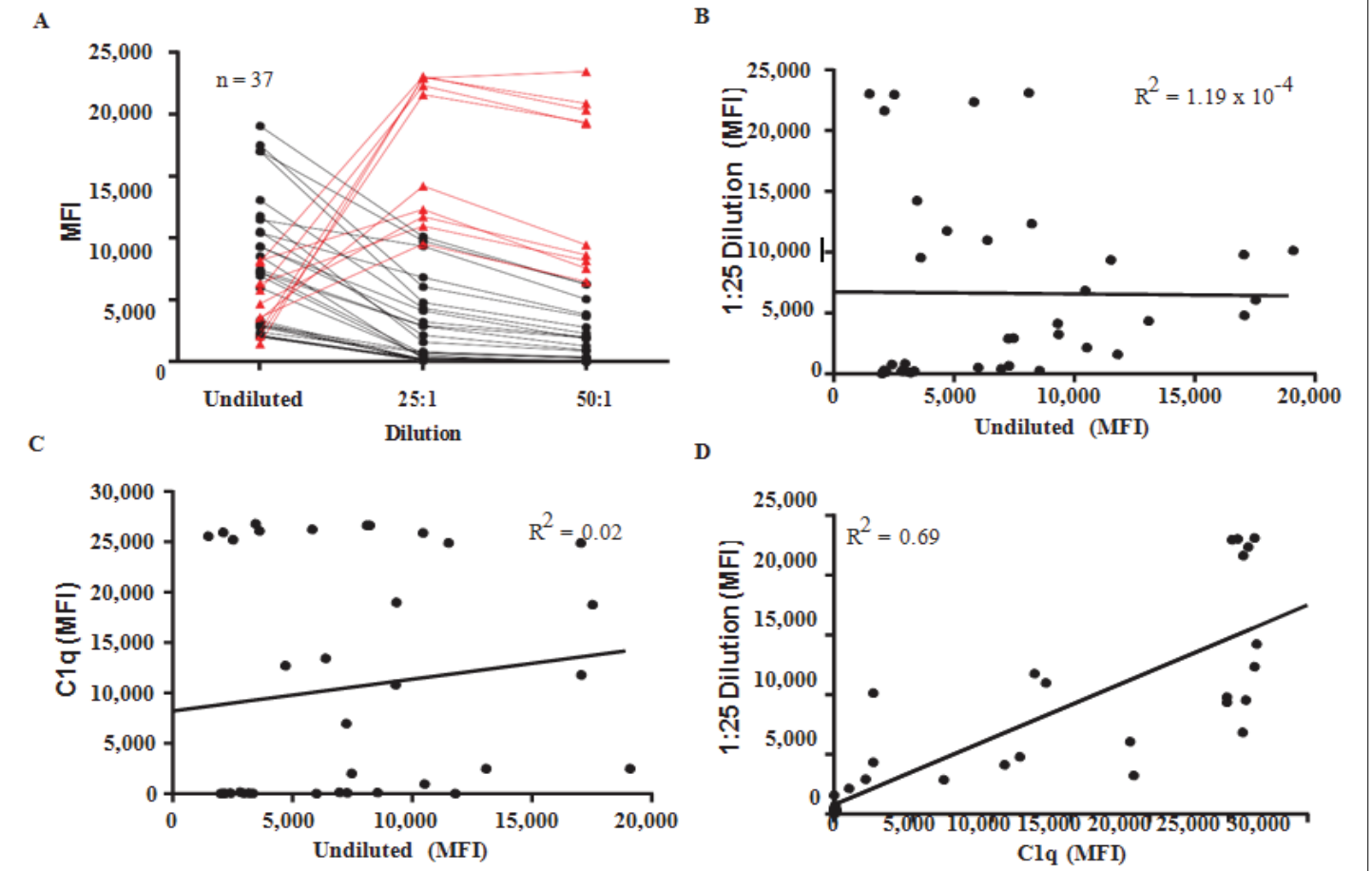

Figure 1: A) Mean fluorescent intensity (MFI) of serial dilutions of patients with DSAs. Linear correlation of single antigen bead (SAB) testing using undiluted serum with **B)** 1:25 diluted serum and **C)** C1q testing. Neither of these correlations is significant. Correlation between C1q testing and SAB with 1:25 diluted serum **D)** was significant and strongly correlated (coefficient of determination = 0.692).

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.

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