Anti-HLA Antibodies & Anti-HLA Eplet Patterns Associated with Platelet Transfusion Refractoriness in Hispanic Patients

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Introduction

Platelet transfusion refractoriness (PTR) increases morbidity and mortality in patients which can be caused by anti-Human Leukocyte Antigen (HLA) Class I antibodies resulting in critically low platelet counts. PTR occurs in approximately 44% of frequently transfused patients.[1] Anti-HLA Class I antibodies contribute to >80-90% of alloimmune PTR cases.[2]

HLA are the most polymorphic human genes, but specific allele and haplotype inheritance patterns are related to race/ethnicity. The Hispanic population has greater diversity and different haplotype patterns compared to Caucasians, but Caucasians are the most frequent platelet donors. Therefore, Hispanic patients are at greater risks for having alloimmune PTR due to increased HLA mismatching.

No published PTR studies have focused on Hispanic patients, the second largest ethnic population which continues to have the greatest absolute growth in the US per the 2024 Census. This study aimed to evaluate anti-HLA Class I antibody detection and HLA eplet patterns associated with PTR in a cohort of Hispanic patients.

Methods and Materials

A single center retrospective study identified 16 adult, self-identified Hispanic patients that were diagnosed with PTR and had Anti-HLA antibody testing from 2016-2024 (Table 1). Patients with ITP or Evan's Syndrome were excluded. Platelet transfusion efficacy was determined by corrected count increment (CCI) calculation and a CCI of < 5,000 was used to categorize patients as having PTR for the platelet transfused immediately prior anti-HLA antibody testing.[3]

Class I anti-HLA antibody testing was performed using the LABScreen Single Antigen (Thermo Fisher) assay with Extended Bead Panel for testing performed 2021-2024. Baseline MFI values for each Class I HLA allele were summed to obtain additive HLA-A, HLA-B, HLA-C, and total additive MFI values. Antibodies were categorized by MFI using clinical cut-points for sub-analysis: Negative (MFI < 1,000), Weak (MFI < 4,000 to ≥ 1,000), Moderate (MFI < 10,000 to ≥ 4,000), Strong (MFI ≥ 10,000). Additionally, Fusion-MatchMaker antibody verified eplets identified for alleles ≥1,000 MFI determined the additive Ellipro scores (Table 2).

Statistical analysis included Spearman Rank Correlation, Jonckheere-Terpstra Test, Mann-Whiteney U Test, and ROC analysis.

Table 1. Characteristics of Hispanic Platelet Transfusion Refractoriness Patients

Characteristic	Number of Patients (Percent)
Demographics	
Female	14 (87.5%)
Male	2 (12.5%)
Age (mean years, SD)	50.50 (12.34)
Anthropometric Parameters (mean, SD)	
Height (meters)	1.606 (0.073)
Weight (kilograms)	73.49 (25.72)
Body Surface Area (meters^2)	1.787 (0.310)
Diagnosis	
Leukemia	5 (31.3%)
Aplastic Anemia or Myelodysplastic Syndrome	4 (25%)
Liver Disease	2 (12.5%)
Cancer	3 (18.8%)
Septic embolism	1 (6.3%)
Thrombotic Thrombocytopenia purpura (TTP)	1 (6.3%)
Co-morbidities Present at Diagnosis of PTR	
Splenomegaly	3 (18.8%)
Disseminated Intravascular Hemolysis	1 (6.3%)
Bleeding	1 (6.3%)
Fever	4 (25.0%)
None	9 (56.3%)
Transfusion History	
Red Blood Cells	15 (93.8%)
Platelets	16 (100%)
Whole Blood	1 (6.3%)
Plasma	4 (25%)
History of Pregnancy (females = 14)	13 (92.9%)
Single pregnancy	1
2-3 Pregnancies	6
4-5 Pregnancies	6

Table 2. Observed Frequency of Potential Antibody Verified Anti-HLA Eplets per HLA Matchmaker

Eplet	Number of Patients (%)	Ellipro Score[4]	Exposition Category[5]	Eplet	Number of Patients (%)	Ellipro Score[4]	Exposition Category[5]
21H	1 (6.3%)	0.404	High	82LR	5 (31.3%)	0.83	High
41T	1 (6.3%)	0.922	High	90D	4 (25%)	0.957	High
44KM	3 (18.8%)	0.642	High	107W	7 (43.8%)	0.591	High
44RMA	4 (25%)	0.661	High	127K	7 (43.8%)	0.593	High
44RT	6 (37.5%)	0.351	High	131S	4 (25%)	0.747	High
45KE	1 (6.3%)	0.275	Intermediate	138K	1 (6.3%)	0.921	High
56R	7 (43.8%)	0.836	High	138MI	2 (12.5%)	0.808	High
62EE	4 (25%)	0.598	High	143S	1 (6.3%)	0.58	High
62GE	7 (43.8%)	0.459	Intermediate	144K	2 (12.5%)	0.736	High
62GK	7 (43.8%)	0.459	Intermediate	144KR	2 (12.5%)	0.736	High
62GRN	8 (50%)	0.459	High	144QL	6 (37.5%)	0.886	High
62LQ	6 (37.5%)	0.486	Intermediate	144TKH	7 (43.8%)	0.797	High
62QE	4 (25%)	0.473	Intermediate	145KHA	7 (43.8%)	0.888	High
62RR	4 (25%)	0.498	High	145RT	5 (31.3%)	0.852	High
65GK	7 (43.8%)	0.416	Intermediate	149TAH	2 (12.5%)	0.852	High
65QIA	5 (31.3%)	0.54	Intermediate	150AAH	7 (43.8%)	0.809	High
65QKR	1 (6.3%)	0.54	Intermediate	151AHA	4 (25%)	0.455	High
65RNA	4 (25%)	0.566	High	156DA	1 (6.3%)	0.259	High
69AA	5 (31.3%)	0.299	Intermediate	158T	5 (31.3%)	0.544	High
69TNT	4 (25%)	0.351	Intermediate	161D	6 (37.5%)	0.547	High
70IAQ	6 (37.5%)	0.299	Low	163EW	4 (25%)	0.365	Intermediate
71ATD	7 (43.8%)	0.345	Intermediate	163LS/G	6 (37.5%)	0.397	Intermediate
71SA	6 (37.5%)	0.276	Low	163LW	5 (31.3%)	0.397	High
71TTS	4 (25%)	0.344	Intermediate	163R	4 (25%)	0.489	High
73AN	2 (12.5%)	0.273	Intermediate	163RG	3 (18.8%)	0.489	High
73TVS	1 (6.3%)	0.273	High	163RW	4 (25%)	0.489	High
76ANT	4 (25%)	0.558	High	166DG	3 (18.8%)	0.594	High
76EG	7 (43.8%)	0.557	High	173K	3 (18.8%)	0.78	High
76ESI	7 (43.8%)	0.557	High	180E	3 (18.8%)	0.455	High
76ESN	3 (18.8%)	0.557	High	193PL	1 (6.3%)	0.936	High
79GT	2 (12.5%)	0.703	High	193PV	5 (31.3%)	0.95	High
80I	5 (31.3%)	0.653	High	219W	1 (6.3%)	0.905	High
80K	2 (12.5%)	0.648	High	248M	2 (12.5%)	0.846	High
80N	3 (18.8%)	0.65	High	253Q	2 (12.5%)	0.941	High
80TLR	4 (25%)	0.634	High				

Results

Significant negative correlations were observed between CCI with total additive MFI (r = -0.656, p =0.008) (Figure 1), allele specific MFI for HLA A*03:01 (r = -0.728, p = 0.002), C*14:02 (r = -0.795, p < 0.001),and C*01:02 (r = -0.727, p = 0.002) and cPRA (r = -.604, p =0.017).

A significant positive correlation between MFI and cPRA (r = 0.944, p < 0.001) was observed (Figure 2). Analysis of cut-points yielded significance between negative and strong MFI for HLA B*07:02 (p =0.002).

No significant difference was observed for additive antibody verified eplet Ellipro scores between patients with and without PTR (Figure 3). Further analysis by ROC yielded a fair area under the curve of 0.716 (95% CI, 0.302, 1.130), but the very broad confidence interval limits utility of additive Ellipro scoring for antibody verified eplets in this small sample size.

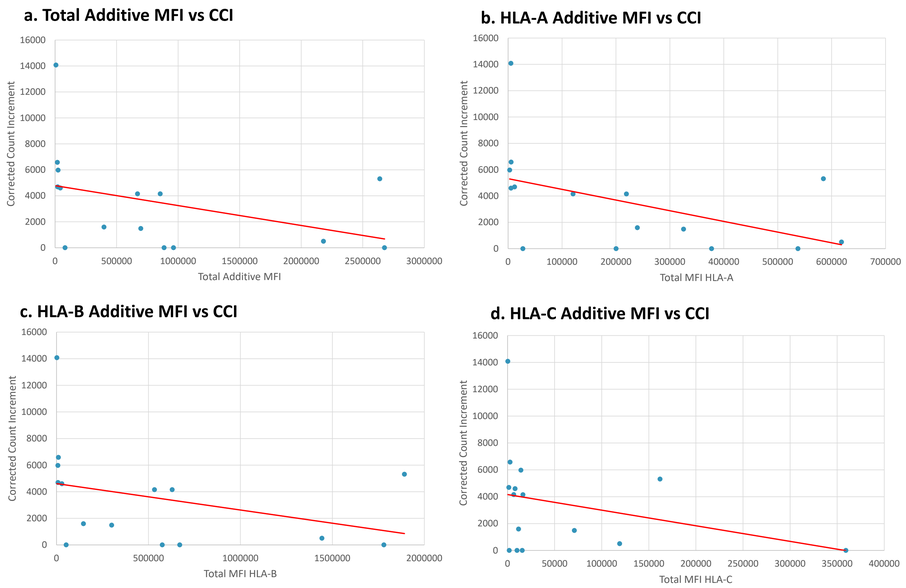

Figure 1 (a-d). Scatterplots of MFI vs Corrected Count Increments (CCI) in Hispanic Patients Diagnosed with PTR. (a) A significant negative correlation (p = 0.008) with a correlation coefficient of -0.656 was determined between total additive MFI and CCI. A sub-analysis for additive MFI for each HLA Antigen (HLA-A, HLA-B, and HLA-C) with CCI was performed using the Spearman Rank Correlation Coefficient. A moderate negative correlation was observed for (b) HLA-A (r = -0.586; p = 0.022) and (c) HLA-B (r = -0.584; p =0.022). No significant correlation was observed with (d) HLA-C (p = 0.150).

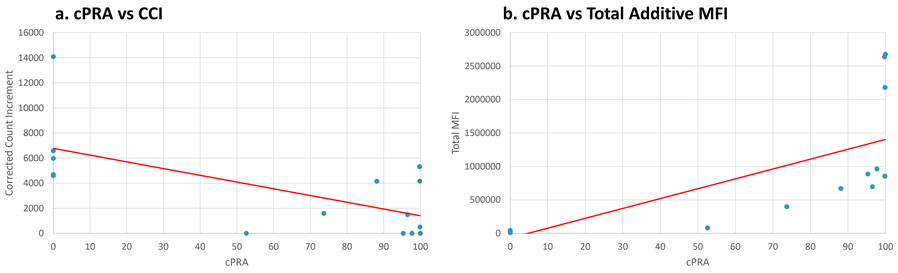

Figure 2 (a-b). Scatterplots of cPRA vs Corrected Count Increments (CCI) and Total Additive MFI in Hispanic Patients Diagnosed with PTR. (a) A significant negative correlation (p = 0.017) with a correlation coefficient of -0.604 was determined between cPRA and CCI. (b) A significant positive correlation (p < 0.001) with a correlation coefficient 0.944 was observed for cPRA and total additive MFI.

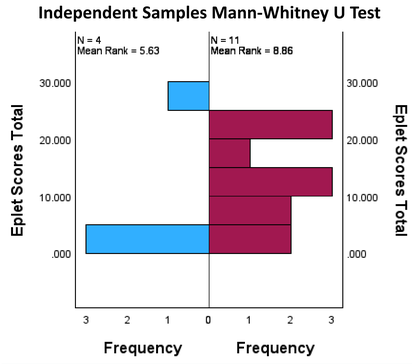

Figure 3. Frequency of Additive Ellipro Eplet Scores Comparison of Patients with Platelet Transfusion Refractoriness (CCI < 5,000) and Patients Without PTR (CCI > 5,000). Although all patients had a PTR diagnosis, classification of Patients with and without PTR was determined by platelet transfusion immediately prior to anti-HLA antibody testing. Frequency distribution reveals most patients with PTR have a total eplet score of above 0.00. Patients without PTR tend to have scores closer to 0.00 except for one outlier that has a total eplet score above all the patients with PTR.

Discussion

Additive MFI values ranged from 7,100 to over 2.6 million. This cohort was primarily multiparous females, supporting that prior sensitizing events increase the risk of alloimmunization to HLA antigens. The patient with the lowest MFI had no history pregnancy but had 28 transfusions.

A significant observation was the inverse correlation of increased additive MFI associated with decreased CCI, suggesting the relative concentration and strength of the antibody directly affects platelet response.

HLA-A*03:01 showed the most significant correlation with CCI suggesting a potential role as a significant determinant of PTR. The HLA-A*03:01 allele is common in both Hispanics and Caucasians; however, Hispanics have a reported frequency of 7.0% while Caucasians almost double the frequency at 13.4% according to CIWD 3.0.[6]

HLA-C antigens revealed the most significant associations, specifically C*14:02 and C*01:02 which may point to a potentially controversial role for anti-HLA-C antibodies in PTR. However, these antibodies may also be targeting a shared eplet/epitope on an HLA-A and/or HLA-B allele due to antibodies against HLA-A or HLA-B with tight linkage disequilibrium to these HLA-C alleles or may be the result of false positive reactivity targeting denatured peptides. Nonetheless, this most likely represents patients with these specific HLA-C antibodies having substantial antibodies to common HLA-A and/or HLA-B antigens or epitopes.

The use of Ellipro scoring for antibody verified eplets provides valuable framework for understanding alloimmunization beyond MFI values or HLA antigen level analyses. The moderate diagnostic value indicated by the ROC curve suggests potential for incorporation into future risk stratification prediction models or matching strategies, particularly in highly sensitized patients.

Conclusions

This study provides valuable insight into anti-HLA antibodies associated with PTR in Hispanic patients. Currently, there is no single parameter used to assess both antibody binding (MFI) and diversity. As additive MFI values increase, CCI values decrease, showing that diversity and binding of anti-HLA antibodies contribute to PTR in Hispanic patients. These results will contribute to development of a preliminary system of more complete immunologic compatibility evaluation in HLA antibody mediated PTR in Hispanic patients.

References

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