

Development of a Calculated Panel Reactive Antibody Web Service with Local Frequencies for Platelet Transfusion Refractoriness Risk Stratification

William J. Gordon^{1,2,3,4}, Layne Ainsworth⁴, Samuel Aronson⁴, Jane Baronas⁵, Richard M. Kaufman^{3,5}, Indira Guleria^{3,6}, Edgar L. Milford^{3,6}, Michael Oates⁴, Rory Dela Paz⁴, Melissa Y. Yeung^{3,6}, William J. Lane^{3,5}

¹Department of Medicine, Division of General Internal Medicine and Primary Care, Brigham and Women's Hospital, Boston, MA, USA, ²Department of Medicine, Division of General Internal Medicine, Massachusetts General Hospital, Boston, MA, USA, ³Harvard Medical School, Boston, MA, USA, ⁴Partners HealthCare, Somerville, MA, USA, ⁵Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA, ⁶Department of Medicine, Division of Renal Medicine, Brigham and Women's Hospital, Boston, MA, USA

Received: 13 May 2019

Accepted: 01 July 2019

Published: 01 August 2019

Abstract

Background: Calculated panel reactive antibody (cPRA) scoring is used to assess whether platelet refractoriness is mediated by human leukocyte antigen (HLA) antibodies in the recipient. cPRA testing uses a national sample of US kidney donors to estimate the population frequency of HLA antigens, which may be different than HLA frequencies within local platelet inventories. We aimed to determine the impact on patient cPRA scores of using HLA frequencies derived from typing local platelet donations rather than national HLA frequencies. **Methods:** We built an open-source web service to calculate cPRA scores based on national frequencies or custom-derived frequencies. We calculated cPRA scores for every hematopoietic stem cell transplantation (HSCT) patient at our institution based on the United Network for Organ Sharing (UNOS) frequencies and local frequencies. We compared frequencies and correlations between the calculators, segmented by gender. Finally, we put all scores into three buckets (mild, moderate, and high sensitizations) and looked at intergroup movement. **Results:** 2531 patients that underwent HSCT at our institution had at least 1 antibody and were included in the analysis. Overall, the difference in medians between each group's UNOS cPRA and local cPRA was statistically significant, but highly correlated (UNOS vs. local total: 0.249 and 0.243, $\rho = 0.994$; UNOS vs. local female: 0.474 and 0.463, $\rho = 0.987$, UNOS vs. local male: 0.165 and 0.141, $\rho = 0.996$; $P < 0.001$ for all comparisons). The median difference between UNOS and cPRA scores for all patients was low (male: 0.014, interquartile range [IQR]: 0.004–0.029; female: 0.0013, IQR: 0.003–0.028). Placement of patients into three groups revealed little intergroup movement, with 2.96% (75/2531) of patients differentially classified. **Conclusions:** cPRA scores using local frequencies were modestly but significantly different than those obtained using national HLA frequencies. We released our software as open source, so other groups can calculate cPRA scores from national or custom-derived frequencies. Further investigation is needed to determine whether a local-HLA frequency approach can improve outcomes in patients who are immune-refractory to platelets.

Keywords: Clinical informatics, human leukocyte antigen, open source, platelet transfusion

INTRODUCTION

Platelet transfusions are an essential component of the clinical care for patients undergoing hematopoietic stem cell transplantation (HSCT).^[1] Platelet refractoriness, or the insufficient rise in platelet count after repeated transfusions, is a common and challenging clinical problem, and can lead to adverse outcomes, including increased bleeding risk, decreased survival, and longer hospitalizations.^[2] The etiology of platelet refractoriness includes nonimmune-mediated

factors such as medication effects, splenomegaly, or sepsis and immune-mediated factors such as alloimmunization

Address for correspondence: Dr. William J. Gordon, Division of General Internal Medicine and Primary Care, Brigham and Women's Hospital, 1620 Tremont Street, Boston, MA 02115, USA. E-mail: wjgordon@partners.org

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Gordon WJ, Ainsworth L, Aronson S, Baronas J, Kaufman RM, Guleria I, *et al.* Development of a calculated panel reactive antibody web service with local frequencies for platelet transfusion refractoriness risk stratification. *J Pathol Inform* 2019;10:26.

Available FREE in open access from: <http://www.jpathinformatics.org/text.asp?2019/10/1/26/263874>

Access this article online

Quick Response Code:



Website:
www.jpathinformatics.org

DOI:
10.4103/jpi.jpi_29_19

to human leukocyte antigen (HLA) or platelet-reactive antibodies.^[3] Despite leukoreduction, alloimmunization to HLA is still a common complication for patients undergoing HSCT.^[4]

After the realization that donor-specific HLA antibodies were associated with hyperacute rejection in renal transplant recipients, understanding which patients would be higher risk for HLA-mediated rejection has been an important component of candidate prioritization in organ allocation algorithms. Panel reactive antibody (PRA) scoring was developed to determine the degree of HLA sensitization. A PRA value is derived using a panel of normal donors to represent the population frequency of HLA – the percentage of this panel of donors a patient has antibodies to is an individual’s PRA score.^[5,6] To improve standardization and take advantage of advances in HLA screening technology, the United Network for Organ Sharing (UNOS) moved toward calculated PRA (cPRA) scores in 2007. cPRA uses a national sample of US kidney donors to estimate the population frequency of unacceptable HLA antigens. Recipient antibodies (and unacceptable antigens) are then compared to this national pool, which results in a cPRA.^[6-8]

Prior work has compared various cPRA and PRA assays in the setting of solid organ transplantation.^[9,10] However, platelets, unlike solid organs, express only Class I HLA antigens (HLA-A, HLA-B, and HLA-C, though HLA-C is not commonly tested).^[11] Because of this, cPRA is often used in clinical practice to estimate the risk of HLA-mediated platelet refractoriness. However, there are several potential limitations to using cPRA to predict platelet refractoriness. First, unlike renal transplantation where a donor can donate at most two organs, platelet donors can continuously re-donate platelets; thus, the true HLA frequency pool is based not on the number of

donors, but the number of units of platelets. Second, the UNOS HLA frequencies are national, but platelet donor pools tend to be regional, potentially leading to important differences in HLA antigen frequency between the two populations. Finally, the UNOS frequencies are renal donors, a different population than platelet donors.

Because of the limitations of using cPRA to predict HLA-incompatibility for platelet transfusion, we hypothesized that building a custom, local HLA frequency pool, where each platelet unit contributed equally (i.e., platelet-driven frequency as opposed to donor-driven frequency), might provide more clinically useful cPRA scores for patients undergoing HSCT. To do this, we constructed a custom HLA frequency data set and compared cPRA scores of patients undergoing HSCT between UNOS and our local, custom calculator.

METHODS

Calculated panel reactive antibody application programming interface

UNOS maintains two methods for calculating cPRA. First, they host a manual calculator.^[12] Users select all recipient antibodies and the website calculates a cPRA. Second, as of November 2016, UNOS maintains an application programming interface (API).^[13] This API requires registration and does not currently allow for custom antigen frequencies. Because of our requirements, notably, a large number of HLA typed patients and the need to create a custom population frequency, we built a RESTful^[14] web service [Figure 1]. This web service supports calculating cPRA using the existing UNOS frequencies, but also allows custom HLA frequencies to be imported and used for calculation. We have released this as

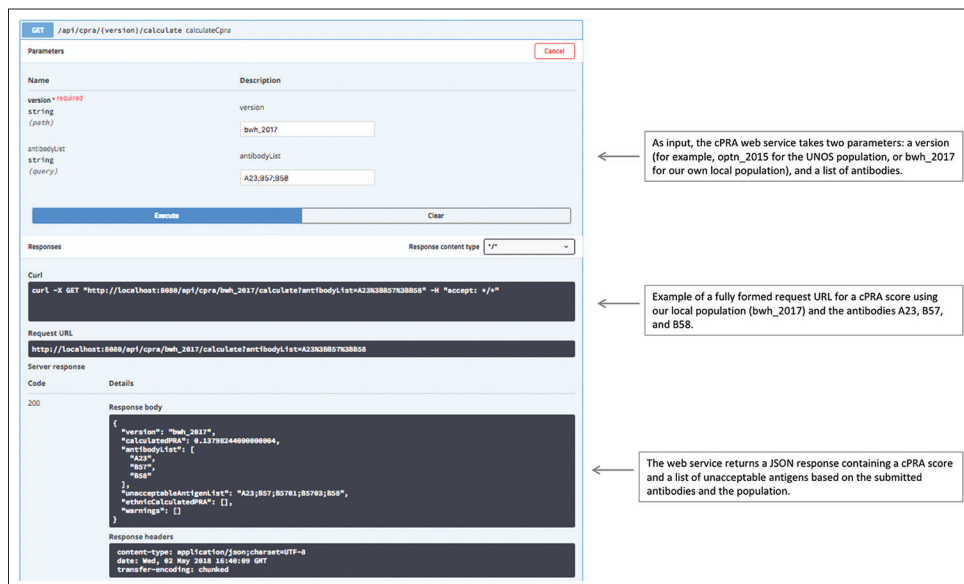


Figure 1: Calculated panel reactive antibody web service. The web service comes packaged with swagger (https://swagger.io), an open-source framework (distributed under the Apache license) for designing and interacting with application programming interfaces (shown). The web service accepts GET requests with a “version” (or population frequency) and a list of antibodies and returns a calculated panel reactive antibody score and corresponding unacceptable antigens

an open-source project, so others can calculate cPRA based on their local population frequencies.^[15] Our API was built using Spring Boot, an open source, microservice-oriented application generator for java applications.^[16] Figure 1 demonstrates how our API works.

Data sources

We collected retrospective Class I HLA antibody data screened at our institution from November 2007 through January 2018. The laboratory uses polymerase chain reaction-based methodologies for HLA typing and Luminex bead-based anti-HLA antibody detection assays to process patient samples.

Generating local frequencies

The current cPRA calculator is rooted in population genetics and based on the HLA and ethnic frequencies of deceased US kidney donors. Specifically, multiple locus haplotype frequencies are estimated using an expectation maximization algorithm, which uses an iterative process to approximate the maximum likelihood estimates of haplotype frequencies in a population of unrelated individuals.^[7,17] The technique is complicated and requires advanced techniques in population genetics, though the Organ Procurement and Transplantation Network has published demonstrations on performing these calculations.^[8]

To generate HLA frequencies based on our local apheresis platelet donor pool, we aggregated class I HLA antigen data for all typed platelet units donated at our institution from September 2016 through June 2019. We picked September 2016 as our start date as this was when our platelet donor center began proactively identifying women who self-reported a current or past pregnancy in line with AABB standard 5.4.1.3.1.^[18] Frequencies were calculated by adding every

unique combination of A and B loci antigens and dividing by the total number of platelet units. Figure 2 demonstrates this methodology in more detail. Source code to generate the frequency file has also been made available online.^[15]

Statistical analysis

For each patient in our data set with at least one HLA antibody, we calculated a cPRA score using the UNOS frequencies and a cPRA score for our local, platelet unit-driven frequencies. We created histograms to examine the distribution of cPRA scores for our population, segmented by gender. To compare differences in the median cPRA scores between groups, we used the Wilcoxon Signed-Rank test. To compare interpopulation median cPRA gender differences, we applied the Mann–Whitney test. We used the Spearman’s rho correlation to examine the relationship between scores from the two calculators. To look at the patient-level magnitude of the difference between calculators, we created histograms, segmented by gender, for the median difference between calculators. Finally, we then placed each cPRA score into one of three buckets: <20% (low cPRA), 20%–90% (medium cPRA), and >90% (high cPRA). We used these buckets based on our clinical experience interpreting cPRA for patients undergoing workups for platelet refractoriness. We looked at intercategory movement of patients between the three cPRA score buckets (low, medium, and high) to assess the clinical impact of using the platelet unit-driven calculator.

All analyses were conducted using R (2016. Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org>). The Partners HealthCare Institutional Review Board reviewed and approved our study (protocol number, 2017P000637).

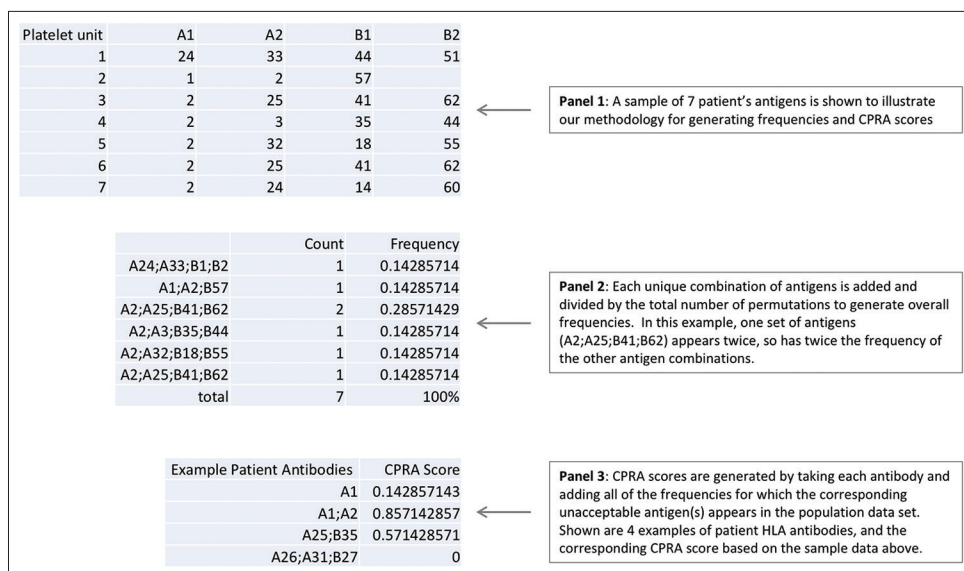


Figure 2: Generating population frequencies using the counting methodology. Panel 1 is a sample set of seven patients with their corresponding human leukocyte antigen. Panel 2 shows how the frequencies for this population would be generated and their corresponding values. Panel 3 shows four other sample patients and their corresponding calculated panel reactive antibody scores based on their antibodies in the left column, and the frequencies of the sample population described in Panel 1 and Panel 2

RESULTS

We calculated cPRA scores for 4,282 samples and 3,124 unique patients undergoing HSCT at our institution using our custom-developed web service [Figure 1]. Of those, there were 2,531 samples with at least one antibody, which we used for the cPRA calculations. The distribution of cPRA scores between the UNOS calculator and our local calculator is shown in Figure 3, segmented by gender, along with median and sample size for each group. There was a statistically significant difference between the median cPRA scores between the UNOS population (median = 0.249, interquartile range [IQR] of 0.067–0.697) and our local population (median = 0.243, IQR of 0.069–0.681) ($P < 0.001$). We also found a statistically significant difference when we segmented by gender. For men, the UNOS median was 0.165 (IQR of 0.029–0.418). The median for our local population was 0.141 (IQR of 0.004–0.408) ($P < 0.001$). For women, the UNOS median was 0.474 (IQR of 0.111–0.872) and the local population median was 0.463 (IQR of 0.112–0.856) ($P < 0.001$), all shown in Table 1.

While the median cPRAs were statistically different between populations, we were unsure of the clinical significance of these findings given the similar values. To further evaluate the implications of these findings, we performed several

analyses. First, we examined the magnitude of the cPRA score difference between the two calculators, segmented by gender, by calculating the absolute difference between the UNOS-calculated cPRA and the local-calculated cPRA scores for every patient in our data set with at least one antibody (e.g., a side-by-side comparison of the two calculators for every patient in our analysis set). As shown in Figure 4, the median difference between the UNOS-calculated cPRA and the local-calculated cPRA was below 0.25 for every patient, with the majority even lower (male median difference 0.014, IQR of 0.004–0.029, female median difference 0.013, IQR of 0.003–0.028). Second, we looked at the correlation between the three comparison groups (total, female, and male). Each group was highly correlated (rho of 0.994, 0.996, and 0.987, $P < 0.001$ for all three comparisons) [Table 2].

Finally, we looked at whether the calculators would place patients into different cPRA groups. Based on our clinical experience, we picked <20%, 20%–90%, and >90% as three clinically significant cPRA scoring groups (mildly, moderately, and highly sensitized, respectively). Overall, the number of patients in each group was similar, with most patients falling in the low or moderately sensitized groups in each population [Figure 5]. At the patient level, there was minimal but important movement between groups. For example,

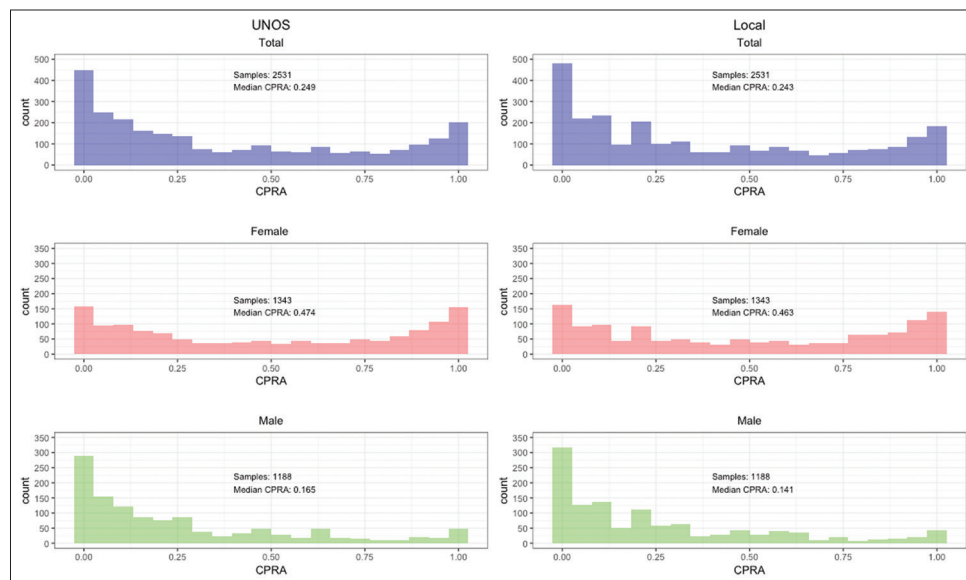


Figure 3: Distribution of calculated panel reactive antibody scores for United Network for Organ Sharing frequencies and local, platelet unit-driven frequencies, total, and segmented by gender, for all patients with at least 1 antibody

Table 1: Median calculated panel reactive antibody scores (interquartile range) for the United Network for Organ Sharing population and our Local population (for all patients with at least one antibody), across the entire population, and segmented by gender

Population	UNOS	Local	P
Total	0.249 (0.067-0.697)	0.243 (0.069-0.681)	<0.001 (UNOS vs. local, total)
Male	0.165 (0.029-0.418)	0.141 (0.004-0.408)	<0.001 (UNOS vs. local, male)
Female	0.474 (0.111-0.872)	0.463 (0.112-0.856)	<0.001 (UNOS vs. local, female)

UNOS: United Network for Organ Sharing

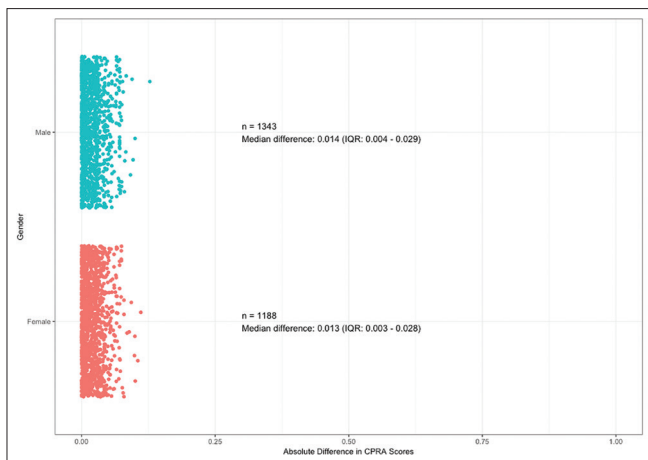


Figure 4: Comparison of median differences between calculated panel reactive antibody scores. Shown are the absolute values of differences in Calculated Panel Reactive Antibody scores between the two populations (united network for organ sharing vs. local) for every patient in our population with at least 1 antibody ($n = 2531$)

25 patients were differentially categorized from the UNOS calculator as compared to the local calculator and 50 patients were differentially categorized from the local calculator to the UNOS calculator, for a total of 2.96% of patients differentially classified (75/2531) [Table 3].

DISCUSSION

Platelet transfusions are a critical supportive therapy for patients undergoing HSCT, though many patients will develop antibodies to HLA making therapeutic platelet transfusion challenging. cPRA scoring is used by clinicians as a population-based method of determining the likelihood of HLA-mediated platelet refractoriness. Because cPRA is currently based on a population frequency of deceased US kidney donors, we hypothesized that using a local frequency that considers the number of platelet units expressing HLA (as opposed to the number of individual donors expressing HLA) might result in different cPRA scores for patients undergoing HSCT at our institution. While we found statistically significant differences between the two calculators, the clinical significance of the results is less clear. The groups were highly correlated, and when we put patients into cPRA “bins” (mildly, moderately, or highly sensitized), there was some, but not great movement between the groups.

These methods and findings are important for several reasons. First, in order to do these calculations at scale, we had to build a cPRA calculator. We have open sourced the computer code for this web service and posted it online.^[15] In addition, we have posted our custom frequency files as well as the code to generate these files, so that others can use our web service to not only calculate cPRAs based on UNOS frequencies for their own population, but also build local, custom frequencies. Second, we calculated cPRA scores based on a platelet

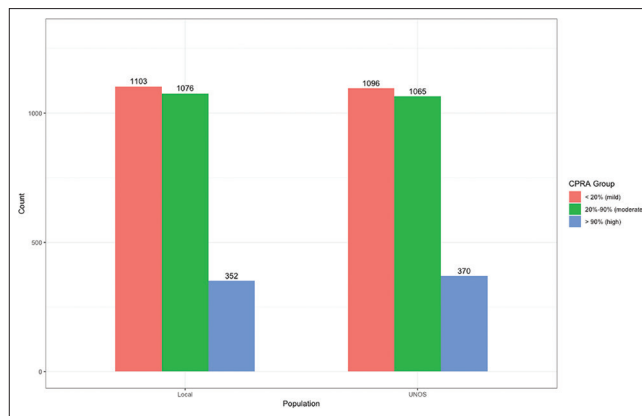


Figure 5: Calculated panel reactive antibody category placement, by population frequency (united network for organ sharing vs. local), for all patients with at least one antibody. Categories of <20% (mildly sensitized), 20%–80% (moderately sensitized), and >90% (highly sensitized) were chosen based on clinical experience

Table 2: Spearman’s rank-order correlation analysis for calculated panel reactive antibody scores generated by the two calculators (United Network for Organ Sharing vs. local) across the entire population, and segmented by gender

Population	Spearman rho	P
Total (UNOS vs. local)	0.994	<0.001
Male (UNOS vs. local)	0.996	<0.001
Female (UNOS vs. local)	0.987	<0.001

UNOS: United Network for Organ Sharing

Table 3: Intergroup movement

	Local population			Total
	<20%	20%-90%	≥90%	
UNOS <20%	1077 (42.6)	19 (0.751)	0 (0)	1096 (43.3)
UNOS 20%-90%	26 (1.03)	1033 (40.8)	6 (0.237)	1065 (42.1)
UNOS ≥90%	0 (0)	24 (0.948)	346 (13.7)	370 (14.6)
Total	1103 (43.6)	1076 (42.5)	352 (13.9)	2531 (100)

Shown are the number of patients who would shift categories if our local calculator were used (columns) instead of the UNOS calculator (rows) for the three calculated panel reactive antibody groups. Groups to the right of the shaded diagonal were placed in higher cPRA groups by our local calculator. UNOS: United Network for Organ Sharing, cPRA: Calculated panel reactive antibody

unit-driven local frequency. Unlike the US kidney donor pool where there is at most a 1:2 ratio between donors and kidneys, many platelet donors give numerous units of platelets over time.^[19] Thus, the true HLA “pool” is a product of platelet donors and platelet units donated. Third, we used a simpler, counting-based method of determining HLA frequencies, as opposed to the more complicated expectation-maximization/maximum likelihood method used by OPTN. The correlation suggests that frequency counting may be sufficient for determining HLA frequencies, though further validation is needed across different populations.

Finally, we note that our population is US based (as is UNOS), and it is possible that different countries will have different results based on their own local frequencies. We believe that our methodology, a mechanism to scale calculation based on population-specific frequencies of genetic information, provides a way to enable more locally precise driven clinical decision support. We believe that this model has value beyond cPRA and HLA.

An additional important finding is the difference in cPRA scores by sex, with females having a statistically and clinically significant higher median cPRA score than males. Given that pregnancy is a known risk factor for HLA sensitization,^[20,21] this is not altogether surprising, and our findings support a lower threshold for contemplating HLA matching for female patients that appear refractory to random platelet transfusions.

There are important limitations to our work. First, this is a single-center study, and our custom frequencies were also derived from a single-center donation site. Second, we did not look at clinical outcomes, for example, how cPRA predicts transfusion response, which we leave to a future study. Third, not all of our platelet donors have undergone HLA typing, given the cost and logistics of performing typing on all donors. While we did not analyze this formally, we suspect this would bias towards frequent donors, as they are more likely to get typed over time. Future work could look at how cPRA predicts platelet count response, as well as how cPRA scores differ across multiple sites, each with their own locally derived frequencies.

CONCLUSIONS

In conclusion, a custom-derived, platelet unit-driven HLA frequency cPRA calculator statistically differed from the UNOS calculator, with unclear clinical implications. Importantly, we have made the tooling available online via an open-source web service, so similar analyses can be done for different population sets. Thrombocytopenia is a dangerous complication for patients undergoing HSCT, and cPRA is one component of managing these patients. Continued efforts to better understand how we can minimize thrombocytopenia will be important for maximizing limited resources such as platelet transfusion products and taking optimal care of these patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Solves P, Sanz J, Freiria C, Santiago M, Villalba A, Gómez I, *et al*. Factors influencing platelet transfusion refractoriness in patients undergoing allogeneic hematopoietic stem cell transplantation. *Ann Hematol* 2018;97:161-7.
- Stanworth SJ, Navarrete C, Estcourt L, Marsh J. Platelet refractoriness – Practical approaches and ongoing dilemmas in patient management. *Br J Haematol* 2015;171:297-305.
- Agarwal N, Chatterjee K, Sen A, Kumar P. Prevalence of platelet reactive antibodies in patient's refractory to platelet transfusions. *Asian J Transfus Sci* 2014;8:126-7.
- Trial to Reduce Alloimmunization to Platelets Study Group. Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. *N Engl J Med* 1997;337:1861-9.
- Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969;280:735-9.
- Cecka JM. Calculated PRA (CPRA): The new measure of sensitization for transplant candidates. *Am J Transplant* 2010;10:26-9.
- Zachary AA, Braun WE. Calculation of a predictive value for transplantation. *Transplantation* 1985;39:316-8.
- Organ Procurement and Transplantation Network. Current CPRA Calculation. Organ Procurement and Transplantation Network; 05 December, 2013. Available from: <https://www.transplantpro.org/wp-content/uploads/sites/3/Current-CPRA-Calculation-2.ppt>. [Last accessed on 2018 Feb 15].
- Asensio E, López-Hoyos M, Romón Í, Ontañón J, San Segundo D. Assessment of Spanish panel reactive antibody calculator and potential usefulness. *Front Immunol* 2017;8:540.
- Tinckam KJ, Liwski R, Pochinco D, Mousseau M, Grattan A, Nickerson P, *et al*. CPRA increases with DQA, DPA, and DPB unacceptable antigens in the Canadian cPRA calculator. *Am J Transplant* 2015;15:3194-201.
- Brown CJ, Navarrete CV. Clinical relevance of the HLA system in blood transfusion. *Vox Sang* 2011;101:93-105.
- Organ Procurement and Transplantation Network. CPRA Calculator. Available from: <https://optn.transplant.hrsa.gov/resources/allocation-calculators/cpra-calculator/>. [Last accessed on 2017 Feb 20].
- New Program Interfaces Available for CPRA Calculator, Donor XML Uploads; 2016. Available from: <https://transplantpro.org/news/technology/new-program-interfaces-available-for-cpra-calculator-donor-xml-uploads/>. [Last accessed on 2019 Mar 02].
- Richardson L, Ruby S. *Restful Web Services*. 1st ed.: O'Reilly Media, Inc.: Sebastopol, CA; 2007.
- Partners Healthcare. cPRA Web Service and Custom Frequency Generator. Partners Healthcare; 2019. Available from: <https://github.com/persistentsystems/open-cpra-calculator>. [Last accessed on 2019 Dec 07].
- Spring Boot. Available from: <https://spring.io/projects/spring-boot>. [Last accessed on 2018 May 31].
- Leffell MS, Cherikh WS, Land G, Zachary AA. Improved definition of human leukocyte antigen frequencies among minorities and applicability to estimates of transplant compatibility. *Transplantation* 2007;83:964-72.
- American Association of Blood Banks. *Standards for Blood Banks and Transfusion Services*. 30th ed. Bethesda, MD: American Association of Blood Banks; 2016.
- Charbonneau J, Cloutier MS, Carrier É. Why do blood donors lapse or reduce their donation's frequency? *Transfus Med Rev* 2016;30:1-5.
- Tait BD, Dandie WJ, Griffiths AP, Esmore DS, Snell GI, Bergin P, *et al*. Covert presensitization to HLA antigens in parous heart and lung transplant recipients may predispose to early allograft rejection. *Transplant Proc* 1995;27:2143-4.
- Velez M, Johnson MR. Management of allosensitized cardiac transplant candidates. *Transplant Rev (Orlando)* 2009;23:235-47.