

Pooled platelet concentrates provide a small benefit over single-donor platelets for patients with platelet refractoriness of any etiology

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Abstract

Background: At our institution, patients with platelet refractoriness (of any etiology) are sometimes switched from apheresis platelets to pooled platelets before human leukocyte antigen (HLA)-matched units become available.

Study design and methods: Seven patients were analyzed. Platelet counts were available from 57 single-unit transfusions (26 pooled, 31 apheresis). A mixed linear effects model was used and significance was determined using a likelihood ratio test.

Results: When analyzed as the only fixed effect in the model, the use of pooled versus single-donor units and time from transfusion to post-transfusion blood sampling each showed a significant effect on platelet count increments. A mixed linear effect model including both factors showed that transfusing a pooled unit correlated with a $4500 \pm 2000/\mu\text{L}$ greater platelet count increment compared with a single-donor unit, and an increase in time from transfusion to post-transfusion blood sampling lowered the platelet count increment by $300 \pm 100/\mu\text{L}$ per hour.

Conclusion: A small but potentially clinically relevant benefit was observed in transfusing pooled random-donor platelets compared with single-donor units for patients with platelet refractoriness (of any etiology).

Keywords

Platelet refractoriness, platelet transfusion, human leukocyte antigen, alloantibody, random donor, pooled, apheresis

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Introduction

Platelet refractoriness is a common clinical problem that can cause significant patient morbidity and increased healthcare costs. Previous studies in multiply transfused hematology/oncology patients have reported an incidence of platelet refractoriness of up to 28% to 34%.^{1,2} An objective definition for refractoriness is based on the corrected count increment (CCI). Calculated by taking into account the post-transfusion increment, the quantity of platelets transfused, and the recipient's body surface area, a CCI of less than 5000/ μ L within 1 hour post-transfusion for two sequential transfusions has been widely used to define refractoriness.^{3,4} In practice, a platelet increment of less than 5000 to 10000/ μ L at 24 hours post-transfusion is often used as an indicator of refractoriness because obtaining a post-transfusion count within 1 hour after transfusion CCI can be logistically challenging and is not reliably accomplished at our institution.

Both immune and non-immune factors contribute to platelet refractoriness. Antibodies against class I human leukocyte antigens (HLA) are the most common cause of immune-mediated refractory status.⁵ Antibodies against other platelet antigens, such as human platelet antigens (HPA) and membrane glycoproteins Ia/IIa, Ib/IX, and IIb/IIIa, have also been reported.⁵⁻⁷ Non-immune conditions that could accelerate platelet consumption include splenomegaly, bleeding, fever, infection, increased height and weight, and vancomycin or heparin therapy.^{3,4} It is common for refractory patients to be concurrently affected by both immune and non-immune causes.

Current treatments for immune refractoriness include HLA-matched or cross-matched products. With most patients presenting with a multifactorial etiology, it

is common that refractoriness persists with HLA-matched transfusion.⁸ To prevent significant hemorrhage during the time that is required to prepare the matched products, an assessment of different platelet product types may be a helpful strategy.

Since August 2014, it has been a policy at our institutional blood bank to provide single-donor (apheresis) products for platelet transfusions to minimize alloimmunization. Under this policy, pooled units are only rarely used. This mirrors the practice across the United States. However, at our institution, when immune refractoriness is suspected and/or when HLA-matched products are pending, some clinicians ask us to switch to pooled random-donor units on the basis of the hypothesis that greater HLA diversity may partially spare transfused platelets from immune-mediated clearance. Our hospital has a long-standing on-site HLA lab, and, for this reason, the platelet antibody screen and platelet cross-match have not been used by our blood bank to date. This study aimed to examine the clinical benefit of switching from single-donor apheresis products to pooled random-donor units.

Materials and methods

Patients and data collection

We performed an retrospective search of institutional blood bank records and identified seven patients who were switched from single-donor units to pooled platelet concentrates from August 2014 to December 2015. The sole criterion for switching (and thus, the sole criterion for inclusion in this review) was hematologist request. No attempt in real time was made by the transfusion medicine service physicians to filter or approve such requests. In each case, the physicians requested the switch because their stated concern was that the patient may show refractoriness

(of any etiology). The University of Wisconsin Health Sciences Institutional Review Board (IRB) Office approved this study and waived the requirement for informed consent.

Refractoriness was defined as a platelet count increase of less than 5000/ μ L after receiving a single-donor unit for more than two consecutive transfusions. Patients who received pooled units without previous laboratory evidence of refractoriness were excluded. For each patient, clinical information was collected including the following: age, sex, previous and active medical diagnoses, anti-HLA antibody workup results, treatment course, transfusion history, and platelet counts during pooled unit transfusion.

Product source and quality control

All single-donor and pooled platelet products were supplied by the American Red Cross (ARC) in Madison, WI, USA. All single-donor units were collected by apheresis. Each pooled unit was prepared by combining five whole blood-derived platelet concentrates. All products were leukocyte-reduced. On the basis of 9 months of available quality control data provided by the ARC, a single-donor platelet unit contained an average platelet yield of 4.2×10^{11} platelets; the average yield in a pooled unit was 4.1×10^{11} platelets.

Statistical analysis

To make comparisons under similar immune and non-immune conditions, analysis of each patient was limited to the period beginning 3 days before the first use of a pooled unit and ending the day when the last pooled unit was transfused during the same admission. The platelet count increment after a single-unit transfusion was calculated if both pre- and post-transfusion data were available. In some

cases, patients received two or more units of platelets between blood samplings, and these data were excluded.

Mixed linear effects analyses were performed to evaluate the effect of each of the following factors on platelet increments: the use of pooled versus single-donor unit, pre-transfusion platelet count, time from pre-transfusion blood sampling to transfusion, time from transfusion to post-transfusion blood sampling, and HLA class I-calculated panel reactive antibody (PRA). Each factor was first evaluated as the only fixed effect in a model that included a random intercept for different patients. Factors generating $P < 0.05$ were further incorporated into a multivariate mixed linear effects model (that also included a random intercept for different patients), and the effect size of each factor was determined. Model assumptions were ensured by visual examination of residual and quantile-quantile plots. Models with and without considering the factor in question were compared using likelihood ratio tests. A P value of < 0.05 was considered significant. The analysis was performed using the lme4 package in R.^{9,10} The analysis method was previously described by Winter.¹¹

Results

Patient characteristics

As shown in Table 1, among the seven patients, four were women and three were men, with a median (range) age of 54 (26 to 70) years. All patients had multiple transfusion histories secondary to hematologic diseases. One patient was 10 days after the initiation of chemotherapy for diffuse large B cell lymphoma and the other six patients were 0 to 52 days post-bone marrow or peripheral blood stem cell transplantation when they received the first pooled platelet unit. Six patients had available anti-HLA antibody testing results with

Table 1. Platelet count increments with single-unit transfusions

Case # (Age/Sex) & History	Transfusion Date	Unit Type	Pre-Tx count in $\times 10^3/\mu\text{L}$ (time to Tx)	Post-Tx count in $\times 10^3/\mu\text{L}$ (time to Tx)
1 (59/F) Multiple myeloma; PBSCT on 5/29/15; PRA ^a = 18%	6/4/15	Single-donor	9 (-4 hours)	7 (+21 hours)
	6/5/15	Single-donor	7 (-6 hours)	4 (+18 hours)
	6/6/15	Single-donor	4 (-2 hours)	2 (+21 hours)
	6/7/15	Single-donor	2 (-3 hours)	8 (+3 hours)
	6/7/15	Pooled	8 (-8 hours)	5 (+11 hours)
	6/8/15	Single-donor	5 (-5 hours)	8 (+4 hours)
	6/10/15	Pooled	9 (-1 hours)	23 (+3 hours)
	6/11/15	Pooled	9 (-1 hours)	9 (+12 hours)
	11/7/15	Single-donor	2 (-7 hours)	2 (+2 hours)
	11/7/15	Single-donor	2 (-0 hours)	2 (+2 hours)
2 (70/F) MGUS with systemic amyloidosis; PBSCT on 10/30/15; PRA ^a unavailable	11/8/15	Pooled	12 (-7 hours)	7 (+7 hours)
	11/9/15	Pooled	7 (-4 hours)	6 (+21 hours)
	11/10/15	Single-donor	6 (-5 hours)	6 (+3 hours)
	11/11/15	Pooled	7 (-4 hours)	7 (+3 hours)
	11/11/15	Pooled	7 (-5 hours)	11 (+3 hours)
	11/12/15	Pooled	11 (-9 hours)	15 (+7 hours)
	11/12/15	Pooled	15 (-1 hours)	12 (+8 hours)
	11/13/15	Pooled	12 (-12 hours)	59 (+1 hours)
	8/9/15	Single-donor	15 (-12 hours)	10 (+11 hours)
	8/12/15	Pooled	23 (-0 hours)	12 (+23 hours)
3 (54/F) Myelodysplastic syndrome; BMT on 8/3/15; PRA ^a = 86%	8/29/15	Single-donor	3 (-10 hours)	1 (+1 hours)
	8/30/15	Single-donor	1 (-4 hours)	1 (+8 hours)
	9/1/15	Pooled	1 (-4 hours)	2 (+7 hours)
	9/1/15	Pooled	2 (-3 hours)	2 (+12 hours)
	12/9/15	Single-donor	11 (-12 hours)	21 (+12 hours)
	12/12/15	Pooled	14 (-24 hours)	17 (+2 hours)
4 (66/M) DLBCL; R-DHAP started on 9/3/15; PRA ^a = 0%	9/10/15	Single-donor	3 (-3 hours)	4 (+6 hours)
	9/10/15	Single-donor	4 (-3 hours)	4 (+3 hours)
	9/10/15	Single-donor	4 (-2 hours)	7 (+4 hours)
	9/11/15	Single-donor	7 (-5 hours)	6 (+7 hours)
	9/12/15	Single-donor	6 (-3 hours)	5 (+21 hours)
	9/13/15	Pooled	5 (-4 hours)	5 (+21 hours)
	9/15/15	Pooled	3 (-6 hours)	8 (+18 hours)
5 (51/F)	9/16/15	Pooled	8 (-5 hours)	14 (+18 hours)
	10/19/15	Single-donor	5 (-4 hours)	6 (+19 hours)
	10/20/15	Single-donor	6 (-5 hours)	5 (+20 hours)
	10/21/15	Single-donor	5 (-2 hours)	4 (+2 hours)
	10/21/15	Single-donor	3 (-9 hours)	4 (+10 hours)

(continued)

Table 1. Continued.

Case # (Age/Sex) & History	Transfusion Date	Unit Type	Pre-Tx count in $\times 10^3/\mu\text{L}$ (time to Tx)	Post-Tx count in $\times 10^3/\mu\text{L}$ (time to Tx)	
Chronic myelogenous leukemia; BMT on 8/31/15; PRA ^a = 74%	10/22/15	Pooled	4 (–5 hours)	10 (+2 hours)	
	10/23/15	Single-donor	5 (–12 hours)	16 (+13 hours)	
	10/25/15	Pooled	10 (–7 hours)	11 (+5 hours)	
	10/26/15	Pooled	8 (–3 hours)	12 (+20 hours)	
	10/28/15	Pooled	6 (–3 hours)	7 (+21 hours)	
	12/4/15	Single-donor	23 (–8 hours)	11 (+10 hours)	
	12/5/15	Single-donor	10 (–4 hours)	7 (+14 hours)	
	12/5/15	Single-donor	11 (–0 hours)	10 (+7 hours)	
	Myelodysplastic syndrome; BMT on 11/16/15; PRA ^a =28%	12/6/15	Single-donor	11 (–3 hours)	10 (+2 hours)
		12/7/15	Pooled	6 (–2 hours)	9 (+5 hours)
12/7/15		Pooled	9 (–6 hours)	12 (+1 hours)	
12/8/15		Pooled	6 (–4 hours)	10 (+3 hours)	
5/27/15		Single-donor	7 (–8 hours)	2 (+21 hours)	
5/28/15		Single-donor	4 (–4 hours)	5 (+2 hours)	
5/28/15		Single-donor	2 (–4 hours)	4 (+2 hours)	
5/29/15		Pooled	4 (–6 hours)	33 (+3 hours)	
7 (26/M)	6/6/15	Single-donor	10 (–10 hours)	7 (+15 hours)	
	6/7/15	Single-donor	7 (–7 hours)	9 (+17 hours)	
	6/8/15	Pooled	9 (–7 hours)	13 (+17 hours)	
PNH; PBSCT on 5/29/15; PRA ^a =86%	6/6/15	Single-donor	10 (–10 hours)	7 (+15 hours)	
	6/7/15	Single-donor	7 (–7 hours)	9 (+17 hours)	
	6/8/15	Pooled	9 (–7 hours)	13 (+17 hours)	

^a HLA class I calculated panel reactive antibody.

Tx, transfusion; PBSCT, peripheral blood stem cell transplantation; PRA, panel reactive antibody; MGUS, monoclonal gammopathy of undetermined significance; BMT, bone marrow transplantation; DLBCL, diffuse large B-cell lymphoma; R-DHAP, chemotherapy including rituximab, dexamethasone, ara-C and cisplatin; PNH, paroxysmal nocturnal hemoglobinuria; HLA, human leukocyte antigens.

an HLA class I calculated PRA ranging from 0% to 86%. Possible non-immune causes of accelerated platelet consumption are summarized in Table 2.

Factor effects on platelet increments

The seven patients received a total of 57 single-unit platelet transfusions (26 pooled and 31 single-donor units) for which platelet count increments could be calculated (Table 1). The median (range) platelet count increment was 0 (–12,000 to 11,000) per μL for a single-donor unit and 3000

(–11,000 to 47,000) per μL for a pooled unit.

Three (9.7%) single-donor and six (23.1%) of pooled unit transfusions achieved a $\geq 5000/\mu\text{L}$ platelet count increment. During the analyzed period, none of the patients experienced clinically significant bleeding.

When analyzed as the only fixed effect in a model, the use of pooled compared with single-donor units ($P=0.04$) and time from transfusion to post-transfusion blood sampling ($P=0.02$) each demonstrated a significant effect on platelet count increments.

Pre-transfusion platelet counts, time from pre-transfusion blood sampling to transfusion, and PRA were not significant predictors (Table 3).

A mixed linear effect model was further built including both the use of pooled compared with single-donor units and time from transfusion to post-transfusion blood sampling as two fixed effects without interaction terms. A random intercept for different patients was also included. In this model, transfusing a pooled unit correlated with a platelet count increment of $4500 \pm 2000/\mu\text{L}$, which was greater than a single-donor unit ($P=0.03$). As expected, the per-

hour increase in the time from transfusion to post-transfusion blood sampling lowered the platelet count increments by $300 \pm 100/\mu\text{L}$ ($P=0.02$; Table 3).

Discussion

In this study, pooled products resulted in greater platelet count increments compared with single-donor apheresis products in platelet transfusion refractory patients. Three (42.9%) patients had $>50\%$ HLA class I calculated PRA. The increased platelet antigen diversity in pooled products might explain the benefit seen in refractory patients because transfused platelets may be partially spared from immune-mediated clearance. However, the use of pooled products incurs a high risk of further alloimmunization.

Moreover, previous studies in hematologic patients without refractoriness have reported no difference in¹² or superior¹³ platelet increments from single-donor units compared with pooled concentrates. One article that focuses on immune refractory patients in particular demonstrated that there was no significant difference in mean the CCI or success rate when transfusing apheresis platelets compared with pooled platelets to HLA-sensitized patients.¹⁴ Our

Table 2. Possible non-immune causes of refractoriness during the analyzed period

Case #	Possible Factors
1	Fever; small subarachnoid hemorrhage
2	Fever
3	Fever; acute graft-versus-host disease; cytomegalovirus viremia
4	Left hand spontaneous hematoma
5	Fever; Henoch–Schönlein purpura; nutritional coagulopathy
6	Fever; Crohn's disease with ileostomy bleeding; splenomegaly
7	Fever; <i>Pseudomonas aeruginosa</i> bacteremia

Table 3. Factors associated with platelet count increment in a mixed linear effects model

Factor	Univariate*		Multivariate**	
	Slope	P-value	Slope	P-value
Unit type: pooled versus single-donor	4.5	0.04	4.5	0.03
Pre-Tx platelet count ($\times 10^3/\mu\text{L}$)	-0.3	0.2		
Time from pre-Tx blood sampling to Tx (hours)	-0.5	0.09		
Time from Tx to post-Tx blood sampling (hours)	-0.3	0.02	-0.3	0.02
HLA class I calculated PRA				
As continuous variable	0	0.66		
$\geq 50\%$ versus $<50\%$	1.2	0.5		

* Factor was analyzed as the only fixed effect in the model.

** Both the use of pooled versus single-donor units and time from transfusion to post-transfusion blood sampling were included as two fixed effects in the model.

Tx, transfusion; PRA, panel reactive antibody; HLA, human leukocyte antigens.

study is different in the following two main ways: 1) it is much smaller; and 2) we did not focus solely on HLA-sensitized patients. Our clinicians used this pooled platelet strategy as soon as we recognized the low CCIs (i.e., often when no HLA antibody results were available or would be available for at least several days). Thus, careful evaluation of patient conditions and etiologies are recommended for interpreting the reported data and for choosing transfusion strategies.

One patient did not have class I HLA antibody test results because the ordering physician did not order the test. We included this patient because our sole criterion for providing pooled platelets was hematologist request regardless of the soundness of the request at the time. Since then, we have introduced a more structured process for approving HLA-matched platelets, which has had variable success. However, at the time, the process was largely “customer service” based, and we fulfilled any request for pooled platelets from a hematologist no matter the timing of the post-transfusion platelet counts or what the antibody testing results (if any) showed.

Limitations of this study include the small subject number, mixed immune and non-immune etiologies of refractoriness among patients, and varying blood sampling times. We attempted to correct for these potential confounding variables by incorporating any factor with a univariate result of $P < 0.05$ into a multivariate model. Because no significant hemorrhage event occurred during the study, it remains unclear whether pooled products offer a benefit over single-donor products in preventing hemorrhage. We concede that some patients had immune refractoriness and some did not show this refractoriness. We included all eligible patients who had two consecutive CCIs < 5 because, as soon as these low CCIs are identified, the clinical team considers the patient “platelet refractory with a *possible* immune

component” and wants to do whatever is possible to increase the platelet count to prevent spontaneous bleeding. This is a relatively aggressive strategy. We also chose to exclude patients who received more than one unit before drawing a post-transfusion platelet count because we could not discern the contributions from each individual unit.

We also concede that pooled platelets have many real or potential disadvantages over apheresis platelets. These include increased donor exposures to infectious disease transmission risk, increased donor exposures to HLAs that may increase patient alloimmunization, and decreased availability. This analysis also does not include a thorough study of patient outcomes. Thus, there may be potential disadvantages to the patients’ clinical outcomes that we did not find because they were beyond the scope of this research.

We do not claim that using pooled platelets is without tradeoffs. The primary aim of our analysis was to answer the question, “Do patients with platelet refractoriness (of any etiology) benefit from pooled platelets?” This question was implicit in our clinicians’ requests for these products as a temporary measure.

Finally, the difference in platelet counts was $4500 \pm 2000/\mu\text{L}$. On the one hand, this can be viewed as a very small difference. On the other hand, nearly all patients with platelet refractoriness (especially bone marrow transplant [BMT] patients) for whom we receive consults had platelet counts of less than $10,000/\mu\text{L}$. Thus, we speculate that this potential benefit of around $4500/\mu\text{L}$ may make a clinically relevant difference if it leads to a count over $10,000/\mu\text{L}$ or if it prevents spontaneous bleeding or stops active bleeding.

Conclusion

We observed a small but potentially clinically relevant benefit in transfusing pooled

random-donor platelets compared with single-donor units for patients with platelet refractoriness (of any etiology). The data (while few) appear to support the practice of switching to the use of pooled products when refractoriness (of any etiology) may be present in patients who are awaiting matched products and class I HLA antibody test results.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

- Legler TJ, Fischer I, Dittmann J, et al. Frequency and causes of refractoriness in multiply transfused patients. *Ann Hematol* 1997; 74: 185–189.
- Klingemann HG, Self S, Banaji M, et al. Refractoriness to random donor platelet transfusions in patients with aplastic anaemia: a multivariate analysis of data from 264 cases. *Br J Haematol* 1987; 66: 115–121.
- Slichter SJ, Davis K, Enright H, et al. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. *Blood* 2005; 105: 4106–4114.
- Stanworth SJ, Navarrete C, Estcourt L, et al. Platelet refractoriness—practical approaches and ongoing dilemmas in patient management. *Br J Haematol* 2015; 171: 297–305.
- Kurz M, Greinix H, Hocker P, et al. Specificities of anti-platelet antibodies in multitransfused patients with haematological disorders. *Br J Haematol* 1996; 95: 564–569.
- Kickler T, Kennedy SD and Braine HG. Alloimmunization to platelet-specific antigens on glycoproteins IIb-IIIa and Ib/IX in multiply transfused thrombocytopenic patients. *Transfusion* 1990; 30: 622–625.
- Laundy GJ, Bradley BA, Rees BM, et al. Incidence and specificity of HLA antibodies in multitransfused patients with acquired aplastic anemia. *Transfusion* 2004; 44: 814–825.
- Rioux-Masse B, Cohn C, Lindgren B, et al. Utilization of cross-matched or HLA-matched platelets for patients refractory to platelet transfusion. *Transfusion* 2014; 54: 3080–3087.
- Bates D, Maechler M, Bolker B, et al. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 2015; 67: 1–48. https://scholar.google.com/scholar?cluster=17803823686299873243&hl=en&as_sdt=0,50
- Bates D, Chambers J, Dalgaard P, et al. R: language and environment for statistical computing and graphics. 1997. Available at <http://www.r-project.org/>
- Winter B. Linear models and linear mixed effects models in R with linguistic applications. arXiv: 1308.5499 2013. https://scholar.google.com/scholar?cluster=10855901729307493922&hl=en&as_sdt=5,50&scioldt=0,50
- Anderson NA, Gray S, Copplesstone JA, et al. A prospective randomized study of three types of platelet concentrates in patients with haematological malignancy: corrected platelet count increments and frequency of nonhaemolytic febrile transfusion reactions. *Transfus Med* 1997; 7: 33–39.
- Gurkan E, Patah PA, Saliba RM, et al. Efficacy of prophylactic transfusions using single donor apheresis platelets versus pooled platelet concentrates in AML/MDS patients receiving allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2007; 40: 461–464.
- Gavva C, Barroso J, Gernsheimer T, et al. Response to random apheresis platelets versus HLA-selected platelets versus pooled platelets in HLA-sensitized patients. *Transfusion* 2019; 59: 2276–2281.