The association between platelet transfusion and idiopathic pneumonia syndrome is unaffected by platelet product type

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BACKGROUND: Methods used to produce platelet (PLT) components, pooling of PLT-rich plasma (PRP-PLT) and apheresis (AP-PLT), may variably contribute to the pathogenesis and severity of idiopathic pneumonia syndrome (IPS).

STUDY DESIGN AND METHODS: We performed a retrospective cohort study of 906 allogeneic hematopoietic cell transplant recipients to examine associations between PLT product type and risks of developing IPS and dying after IPS onset. Proportional hazards models included separate terms for the sum of all PLT transfusions and the sum of PRP-PLT units received in the 3 or 7 days before IPS onset. Similarly constructed models analyzed the outcome of time to death after IPS onset. All analyses were adjusted for known IPS risk factors.

RESULTS: Patients received a median of three PRP-PLT transfusions (interquartile range [IQR], 0-6) and five AP-PLT transfusions (IQR, 1-13) while at risk for IPS. Seventy-five patients (8%) developed IPS by Posttransplant Day 120. The proportion of PRP-PLT transfusions was not associated with risk of developing IPS (3-day hazard ratio [HR] 0.98, 95% CI 0.74-1.29, p = 0.86; 7-day HR 1.00, 95% CI 0.86-1.15, p = 0.95) or dying after IPS onset (3-day HR 0.99, 95% CI 0.75-1.31, p = 0.97; 7-day HR 0.98, 95% CI 0.78-1.12, p = 0.47).

CONCLUSION: The association between PLT transfusions and risk of developing IPS or dying after IPS onset does not differ according to PLT product type. Further research is required to identify potentially modifiable steps in PLT component production that contribute to IPS.

Idiopathic pneumonia syndrome (IPS) is a morbid pulmonary process that complicates 3% to 15% of allogeneic hematopoietic cell transplants (HCTs).[1] IPS includes several types of inflammatory lung injury, some of which have unique clinical manifestations, such as diffuse alveolar hemorrhage[2] and engraftment syndrome.[3,4] Risk factors for IPS include the patient's age, the disease for which the patient is transplanted, and the intensity of pre-HCT conditioning therapy.[5-8] Experimental models highlight graft-versus-host disease (GVHD),[9-11] circulating lipopolysaccharide,[12,13] and oxidative stress from chemoradiation therapy[14,15] as potential causes for IPS. However, the mechanisms underlying IPS and its phenotypic heterogeneity are not well understood. Recently, we demonstrated that an increased number of platelet (PLT) transfusions administered to HCT patents resulted in an increased risk of IPS onset within the

ABBREVIATIONS: AP = apheresis; HCT(s) = hematopoietic cell transplant(s); HR = hazard ratio; IPS = idiopathic pneumonia syndrome; IQR = interquartile range; PRP = platelet-rich plasma.

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following week.[16] Our findings may either represent causal links between PLT transfusions and IPS or reflect that transfusions are a marker for illness severity, which itself increases the risk of IPS.

In the United States, PLT components are collected from a single donor via apheresis (AP-PLT) or are produced by pooling multiple donors' PLT-rich plasma (PRP-PLT). These two production techniques affect PLT hemostatic and immune functions differently; however, which method yields the most in vivo proinflammatory potential is unknown.[17-22] Conceivably, PLT product types may differentially stimulate PLT-directed inflammatory pathways that contribute to the development and severity of IPS. Whether IPS risk differs by PLT product type is not well studied.[23] In this study, we tested two hypotheses: 1) mortality after HCT increases with the number of PLT transfusions received before IPS onset and 2) PRP-PLT transfusions specifically increase the risk of developing IPS and dying after IPS onset relative to AP-PLTs.

MATERIALS AND METHODS

Cohort description

We studied a cohort of 906 patients who received at least one PLT transfusion within 120 days of undergoing myeloablative allogeneic HCT at Fred Hutchinson Cancer Research Center (Seattle, WA). This cohort was a subset of 1100 patients who underwent HCT between 1997 and 2001 and were previously studied to identify IPS risk factors.[5] Transplantation protocols were similar throughout this time period. We excluded patients who underwent autologous HCT and nonablative allogeneic HCT as the mechanisms underlying IPS in these settings may importantly differ. We defined the day of hematopoietic stem cell infusion as HCT Day 0.

Study exposures, potential confounders, and outcomes

During the period of this study, our standard institutional practice was to transfuse 1 PLT unit composed of either six PRP pools or one AP component when the patient's PLT count was less than 20×10^9/L and in response to clinically significant bleeding. However, blood component transfusions were administered at the providers' discretion.

All blood products transfused to this cohort were produced at our regional blood center (Puget Sound Blood Center, Seattle, WA) following Food and Drug Administration standards. Per standard local practice, cytomegalovirus (CMV)-negative patients received CMV-negative blood components throughout their transplant course. Before HCT, all blood components were rendered "CMV-safe" by leukoreduction. HCT Day 0 and beyond, blood components were either leukoreduced or CMV negative by serologic testing. CMV-positive patients were only guaranteed to receive leukoreduced units before transplant. When indicated, leukofiltration occurred at the time of issue after a period of storage, including for AP-PLT units not meeting standard white blood cell counts at the time of collection. Due to limited inventory, patients occasionally received volume-reduced PLTs with an ABO blood group that did not match their own and/or their HCT donors' blood type. Our prior study showed that associations between PLT transfusions and IPS risk did not differ according to whether or not the transfusions were ABO matched.[16]

We defined a PLT transfusion as a single AP-PLT component or a single pooled PRP-PLT component. Studies investigating links between transfusions and lung injury demonstrate that lung injury risk is temporally related to recent transfusions.[24,25] However, the exact time frame during which transfusions mechanistically contribute to lung injury is unknown. Therefore, we examined the number of PLT components transfused in the prior 3 days and prior 7 days in separate analyses. We defined the at-risk period for IPS to start on HCT Day 0 and end on Day 120; therefore, transfusions given up to 6 days before HCT could be included in weekly sums.

We considered all previously identified IPS risk factors to be potential confounders. This included patient age (<40 years vs. 40 years and older), HCT indication (acute myelogenous leukemia and myelodysplastic syndrome vs. other indications), total body irradiation dose (<12, 12, and >12 Gy), and consensus criteria–defined[26] severe (Grade III-IV) acute GVHD.[6,10,11,27]

Our approach to identifying IPS cases is detailed elsewhere.[5] Briefly, two physicians reviewed relevant clinical, radiographic, microbiology, and pathology data to identify IPS cases according to the 1993 National Lung, Heart, and Blood Institute Consensus definition.[1] To fulfill these criteria, a patient must have multilobar opacities on chest imaging not attributed to heart failure or other organ dysfunction. Additionally, there cannot be evidence of bacterial, viral, fungal, or atypical pneumonia on thorough evaluation of bronchoalveolar lavage fluid sampled via bronchoscopy. When obtained, histologic examination of transbronchial lung biopsies cannot reveal viral inclusions or invasive organisms. Patients meeting these criteria were not considered IPS cases if they responded to diuretics or antibiotic therapy. The date of IPS onset was the first day that diagnostic radiographic abnormalities coincided with impaired pulmonary physiology, defined as the presence of hypoxemia or restrictive abnormality on pulmonary function testing.

Statistical analysis

We compared baseline characteristics between individuals who went on to develop IPS and those that never developed IPS using t tests with unequal variances, Wilcoxon

rank-sum tests, and chi-square tests of homogeneity, where appropriate.

PLT transfusions and IPS

To confirm that both types of PLT transfusions are associated with IPS risk, we performed two Cox proportional hazards regressions[28] that included separate terms for the sum of PRP-PLT transfusions and sum of AP-PLT transfusions received in the prior 3 or 7 days. Time to IPS started on HCT Day 0. To examine associations between PLT transfusions and mortality among patients with IPS, we performed similarly modeled proportional hazards analyses using 120-day and 2-year mortality as outcomes. To minimize overfitting due to the small sample size,[29] the mortality analyses excluded GVHD as a potential confounder. We performed sensitivity analyses adjusting for severe acute GHVD as a dichotomous time-varying covariate[30] indicating onset any time before the analysis day.

PLT product type and IPS

We performed two proportional hazards analyses to examine associations between the proportion of recent PLT transfusions that were produced by PRP pooling and IPS. The first model included separate continuous variables equal to the sums of PRP-PLT units and total PLT units transfused in the prior 3 days. The second model examined 7-day transfusion sums. All analyses were adjusted for the complete list of potential confounders. Severe acute GVHD was modeled as previously described. Receipt of other blood component types may confound the relationship between PLT transfusions and IPS. However, the number of recent PLT transfusions is correlated with the number of other recently transfused components, such as red blood cells (RBCs). Due to multicollinearity, adjusting for sums of other transfusions may introduce bias.[31] For comparison, we performed each analysis with and without adjustment for sums of RBC and other transfusions.

We fit similarly constructed models to examine associations between PRP-PLT transfusions and death occurring after IPS onset and within 120 days and 2 years of HCT. For mortality analyses, observation started the day of IPS onset. The mortality models again excluded adjustments for GVHD due to sample size.

We evaluated the proportional hazards assumption of all models by testing whether the relationship between Schoenfeld residuals and analysis time is linear.[32] Any covariate violating the proportional hazards assumption (total body irradiation dose) was modeled as a multiplicative interaction with a function of analysis time to resolve violations. We used statistical software (StataIC 12.0, StataCorp, College Station, TX) for all analyses. Results were interpreted using a two-sided p value of less than 0.05 to allow for the possibility that either PRP- or AP- PLTs could cause greater risk. This research was approved by the institutional review board. Some of the results have been published elsewhere in abstract form.[33]

RESULTS

The patients primarily received bone marrow grafts and underwent HCT for hematologic malignancy or myelodysplastic syndrome (Table 1). Ninety-eight percent of patients were undergoing their first HCT and 2% their second. The patients received a total of 23,650 blood component transfusions between 6 days before HCT and Day 120. Sixty-four percent of the transfused components were PLTs (70% AP-PLTs and 30% PRP-PLTs), 33% were RBC units, and 3% were other types (cryoprecipitate, granulocyte infusions, plasma, and whole blood transfusions). Seventy-five percent of patients received at least one PRP-PLT unit, 90% received at least one AP-PLT unit, and 65% received both types. The median number of PLT units transfused while at risk for IPS was eight (interquartile range [IQR], 4-19) and the range was 1 to 127. The median number of AP-PLT units transfused to patients while at risk for IPS was five (IQR, 1-13) and the range was 0 to 108. The median number of PRP-PLT units transfused to patients while risk for IPS was three (IQR, 0-6) and the range was 0 to 49. The mean number of donors per PRP-PLT unit was 5.7 (SD, 0.7).

The cohort was observed for a total of 93,873 person-days through Posttransplant Day 120. Seventy-five subjects (8%) developed IPS. Severe acute GVHD developed in 37% of patients who subsequently developed IPS, compared to 24% of patients who never developed IPS ($p = 0.0001$). The median time to IPS onset was 23 days (IQR 13-48 days, range 4-106 days). Mortality by Posttransplant Day 120 was 21% overall, 16% among patients that did not develop IPS, and 76% among patients who developed IPS ($p < 0.0001$). Eighty-five percent of patients with IPS and 41% of patients without IPS died within 2 years of HCT (Fig. 1). The median number of days between IPS onset and death was 14 (IQR, 6-27 days).

Both PRP-PLT units and AP-PLT units transfused in the prior 3 days were associated with IPS risk after adjusting for potential confounders and other transfusions received in the same time interval (hazard ratio [HR] 1.36, 95% CI 1.06-1.74, $p = 0.016$; and HR 1.36, 95% CI 1.21-1.54, $p < 0.001$, respectively). When examined over the prior 7 days, the associations were attenuated (HR 1.16, 95% CI 1.02-1.33, $p = 0.027$; and HR 1.17, 95% CI 1.09-1.25, $p < 0.001$, respectively) and consistent with our prior study.[16] Sensitivity analyses that also adjusted for GVHD produced similar results.

The sums of PLT transfusions received in the 3 and 7 days before IPS onset were associated with 120-day mortality when adjusted for nontransfusion potential confounders (HR 1.28, 95% CI 1.13-1.46, $p < 0.001$; and HR 1.10, 95% CI 1.03-1.17, $p = 0.004$, respectively). However,

TABLE 1. Characteristics of allogeneic HCT recipients with and without IPS*

Baseline characteristics	All patients (n = 906)	IPS		p value†
		Absent (n = 831)	Present (n = 75)	
Age (years)	39 (±14)	38 (±14)	44 (±12)	0.0003
Male	498 (55)	454 (55)	45 (60)	0.36
White race	736 (81)	672 (81)	64 (85)	0.37
Disease indicating transplant				
Acute leukemia	351 (39)	316 (38)	35 (47)	0.18
Myelodysplastic syndrome	150 (17)	136 (16)	15 (20	
Chronic leukemia	333 (37)	310 (37)	23 (31)	
Lymphoma	37 (4)	35 (4)	2 (3)	
Other	43 (5)	40 (5)	3 (4)	
Total body irradiation dose (Gy)				
None	381 (42)	360 (43)	21 (28)	<0.001
12	255 (28)	220 (26)	35 (47)	
>12	270 (30)	251 (30)	19 (25)	
Stem cell source				
Bone marrow	594 (66)	545 (66)	49 (65)	0.79
Peripheral blood	307 (34)	281 (34)	26 (35)	
Both	5 (<1)	5 (<1)	0	
Donor type				
HLA matched, related	424 (47)	391 (47)	33 (44)	0.32
HLA mismatched, related	54 (6)	52 (6)	2 (3)	
Unrelated donor	428 (47)	388 (47)	40 (53)	
Cytomegalovirus infection				
Donor +/recipient +	235 (26)	218 (26)	17 (23)	0.44
Donor +/recipient –	132 (15)	122 (15)	10 (13)	
Donor –/recipient +	205 (23)	191 (23)	14 (19)	
Donor –/recipient –	331 (37)	298 (36)	34 (45)	

* Data are reported as mean (±SD) or number (%).
† Means are compared with t tests (unequal variances), medians with Wilcoxon rank-sum test, and categorical variables with chi-square tests of homogeneity; p values are two-sided.

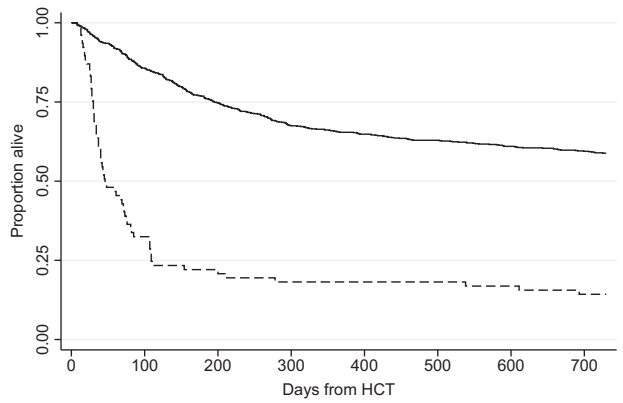

Fig. 1. Two-year posttransplant survival. Kaplan-Meier survival among patients who did (—) and did not (– – –) develop IPS after myeloablative allogeneic HCT.

only the association for 3-day sums remained after adjusting for other transfusions (HR 1.26, 95% CI 1.06-1.51, p = 0.009). Three- and 7-day sums were also associated with 2-year mortality among IPS patients when adjusted for nontransfusion potential confounders (HR 1.26, 95% CI 1.11-1.43, p < 0.001; and HR 1.09, 95% CI 1.03-1.16, p = 0.004, respectively). Only the association for 3-day sums remained after adjusting for other transfusions (HR 1.24, 95% CI 1.05-1.48, p = 0.0014).

After the total number of PLT transfusions was adjusted for, the proportion of PRP-PLTs received in the prior 3 days was not associated with IPS development (Table 2). Likewise, there was no association between PLT product type and IPS when examining transfusions received in the prior 7 days. Neither 3- nor 7-day sums of PRP-PLT transfusions were associated with post-IPS mortality.

DISCUSSION

In this study, the transfusion of either PRP- or AP-PLT components within 120 days of myeloablative allogeneic HCT was associated with greater risks of developing IPS and dying after IPS onset. These risks did not differ between the PLT product types produced by US blood banks. The sums of all PLT transfusions received in the 3 or 7 days before IPS onset were risk factors for 120-day and 2-year mortality. The associations with IPS development and subsequent mortality were stronger for units transfused in the prior 3 days than units transfused in the prior 7 days. PLT components transfused closer to IPS onset may be more relevant to IPS risk.

TABLE 2. Estimated associations between receiving PRP-PLT components and developing IPS and dying after IPS onset

Exposure	Model covariates	HR	95% CI	p value
IPS risk per additional PRP unit				
3-day sum	Potential confounders*	0.99	0.76-1.29	0.93
	Confounders and transfusions†	0.98	0.74-1.29	0.86
7-day sum	Potential confounders*	1.00	0.87-1.15	0.99
	Confounders and transfusions†	1.00	0.86-1.15	0.95
Risk of death after IPS per additional PRP unit‡				
3-day sum	Potential confounders*	0.99	0.76-1.29	0.95
	Confounders and transfusions†	0.99	0.75-1.31	0.97
7-day sum	Potential confounders*	0.94	0.78-1.12	0.47
	Confounders and transfusions†	0.98	0.81-1.18	0.83

* Model adjusted for age, transplant indication, radiation dose, and sum of PLT transfusions. IPS risk models also adjusted for severe acute GVHD as a time-varying covariate.
† Model also adjusted for sums of RBC and other transfusions given in same period.
‡ Outcome of death after IPS onset and within 120 days of transplant.

Blood component transfusions contain immunomodulatory substances, collectively referred to as the storage lesion,[21,34] that mechanistically contribute to acute lung injury in animal models.[35-41] PLTs play both direct and indirect roles in mediating experimental acute lung injury.[40-44] Studies across diverse patient populations consistently identify transfusions as clinical risk factors for acute lung injury or acute respiratory distress syndrome,[45-51] which shares clinicopathologic features and can coincide with IPS.[1] Given the breadth and strength of the evidence for transfusion-related acute lung injury, it remains plausible that PLT transfusions are a potentially modifiable contributor to IPS pathogenesis. Our study suggests this possible biologic relationship is independent of PLT product type, which has important implications for blood banking and transfusion strategies.

Posttransplant thrombocytopenia is associated with death from causes other than bleeding and relapse.[52,53] One potential link is through receipt of PLT transfusions. Recently, Christou and colleagues[54] observed that the risk of intensive care unit admission and 100-day nonrelapse mortality increases with the number of PLT transfusions after HCT. Randomized trials of various PLT transfusion strategies in patients with thrombocytopenia either were not designed or were underpowered to examine relationships between number of PLTs transfused and mortality.[55-61] Our study suggests that PLT transfusions may contribute to mortality in part by increasing the incidence and severity of IPS. An alternative interpretation of our results is that PLT transfusions may play a role in other fatal HCT complications. In contrast, PLT transfusions may be correlated with true causes of death, such as graft failure or relapse of malignancy, without playing a causal role.

This study has several noteworthy limitations. The patients in this study received transplants between 13 and 17 years ago. We studied this patient population because it is one of the largest cohorts of IPS patients studied to date and IPS was carefully adjudicated. There have been many evolutions in HCT since that time, such as use of umbilical cord stem cells,[62] reduced-intensity conditioning,[63] and new prophylactic regimens for infection[64] and GVHD[65] that have improved overall outcomes[66,67] and may impact the epidemiology of IPS. Collectively, our data suggest that PLT transfusion is one of several important risk factors for IPS. The mechanisms responsible for this association are unclear. Moreover, many of these risk factors were identified using dated cohorts. Like other IPS risks, the association between transfusion and IPS requires further study in contemporary cohorts. Next, like all observational studies, our results are subject to bias by residual confounding. A particularly relevant potential source of error is indication bias, meaning that patients receiving PRP-PLTs may be importantly different from those receiving AP-PLT units. Whereas most patients received PLT components produced by both methods, transfusion-refractory patients theoretically may have preferentially received PLTs produced using a specific method. PLT refractoriness may be correlated with other clinical characteristics that influence IPS risk,[68,69] including prior transfusions;[70,71] however, refractoriness may not be completely accounted for in our model adjustments. The association between PLT transfusions and 2-year mortality may be less influenced by indication bias given the increased time separating the exposure and outcome. Third, we lack data to examine other factors that may modify the relationship between PLT product type and IPS, including storage age and exact timing of leukoreduction.

Another potential limitation of our study is that some IPS cases may be misclassified. IPS encompasses a spectrum of noninfectious lung injuries occurring after HCT.[1] There are several proposed mechanisms by which IPS develops and it is possible that there are distinct intermediate phenotypes with unique risk factors, mechanisms, relationships with blood transfusions, and outcomes.

Additionally, some individuals suspected to have IPS may instead have occult pneumonia.[72,73] These potential sources of error should be accounted for in future studies of IPS epidemiology and pathobiology.

Despite its limitations, our study remains important because it shows that increased frequency of PLT transfusions may contribute to mortality after HCT. The association between PLT transfusions and death after IPS onset suggests that there may be a true mechanistic relationship between blood transfusions and IPS. This potentially causal and modifiable mechanism underlying a devastating disease warrants future investigation in the modern HCT era.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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