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Organ System Bleeding in the PLADO Trial

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Abstract

In the multi-institutional platelet dose trial (PLADO Trial), there were 1,077 hematology/oncology patients ≥18 years of age who received at least one platelet transfusion. These patients were analyzed for types and severity of organ system bleeding based on baseline patient characteristics, laboratory assays, primary diagnosis, and type of treatment. Patients were randomly assigned to receive one of three different prophylactic platelet doses for morning platelet counts of ≤10 × 10^9 /L. Daily assessments of bleeding in nine organ systems were performed and bleeding severity was based on a WHO bleeding scale. For the purposes of analyses, Grade 2A bleeding was defined to be WHO Grade 2 bleeding that was not solely due to purpura. Grade 2A or greater bleeding occurred in 616 patients (57.2%) on 13.3% of study days predominately in the GI (31.2% of patients on 4.4% of days), GU (21.1%

of patients on 5.1% of days), pulmonary (17.6% of patients on 2.7% of days), and oral/nasal systems (14.6% of patients on 2.0% of days). Grade 3 or greater bleeding occurred in 102 patients (9.5%) on 0.8% of study days. CNS bleeding occurred in 2.7% of patients on 0.2% of study days and was independent of platelet count. Treatment stratum (ALLO, CHEMO, AUTO) impacted degree of bleeding and onset; and distribution of involved organs systems. Secondary analyses of the PLADO Trial patient data, the largest patient dataset curated to date, showed that patients with hypoproliferative thrombocytopenia experience significant bleeding in multiple organ systems, which varies by treatment group, but is independent of platelet dose.

Keywords
Bone marrow transplant; bleeding; platelet counts; platelet transfusions; platelet dose

1. Introduction

One of the major concerns for physicians who manage hematologic patients with thrombocytopenia is the likelihood of bleeding and which organ systems are most vulnerable. Both observational studies and randomized controlled clinical trials have attempted to gain a clearer understanding of the clinical risk factors for and patterns of bleeding during periods of hypoproliferative thrombocytopenia [1-6]. Most studies use defined bleeding scales, some of which have been modified for the purposes of the clinical trial [7-9]. One commonly used bleeding scale is the WHO bleeding scale which defines grades of bleeding for each of nine organ systems [10]. As part of the PLADO trial [11], which sought to examine the impact of platelet dosing strategy on clinical bleeding, data were collected daily on the occurrence of bleeding in each organ system while the patients were hospitalized. Reported here are post-hoc analyses of the types of organ system bleeding that were observed both before and after the date of a patient's first platelet transfusion and whether bleeding in a specific organ system was associated with any recorded parameter (i.e., gender, age, primary diagnosis, disease treatment, laboratory assays, or platelet dose), the goal being to increase clinicians' knowledge regarding the characteristics of bleeding in at-risk adult patient populations.

2. Materials and Methods

The PLADO study was a multicenter randomized controlled trial conducted by the NHLBI/TMH Network and has been previously described in detail [11].

2.1 Patients

Patients were eligible for enrollment if they were expected to have platelet counts of $\leq 10 \times 10^9$ /L for at least five days. They were prospectively randomized to one of three platelet dose groups; i.e., low, medium, or high dose (1.1×10^{11}, 2.2×10^{11}, or 4.4×10^{11} platelets per square meter of body surface area, respectively) with randomization stratified by treatment [autologous or syngeneic hematopoietic stem cell transplant (HSCT) (AUTO), allogeneic HSCT (ALLO), and

chemotherapy for hematologic malignancy without HSCT (CHEMO)]. This paper is restricted to the 1,077 PLADO study patients who were 18 years or older and received at least 1 platelet transfusion during the study period. The 1,077 patients included 352 patients in the low, 355 patients in the medium, and 370 patients in the high dose groups. There were 378, 413, and 286 patients in the AUTO, ALLO, and CHEMO treatment categories, respectively.

2.2 Bleeding Grades

Bleeding data were collected for nine organ systems that included oral/nasal (ON), skin/soft tissue/musculoskeletal (ST), gastrointestinal (GI), genitourinary (GU), pulmonary (PL), body cavity (BC), central nervous system (CNS), invasive sites (IS), and hemodynamic instability (HD) (moderate or severe). Organ system bleeding grades were defined using the World Health Organization (WHO) scale (Table S1) [10].

Not every bleeding grade (1, 2, 3, or 4) had criteria defined for every organ system. For example, hemodynamic instability could only be Grade 3 or 4. Because Grade 1 bleeding and Grade 2 skin bleeding are not usually considered clinically significant, we defined a new bleeding category called Grade 2A, which included all Grade 2 bleeding except skin bleeding.

2.3 Bleeding Assessments

Research staff performed daily bleeding assessments using physical examinations, patient interviews, and chart reviews for any bleeding events noted by hospital staff. Using collected bleeding data, a computerized system calculated each patient's daily bleeding grade. Daily platelet counts, hematocrit values, and hemoglobin levels were measured.

At enrollment, all patients had to have prothrombin (PT) and partial thromboplastin times (aPTT) not more than 1.3 times the upper limit of normal and a fibrinogen concentration of at least 100 mg/dL. Any subsequent coagulation testing obtained as part of patient care was recorded.

2.4 Transfusions

Platelets were transfused prophylactically if the morning platelet count was $\leq 10 \times 10^9$ /L. The patient's physician could change the transfusion threshold or platelet dose based on clinical reasons, with resumption of study guidelines as soon as possible.

RBC transfusions were given according to local practice guidelines.

2.5 Study Duration

Patients were considered on study until 30 days after their first platelet transfusion, after a 10-day period without a platelet transfusion, at hospital discharge, study withdrawal, or death, whichever occurred first.

2.6 Statistical Analysis

For this report, analyses were limited to the 1,077 adult PLADO patients (≥18 years) who received at least one platelet transfusion. Baseline characteristics were summarized using frequency (percentage) for categorical variables and mean (standard deviation) for continuous variables. One

variable of interest was primary diagnosis, which might affect bleeding by itself or by type of treatment given. Seven diagnostic categories (Chronic Lymphocytic Leukemia, Chronic Myelomonocytic Leukemia, Non-Hematopoietic Solid Tumors Carcinoma, or Sarcoma, Aplastic Anemia, and Other diagnosis) had such a small number of adult patients (47 total), they were excluded from analyses that included primary diagnosis. Associations between organ system bleeding and stratum, dose group, gender, age, and laboratory assays were evaluated using both univariate, and multiple logistic regression models taking into account the within patient correlation. Kaplan-Meier curves were used to explore Grade 2A+ organ system bleeding event rate. The time to Grade 2A+ bleeding onset between strata were tested using log-rank test statistics. All statistical analyses were performed in SAS v9.4 and figures were plotted in R v4.0.2. The PLADO trial, for which the data are further analyzed herein, complied with the Research Ethics Guidelines. Participating institutional review boards approved the study. Participating adult study subjects provided written informed consent; for participating child subject, a parent or legal guardian provided written informed consent. Children provided assent if required by local site policy. A data and safety monitoring board reviewed data twice a year.

3. Results

3.1 Days on Study

Of the 1,077 study patients, 1,035 had 3,791 days after enrollment before their first platelet transfusion with an overall median of 3 pre-transfusion days, and medians of 2, 3 and 3 days for the AUTO, ALLO and CHEMO strata, respectively. The remaining 42 patients had their first platelet transfusion at randomization. On or after their first platelet transfusion, the 1,077 patients had a total of 16,320 days on study with an overall median of 14 days, and medians of 8, 16, and 19 days for the AUTO, ALLO and CHEMO strata, respectively. On or after their first platelet transfusion, the lower, medium, and high dose platelet transfusion groups had medians of 14, 13, and 14 days on study, respectively.

3.2 Grade 2A+ Organ System Bleeding

Table 1 shows the number of patients and patient-days with each type of Grade 2A or greater (Grade 2A+) or Grade 3 or greater bleeding (Grade3+); before the date of the first platelet transfusion or on or after the date of the first platelet transfusion. Overall, 616 patients (57.2% of patients) had Grade 2A+ bleeding on 2,681 days (13.3% of study days). Before the date of their first platelet transfusion, 155 patients (15.0%) had Grade 2A+ bleeding. On or after the date of their first platelet transfusion, 577 patients (53.6%) had Grade 2A+ bleeding. In decreasing frequency during both time periods, GI, GU, PL, and ON bleeding predominated. Overall, 336 patients (31.2%) had GI bleeding on 876 days (4.4% of days), 227 patients (21.1%) had GU bleeding on 1,021 days (5.1%), 190 patients (17.6%) had PL bleeding on 536 days (2.7%), and 157 patients (14.6%) had ON bleeding on 393 days (2.0%).

Table 1 Organ system bleeding.

	ALL STUDY DAYS		BEFORE THE DATE OF THE FIRST PLATELET TRANSFUSION		ON OR AFTER THE DATE OF THE FIRST PLATELET TRANSFUSION	
GRADE 2A+ BLEEDING CRITERIA	**Patients (N = 1077)**	**Days (N = 20111)**	**Patients (N = 1035)**	**Days (N = 3791)**	**Subjects (N = 1077)**	**Days (N = 16320)**
All Patients*	616 (57)	2681 (13)	155 (15)	243 (6)	577 (54)	2438 (15)
• Gastrointestinal (GI)	336 (31)	876 (4)	84 (8)	107 (3)	293 (27)	769 (5)
• Genitourinary (GU)	227 (21)	1021 (5)	49 (5)	86 (2)	212 (20)	935 (6)
• Pulmonary (PL)	190 (18)	536 (3)	18 (2)	25 (1)	184 (17)	511 (3)
• Oral and Nasal (ON)	157 (15)	393 (2)	7 (1)	11 (<1)	155 (14)	382 (2)
• Invasive Sites (IS)	79 (7)	147 (1)	16 (2)	21 (1)	71 (7)	126 (1)
• Soft Tissue, and Musculoskeletal (ST)	34 (3)	40 (<1)	4 (<1)	4 (<1)	31 (3)	36 (<1)
• Body Cavity (BC)	28 (3)	35 (<1)	0 (0)	0 (0)	28 (3)	35 (<1)
• Central Nervous System (CNS)	29 (3)	38 (<1)	1 (<1)	1 (<1)	28 (3)	37 (<1)
GRADE 3 OR GREATER BLEEDING CRITERIA						
All Patients**	102 (9)	162 (1)	3 (<1)	3 (<1)	100 (9)	159 (1)
• RBC Transfusion to Treat Bleeding	81 (8)	128 (1)	2 (<1)	2 (<1)	80 (7)	126 (1)
• Central Nervous System (CNS)	21 (2)	28 (<1)	1 (<1)	1 (<1)	20 (2)	27 (<1)
• Hemodynamic Instability - Moderate or Severe (HD)	16 (1)	21 (<1)	0 (0)	0 (0)	16 (1)	21 (<1)
• Body Cavity (BC)	7 (1)	10 (<1)	0 (0)	0 (0)	7 (1)	10 (<1)
• Fatal Bleeding	1 (<1)	1 (<1)	0 (0)	0 (0)	1 (<1)	1 (<1)

Data in parentheses are the percentage of patients or days. Patients or days with bleeding in more than one organ system are counted in each of the corresponding rows in the table.

*All patients with Grade 2A or greater bleeding (Grade 2A+).

**All patients with Grade 3 or greater bleeding (Grade 3+).

3.3 Simultaneous Bleeding in Multiple Organ Systems

Of the 616 patients with Grade 2A+ bleeding, 164 (26.6%) had at least one day with bleeding in more than one organ system on 399 days (14.9%). The most common bleeding combination was GI and GU bleeding in 53 (8.6%) of patients on 101 days (3.8%).

3.4 Grade 3+ Organ System Bleeding

The only organ systems with specific criteria for Grade 3 or higher (Grade 3+) bleeding are CNS, hemodynamic instability, and body cavity. Grade 3+ bleeding criteria were rarely met for these

organs. There were 102 patients (9.5% of patients) on 162 days (0.8%) with Grade 3+ bleeding (Table 1). Only three had Grade 3+ bleeding before their first platelet transfusion, and one had a CNS bleed. Most days with Grade 3+ bleeding reached that Grade because the patient received a red cell transfusion to treat active bleeding. No data were collected on the specific bleeding symptoms which prompted a RBC transfusion. A pulmonary hemorrhage was the only fatality (Grade 4 bleeding).

3.5 CNS Bleeding

Because CNS bleeding is of such concern, available study data for each patient-day with CNS bleeding are presented (Table 2). Overall, CNS bleeding occurred in 29 (2.7%) patients on 38 days (0.2%). Seven patients had more than one day of CNS bleeding, and 3 patients had 2 types of CNS bleeding. Grade 2A CNS bleeding occurred in 10 patients, Grade 3 CNS bleeding occurred in 3 patients, and Grade 4 CNS bleeding occurred in 18 patients on 25 days (patient 27 had 3 days of CNS bleeding; patients 6, 15, 18, 25, and 29 had each 2 days of CNS bleeding.).

Table 2 CNS bleeding by bleeding type (individual patients' bleeding days).

Subj ID*	Stratum	Primary Diagnosis**	Dose Group	Days from 1st Platelet Txn	Days from Most Recent Platelet Txn Most Recent ***	2nd Most Recent	Platelet Count (x10^9/L)	HCT (%)	Fibrinogen(mg/dL)	PTT (sec)	INR	Type of CNS bleeding
1	ALLO	AML	Low	1	1		16	26				Retinal Bleeding w/o Visual Impairment (G2A)
2	ALLO	MDS	Low	20	0	1	18	33		29	1.2	
3	ALLO	AML	High	14	0	1	28	28	434	33	1.4	
4	CHEM	AML	Low	3	0	3	11	27				Bloody Lumbar Puncture; Microscopic Blood No Symptoms(G2A)
5	CHEM	AML	High	5	1	5	74	25				
6	CHEM	AML	Low	15	0	2	10	34	786	26	1.3	
7	CHEM	AML	High	5	0	3	45	25	1659	39	1.0	
8	CHEM	AML	High	10	0	5	8					
9	CHEM	AML	Med	22	6	8	107	31				
10	CHEM	AML	Med	29	0	7	18	26	624	36	1.2	
11	CHEM	AML	High	-5			54	22	477	36	1.1	Bloody Lumbar Puncture; Visible Blood No Symptoms(G3)
12	CHEM	ALL	High	5	0	5	18	24				
13	CHEM	AML	High	21	2	9	55	29				
14	ALLO	AML	Low	7	0	2	7	21				Retinal Bleeding with Visual Impairment (G4)
15	ALLO	AML	High	27	3	4	30	24		26	1.2	
16	ALLO	Systemic Mastocytosis	Med	13	1	4	18	25				
17	ALLO	CML	High	13	0	3	21	25			1.4	

18	ALLO	MDS	Med	9	0	1	10	23			1.4	
18	ALLO	MDS	Med	10	0	1	20	26				
19	CHEM	AML	Low	6	3	6	30	24				
20	CHEM	AML	High	6	0	1	11	22				
21	AUTO	PCD	Low	4	2	4	16	32				
22	ALLO	AML	Low	9	4	5	89	32		40	1.1	Bloody Lumbar Puncture with CNS Symptoms (G4)
15	ALLO	AML	High	28	0	4	11	42		28	1.2	
23	CHEM	AML	Med	13	0	1	30	24				
24	AUTO	HL	High	7	0	1	45	32				
25	ALLO	ALL	Low	18	0	1	72	27		58	1.2	
25	ALLO	ALL	Low	21	0	1	71	27				
26	ALLO	MDS	Low	8	6	8	226	29				
3	ALLO	AML	High	15	0	1	31	28	460	31	1.4	
27	ALLO	AA	Med	4	0	1	16	23				
27	ALLO	AA	Med	15	1	2	107	22				CNS Bleeding on Imaging Study (G4)
27	ALLO	AA	Med	18	0	1	108	30		20	1.0	
6	CHEM	AML	Low	20	0	5	54	29	634	25	1.2	
6	CHEM	AML	Low	21	0	1	109	26		25	1.4	
28	CHEM	AML	Low	11	0	1	47	29	914	29	1.2	
29	AUTO	PCD	High	2	0	2	57	22				
29	AUTO	PCD	High	3	0	1	51	22				

*Each number indicates a unique subject. Patients 3, 6, 15, 18, 25, 27, and 29 had more than one day of CNS bleeding. Three patients had more than one type of CNS bleeding: 3 (retinal bleeding without visual impairment and CNS bleeding on imaging study), 6 (bloody lumbar puncture without CNS symptoms and CNS bleeding on imaging study), and 15 (retinal bleeding with visual impairment and bloody lumbar puncture with CNS symptoms) had more than one type of CNS bleeding. ** ALL=Acute Lymphocytic Leukemia, AML=Acute Myelogenous Leukemia, CML=Chronic Myelogenous Leukemia, HL=Hodgkins Lymphoma, NHL=Non-Hodgkins Lymphoma, MDS=Myelodysplastic Syndromes, PCD=Plasma Cell Disorders, AA=Aplastic Anemia Congenital or Acquired. ***A zero in this column means the patient had a platelet transfusion on the day of CNS bleeding, but we do not know if the platelet transfusion was given before or after the onset of bleeding.

The most common types of CNS bleeding were those found by imaging study, followed by retinal bleeding with visual impairment, and lumbar puncture with microscopic blood without CNS symptoms.

The average platelet count at onset of CNS bleeding was 46 ± 43 × 10^9/L (range of 7 × 10^9 /L to 226 × 10^9 /L). There were no differences in platelet counts based on the severity of bleeding; platelet counts averaged 34 × 10^9/L ± 33 × 10^9/L (range 8 × 10^9 /L to 107 × 10^9 /L) on the 10 days with Grade 2A bleeding, 42 × 10^9 ± 21 × 10^9 /L (range 18 × 10^9 /L to 55 × 10^9 /L) on the 3 days with Grade 3 bleeding, and 51 × 10^9 ± 49 × 10^9 /L (range 7 × 10^9/L to 226 × 10^9 /L) on the 25 days with Grade 4 bleeding (p = 0.34 for 3 group comparison). The types of bleeding with the lowest platelet counts were retinal bleeding without visual impairment (Grade 2A) [mean platelet count 21 × 10^9 /L ± 6 × 10^9 /L, range of 16 × 10^9 /L to 28 × 10^9 /L)] or with visual impairment (Grade 4) [mean platelet count 18 × 10^9 /L ± 8 × 10^9 /L, range of 7 × 10^9 /L to 30 × 10^9/L)] (Figure 1).

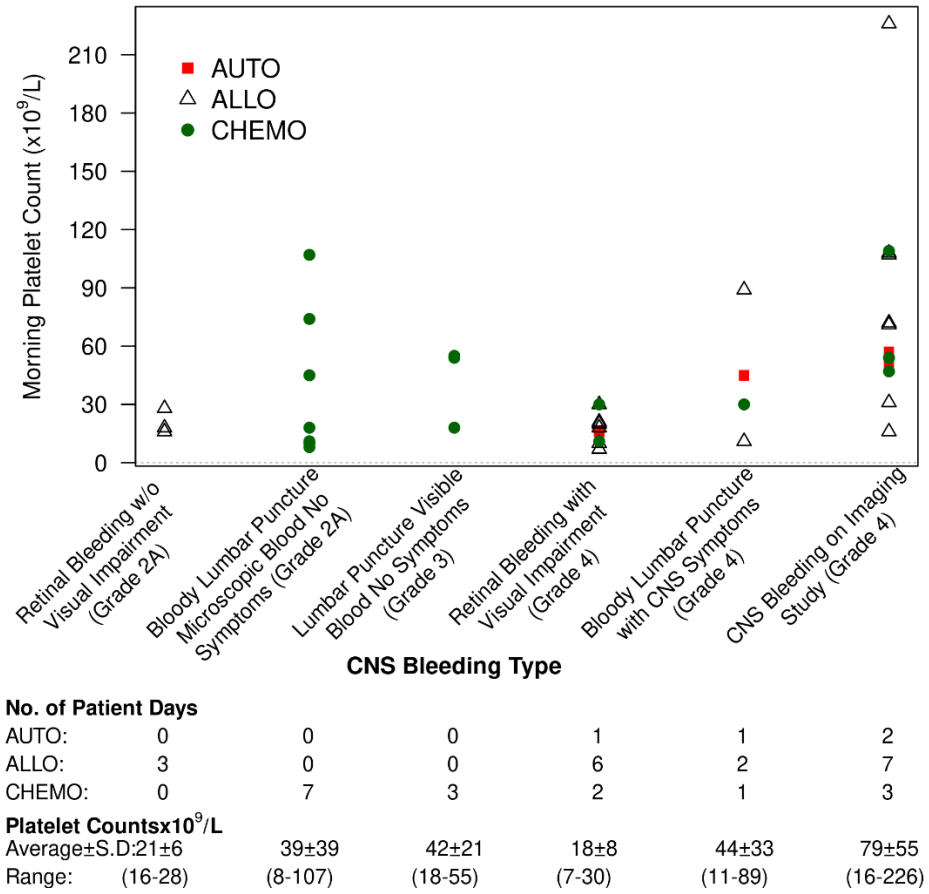


Figure 1 CNS bleeding types by morning platelet count and stratum.

These data suggest that bleeding can occur at any platelet count and at any time during a patient's thrombocytopenic episode. Only one CNS bleed occurred before the patient's first platelet transfusion. Of the 38 days with CNS bleeding, a platelet transfusion was given on 26 days (68%) (Table 2). Data are not available on whether these transfusions were given before or after the onset of CNS bleeding. However, only 7 of these transfusions may have been given solely because of a platelet count of ≤11 × 10^9 /L, and only 4 had platelet counts meeting the transfusion trigger of ≤10 × 10^9 /L. Excluding those days with a platelet transfusion given on the same day of a CNS bleed (day 0), or before the first platelet transfusion, the average days from the most recent platelet

transfusion to onset of CNS bleeding was 2.7 ± 1.9 days (range 1 to 6 days), and the mean days from the second most recent transfusion to the date of CNS bleeding was 3.1 ± 2.4 days (range 1 to 9) (Table 2). Of the 12 patients not given a platelet transfusion on the day of CNS bleeding, 3 had Grade 2A CNS bleeding, 2 had Grade 3, and 7 had Grade 4 (4 of these had retinal bleeding with visual impairment, 1 had a bloody lumbar puncture with CNS symptoms with a platelet count of 89×10^9 /L, and 2 had CNS bleeding on imaging study with platelet counts of 107×10^9 /L and 226×10^9 /L).

The average hematocrit at onset of CNS bleeding was 27 ± 4% (range of 21% to 42%). There were no differences in hematocrits based on the severity of bleeding; hematocrits averaged 28 ± 3% (range 25% to 34%) on the 9 days with Grade 2A bleeding with hematocrit measurements, 25 ± 4% (range 22% to 29%) on the 3 days with Grade 3 bleeding, and 27 ± 5% (range 21% to 42%) on the 25 days with Grade 4 bleeding ($p = 0.30$ for 3 group comparison).

The risk of CNS bleeding differed by stratum, 3 of 365 AUTO patients (0.8%), 12 of 399 ALLO patients (2.9%), and 14 of 271 CHEMO patients (4.9%) experienced any CNS bleeding ($p=0.02$ for the three-group comparison). Of the 10 patients with a bloody lumbar puncture without CNS symptoms, all were CHEMO patients (9 AML and 1 ALL). These patients may have received intrathecal chemotherapy necessitating an asymptomatic lumbar puncture. Retinal bleeding with or without visual impairment occurred in 11 patients, 8 (73%) were ALLO patients. Of the 7 patients with CNS bleeding on imaging study, 4 (57%) were ALLO patients, 2 (29%) were CHEMO, and 1 (14%) was AUTO. However, the risk did not differ by dose group.

3.6 Predictors of Bleeding in Each Organ System

We assessed whether the following variables were associated with bleeding in each of the nine organ systems, individually and as part of a multi-predictor model: stratum, gender, age, randomized platelet dose group (for days on or after the first platelet transfusion), primary diagnosis, morning platelet count category, and hematocrit category.

As shown in Table 1, only 155 of 1,035 patients (15.0%) whose bleeding was assessed for a total of 243 days prior to their first transfusion had any evidence of bleeding. The small number of patients and the few days of bleeding assessments limited our ability to model for relevant risk factors for bleeding prior to their first transfusion. Therefore, analysis of risk factors was only determined for patients on or after their first platelet transfusion.

For predictors of bleeding, the univariate models with patient as the unit of analysis are shown in Table 3. The most common type of bleeding in almost every diagnostic group of patients was GI bleeding, followed by GU bleeding. However, the most common type of bleeding for patients with ALL was pulmonary (36.0% of patients), and it was also high for patients with AML (19.9%), CML (24.4%), and MDS (18.9%).

Table 3 Baseline patient characteristics as individual predictors of organ system bleeding, on or after the date of first platelet transfusion.

	ON N (%)	ST N (%)	GI N (%)	GU N (%)	PL N (%)	BC N (%)	CNS N (%)	IS N (%)	HD N (%)
Stratum									
AUTO (N = 378)	25 (6.6)	8 (2.1)	64 (16.9)	34 (9.0)	38 (10.1)	1 (0.7)	3 (0.8)	16 (4.2)	5 (1.3)
ALLO (N = 413)	95 (23.0)	14 (3.4)	162 (39.2)	116 (28.1)	104 (25.2)	7 (1.7)	12 (2.9)	30 (7.3)	5 (1.2)
CHEMO (N = 286)	35 (12.2)	9 (3.2)	67 (23.4)	62 (21.7)	42 (14.7)	19 (6.6)	13 (4.6)	25 (8.7)	6 (2.1)
Overall P-value	*<0.001*	*0.54*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.02*	*0.06*	*0.61*
Primary Diagnosis*									
AML (N = 413)	65 (15.7)	13 (3.2)	120 (29.1)	103 (24.9)	82 (19.9)	18 (4.4)	17 (4.1)	32 (7.8)	7 (1.7)
NHL (N = 191)	23 (12.0)	3 (1.6)	46 (24.1)	28 (14.7)	27 (14.1)	2 (1.1)	0 (0)	9 (4.7)	4 (2.1)
PCD (N = 157)	10 (6.4)	6 (3.8)	29 (18.5)	12 (7.6)	14 (8.9)	0 (0)	2 (1.3)	8 (5.1)	1 (0.6)
ALL (N = 75)	22 (29.3)	3 (4.0)	26 (34.7)	17 (22.7)	27 (36.0)	1 (1.3)	2 (2.7)	7 (9.3)	1 (1.3)
HL (N = 75)	7 (9.3)	0 (0)	14 (18.7)	12 (16.0)	7 (9.3)	2 (2.7)	1 (1.3)	3 (4.0)	1 (1.3)
MDS (N = 74)	10 (13.5)	2 (2.7)	30 (40.5)	17 (22.0)	14 (18.9)	1 (1.4)	3 (4.1)	2 (2.7)	0 (0)
CML (N = 45)	8 (17.8)	2 (4.4)	19 (42.2)	9 (20.00)	11 (24.4)	3 (6.7)	1 (2.2)	6 (13.3)	2 (4.4)
Overall P-value	<0.001	-	<0.001	<0.001	<0.001	-	-	0.18	-
Gender									
Male (N = 645)	93 (14.4)	16 (2.5)	164 (25.4)	87 (13.5)	107 (16.6)	17 (2.6)	19 (3.0)	42 (6.5)	8 (1.2)
Female (N = 432)	62 (14.4)	15 (3.5)	129 (29.9)	125 (28.9)	77 (17.8)	10 (2.3)	9 (2.1)	29 (6.7)	8 (1.9)
Overall P-value	*0.98*	*0.34*	*0.11*	*<0.001*	*0.60*	*0.74*	*0.39*	*0.90*	*0.42*
Dose Group									
Low (N = 352)	59 (16.8)	13 (3.7)	90 (25.6)	71 (20.2)	60 (17.1)	7 (2.0)	11 (3.1)	26 (7.4)	7 (2.0)
Medium (N = 355)	46 (13.0)	14 (3.9)	103 (29.0)	69 (19.4)	57 (16.1)	12 (3.4)	6 (1.7)	25 (7.0)	5 (1.4)
High (N = 370)	50 (13.5)	4 (1.1)	100 (27.0)	72 (19.5)	67 (18.1)	8 (2.2)	11 (3.07)	20 (5.4)	4 (1.1)
Overall P-value	*0.30*	*0.06*	*0.59*	*0.96*	*0.76*	*0.44*	*0.43*	*0.52*	*0.60*
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
Age									

Grade 2A+ bleeding**	48±13	51±14	48±13	48±13	49±13	49±13	49±14	52±14	58±13
No Grade 2A+ bleeding***	51±14	50±14	51±14	51±14	51±14	51±14	51±14	50±14	50±14
Overall P-value	*0.003*	*0.81*	*0.002*	*<0.001*	*0.21*	*0.48*	*0.58*	*0.41*	*0.03*

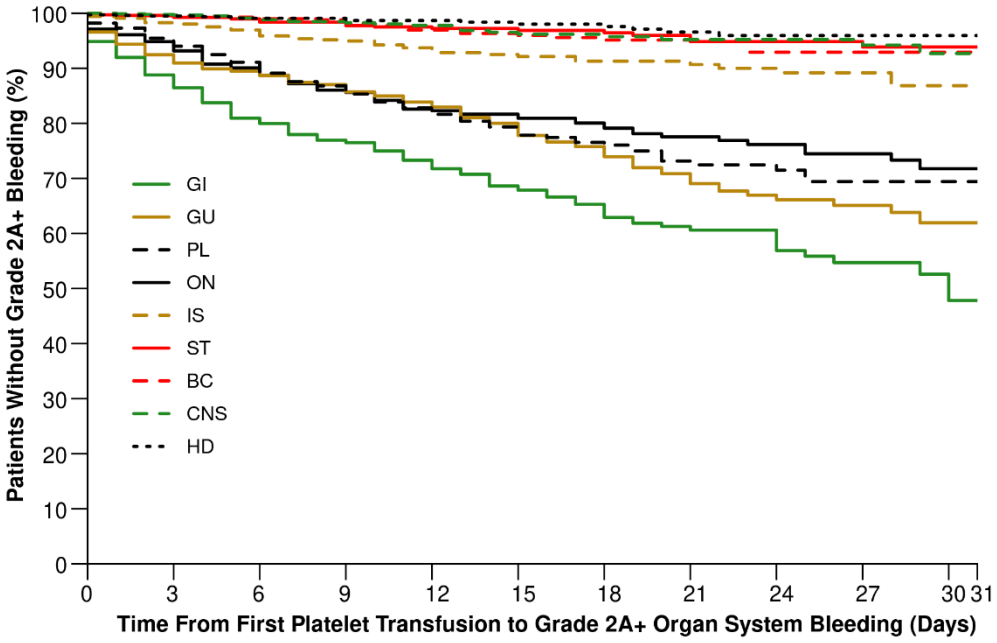
The multi-predictor models not including primary diagnosis are provided in Table S2A and Table S2B. After adjustment for other variables in the models, the following associations were statistically significant.

- Oral/Nasopharyngeal (ON) bleeding: ALLO patients are at higher risk than AUTO and CHEMO patients. Older patients were at lower risk than younger patients. Days with morning platelet counts of 1-10 × 10^9 /L, 11-20 × 10^9 /L, 21-40 × 10^9 /L, and 41-60 × 10^9 /L were all at higher risk than days with platelet counts >60 × 10^9 /L.
- Soft Tissue (ST) bleeding: Patients in the high platelet dose group were at lower risk than patients in the medium platelet dose group. Days with morning platelet counts of 1-10 × 10^9 /L and 11-20 × 10^9 /L were at high risk compared to 21-40 × 10^9 /L.
- Gastrointestinal (GI) bleeding: ALLO patients were at higher risk than AUTO and CHEMO patients. Days with morning platelet counts of 1-10 × 10^9 /L, 11-20 × 10^9 /L, and 41-60 × 10^9 /L were at higher risk than days with platelet counts >60 × 10^9 /L.
- Genitourinary (GU) bleeding: ALLO patients were at higher risk than AUTO and CHEMO patients, and CHEMO patients were at higher risk than AUTO patients. Males were at lower risk. Older patients were at lower risk. Days with morning platelet counts of 1-10 × 10^9 /L, 11-20 × 10^9 /L, 21-40 × 10^9 /L, and 41-60 × 10^9 /L were all at higher risk than days with platelet counts >60 × 10^9 /L.
- Pulmonary (PL) bleeding: ALLO patients were at higher risk than AUTO and CHEMO patients.
- Body Cavity (BC) bleeding: ALLO and CHEMO patients were at higher risk than AUTO patients.
- Invasive Sites (IS) bleeding: Days with hematocrits ranging between 26 and 29% had the lowest risk and days with hematocrit ≤25% had the highest risk.
- Hemodynamic Instability (HD) bleeding: Older patients were at higher risk. Days with hematocrit ≤25% had the highest risk.

These associations remained similar for GI, GU, PL and IS bleeding after adding primary diagnosis to the models. This was not the case for ST, CNS, and HD bleeding due to paucity of data.

3.7 Time to Onset of Grade 2A+ Organ System Bleeding

If a patient met criteria for Grade 2A+ or greater bleeding in a specific organ system between first platelet transfusion and end of study, the date of the first such bleeding was considered as the event date for the patient. Otherwise, the patient was censored on the last study day. Kaplan-Meier curves for the time to onset of Grade 2A+ organ system bleeding on or after the date of the first platelet transfusion are shown in Figure 2.



No. of patients at risk

GI	993	689	498	347	237	182	137	93	65	43	11 2
GU	1028	738	569	394	278	217	165	118	81	56	15 2
PL	1059	792	598	409	284	212	157	105	75	53	12 2
ON	1070	792	597	407	284	221	172	127	97	73	17 1
IS	1061	809	630	447	322	254	202	149	110	85	21 2
ST	1073	835	657	473	348	275	223	169	127	97	23 2
BC	1077	836	661	475	345	269	214	158	115	87	19 2
CNS	1076	837	661	474	348	272	219	162	124	94	22 2
HD	1077	835	659	477	351	278	224	166	124	93	21 2

Figure 2 Time from first platelet transfusion to G2A+ bleeding.

Kaplan-Meier curves of the time from first platelet transfusion to Grade 2A+ organ system bleeding by stratum are shown in Figures S1A-S1I. The log-rank test results show stratum was significantly associated with time to onset of ON, GI, GU, PL, and BC bleeding ($p < 0.05$). ALLO patients had shorter time to onset of ON, GI, GU, and PL bleeding compared to both AUTO and CHEMO patients. For BC bleeding, CHEMO patients bled sooner than AUTO and ALLO patients.

4. Discussion

The secondary analyses of the PLADO trial data reported here, which are derived from the largest study population analyzed to date, sought to examine the relationship between multiple factors and the patterns of organ bleeding in patients with hypoproliferative thrombocytopenia. Direct comparison of these secondary analyses with other studies examining bleeding in the setting of hypoproliferative thrombocytopenia is not possible due to differences in study protocols and bleeding assessment tools employed as well as the lack of comparable granularity of the analyses (e.g. analysis of specific factors associated with bleeding by organ type) [5, 9, 12, 13]. However, using broad brush comparisons, our results are consistent with other reports in that clinically significant bleeding (WHO Grade 2A+) occurred in a high percentage of study patients (57.2%); severe/life threatening hemorrhage (WHO Grade 3-4) occurred in a smaller, but clinically relevant, percentage of patients (9.5%) [5, 14, 15].

Overall, the most common type of bleeding occurred in the GI tract (31.2% of patients) followed by GU (21.1%), pulmonary (17.6%) and oral/nasal (14.6%). Of note, the distribution of organ

bleeding observed in this analysis of adults was somewhat different from what was reported in the analysis of pediatric patients enrolled in the PLADO trial; oral/nasal bleeding represented the most common type of bleeding in the pediatric patient (<19 years) [6]. As pointed out by these authors and others, site-specific bleeding risk in the setting of thrombocytopenia may be related to differences in endothelial integrity secondary to the type and intensity of treatment regimens as well as the inflammatory milieu [6, 16-18]. And, as has been cautioned by Crighton and colleagues [13], findings reported from studies of adult patient populations may not be applicable to pediatric patient populations.

In multi-predictor models including gender, age, treatment stratum, and with and without primary diagnosis, significant associations were found for several types of bleeding. Overall, ALLO patients were at higher risk for bleeding in most organ systems and had a shorter time from their first transfusion to onset of bleeding, in keeping with reports by others [5, 15]. With respect to influence of gender, our analyses showed females were more likely to have genitourinary bleeding ($p < 0.001$); but gender did not appear to impact bleeding differences for any other organ system. This is in contrast to Stanworth et al [19] where they found female sex was significantly associated with WHO grade 2-4 bleeding even after excluding vaginal-only bleeding.

As previously reported by PLADO study investigators and others, hematocrit is inversely associated with risk of bleeding [20-22]. In the present data analysis, patients with hematocrits $\leq$ 25% were at higher risk for bleeding at invasive sites and bleeding associated with hemodynamic instability. Thakar and colleagues, in a recent review, nicely synthesize data from animal studies and clinical trials which support the concept of altered red blood cell rheology in the face of reduced red cell mass as a consequence of the anemic state. This altered rheology, in turn, impairs platelets' important participation in primary hemostasis thereby contributing to increased risk for bleeding [23].

Because CNS bleeding is of substantial interest to clinicians despite the low frequency of occurrence, we thoroughly examined all study collected data for patients with recorded CNS bleeding. Important observations derived from data analysis included the fact that the risk of CNS bleeding varied by stratum, occurring in 12 ALLO patients (2.9%), 14 CHEMO (4.9%), and 3 AUTO patients (0.8%). Additionally, platelet counts at onset of CNS bleeding averaged $46 \pm 43 \times 10^9$ /L with a range of 7×10^9/L to 226×10^9 /L; bleeding was not associated with platelet count nor platelet dose and occurred any time during hospitalization (Figure 1). The absence of a relationship between platelet dose and CNS bleeding is in direct contrast to the findings of the Strategies for Transfusion of Platelets (SToP) study (NCT00420914) where 3 subjects randomized to the lower dose strategy (1.5×10^{11}-3.0×10^{11} platelets per transfusion) experienced grade 4 bleeding compared to no patients randomized to standard dose arm ($3.0 \times 10^{11} - 6.0 \times 10^{11}$ platelets per transfusion) triggering the predefined stopping rule and discontinuation the trial [4]. As reported in the primary PLADO manuscript, a possible explanation for this may relate to differences in dosing strategy: the PLADO trial dosing strategy adjusted for body-surface area whereas in the SToP study, a fixed dose range was used for patients randomized to their respective treatment arm [11].

Of all the CNS bleeding observed in our study, retinal bleeding had the lowest platelet counts of any type of CNS bleeding (Figure 1). Interestingly, the Grade 4 bleeding observed in 2 of the 3 patients in the SToP trial was attributable to a retinal bleed [4]. The occurrence of retinal bleeding at very low platelet counts has been observed in numerous reports on ocular complications following ALLO and AUTO HSCT; it is reported to be multifactorial in nature, secondary to retinal

damage in the setting of CMV retinitis or pre-transplant conditioning and immunosuppressive regimens [24-26].

The authors acknowledge several limitations of this study, which are common to reports on post-hoc analyses [19]. These include the fact that modeling of risk factors and other observations were restricted to data collected during the primary trial. In particular, we did not have data on timing of bleeding events in relation to transfusion, precluding the determination as to whether platelet or RBC transfusion were given in response to the bleeding event. Additionally, as noted in previous publications, the lack of detailed information on chemotherapeutic regimens or preparative transplant protocols does not allow for analysis of their potential contribution to the observed bleeding event and predilection of vulnerability to a particular organ system [11, 20].

In summary, the secondary analyses presented in this paper show that patients with hypoproliferative thrombocytopenia have a significant risk of bleeding in multiple organ systems. Prominently, the risk for bleeding is affected by treatment category with ALLO patients having the highest risk for most types of bleeding. Moreover, the secondary analyses of this large cohort revealed the most common types of organ system bleeding are GI, GU, pulmonary, and oral/nasal; and reinforced previous reports on risk for CNS bleeding: CNS bleeding is a relatively rare, but clinically relevant event, which occurs across a wide range of platelet counts [4, 5, 27]. It is hoped that the observations we report will assist clinicians caring for patients experiencing hypoproliferative thrombocytopenia, as well as inform future studies with regard to protocol design and patient stratification in order to further refine our understanding of optimal platelet transfusion management.

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Author Contributions

L.U., S.J.S., T.G. and S.F.A. designed the research; L.U., S.J.S. and T.G. performed the research; L.U., S.J.S, T.H.H., T.G. and S.F.A. analyzed the data; and L.U., S.J.S, T.H.H., T.G. and S.F.A. all contributed to writing of this manuscript.

Competing Interests

The authors declare no competing financial interests.

Additional Materials (if any)

Trial: The Platelet Dose Trial (PLADO): A Transfusion Medicine/Hemostasis (TMH) Clinical Trials Network Study: Registered as Clinical Trials.gov identifier-NCT00128713.

The following additional materials are uploaded at the page of this paper.

1. Table S1: Who grading system for bleeding.
2. Table S2A: Multi-predictor logistic regression analyses of predictor variables for ON, ST, GI, GU and PL organ system bleeding.
3. Table S2B: Multi-predictor logistic regression analyses of predictor variables for BC, CNS, IS, and HD organ system bleeding.
4. Figure S1A: Time from first platelet transfusion to G2A+ GI bleeding.
5. Figure S1B: Time from first platelet transfusion to G2A+ GU bleeding.
6. Figure S1C: Time from first platelet transfusion to G2A+ PL bleeding.
7. Figure S1D: Time from first platelet transfusion to G2A+ ON bleeding.
8. Figure S1E: Time from first platelet transfusion to G2A+ IS bleeding.
9. Figure S1F: Time from first platelet transfusion to G2A+ ST bleeding.
10. Figure S1G: Time from first platelet transfusion to G2A+ BC bleeding.
11. Figure S1H: Time from first platelet transfusion to G2A+ CNS bleeding.
12. Figure S1I: Time from first platelet transfusion to G2A+ HD bleeding.

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