Bleeding Risks Are Higher In Children versus Adults Given Prophylactic Platelet Transfusions for Treatment-Induced Hypoproliferative Thrombocytopenia

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Trial: Determination of the Optimal Platelet Dose Strategy to Prevent Bleeding in Thrombocytopenic Patients (Platelet Dose Trial): A Transfusion Medicine/Hemostasis (TMH) Clinical Trials Network Study. Registered as Clinical Trials.gov identifier-NCT00128713

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Abstract

Age-group analyses were conducted of patients in the prophylactic platelet dose trial (PLADO), which evaluated the relationship between platelet dose per transfusion and bleeding. Hospitalized patients with treatment-induced hypoproliferative thrombocytopenia were randomized to one of three platelet doses: $1.1 \times 10^{11}$, $2.2 \times 10^{11}$, or $4.4 \times 10^{11}$ platelets/m$^2$/transfusion, given for morning counts of $\leq 10,000$ platelets/$\mu l$. Daily hemostatic assessments were performed. The primary endpoint (percentage of patients who developed Grade 2 or higher World Health Organization bleeding) was evaluated in 198 children (0-18 years) and 1,044 adults. Although platelet dose did not predict bleeding for any age group, children overall had a significantly higher risk of Grade 2 or higher bleeding than adults (86%, 88%, 77% versus 67% of patients aged 0-5 years, 6-12 years, 13-18 years, versus adults, respectively) and more days with Grade 2 or higher bleeding (median 3 days in each pediatric group versus 1 day in adults, $p<0.001$). The effect of age on bleeding differed by disease treatment category, and was most pronounced among autologous transplant recipients. Pediatric subjects were at higher risk of bleeding over a wide range of platelet counts, indicating that their excess bleeding risk may be due to factors other than platelet counts.

Registered as Clinical Trials.gov identifier-NCT00128713.
Introduction

Both pediatric and adult patients undergoing hematopoietic stem cell transplants (HSCT) and/or chemotherapy for leukemia and other malignant conditions experience hypoproliferative thrombocytopenia, placing them at risk for bleeding. However, the incidence of bleeding in children compared to adults has not been well characterized. Likewise, the precise relationships between bleeding and patient platelet count or prophylactic platelet transfusion dose in children are unknown.

One of the few studies that analyzed the relationship between bleeding and platelet counts in children with leukemia was a retrospective review by Roy et al conducted between 1950 and 1955, when platelets were transfused to treat, rather than to prevent, overt bleeding. When patient platelet counts were either < 11,000/µl or between 11,000 – 20,000/µl, minor bleeding occurred on 53% and 47% of days, respectively, and clinically significant bleeding (gross gastrointestinal bleeding or hematuria) occurred on 26% and 10% of days, respectively. However, current relevance of these data is limited since children in that study undoubtedly received aspirin; none received modern chemotherapy, radiation treatment or a HSCT; and retrospective review may have failed to detect some bleeds.

Subsequent studies suggested that prophylactic platelet transfusions could reduce bleeding in thrombocytopenic patients with leukemia, both in adults and children. In the 1970’s, Murphy and colleagues found that children with leukemia experienced an average of 7.9 significant bleeds (nasal/oral bleeding requiring packing, gross gastrointestinal or genitourinary bleeding, central nervous system bleeding, or bleeding requiring red cell transfusion) per 100 months of
observation without prophylactic platelet transfusions versus 1.9 for children given prophylactic platelet transfusions.\textsuperscript{3} However, relevance of these data is still questionable for the reasons discussed above, and a prophylactic platelet transfusion trigger of 20,000 platelets/µl was used versus the 10,000 platelets/µl trigger that is currently recommended\textsuperscript{4}. Furthermore, the source of platelet concentrates (apheresis versus whole blood derived), and the quality and quantity of platelets transfused, were probably quite different and perhaps inferior, compared to current practice.

While there is evidence that prophylactic platelet transfusions reduce the incidence of bleeding\textsuperscript{3}, the optimal dose of platelets to transfuse is controversial.\textsuperscript{5} Roy et al randomized 62 patients to receive one of two doses, based on units per body weight, when the platelet count was ≤ 25,000/µl.\textsuperscript{1} Both doses were equally efficacious in reducing bleeding incidence, but the small sample size precluded definitive dosing conclusions.

Based on the paucity of data, and the difficulty of applying these historic studies to current practice, a secondary analysis of the recently reported Determination of the Optimal Platelet Dose Strategy to Prevent Bleeding in Thrombocytopenic Patients (PLADO) clinical trial\textsuperscript{6} was conducted to determine if bleeding outcomes differed among the three pediatric age groups, or between any pediatric age group versus adults.
Methods

The methodology and overall results of the PLADO study have previously been reported.\textsuperscript{6} PLADO was registered on clinicaltrials.gov with identifier NCT00128713.

Study population

Briefly, the PLADO study was a prospective, multi-center, randomized controlled trial of patients who were hospitalized and expected to become thrombocytopenic (platelet counts of \( \leq 10,000 \) platelets/µl for \( \geq 5 \) days) as a result of HSCT or chemotherapy for hematologic malignancy or solid tumor. The Institutional Review Boards at participating hospitals approved the PLADO study. Adult patients were required to provide written informed consent in accordance with the Declaration of Helsinki, and, for children, a parent or legal guardian provided consent. Assent from children was obtained in accordance with local policy.

Other eligibility criteria included a body weight of 10 to 135 kilograms (kgs), prothrombin (PT) and partial-thromboplastin (PTT) times no more than 1.3 times the upper limit of normal for the laboratory, a fibrinogen of at least 100 mg per deciliter, and no previous platelet transfusions during the current hospitalization. Patients were ineligible if they currently had WHO Grade 2 or higher bleeding\textsuperscript{7} (Supplemental Table 1), platelet refractoriness within the past 30 days, known panel reactive HLA antibodies \( \geq 20\% \), acute promyelocytic leukemia, idiopathic or thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, planned prophylactic platelet transfusions at platelet counts of \( > 10,000 \) platelets/µl, planned use of bedside-leukoreduced platelets, major surgery within 2 weeks, use of prothrombotic or anti-thrombotic drugs, pregnancy, or previous enrollment in the PLADO trial.
Stratification, randomization, and blinding

Randomization was stratified using 4 disease treatment categories (autologous or syngeneic HSCT, allogeneic HSCT, chemotherapy without HSCT for hematologic malignancy, or chemotherapy without HSCT for solid tumor), and was balanced by hospital. Randomization did not take patient age into account. Enrolled patients were randomly assigned in a 1:1:1 ratio, by means of computer-generated permuted blocks within disease treatment categories, to receive prophylactic platelet transfusions using one of three doses, based on the patient’s body surface area (BSA): a medium dose (MD), $2.2 \times 10^{11}$ platelets/m$^2$; a lower dose, $1.1 \times 10^{11}$ platelets/m$^2$ (½ the MD); or a higher dose, $4.4 \times 10^{11}$ platelets/m$^2$ (2x the MD). The medium dose was selected to correspond to the usual adult platelet dose of either one pool of whole blood derived platelets from 4-6 donors or one apheresis collection per transfusion. Blood bank technical staff knew both the patient’s target dose and the acceptable dose range (0.75 to 1.25 times the target dose) but were not told the patient’s randomization assignment. Staff who performed the daily hemostatic assessments were not given any information about the patient’s assigned dose, but may not have been completely blinded due to differences in transfusion volumes between the dose groups.

Transfusions

The prophylactic platelet transfusion trigger was a morning platelet count of $\leq 10,000$ platelets/µl. Platelets could also be administered at any time for acute bleeding or in association with an invasive procedure. If clinically indicated, the dose or trigger for prophylactic platelet transfusions could be altered at the discretion of the attending physician. However, once the patient stabilized, the study dose and trigger guidelines were to be
reinstiuted. HLA-selected platelet units were transfused completely, regardless of the patient’s assigned dose, to avoid wasting the product. Red blood cells (RBC) were transfused according to local guidelines. Both platelets and RBCs were leukoreduced.

Hemostatic Assessments

Daily assessments were performed by trained research staff and included physical assessments, patient/family interviews, and medical record reviews. Data were collected on all bleeding manifestations, excluding urine dipstick and stool guaiac tests. A computer algorithm then calculated each patient’s daily bleeding grade, based on both the bleeding signs and symptoms and red cell transfusion data. Daily platelet counts and hematocrits were collected.

Study Completion

Patients completed study participation the first time any of the following occurred: a) thirty days after their first platelet transfusion, b) 10 days without a platelet transfusion, c) hospital discharge, d) death, or e) study withdrawal.

Statistical Analysis

Analyses are restricted to the 1,272 PLADO patients who received at least one platelet transfusion while on study. Patients were analyzed in their assigned dose group, even if some or all of their platelet transfusions were outside their dose range. For these current post-hoc analyses of patient age, patients were divided into four age groups based on the likelihood of biologic and physiologic similarities within each group: 0-5 years; 6-12 years; 13-18 years; and
≥ 19 years. The age groups were compared with respect to baseline characteristics, bleeding outcomes, morning platelet counts, number of transfusion episodes, total quantity of blood products received, and compliance with both the study dose and the 10,000 platelets/µl prophylactic platelet transfusion trigger. The data were analyzed using SAS (version 9.2).9

For unadjusted age-group comparisons, Fisher’s exact test was used for binary and categorical variables; ANOVA was used for variables with approximately normal distributions in each age group, and the Kruskal-Wallis test was used for continuous variables that were not normally distributed. The time from stem cell transplant to onset of Grade 2 or higher bleeding was compared using Cox proportional hazards regression.10 Because many outcome variables were skewed, medians and quartiles are presented rather than means and standard deviations.

For multi-predictor models, logistic regression was used for binary outcomes and generalized linear models were used for continuous outcomes. For analyses with patient-day as the unit of analysis, within-person correlation was taken into account. Interaction tests were used to assess whether the age-group effect differed by disease treatment category or by randomized dose group. For variables that were not normally distributed, ANCOVA on the ranks was used for interaction tests. The group receiving chemotherapy without HSCT for solid tumor was omitted from interaction analyses by disease treatment category due to the very small number of patients (n=7).
For each age-group comparison, a p-value is reported for the 3-degrees-of-freedom test where the null hypothesis is that there are no differences between the four age groups and the alternative hypothesis is that at least one pair of age groups differs. When this overall comparison was significant at the 0.05 level, p-values for all six pairwise comparisons of age groups were calculated, and pairwise comparisons that were significant at the 0.05 level are reported.

No adjustment was made for analyzing multiple outcomes.

Results

The PLADO trial enrolled 1,351 patients during 2004-2007 at 26 hospitals across the United States. Overall, 1,272 patients, including 200 pediatric patients, had at least one study platelet transfusion. At 18 hospitals, more than 90% of patients were adults, including 11 hospitals that solely enrolled adults. At 6 hospitals, more than 90% of patients were children, including 3 hospitals that exclusively enrolled children. Similar numbers of patients were enrolled in each of the pediatric groups: 0-5 years (n=66); 6-12 years (n=69); and 13-18 years (n=65), while the majority of patients were adults aged ≥ 19 years (n=1072). The minimum subject’s age was 9 months and the maximum age was 83 years.
Baseline Characteristics

Comparisons of baseline characteristics among the four age groups are presented in Table 1. There were no significant differences in platelet dose assignments, gender, spleen status, or baseline hemoglobin value. Children in all 3 age groups were more likely than adults to have received prior platelet and RBC transfusions. Of particular note are the differences in disease treatment categories among the age groups: the percentage of patients undergoing autologous or syngeneic HSCT varied widely between age groups (44% ages 0-5, 17% ages 6-12, 18% ages 13-18, and 35% of adults) as did the percentage of subjects undergoing allogeneic HSCT (41%, 65%, 60%, and 38%, respectively). Chemotherapy without HSCT for hematologic malignancy was less common in the pediatric age groups than in adults (11%, 16%, 17%, and 26%, respectively). No adults and few patients in each pediatric age group received chemotherapy without HSCT for solid tumors (n=7).

Furthermore, within each HSCT disease treatment category, the primary diagnoses differed significantly by age group (Supplemental Table 2). Pediatric patients undergoing autologous or syngeneic HSCT usually had non-hematologic solid tumors (e.g., neuroblastoma, Wilm’s tumor, brain tumors), while adults (>90%) had multiple myeloma and lymphoma. Among subjects undergoing allogeneic transplants, acute myelogenous leukemia (AML) was common in all age groups, while acute lymphocytic leukemia (ALL) and aplastic anemia were more common in pediatric patients, and leukemias and myelodysplastic syndromes were more common in adults. In both pediatric and adult patients receiving chemotherapy without HSCT for hematologic malignancy, ALL and AML were the most common diagnoses.
Protocol compliance

Compliance with Randomized Prophylactic Platelet Transfusion Doses

A total of 6,030 prophylactic platelet transfusions were given. After excluding 588 transfusions that were HLA-selected or volume-reduced, and 88 transfusions with missing data, 5,384 transfusions were analyzed for compliance with the assigned target dose. Of these, 4,925 transfusions (91%) were compliant. The predominant non-compliance in all age groups was issuing more platelets than the patient was randomized to receive. For age groups 0-5, 6-12, 13-18, and 19+ years, doses were compliant for 85%, 93%, 77%, and 93% of transfusions, respectively (p=0.06, adjusting for within-person correlation). In models that also adjusted for randomized dose assignment or for disease treatment category, age group was still not a significant predictor of dose compliance.

Compliance with 10,000 platelets/µl prophylactic platelet transfusion trigger

Based on clinical events, the protocol allowed either a temporary or permanent increase in the transfusion trigger at the discretion of the patient's physician. Of 24,690 patient-days on study, the 10,000 trigger was adhered to on 22,704 days (92%). In all age groups, nearly all non-compliance was due to providing prophylactic platelets to a patient with a morning platelet count >10,000 platelets/µl. For age groups 0-5, 6-12, 13-18, and 19+ years, the trigger was adhered to on 93%, 92%, 89%, and 92% of study days, respectively (p= 0.22, adjusting for within-person correlation). In models that also adjusted for randomized dose group or for disease treatment category, age group was still not a significant predictor of trigger compliance.
Bleeding Outcomes

Age group was a significant predictor of all but one of the bleeding outcomes analyzed. In addition, interaction tests showed that for many of the outcomes related to bleeding of Grade 2 and higher, the effect of age differed by disease treatment category, and was more pronounced in the group of patients receiving autologous/syngeneic HSCT.

WHO Bleeding Grades

As shown in Figure 1A, younger children were significantly more likely than adults to have at least one day of Grade 2 or higher bleeding while on study (86%, 88%, 77%, and 67%, for ages 0-5, 6-12, 13-18, and 19+ years respectively) (p<0.001). This effect differed by disease treatment category (interaction p=0.04). In patients receiving autologous/syngeneic HSCT, bleeding of Grade 2 or higher occurred in 93%, 83%, 83% and 53% of patients in the four age groups, respectively (p<0.001). In patients who received allogeneic HSCT, bleeding of Grade 2 or higher occurred in 85%, 91%, 85%, and 77% of the patients in the four age groups, respectively (p=0.10). In the small number of patients receiving chemotherapy without HSCT for hematologic malignancy, age group was not a significant predictor of Grade 2 or higher bleeding (71%, 80%, 55%, and 73% respectively, p=0.53).

The percentage of subjects who experienced Grade 3 or higher bleeding also differed by age group (p=0.02, Figure 1B). Only 28 patients experienced Grade 4 bleeding, and this did not differ by age group (p=0.12, Figure 1C).
The median number of days with Grade 2 or higher bleeding was 3 in each pediatric age group versus 1 in adults (Table 2, p<0.001). Among patients who received HSCT, children had significantly shorter times from transplant to Grade 2 or higher bleeding than adults (median days 3.0, 5.5, 6.0, and 11.0 for the four age groups, respectively, p<0.001, Figure 2A). This was true both for patients receiving autologous/syngeneic HSCT (Figure 2B) and patients receiving allogeneic HSCT (Figure 2C). Further analyses showed that the effect of age on the bleeding outcomes was not impacted by the assigned platelet dose. In models that included age group and platelet dose assignment, age group remained a significant predictor of the bleeding outcomes, but dose was not a significant predictor of any of the bleeding outcomes.

**Relationship between morning platelet count and the occurrence of Grade 2 or higher bleeding**

On average, morning platelet counts in pediatric subjects (0-18 years) were approximately 12,000 platelets per µL higher than in adults (p<0.001). Figure 3A depicts the relationship between the morning platelet count and whether or not Grade 2 or higher bleeding occurred during that day, treating each patient-day as the unit of analysis. Platelet counts were divided into categories each spanning 5,000 platelets per µL. Some combinations of age group and morning platelet count categories were rare; therefore, the three pediatric age groups were combined for these analyses. In both pediatric and adult patients, the percentage of days with bleeding for both groups was fairly constant for morning platelet counts over the wide range of 6,000 to 80,000 platelets/µL. However, over this wide range, children were significantly more likely to experience bleeding compared to adults (p <0.001 for age group, adjusting for morning count category and within-person correlation).
In further analyses of morning platelet count as a predictor of Grade 2 or higher bleeding, the age group effect differed by disease treatment category (interaction p<0.001). Among both types of HSCT patients, children had higher bleeding risk than adults, although this reached statistical significance only for patients who received autologous/syngeneic HSCT (p<0.001) (Figure 3B), and approached significance for patients who received allogeneic HSCT (p=0.053) (Figure 3C). The logistic model did not converge for patients who received chemotherapy without HSCT for hematologic malignancies because some combinations of age group and morning platelet count had no days with bleeding, but it appears that in this disease treatment category, children have similar or somewhat lower risk of bleeding than adults over a wide range of platelet counts (Figure 3D).

**Organ system bleeding**

The WHO bleeding score is based on bleeding manifestations in eight organ systems and on hemodynamic instability (Supplemental Table 1). Figure 4 depicts the percentage of patients in each age group that experienced Grade 2 or higher bleeding in each organ system or bleeding that resulted in hemodynamic instability. Supplemental Table 3 provides further detail on specific bleeding manifestations in each age group.

Oral/nasal bleeding was more common in children than adults (35%, 32%, 32%, and 14% in the four age groups, respectively, p<0.001). Gastrointestinal bleeding, independent of swallowed nasal blood, was also more common in children than adults (71%, 61%, 57%, and
31%, respectively, p<0.001). Skin/soft tissue/musculoskeletal bleeding was less common in children than adults (14%, 16%, 12%, and 30% of patients, respectively, p<0.001).
Pulmonary bleeding was most common in patients aged 13-18 years (18%, 17%, 35%, 18%, p=0.01), as was visible blood in a body cavity (2%, 0%, 8%, 2%, p=0.05), central nervous system bleeding (6%, 6%, 9%, 3%, p=0.009), and moderate to severe hemodynamic instability (48%, 43%, 55%, 33%, p<0.001).

Resource Utilization Outcomes

Data on platelet and RBC utilization outcomes by age group are shown in Figures 5A-5D. For many of the resource utilization outcomes, interaction tests showed that the effect of age group differed by disease treatment category.

In the 1249 patients with complete data on number of platelet transfusion episodes, age was a significant predictor of this outcome (Figure 5A, p<0.001). Of particular note, children ages 0-5 received significantly fewer platelet transfusions as compared to subjects ages 13-18, while children ages 6-12 and ages 13-18 had significantly more platelet transfusions than those ≥19 years. After adjusting for the assigned platelet dose arm, the same age comparisons remained statistically significant. The assigned dose was also a significant predictor for the number of platelet transfusions given for all ages. For all ages, there was a trend towards more platelet transfusions in the lower dose arm. Further analyses demonstrated that the effect of age on the number of platelet transfusions did not differ by assigned dose arm (interaction p-value=0.80).
The 1000 patients with no missing data on product platelet counts were evaluated for the total number of platelets transfused per m² BSA. In an unadjusted analysis, age was a significant predictor of the total number of platelets transfused per m² BSA (Figure 5B, p<0.001). Children ages 0-5 and 6-12 had significantly more platelets transfused per BSA than patients aged ≥ 19 years. When adjusted for the assigned platelet dose, the same age comparisons remained statistically significant (p=0.004 and p=0.001, respectively).

More than 90% of patients required at least one RBC transfusion while on study, and this percentage did not significantly differ by age group (Figure 5C, p=0.11). However, there was a small, but statistically significant, difference in the number of RBC transfusions per patient, which was lower in the two youngest age groups (Figure 5D, p=0.045).

**Discussion**

The current secondary analysis of the PLADO study was conducted to determine whether either bleeding outcomes or the relationship between platelet dose per transfusion and bleeding might vary with age, despite the primary finding of the original report that, when all age groups were combined, there was no such platelet dose effect overall or within any disease treatment category. For this secondary analysis, patients were divided into three pediatric age groups based on the likelihood of biologic and physiologic similarities of subjects within each group (0-5 yrs; 6-12 yrs; 13-18 yrs), as well as an adult group (19+ yrs) to determine whether there were age related differences in study outcomes. The four age groups differed significantly in many baseline characteristics, including disease treatment categories and primary diagnosis (Table 1).
Importantly, large and statistically significant differences were found between age groups in the primary endpoint of the PLADO study, i.e., the percentage of patients having one or more days of Grade 2 or higher bleeding. This outcome occurred in 86% of patients ages 0-5 years, 88% ages 6-12, 77% ages 13-18, and 67% of adults. Similar to the overall results of the PLADO trial,6 there was no effect of prophylactic platelet transfusion dose in any age group. The PLADO data do not indicate that pediatric patients would benefit from a higher prophylactic transfusion trigger than the 10,000 platelets/µl trigger used in this study, because children, particularly those undergoing HSCT, were at higher risk of bleeding than adults over a wide range of morning platelet counts (Figures 3A, B, and C), suggesting that factors other than platelet count are responsible for the higher incidence of bleeding in children.

Patient age was also found to be a significant predictor of whether or not ≥ Grade 3 bleeding occurred (p=0.02), and this outcome was most common in children ages 6-12 and 13-18 yrs. Nearly all patient-days with bleeding Grade 3 or higher were assigned that grade because the patient received a RBC transfusion for which the stated indication was to treat active bleeding; other types of Grade 3 and 4 bleeding were very rare. It is possible that there were differences among age groups in RBC transfusion guidelines or in how it was determined whether RBC transfusions were given for active bleeding versus anemia for patients with similar clinical situations. However, the PLADO study did not collect data to address these possible explanations. In contrast to Grade 3 or higher bleeding, patient age was not found to be predictive of Grade 4 bleeding. This is not surprising, given the overall low percentage of patients experiencing this level of bleeding. Additionally, age group was a significant predictor
of the number of platelet transfusion episodes and the total number of platelets transfused per m². Platelet utilization was also generally higher in children than adults.

Further analyses found that the increased risk of bleeding in pediatric patients was most pronounced in the patients who received autologous/syngeneic HSCT. Among adult patients, the autologous/syngeneic HSCT group was at markedly lower risk of bleeding than the allogeneic or chemotherapy groups (53%, 77%, and 73% respectively). However, in the pediatric age groups, the autologous/syngeneic HSCT patients had similar or higher bleeding risk compared to patients in the other disease treatment categories (Figure 1A).

This secondary analysis provided insight into the potential role of factors that might contribute to the findings of increased bleeding risk in children versus adults. The number of platelets transfused during each transfusion episode and the concentration of circulating platelets are not likely to be responsible for increased bleeding in pediatric versus adult patients, since the increased risk of bleeding in pediatric patients was similar in all three randomized dose groups, and was present across a wide range of morning platelet counts. Furthermore, the types of bleeding that occurred differed between children and adults. Compared to adults, a higher percentage of children experienced oral, nasal, and gastrointestinal bleeding, and a lower percentage experienced skin, soft tissue, and musculoskeletal bleeding. This may reflect their degree of mucositis due to intense chemotherapeutic treatment regimens for their underlying disease.
One hypothesis to explain the increased bleeding risk in pediatric patients within specific organs is that there may be functional differences between age groups in the interaction between platelets and vascular endothelium in certain tissues. Endothelial structure and function likely vary with age and also may be affected pathophysiologically by age group differences in the expression of the underlying diseases present, in the differing intensity of chemotherapeutic treatments and HSCT conditioning regimens, and in the interplay between these factors, all present within the context of hypoproliferative thrombocytopenia. These hypothetical age-dependent effects on endothelial function and structure likely vary among different organ systems.

First, endothelial cell injury and multiple factors involved in endothelial regeneration in allogeneic HSCT pediatric patients have recently been found by McPherson et al\textsuperscript{11} to be associated with a poor response to platelet transfusions. In preliminary studies, these investigators found that angiopoietin-2 (ang-2) and vascular endothelial growth factor (VEGF) increased, whereas platelet-derived growth factor-BB (PDGF-BB) and soluble platelet endothelial cell adhesion molecule (sPECAM) decreased following conditioning regimens utilizing high-dose chemotherapy, often including total body irradiation. These investigators observed both an increased number of bleeding events and accelerated platelet consumption during a period of “vascular regeneration.”

Second, normal (not elevated) levels of VEGF are thought to be required for maintenance of endothelial cell stability and function\textsuperscript{12} as well as for regulation of vascular filterability (absence of leakiness to plasma water)\textsuperscript{13}. Indeed, platelets are rich sources of VEGF, sphingosine-1
phosphate, and angiopoietins$^{14-17}$, which are released at low levels in the microvasculature to mediate endothelial cell survival and permeability. Consistent with this, Kitchens$^{18}$ demonstrated by electron microscopy that, compared to normal rabbits, tongue capillaries in rabbits made severely thrombocytopenic by busulfan had thinner endothelial cell walls with focal gaps or fenestrations.

Another hypothesis is that the greater risk of bleeding in children may be due to one or more important functional or structural differences among the endothelium of younger children, older children and adults - variations that may render younger children more susceptible than adults to bleeding in the setting of chemotherapy- and radiation therapy-induced thrombocytopenia. There may be an age difference in endothelial structure, as suggested by measurements of the pulmonary capillary filtration and reflection coefficients in newborn versus adult rabbits$^{19}$. These measurements demonstrate that the permeability to water (filtration coefficient) of newborn rabbits is twice that of adults, and that the number of vascular endothelial pores increases, and the pore radius decreases, continuously even beyond 4 weeks of age$^{20}$. These animal studies support the possibility of age-dependent differences in structure and function of the human endothelium.

Compounding this vulnerability, some chemotherapeutic regimens used to manage pediatric patients, especially for neuroblastoma and brain tumors during autologous HSCT, may be significantly more toxic to naïve endothelium than the regimens administered in adult autologous HSCT$^{21}$. In addition, children are frequently prescribed relatively higher doses per m$^2$ BSA of chemotherapeutic agents compared to adults. These high intensity
Chemotherapeutic regimens have increased cure rates in some pediatric malignancies to as high as 95%, and above 30% in more recalcitrant malignancies such as Stage IV Neuroblastoma. However, relatively high doses of chemotherapy may contribute to increased bleeding in children by disrupting and more severely damaging endothelial cells that line vessel walls of various organs in comparison to the relatively lower doses recommended for adults.

These differences in treatment regimen and intensity of treatment support the speculation that the increased risk of bleeding in pediatric patients undergoing HSCT or chemotherapy may be related to difficulty in maintaining a sufficient level of integrity of the vascular endothelium, as an organ system, to provide optimal hemostasis.

While the PLADO trial included more children undergoing prophylactic platelet transfusions than prior studies, these post hoc analyses of the PLADO trial are, nonetheless, limited by the relatively small sample size in each of the pediatric age groups, particularly within individual disease treatment categories. Although the p-values indicate that the higher bleeding risk in children is very unlikely to be due to chance, especially when the p-values are < 0.01, chance is still a possible explanation. Another important limitation is that data on specific treatment regimens and doses of chemotherapeutic agents and radiation were not collected in the PLADO trial. Therefore, no analyses could be carried out to assess whether specific treatment regimens may have contributed to bleeding risk, making it difficult to extrapolate our findings to predict bleeding risks for individual patients. While Graft vs. Host Disease (GVHD) may affect bleeding, this study did not collect data on GVHD diagnosed prior to study entry and only three
subjects developed GVHD while on study. Therefore, no analyses could be carried out to assess whether the degree of GVHD explained differences in bleeding between age groups. Information regarding allergic rhinitis history was not collected and therefore could not be included or excluded from contributing to epistaxis. Future studies are needed to determine which treatment regimens and other factors are most likely to increase the risk of bleeding in patients of different ages, and to better characterize the physiology of normal pediatric endothelial cells and their response to injury.
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Authorship


Conflict of Interest

The authors declare that there are no conflicts of interest relevant to the manuscript submitted to BLOOD.

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Table 2. Number of days with Grade 2 or higher bleeding by age group, overall and within each disease treatment category

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Figure 1. Relationship between age group and percent of patients experiencing bleeding of various grades, in all patients and stratified by disease treatment category. Age group comparisons with pairwise p-values < 0.05 are noted (A: 0-5 years vs. 6-12 years; B: 0-5 years vs. 13-18 years; C: 0-5 years vs. 19+ years; D: 6-12 years vs. 13-18 years; E: 6-12 years vs. 19+ years; F: 13-18 years vs. 19+ years). A) at least one day with Grade 2 or higher bleeding (overall test for age group p<0.001, interaction of age group with disease treatment category=0.04), B) at least one day with Grade 3 or higher bleeding (overall test for age group p = 0.02, interaction p = 0.13), C) at least one day with Grade 4 bleeding (p = 0.12, interaction p not estimable).

Figure 2. Relationship between age group and time from hematopoietic stem cell transplant (HSCT) to Grade 2 or higher bleeding, in all HSCT patients and by type of HSCT. A) all HSCT patients (overall test for age group p <0.001, each pediatric age group significantly different from adults, interaction of age group with type of transplant p < 0.001), B) autologous/syngeneic HSCT patients (overall test for age group p <0.001, each pediatric age group significantly different from adults), C) allogeneic HSCT patients (overall test for age group p <0.001, 6-12 and 13–18 significantly different from adults).

Figure 3. Relationship between morning platelet count category and the occurrence of Grade 2 or higher bleeding on that day, in pediatric and adult age groups A) all patients (p = <0.001 for age group and <0.001 for platelet count category), B) autologous/syngeneic hematopoietic stem cell transplant (HSCT) (p = <0.001 for age group and <0.001 for platelet count category), C) allogeneic HSCT(p = 0.053 for age group and 0.004 for platelet count category), D) chemotherapy without HSCT for hematologic malignancy (logistic model did not converge due to some categories having no days with bleeding).

Figure 4. Percentage of patients in each age group that experienced Grade 2 or higher bleeding in each organ system. Age group comparisons with pairwise p-values < 0.05 are noted (A: 0-5 years vs. 6-12 years; B: 0-5 years vs. 13-18 years; C: 0-5 years vs. 19+ years; D: 6-12 years vs. 13-18 years; E: 6-12 years vs. 19+ years; F: 13-18 years vs. 19+ years).
Figure 5. **Relationship between age group and transfusion resource requirements, in all patients and within disease treatment category.** Age group comparisons with pairwise p-values < 0.05 are noted (A: 0-5 years vs. 6-12 years; B: 0-5 years vs. 13-18 years; C: 0-5 years vs. 19+ years; D: 6-12 years vs. 13-18 years; E: 6-12 years vs. 19+ years; F: 13-18 years vs. 19+ years) A) number of platelet transfusions per patient (overall test for age group p < 0.001, interaction between age group and disease treatment category < 0.001), B) total number of platelets transfused per BSA, based on at-issue count (overall test for age group p < 0.001, interaction between age group and disease treatment category < 0.001), C) percentage of patients who received at least one red cell transfusion (overall test for age group p=0.11, interaction p not estimable), D) total number of RBC transfusion events (overall test for age group p=0.045, interaction between age group and disease treatment category < 0.001).
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<tr>
<th>Characteristic</th>
<th>0 - 5 years</th>
<th>6 - 12 years</th>
<th>13 - 18 years</th>
<th>19+ years</th>
<th>Total</th>
<th>P for overall comparison of the four age groups</th>
<th>Significant pairwise comparisons*</th>
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<tbody>
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<td>No. of patients</td>
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<td>65</td>
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<td>1272</td>
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<td>431 (40)</td>
<td>504 (40)</td>
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<td>Age, years</td>
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<td>52.3 (41.6, 60.4)</td>
<td>48.8 (31.9, 59.0)</td>
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<td>Weight, kg</td>
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<td>Height, cm</td>
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<td>12 (17)</td>
<td>12 (18)</td>
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<td>429 (34)</td>
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<td>523 (41)</td>
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<td>C, E, F</td>
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<td>1 (2)</td>
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<tr>
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<td>36 (25.0, 54.0)</td>
<td>38 (25.0, 61.0)</td>
<td>&lt;0.001</td>
<td>B, C, E, F</td>
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<td>10 (8.9, 10.7)</td>
<td>9.8 (8.9, 11.5)</td>
<td>9.8 (9.1, 10.7)</td>
<td>9.8 (9.0, 10.7)</td>
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<tr>
<td>Hemoglobin, g/dL</td>
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<td>28 (26.0, 30.8)</td>
<td>27.6 (25.9, 32.6)</td>
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<td>28 (26.0, 31.0)</td>
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<td></td>
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<td>0</td>
<td>0</td>
<td>3</td>
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<tr>
<td></td>
<td>32.2 (29.0, 34.8)</td>
<td>31.3 (28.9, 35.0)</td>
<td>32.7 (29.9, 36.7)</td>
<td>28.7 (26.0, 32.0)</td>
<td>29 (26.2, 32.7)</td>
<td>&lt;0.001</td>
<td>C, E, F</td>
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<td>0</td>
<td>11</td>
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<td></td>
<td>306 (29.0, 357.0)</td>
<td>328 (266.0, 422.0)</td>
<td>344 (276.0, 410.0)</td>
<td>369 (283.0, 480.0)</td>
<td>360 (278.5, 466.5)</td>
<td>&lt;0.001</td>
<td>B, C, E, F</td>
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### Lymphocytotoxic antibody screen

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<th>0</th>
<th>15</th>
<th>16</th>
<th>0.04</th>
<th>A</th>
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<td>50 (79)</td>
<td>55 (83)</td>
<td>53 (85)</td>
<td>736 (74)</td>
<td>894 (75)</td>
<td>0.04 A</td>
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<tr>
<td>1% - 19%</td>
<td>4 (6)</td>
<td>9 (14)</td>
<td>6 (10)</td>
<td>156 (16)</td>
<td>175 (15)</td>
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<tr>
<td>Greater than or equal to 20%</td>
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<td>3</td>
<td>73</td>
<td>82</td>
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Values for categorical variables are presented as n (%); values for continuous variables are presented as median (quartile 1, quartile 3).

BSA indicates body surface area; RBC, red blood cells; PT, prothrombin time; INR, international normalized ratio; and PTT, partial thromboplastin time

* A: 0-5 years vs. 6-12 years; B: 0-5 years vs. 13-18 years; C: 0-5 years vs. 19+ years; D: 6-12 years vs. 13-18 years; E: 6-12 years vs. 19+ years; F: 13-18 years vs. 19+ years

† Excluded from analyses of interaction between age group and disease treatment category due to very small sample sizes

‡ Patients already known to have panel reactive antibody greater than or equal to 20% were excluded from PLADO. However, some patients were found to have values greater than or equal to 20% on the study's baseline test.
Table 2. Number of days with grade 2 or higher bleeding by age group, overall and within disease treatment category

<table>
<thead>
<tr>
<th>Outcome</th>
<th>P for interaction between age group and disease treatment category</th>
<th>0 - 5 years</th>
<th>6 - 12 years</th>
<th>13 - 18 years</th>
<th>19+ years</th>
<th>Total</th>
<th>P for overall comparison of the four age groups</th>
<th>Significant pairwise comparisons*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>&lt;0.001</td>
<td>66</td>
<td>69</td>
<td>65</td>
<td>1072</td>
<td>1272</td>
<td>&lt;0.001</td>
<td>C, E, F</td>
</tr>
<tr>
<td>Number of days with bleeding of grade 2 or higher, all patients, median (quartile 1, quartile 3)</td>
<td>3 (1, 6.5)</td>
<td>3 (1, 6)</td>
<td>3 (0, 9.5)</td>
<td>1 (0, 4)</td>
<td>1 (0, 4)</td>
<td>147</td>
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<td>C, E, F</td>
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<td>13</td>
<td>13</td>
<td>115</td>
<td>147</td>
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<td></td>
<td></td>
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<tr>
<td>Number of days with bleeding of grade 2 or higher, within disease treatment category</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Autologous or syngeneic stem cell transplant, median (quartile 1, quartile 3)</td>
<td>4 (2, 7)</td>
<td>2 (1, 8)</td>
<td>2 (1, 8)</td>
<td>0 (0, 1)</td>
<td>1 (0, 2)</td>
<td>&lt;0.001</td>
<td>C, E, F</td>
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<tr>
<td>Unknown</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>31</td>
<td>37</td>
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<tr>
<td>Allogeneic stem cell transplant, median (quartile 1, quartile 3)</td>
<td>3 (1, 6.5)</td>
<td>3.5 (1.5, 6)</td>
<td>5 (2, 17)</td>
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<td>1 (0, 1)</td>
<td>0 (0, 3)</td>
<td>2 (0, 4)</td>
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<tr>
<td>Chemotherapy for solid tumor, median (quartile 1, quartile 3)</td>
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<td>0 (0, 0)</td>
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<td>0 (0, 2)</td>
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<td>1</td>
<td></td>
<td>2</td>
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</table>

Values are n (%) unless otherwise indicated. The percentages were calculated from the patients with available data on each parameter.

* A: 0-5 years vs. 6-12 years; B: 0-5 years vs. 13-18 years; C: 0-5 years vs. 19+ years; D: 6-12 years vs. 13-18 years; E: 6-12 years vs. 19+ years; F: 13-18 years vs. 19+ years
Figure 1A

Percent of Subjects With Grade 2 or Higher Bleeding

- 0-5 yrs
- 6-12 yrs
- 13-18 yrs
- 19+ yrs

Significant Pairwise Comparisons:
- All Patients: C, E
- Autologous/Syngeneic SCT: C, E, F
- Allogeneic SCT: C, E
- Chemotherapy Hematologic Malignancy: C, E
- Chemotherapy Solid Tumor: C, E, F

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Figure 1B

Percent of Subjects With Grade 3 or Higher Bleeding

- All Patients
- Autologous/Syngeneic SCT
- Allogeneic SCT
- Chemotherapy Hematologic Malignancy
- Chemotherapy Solid Tumor

Significant Pairwise Comparisons:
- B, F
- A, E
- F
Figure 1C

Percent of Subjects With Grade 4 Bleeding

- All Patients
- Autologous/Syngeneic SCT
- Allogeneic SCT
- Chemotherapy Hematologic Malignancy
- Chemotherapy Solid Tumor

Significant Pairwise Comparisons

None at 0.05 level
Figure 2A

Cumulative Incidence of Grade 2 or Higher Bleeding

Days After HSCT

No. Patients At Risk

0 - 5 yrs  45  37  22  18  10  9  8  6  6  5  3  2  2  1  0
6 - 12 yrs  48  41  35  24  15  9  5  4  4  3  0
13 - 18 yrs  46  41  34  26  17  11  10  10  8  4  3  1  1  1  0
19+ yrs  707  677  649  581  486  405  321  218  149  94  53  28  18  13  5  4  2  1  0
Figure 2B

Days After Autologous/Syngeneic HSCT

Cumulative Incidence of Grade 2 or Higher Bleeding

No. Patients At Risk

- 0 - 5 years
  - 0 - 5 yrs: 23
  - 6 - 12 yrs: 11
  - 13 - 18 yrs: 11
  - 19+ yrs: 350

- 6 - 12 years
  - 0 - 5 yrs: 16
  - 6 - 12 yrs: 10
  - 13 - 18 yrs: 9
  - 19+ yrs: 343

- 13 - 18 years
  - 0 - 5 yrs: 7
  - 6 - 12 yrs: 8
  - 13 - 18 yrs: 7
  - 19+ yrs: 339

- 19+ years
  - 0 - 5 yrs: 4
  - 6 - 12 yrs: 5
  - 13 - 18 yrs: 6
  - 19+ yrs: 306

- 0 - 5 years
  - 0 - 5 yrs: 2
  - 6 - 12 yrs: 2
  - 13 - 18 yrs: 3
  - 19+ yrs: 265

- 6 - 12 years
  - 0 - 5 yrs: 2
  - 6 - 12 yrs: 4
  - 13 - 18 yrs: 2
  - 19+ yrs: 226

- 13 - 18 years
  - 0 - 5 yrs: 1
  - 6 - 12 yrs: 4
  - 13 - 18 yrs: 2
  - 19+ yrs: 179

- 19+ years
  - 0 - 5 yrs: 1
  - 6 - 12 yrs: 2
  - 13 - 18 yrs: 2
  - 19+ yrs: 93

- 0 - 5 years
  - 0 - 5 yrs: 0
  - 6 - 12 yrs: 2
  - 13 - 18 yrs: 2
  - 19+ yrs: 45

- 6 - 12 years
  - 0 - 5 yrs: 1
  - 6 - 12 yrs: 1
  - 13 - 18 yrs: 1
  - 19+ yrs: 22

- 13 - 18 years
  - 0 - 5 yrs: 1
  - 6 - 12 yrs: 1
  - 13 - 18 yrs: 1
  - 19+ yrs: 9

- 19+ years
  - 0 - 5 yrs: 1
  - 6 - 12 yrs: 0
  - 13 - 18 yrs: 0
  - 19+ yrs: 4
Figure 2C

Cumulative Incidence of Grade 2 or Higher Bleeding

Days After Allogeneic HSCT

0 - 5 years
6 - 12 years
13 - 18 years
19+ years

No. Patients At Risk
0 - 5 yrs  22  21  15  14  8  7  6  5  5  4  3  2  2  1  0
6 - 12 yrs 37  31  27  19  11  5  2  2  2  2  0
13 - 18 yrs 35  32  27  20  14  9  8  8  8  7  4  3  1  1  1  0
19+ yrs 357 334 310 275 221 179 142 125 104 72 44 24 15 11 5 4 2 1 0
Figure 3A

Morning Platelet Count Category

- Percent of Days With Grade 2 or Higher Bleeding

No. Of Days

<table>
<thead>
<tr>
<th>Morning Platelet Count Category</th>
<th>0 - 18 yrs</th>
<th>19+ yrs</th>
</tr>
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<tbody>
<tr>
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Figure 3B

Morning Platelet Count Category

Percent of Days With Grade 2 or Higher Bleeding

0 - 18 years

19+ years

No. Of Days

0 - 18 yrs

19+ yrs

19 95 125 122 88 70 47 39 37 35 28 20 23 17 25 7 5 7 15 146

128 632 724 617 470 358 281 203 199 139 123 102 81 79 63 41 34 27 20 15 165
Figure 3D

Morning Platelet Count Category

Percent of Days With Grade 2 or Higher Bleeding

No. Of Days

0 - 18 yrs
19+ yrs

11 70 81 68 48 39 27 44 20 14 11 13 10 12 8 6 4 5 2 3 72
242 1068 1128 892 660 541 368 288 247 200 142 108 107 79 55 35 24 23 280
Figure 4

Percent of Subjects With Grade 2 or Higher Bleeding

Significant Pairwise Comparisons
- Oral and Nasal: C, E, F
- Skin, Soft Tissue, and Musculoskeletal: C, E, F
- Gastrointestinal: C, E, F
- Genitourinary: B, D, F
- Pulmonary: D, F
- Body Cavity: F
- Central Nervous System: C, F
- Invasive Sites: C, F
- Hemodynamic Instability - Moderate or Severe: C, F

Legend:
- 0-5 yrs (N=66)
- 6-12 yrs (N=69)
- 13-18 yrs (N=65)
- 19+ yrs (N=1072)
Figure 5B
Figure 5C

Percent of Subjects With At Least One Red Cell Transfusion

- All Patients
- Autologous/Syngeneic SCT
- Allogeneic SCT
- Chemotherapy Hematologic Malignancy
- Chemotherapy Solid Tumor

Significant Pairwise Comparisons

0-5 yrs 6-12 yrs 13-18 yrs 19+ yrs
Figure 5D

This figure presents a box plot showing the total number of RBC transfusion events per patient across different age groups and treatment types. The x-axis represents age groups (0-5, 6-12, 13-18, 19+) and the y-axis represents the total number of transfusion events. The box plots are color-coded for different treatment types: All Patients, Autologous/Syngeneic SCT, Allogeneic SCT, Chemotherapy Hematologic Malignancy, and Chemotherapy Solid Tumor.

Significant pairwise comparisons:
- All Patients: B, C, D
- Autologous/Syngeneic SCT: C
- Allogeneic SCT
- Chemotherapy Hematologic Malignancy: C, E, F
- Chemotherapy Solid Tumor
Bleeding risks are higher in children versus adults given prophylactic platelet transfusions for treatment-induced hypoproliferative thrombocytopenia

Cassandra D. Josephson, Suzanne Granger, Susan F. Assmann, Marta-Ínés Castillejo, Ronald G. Strauss, Sherrill J. Slichter, Marie E. Steiner, Janna M. Journeycake, Courtney D. Thornburg, James Bussel, Eric F. Grabowski, Ellis J. Neufeld, William Savage and Steven R. Sloan