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LABORATORY PREDICTORS OF BLEEDING, AND EFFECT OF PLATELET AND RBC TRANSFUSIONS ON BLEEDING OUTCOMES, IN THE PLADO TRIAL

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Key Points:

1. Laboratory parameters associated with increased bleeding were platelet counts ≤5,000/μL; hematocrits ≤25%; INR >1.2; and aPTT >30 sec.
2. Platelet and RBC transfusions on days with bleeding are often not sufficient to change bleeding outcomes on the following day.

ABSTRACT

Bleeding remains a significant problem for many thrombocytopenic hematology/oncology patients in spite of platelet transfusions. Factors that might contribute to bleeding were analyzed for 16,320 patient-days on or after their first platelet transfusion in 1077 adult patients enrolled in the Platelet Dose Trial (PLADO). All patients had greatly increased risk of bleeding at platelet counts of ≤5,000/µl (OR 3.1, 95% CI 2.0-4.8) compared to platelet counts ≥81,000/µL. Platelet counts between 6,000/µl and 80,000/µL were also associated with somewhat elevated bleeding risk in patients receiving allogeneic SCT or chemotherapy, but not in those undergoing autologous SCT. Other significant laboratory predictors of bleeding were hematocrit ≤25% (OR 1.29, CI 1.11-1.49); aPTT of 30 to ≤50 seconds (OR 1.40, CI 1.08-1.81, p=0.01), aPTT >50 sec (OR 2.34, CI 1.54-3.56); INR of 1.2 to 1.5 (OR 1.46, CI 1.17-1.83); and INR >1.5 (OR 2.05, CI 1.43-2.95). Transfusion of either platelets or red cells on days with bleeding was often not sufficient to change bleeding outcomes on the following day. Because bleeding occurred over a wide range of platelet counts among patients undergoing allogeneic stem cell transplant or chemotherapy, and platelet transfusions may not prevent bleeding, other risk factors may be involved. These may include low hematocrit and coagulation abnormalities.

INTRODUCTION

Prophylactic platelet transfusion is a mainstay of clinical care for patients with secondary hypoproliferative thrombocytopenia. However, the effectiveness of such therapy is variable, and as shown in prior studies, platelet transfusions may not always eliminate bleeding.[1-3] Except at extremely low platelet counts, the degree of thrombocytopenia is not clearly associated with bleeding.[2-6] Other factors may contribute to an increased risk of bleeding in thrombocytopenic patients, including the cause of thrombocytopenia, medications, underlying infection, and sepsis.[7-9] Bleeding risk may also be related to coagulation abnormalities[10], and low hematocrits.[11-12]

The Platelet Dose Study (PLADO) enrolled over 1200 patients with hypoproliferative thrombocytopenia due to stem cell transplant (SCT) or chemotherapy for malignancy. PLADO's dataset afforded a unique opportunity to examine the associations between platelet count, hematocrit, and coagulation factors (as assessed by INR, aPTT and fibrinogen concentration), and the occurrence and grade of bleeding. In actively bleeding patients, the effects of platelet or red cell transfusion on bleeding outcomes on the following day could also be evaluated.

METHODS

As previously described[2], the PLADO study, conducted by the NHLBI/TMH Network, was a multicenter randomized controlled trial of hospitalized patients expected to experience a period of hypoproliferative thrombocytopenia secondary to chemotherapy or SCT. Only patients ≥18 years of age were included in this secondary analysis. Randomization was stratified by cause of thrombocytopenia: autologous or syngeneic SCT (AUTO stratum), allogeneic SCT (ALLO stratum), or chemotherapy for hematologic malignancy without SCT (CHEMO stratum). Patients were randomized to one of three

prophylactic doses: 1.1 x 10^{11} (low dose, LD), 2.2 x 10^{11} (medium dose, MD), and 4.4 x 10^{11} (high dose, HD) platelets per square meter BSA for each platelet transfusion. Additional eligibility criteria included INR and aPTT ≤1.3 X the upper limit of normal for the laboratory, fibrinogen ≥100 mg/dl, no prior platelet transfusions for thrombocytopenia during the current hospitalization, and no World Health Organization (WHO) bleeding Grade 2 or greater at eligibility assessment.

Transfusions

Platelet transfusions were given prophylactically for morning platelet counts of ≤10,000/μl. The patient's physician could change the transfusion trigger or dose based on clinical indications, with return to study guidelines as soon as possible. Local practice determined indications for RBC transfusion.

Clinical Assessments

Supplemental Table 1 gives the definitions of WHO bleeding from Grades 1 to 4. Grade 1 is minor bleeding; Grade 2 bleeding is more than minor but not requiring RBC transfusion; Grade 3 bleeding includes gross body cavity bleeding with organ dysfunction, visible blood with lumbar puncture but no central nervous system symptoms (CNS), moderate hemodynamic instability, or RBC transfusion given to treat active bleeding; and Grade 4 bleeding includes retinal bleeding with visual impairment, CNS symptoms with bloody lumbar puncture, CNS bleeding on imaging study, severe hemodynamic instability, and fatal bleeding. Research staff performed daily bleeding assessments using physical examination, patient interview, and chart review, and collected data using WHO criteria, except for urine dipstick and stool guaiac tests. These data were used to generate each patient's daily bleeding grade using a computer algorithm. For the analyses reported here, Grade 2A bleeding was defined to be WHO Grade 2 bleeding that was not solely due to purpura. For a few patient-days, one or more of the daily bleeding outcomes could not be determined.

Daily platelet counts, hematocrits, and hemoglobins were obtained. Fibrinogen, aPTT, and INR were required only at baseline to determine study eligibility, but if any of these were done as part of the patient's management, the results were collected.

Study Completion

Patients completed the study at 30 days from their first platelet transfusion, ten days without a platelet transfusion, hospital discharge, death, or study withdrawal, whichever occurred first.

Statistical Considerations

For this report, analyses were limited to the 1,077 adult PLADO patients (≥18 years) who received at least one platelet transfusion. Analyses were also restricted to patient-days on or after the patient's first platelet transfusion.

Baseline characteristics were summarized and tested for differences across the three dose groups and across the three strata with Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables. The distributions were summarized using frequency (percentage) for categorical variables and mean (standard deviation) for continuous variables. Associations between bleeding grades (Grade 2A or greater (G2A+), Grade 3 or greater (G3+)) and dose group, stratum, morning platelet count, hematocrit, fibrinogen, aPTT or INR were examined using logistic regression taking into account within patient correlations. All statistical analyses were performed in SAS v9.4 and figures were plotted in R v3.1.1.

Human Subjects Protection

IRBs at each clinical site and the Data Coordinating Center approved the study, and each patient signed informed consent. A Data and Safety Monitoring Board reviewed data twice a year.

RESULTS

Study Population

Between 2004 and 2007, 26 sites enrolled 1,351 patients, of which 1,077 were $\geq$ 18 years old and received at least one platelet transfusion. These 1,077 patients included 352, 355, and 370 patients in the LD, MD, and HD groups respectively. There were 378 patients in the AUTO stratum, 413 in the ALLO stratum, and 286 in the CHEMO stratum. Baseline characteristics were generally well-balanced between dose groups[2], but the strata differed on several baseline characteristics (Table 1). The large sample size resulted in statistically significant differences among baseline characteristics but none were considered clinically relevant.

Daily Bleeding Grades

Bleeding data were collected on 16,320 patient-days on or after their first platelet transfusion, with median days of 14, 13, and 14 for the LD, MD, and HD groups respectively ($p>0.05$ for all pairwise comparisons). The median assessed days differed by stratum; 8, 16, and 19 for the AUTO, ALLO, and CHEMO strata, respectively ($p <0.001$ for all two way comparisons).

There were no differences between dose groups for any bleeding outcomes. Table 2 shows daily bleeding outcomes by patient-day for each stratum. There were significant differences between some strata for Grade 2A+ bleeding. The ALLO group had bleeding on 21% of patient-days, compared to 10% in the AUTO group and 11% in the CHEMO group (both $p<0.001$); but AUTO and CHEMO did not differ ($p=0.13$). For Grade 3+ and Grade 4 bleeding, there were no significant differences between strata.

Associations Between Morning Platelet Count and Daily Bleeding Outcomes

The median morning platelet counts for patient-days with and without Grade 2A+ bleeding were 21,000/μL and 20,000/μL, respectively with identical 25th and 75th percentiles of 12,000/μL and 37,000/μL. Figure 1A shows that the percentage of patient-days with Grade 2A+ bleeding ranged from 8 to 21 percent based on the morning platelet count category. These percentages do not take into account within-person correlation (the tendency for patient-day outcomes to be more similar within a patient than between patients). Figure 1B shows the odds ratios (OR) comparing each lower platelet count category to the reference category of ≥81,000/μL. At platelet counts ≤80,000/μL, most platelet count categories had significantly higher risk of Grade 2A+ bleeding compared to the reference category, usually with $p < 0.001$. The risk of Grade 2A+ bleeding was highest for the 1,000-5,000/μL category (OR 3.1, CI 2.0-4.8). ORs ranged from 1.3 to 2.6 for categories between 6,000 and 80,000/μL, with no clear pattern of decreasing risk with increasing platelet counts. The associations between category of morning platelet count and Grade 2A+ bleeding were similar for all three dose groups (interaction p-value=0.84).

The median morning platelet count for patient-days without Grade 3+ bleeding was 20,000/μL, with 25th and 75th percentiles of 12,000/μL and 37,000/μL. For patient-days with Grade 3+ bleeding, the median platelet count was 19,000/μL, with 25th and 75th percentiles of 11,000/μL and 38,000/μL. There was no statistically significant relationship between morning platelet count category and the occurrence of Grade 3+ bleeding (p=0.85, Figures 1C and 1D).

Association Between Morning Platelet Count and Daily Bleeding within Strata

There was a significant interaction (p=0.03) between morning platelet count categories and stratum. Figure 2A shows the relationship between platelet count and Grade 2A+ bleeding by stratum, not taking into account within-person correlations. Over a wide range of platelet counts, the ALLO stratum had a higher risk of bleeding than other strata. Figures 2B-D show ORs for each morning platelet count category vs. the reference category of ≥81,000 platelets/μL for the ALLO, AUTO, and CHEMO strata respectively. In both the ALLO and CHEMO strata, most categories had significantly higher risk than the reference category, but there was no clear pattern of decreasing bleeding with increasing platelet count in the range of 6,000-80,000/μL. For the AUTO stratum, only the 1,000-5,000/μL group had significantly higher bleeding risk than the reference category (p=0.03).

Associations Between Morning Hematocrit and Daily Bleeding Outcomes

The median morning hematocrit for patient-days without Grade 2A+ bleeding was 28%, with 25th and 75th percentiles of 26% and 30% and for patient-days with Grade 2A+ bleeding, the median hematocrit was 27%, with 25th and 75th percentiles of 25% and 29%. Figure 3A shows the relationship between morning hematocrit and whether Grade 2A+ bleeding occurred that day. Figure 3B shows the ORs for each lower hematocrit category compared to the reference category of hematocrit > 29%. Hematocrit

was a significant predictor of Grade 2A+ bleeding (overall 2 d.f. p=0.002). Hematocrits of ≤ 25% had OR 1.29, CI 1.11-1.49, compared to the reference category. Hematocrits of 26-29% had OR 1.11, CI 0.98-1.26. There were no significant interactions between hematocrit category and stratum, or between hematocrit category and dose group (interaction p-values 0.82 and 0.18 respectively).

The median morning hematocrit value for patient-days without Grade 3+ bleeding was 28%, with 25th and 75th percentiles of 26% and 30% and for patient-days with Grade 3+ bleeding, the median hematocrit was 25%, with 25th and 75th percentiles of 23% and 28%. Figure 3C shows the unadjusted relationship between morning hematocrit and whether Grade 3+ bleeding occurred. Figure 3D shows the OR for each category compared to the reference category of hematocrit >29%. Hematocrit was a significant predictor of Grade 3+ bleeding (overall 2 d.f. p<0.001). Similar to G2A+ bleeding, only days with hematocrit ≤25% were significantly different from the reference category with OR of 4.88 (CI 2.61-9.94). Hematocrits of 26%-29% had OR of 1.54 (CI 0.81-2.96). The effect of hematocrit was similar across dose groups (interaction p-value=0.13) and across strata (interaction p-value=0.18).

Coagulation assays

Fibrinogen, aPTT, and INR were more likely to be performed on days when patients were bleeding (Figure 4). Compared to days with <G2A bleeding, each assay was more likely to be ordered on a day with G2A bleeding (p<0.05), and even more likely to be ordered on a day with G3+ bleeding (p<0.003).

There were 949 patient-days with fibrinogen tests, among 264 subjects. Out of these 949 patient-days, 46 had fibrinogen ≤200 mg/dL, 243 had fibrinogen between 200 and ≤400 mg/dL, 294 had fibrinogen between 400 and ≤600 mg/dL, and 366 had fibrinogen >600 mg/dL. There were no significant

differences between the fibrinogen categories with respect to Grade 2A+ bleeding (p-value = 0.86) or Grade 3+ bleeding (p-value = 0.81) (data not shown).

There were 3,697 patient-days with aPTT tests, among 676 subjects. aPTT category was significantly related to the occurrence of Grade 2A+ bleeding (p=0.002). Figure 5A shows the percentage of days with Grade 2A+ bleeding by aPTT category and Figure 5B shows the OR for each category compared to the reference category of aPTT ≤30 seconds. Compared to the reference category, aPTT of 30 to ≤50 seconds was associated with a higher risk of bleeding (OR 1.40, CI 1.08-1.81), and aPTT>50 seconds was associated with a still higher risk of bleeding (OR 2.34, CI 1.54-3.56). aPTT category was not significantly associated with Grade 3+ bleeding (p=0.40, Figure 5C-D) although there was some indication that aPTT > 50 seconds may confer a higher risk (OR 2.55, CI 0.90-7.21). However, there were very few patient-days with Grade 3+ bleeding in this category.

There were 4,096 patient-days with INR results, among 724 subjects. INR category was significantly related to the occurrence of G2A+ bleeding (p<0.001). Figure 6A shows the percentage of days with Grade 2A+ bleeding by INR category and Figure 6B shows the OR for each category compared to the reference category of INR ≤1.2. Compared to the reference category of INR≤1.2, 1.2<INR≤1.5 was associated with higher risk of Grade 2A+ bleeding (OR 1.46, CI 1.17-1.83) and INR>1.5 was associated with a still higher risk of bleeding (OR 2.05, CI 1.43-2.95). INR category was also significantly associated with Grade 3+ bleeding (p=0.04, Figures 6C-D), but the comparison with the reference group was only significant for the highest INR category. INR between 1.2 and ≤1.5 had an OR 0.79 with CI 0.38-1.64. However, INR>1.5 had an OR of 4.08 (CI 1.90-8.78).

Multi-predictor Models

Table 3 shows two multi-predictor models for Grade 2A+ bleeding. Model 1 includes stratum, platelet count category, and hematocrit (the two laboratory tests which were expected for every patient-day). Stratum, platelet count, and hematocrit remained significant predictors of bleeding, and the odds ratios for platelet count and hematocrit categories were similar to those in Figures 1B and 3B.

Model 2 adds aPTT and INR to the model. In this model, which is limited to patient-days with all four laboratory tests available, only stratum, aPTT, and INR are statistically significant.

Table 4 shows a multi-predictor model for Grade 3+ bleeding, including stratum, platelet count, and hematocrit. Similar to the one-predictor models, stratum and platelet count were not significantly associated with Grade 3+ bleeding, but hematocrit was, with the lowest category at greatly elevated risk.

Platelet Transfusion

There were 2,181 patients-days with exactly Grade 2A bleeding that had bleeding data available on the following day. As shown in Table 5, on 1327 (61%) of these days the patient received at least one platelet transfusion. Among these 1327 patient-days 790 (60%) of the patients still had Grade 2A+ bleeding the following day. Among the 854 patient-days without a platelet transfusion, only 445 (52%) of the patients still had Grade 2A+ bleeding the following day. Taking into account within-person correlation, platelet transfusion was associated with significantly higher risk of continued G2A+ bleeding (OR 1.28, CI 1.06-1.54). Adjusting for stratum or dose group did not change the results regarding the association of platelet transfusion with the next day's bleeding grade. As shown in the remainder of Table 5, platelet transfusions were more likely to be given on days with lower platelet

counts. However, the relationship between platelet transfusion and next-day bleeding did not differ significantly between platelet count categories (interaction p=0.34), and platelet transfusion was not associated with a significant improvement in next-day bleeding for any platelet count category.

There were 147 patient-days with Grade 3+ bleeding that had bleeding data available for the following day. For the 113 patient-days (77%) with at least one platelet transfusion, 22% had Grade 3+ bleeding the following day, compared to 12% of the 34 patient-days without a platelet transfusion. This association between platelet transfusion and the following day's bleeding grade was not statistically significant (p-value=0.31).

RBC Transfusion

Of the 2,181 patient-days with exactly Grade 2A bleeding and bleeding data available for the following day, 455 patient-days (21%) had at least one RBC transfusion on the day of Grade 2A bleeding. As shown in the first row of Table 6, on 276 (61%) of these 455 days the patient still had Grade 2A+ bleeding on the following day. Among the 1,726 patient-days without a RBC transfusion on the day of Grade 2A bleeding, only 56% of patients still had Grade 2A+ bleeding the following day. However, the association between whether or not any RBC transfusion was given on the day of Grade 2A bleeding and the following day bleeding grade was not statistically significant in a model taking into account within-person correlation (OR=1.20, CI 0.98-1.47). As shown in the remainder of Table 6, RBC transfusions were more likely to be given on days with lower hematocrits, but there was no difference between hematocrit categories in the relationship between RBC transfusion and next-day bleeding (interaction p=0.60), and RBC transfusion was not associated with reduced next-day bleeding in any hematocrit category.

Among the 147 patient-days with Grade 3+ bleeding that had bleeding data available for the following day, a red cell transfusion was given on 127 days (86%) and Grade 3+ bleeding continued on 21% of the following days compared to 2 (10%) of the 20 days with no RBC transfusion. The association between RBC transfusion given on the day of Grade 3+ bleeding and the following day bleeding grade was not statistically significant (OR=2.14, CI 0.50-11.59).

DISCUSSION

Hypoproliferative thrombocytopenia is a well-recognized complication of high dose chemotherapy and hematopoietic stem cell transplantation. Use of prophylactic platelet transfusions is widely accepted as standard of care to reduce the risk of clinically significant bleeding. However, recent examinations of this practice have raised several questions including whether or not a prophylactic platelet transfusion strategy to prevent bleeding is superior to therapeutic-only platelet transfusions to treat active bleeding.[5,14] Also, there are other factors (either laboratory or clinical) which may contribute to the risk of bleeding in thrombocytopenic patients. Our secondary analyses of PLADO Trial data using models with single laboratory predictors and models including multiple laboratory predictors suggest that platelet counts, hematocrits, coagulation factors and clinical treatment categories may all predict increased risk of bleeding.

At morning platelet counts between 6,000/µL and 80,000/µL, there were fairly similar risks of Grade 2A+ bleeding within each treatment stratum. Patients in the ALLO stratum had higher risks of Grade 2A+ bleeding compared to patients in the AUTO and CHEMO strata, likely reflecting direct toxicity to the vascular endothelium and co-morbid side effects of therapeutic regimens. At very low platelet counts

of ≤5,000/μL, patients in all three strata had a significantly higher bleeding risk, compared to the reference category. These data are in direct contrast to the reports by Webert et al.[9] and Estacourt et al.[15] who observed in smaller cohorts of patients with hematologic malignancies a reduction in bleeding risk for every 10,000/uL increase in platelet count.

As has been reported by others, hematocrit was inversely associated with risk of bleeding[9,15]. On patient days with hematocrit values ≤25% there were increased risks for Grade 2A+ and Grade 3+ bleeding compared to patient days with hematocrit values >29%. These observations are entirely consistent with animal models as well as human studies demonstrating correlation between prolonged bleeding times and hematocrit values.[11-13,16] A caveat to these data is the fact that an RBC transfusion given for bleeding qualifies for Grade 3+ bleeding, thus one must be circumspect about causality.

Analysis of coagulation assays demonstrated an increased overall risk for bleeding for patients with abnormal INR and aPTT. However, these data should be interpreted with caution as the study did not require any coagulation assays after enrollment. This makes it difficult to accurately assess the association of these parameters with bleeding in the PLADO patients, or the extent to which adjusting for them impacts the apparent association of other parameters with bleeding. Others have demonstrated no association between bleeding risk and functional assessment of coagulation by either standard coagulation assays or thromboelastography after adjustment for platelet count[15]. Therefore, the observed association between increased bleeding risk and physician ordered coagulation assays may simply relate to the clinical team's assessment of a patient's current bleeding triggering the laboratory assessment.

There is increasing evidence supporting the consideration for a risk-stratified approach to platelet transfusion management of hypoproliferative thrombocytopenia.[5,6,17] Our secondary analysis demonstrated that patients in the AUTO strata had lower risk for bleeding and thus represent a patient population for which one could consider a therapeutic-only platelet transfusion approach. However, even AUTO patients had high risk of bleeding at platelet counts ≤ 5,000/μL. In addition, all patients in PLADO received prophylactic platelet transfusions for morning counts ≤10,000/μL, so we cannot determine what the effect of a therapeutic-only strategy would have been, overall or in any of the three strata.

The reports by Wandt et al[5] and Stanworth et al[6] show remarkably similar findings to ours with respect to increased bleeding risk in patients treated with chemotherapy or allogeneic transplant vs. patients undergoing autologous stem-cell transplantation. In both studies, the proportion of patients randomized to therapeutic platelet transfusion experienced more days with bleeding compared to those receiving prophylactic treatment, but higher rates of grade 3 or 4 bleeding were observed in patients receiving chemotherapy or undergoing allogeneic transplant. These studies show that prophylactic platelet transfusions are effective, at least in certain patient populations; but clearly, treatment related factors contribute to increased bleeding risk. Until these are better understood, the continuing use of a prophylactic platelet transfusion strategy for all patients with hypoproliferative thrombocytopenia seems prudent.

We sought to assess the impact of platelet and RBC transfusion on bleeding outcomes. Patients who received a platelet transfusion on a day with Grade 2A bleeding were significantly more likely to have bleeding of at least Grade 2A the next day, compared to patients who did not receive a platelet

transfusion on a day with Grade 2A bleeding. However, these results cannot be interpreted as proof that platelet transfusions do not reduce bleeding on the following day. Clinical teams may have elected to order platelet transfusions for subjects judged to be at higher risk of continued or worsening bleeding the next day than for subjects judged to be at lower risk. This introduces "confounding by indication". Some patients who did receive a platelet transfusion on a day with Grade 2A bleeding may have experienced better outcomes than they would have had if they had not received the platelet transfusion. PLADO collected no data on the reasons for deciding whether or not to order a platelet transfusion on a particular patient-day, nor did it collect data on other daily risk factors for bleeding except for hematocrits. Therefore, we could not perform analyses to address these potential confounders. Similar observations apply to the relationship between RBC transfusion and improvement of bleeding grade. Furthermore, as the bleeding risk was constant over a broad range of platelet counts, it is not surprising that a minor improvement in platelet count associated with a platelet transfusion may not affect the next day's bleeding grade. Similarly, minor improvement in hematocrit through RBC transfusion may not affect the next day's bleeding grade. This is further supported by the observation that the median platelet counts and hematocrits on days with and without bleeding were similar.

The authors acknowledge several limitations of this study, including the small sample size for patients experiencing Grades 3 and 4 bleeding. This precluded further analysis of the potential risk factors for higher bleeding grades, serving to remind us that further studies are required to elucidate underlying pathologies that may contribute to these rare occurrences.[18] Another limitation relates to the fact that detailed information on chemotherapeutic treatment regimens and preparative transplant protocols were not collected, thus precluding the analysis of their possible contribution to the observed bleeding events. However, bleeding outcomes differed between treatment strata, suggesting that these factors resulted in

significant effects on bleeding risks. Finally, the timing of bleeding events on a given day was not recorded, so it is not possible to tell whether a bleeding event occurred before or after a particular platelet or RBC transfusion or laboratory test.

In conclusion, the secondary analyses presented in this manuscript attempt to further elucidate risk factors for bleeding in the setting of hypoproliferative thrombocytopenia. As described, increased overall risk for bleeding correlated with treatment stratum, profoundly low platelet counts (≤5,000/μL), hematocrit of ≤25%, INR > 1.2 and aPTT > 30 sec. The findings echo those reported by others in demonstrating that a large percentage of patients with hypoproliferative thrombocytopenia experience Grade 2A bleeding[15] and a minority experience Grade 3 and/or 4 bleeding. Given the attendant risks of platelet transfusion [adverse reactions, including septic transfusion reactions], further exploration of the impact of treatment related risk factors and careful assessment of post-transfusion impact on bleeding is encouraged. Additionally, we are in agreement with others regarding the need to develop alternatives to platelet transfusion therapy to improve hemostasis during the period of hypoproliferative thrombocytopenia experienced by patients undergoing therapy for hematologic malignancies.[17]

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AUTHORSHIP

Contribution: L.U., S.A., T.G., S.S. designed research; L.U., T.G., S.S. performed research; L.U., S.A., T.G., T.H., R.H., S.S. analyzed data; L.U., S.A., T.G., T.H., S.S. wrote the paper.

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REFERENCES

1. Friedmann AM, Sengul H, Lehmann H, Schwartz C, Goodman S. Do basic laboratory tests or clinical observations predict bleeding in thrombocytopenic oncology patients? A reevaluation of prophylactic platelet transfusions. *Transfus Med Rev*. 2002;16(1):34-45.
2. Slichter SJ, Kaufman RM, Assmann SF, et al. Dose of prophylactic platelet transfusions and prevention of hemorrhage. *N Engl J Med*. 2010;362(7):600-13.
3. Josephson CD, Granger S, Assmann SF, et al. Bleeding risks are higher in children versus adults given prophylactic platelet transfusions for treatment-induced hypoproliferative thrombocytopenia. *Blood*. 2012;120(4):748-60.
4. Heddle NM, Cook RJ, Tinmouth A, et al. A randomized controlled trial comparing standard- and low-dose strategies for transfusion of platelets (SToP) to patients with thrombocytopenia. *Blood*. 2009;113(7):1564-73.
5. Wandt H, Schaefer-Eckart K, Wendelin K, et al. Therapeutic platelet transfusion versus routine prophylactic transfusion in patients with haematological malignancies: an open-label, multicentre, randomised study. *Lancet*. 2012;380(9850):1309-16.
6. Stanworth SJ, Estcourt LJ, Powter G, et al. A no-prophylaxis platelet-transfusion strategy for hematologic cancers. *N Engl J Med*. 2013;368(19):1771-80.
7. Goerge T, Ho-Tin-Noe B, Carbo C, et al. Inflammation induces hemorrhage in thrombocytopenia. *Blood*. 2008;111(10):4958-64.
8. Slichter SJ. Relationship between platelet count and bleeding risk in thrombocytopenic patients. *Transfusion Med Rev*. 2004;18(3):153-67.
9. Webert K, Cook RJ, Sigouin CS, Rebulla P, Heddle NM. The risk of bleeding in thrombocytopenic patients with acute myeloid leukemia. *Haematologica*. 2006;91(11):1530-7.
10. Franchini M, Frattini F, Crestani S, Bonfanti C. Bleeding complications in patients with hematologic malignancies. *Semin Thromb Hemost*. 2013;39(1):94-100.
11. Valeri CR, Cassidy G, Pivacek LE, et al. Anemia-induced increase in the bleeding time: implications for treatment of nonsurgical blood loss. *Transfusion*. 2001;41(8):977-83.
12. Eugster M, Reinhart WH. The influence of the haematocrit on primary haemostasis in vitro. *Thromb Haemost*. 2005;94(6):1213-8.
13. Ho CH. The hemostatic effect of packed red cell transfusion in patients with anemia. *Transfusion*. 1998;38(11-12):1011-4.
14. Estcourt LJ, Stanworth SJ, Murphy MF. Platelet transfusions for patients with haematological malignancies: who needs them? *Br J Haematol*. 2011;154(4):425-40.
15. Estcourt LJ, Stanworth SJ, Harrison P, Powter G, McClure M, Murphy MF, Mumford AD. Prospective observational cohort study of the association between thromboelastometry, coagulation and platelet parameters and bleeding in patients with haematological malignancies – the ATHENA study. *Br J Haematol*. 2014;166(4):581-91.
16. Blajchman MA, Bordin JO, Bardossy L, Heddle NM. The contribution of the haematocrit to thrombocytopenic bleeding in experimental animals. *Br J Haematol*.1994;86(2):347-50.

17. Stanworth SJ, Hudson CL, Estcourt LJ, Johnson RJ, Wood EM; TOPPS study investigators. Risk of bleeding and use of platelet transfusions in patients with hematologic malignancies: recurrent event analysis. *Haematologica*. 2015;100(6):740-7.

18. Schiffer CA. They took a mulligan and mostly got it right … the issue of prophylactic platelet transfusion for patients receiving autologous stem cell transplantation. *Transfusion*. 2014;54(10):2372-4.

TABLES

Table 1: Baseline characteristics by stratum.

	Stratum			
	AUTO (N=378)	**ALLO (N=413)**	**CHEMO (N=286)**	**p-value***
Male Gender N (%)	238 (63%)	252 (61%)	155 (54%)	0.06
Age (yrs) Mean (SD)	53 (13)	46 (12)	54 (15)	<0.001
Body Surface Area (m^2) Mean (SD)	1.95 (0.23)	1.95 (0.23)	1.91 (0.23)	0.02
Platelet Count ($x10^3/\mu L$) Mean (SD)	47 (36)	50 (38)	37 (25)	<0.001
Hematocrit (%) Mean (SD)	29 (3)	29 (4)	28 (4)	0.002
INR Mean (SD)	1.05 (0.11)	1.07 (0.11)	1.10 (0.13)	<0.001
aPTT (sec) Mean (SD)	30 (7)	29 (5)	30(11)	0.34
Fibrinogen (mg/dL) Mean (SD)	394 (155)	389 (155)	425 (214)	0.31

* The p-values were calculated using Fisher exact test for categorical variables and Kruskal-Wallis test for continuous variables.

Table 2. Daily bleeding outcomes by stratum.

	Stratum			
	AUTO (N=3442)	**ALLO (N=7143)**	**CHEMO (N=5735)**	**p-value* (2 d.f.)**
Grade 2A+				<0.001
Yes	341 (10%)	1454 (21%)	643 (11%)	
No	3070 (90%)	5610 (79%)	5024 (89%)	
Missing data	31	31	68	
Grade 3+				0.88
Yes	30 (1%)	72 (1%)	57 (1%)	
No	3406 (99%)	7065 (99%)	5665 (99%)	
Missing data	6	6	13	
Grade 4				0.34
Yes	4 (<1%)	18 (<1%)	7 (<1%)	
No	3435 (>99%)	7117 (>99%)	5717 (>99%)	
Missing data	3	3	11	

*P-values take into account within-person correlation.
The percentages expressed are based on the total days with non-missing data.

Table 3. Multi-predictor models of laboratory predictors of Grade 2A+ bleeding, taking into account within-person correlation.

Predictor	Model 1 (Grade 2A+ bleeding days / total days= 2,403 / 15,979 = 15%)			Model 2 (Grade 2A+ bleeding days / total days= 612 / 3,604 = 17%)		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Stratum			**<0.001**			**<0.001**
ALLO	1.00	Ref.		1.00	Ref.	
AUTO	0.44	(0.34,0.56)	<0.001	0.38	(0.26,0.55)	<0.001
CHEMO	0.55	(0.43,0.71)	<0.001	0.55	(0.39,0.77)	<0.001
Platelet count			**<0.001**			**0.55**
1-5 K	2.96	(1.93,4.54)	<0.001	1.79	(0.86,3.75)	0.12
6-10 K	2.02	(1.41,2.87)	<0.001	1.62	(0.98,2.68)	0.06
11-15 K	2.34	(1.65,3.33)	<0.001	1.61	(0.95,2.72)	0.08
16-20 K	2.21	(1.56,3.13)	<0.001	1.62	(0.94,2.78)	0.08
21-25 K	1.96	(1.37,2.79)	<0.001	1.52	(0.91,2.54)	0.11
26-30 K	1.89	(1.33,2.68)	<0.001	1.86	(1.12,3.09)	0.02
31-35 K	2.06	(1.45,2.94)	<0.001	1.68	(0.96,2.94)	0.07
36-40 K	1.82	(1.26,2.63)	0.001	1.50	(0.86,2.61)	0.16
41-45 K	1.91	(1.33,2.74)	<0.001	1.57	(0.89,2.75)	0.12
46-50 K	2.01	(1.33,3.04)	<0.001	1.81	(0.97,3.37)	0.06
51-55 K	2.10	(1.38,3.19)	<0.001	1.76	(0.96,3.23)	0.07
56-60 K	1.82	(1.18,2.80)	0.01	1.56	(0.78,3.12)	0.21
61-65 K	1.25	(0.74,2.10)	0.41	0.91	(0.41,2.01)	0.82
66-70 K	2.54	(1.60,4.02)	<0.001	2.28	(0.97,5.34)	0.06
71-75 K	1.64	(0.91,2.98)	0.10	0.96	(0.41,2.25)	0.93
76-80 K	1.67	(0.91,3.04)	0.10	1.46	(0.60,3.55)	0.40
81+ K	1.00	Ref.		1.00	Ref.	
Hematocrit			**0.02**			**0.35**
≤25%	1.20	(1.03,1.39)	0.02	1.08	(0.84,1.38)	0.56
26-29%	1.06	(0.94,1.19)	0.36	0.93	(0.74,1.18)	0.57
>29%	1.00	Ref.		1.00	Ref.	
aPTT						**0.04**
≤30				1.00	Ref.	
31-50				1.17	(0.90,1.52)	0.24
>50				1.90	(1.24,2.91)	0.003
INR						**0.01**
≤1.2				1.00	Ref.	
1.3-1.5				1.35	(1.06,1.71)	0.01
>1.5				1.79	(1.28,2.52)	<0.001

Table 4. Multi-predictor model of laboratory predictors of Grade 3+ bleeding, taking into account within-person correlation.

Predictor	**Model 1** (Grade 2A+ bleeding days / total days= 155 / 16,129 = 1%)		
	OR	**95% CI**	**p-value**
Stratum			**0.91**
ALLO	1.00	Ref.	
AUTO	1.09	(0.62,1.91)	0.76
CHEMO	1.11	(0.66,1.88)	0.69
Platelet count			**0.85**
1-5 K	1.10	(0.35,3.44)	0.86
6-10 K	0.67	(0.30,1.50)	0.33
11-15 K	0.70	(0.31,1.56)	0.38
16-20 K	0.77	(0.36,1.64)	0.51
21-25 K	0.87	(0.37,2.03)	0.75
26-30 K	0.94	(0.41,2.17)	0.89
31-35 K	0.39	(0.12,1.30)	0.13
36-40 K	0.36	(0.09,1.37)	0.13
41-45 K	0.84	(0.31,2.31)	0.74
46-50 K	0.58	(0.15,2.24)	0.43
51-55 K	1.01	(0.33,3.07)	0.98
56-60 K	1.27	(0.46,3.51)	0.65
61-65 K	1.09	(0.27,4.34)	0.90
66-70 K	0.98	(0.22,4.29)	0.97
71-75 K	1.85	(0.31,11.07)	0.50
76-80 K	0.70	(0.06,8.75)	0.78
81+ K	1.00	Ref.	
Hematocrit			**<0.001**
≤25%	5.09	(2.65,9.79)	<0.001
26-29%	1.55	(0.80,3.01)	0.20
>29%	1.00	Ref.	

Table 5. Association between platelet transfusion on the day of Grade 2A bleeding and Grade 2A+ bleeding the following day, within morning platelet count sub-groups, taking into account within-person correlation.

Morning Platelet Count	Days with Grade 2A Bleeding	Days with Platelet Transfusion on the Day of Grade 2A Bleeding (%)	Next Day Bleeding Grade 2A+ after Days with Platelet Transfusion (%)	Days without Platelet Transfusion on the Day of Grade 2A Bleeding (%)	Next Day Bleeding Grade 2A+ after Days without Platelet Transfusion (%)	Odds Ratio**	95% CI	P-value
All platelet counts	2,181*	1327 (61%)	790 (60%)	854 (39%)	445 (52%)	1.28	(1.06, 1.54)	0.01
1-10 K	420	402 (96%)	228 (57%)	18 (4%)	8 (44%)	2.10	(0.74, 5.91)	0.16
11-20 K	666	410 (62%)	224 (55%)	256 (38%)	123 (48%)	1.20	(0.86, 1.69)	0.28
21-30 K	379	201 (53%)	126 (63%)	178 (47%)	93 (52%)	1.45	(0.93, 2.25)	0.10
31-40 K	245	113 (46%)	77 (68%)	132 (54%)	72 (55%)	1.61	(0.90, 2.89)	0.11
41-50 K	171	92 (54%)	63 (68%)	79 (46%)	45 (57%)	0.98	(0.47, 2.06)	0.96
51-60 K	125	59 (47%)	43 (73%)	66 (53%)	38 (58%)	1.62	(0.78, 3.39)	0.20
61-70 K	63	21 (33%)	15 (71%)	42 (67%)	25 (60%)	2.10	(0.80, 5.47)	0.13
71-80 K	38	13 (34%)	5 (38%)	25 (66%)	13 (52%)	0.53	(0.12, 2.27)	0.39
81+ K	67	12 (18%)	6 (50%)	55 (82%)	26 (47%)	1.33	(0.42, 4.16)	0.63

*7 days had missing data for morning platelet count and are not included in the rows below.

** Comparing platelet transfusion to no platelet transfusion

The interaction between the nine morning platelet count categories and platelet transfusion was not significant (p-value = 0.34).

Table 6. **Association between RBC transfusion on the day of Grade 2A bleeding and Grade 2A+ bleeding the following day,within Hematocrit sub-groups, taking into account within-person correlation.**

Hematocrit category	Days with Hematocrit Data on Day of Grade 2A Bleeding	Days with RBC Transfusion on the Day of Grade 2A Bleeding (%)	Next Day Bleeding Grade 2A+ after Days with RBC Transfusion (%)	Days without RBC Transfusion on the Day of Grade 2A Bleeding (%)	Next Day Bleeding Grade 2A+ after Days without RBC Transfusion (%)	Odds Ratio**	95% CI	P-value
All hematocrit values	2,181*	455 (21%)	276 (61%)	1726 (79%)	959 (56%)	1.20	(0.98, 1.47)	0.08
≤ 25	524	296 (56%)	182 (61%)	228 (44%)	144 (63%)	1.02	(0.74,1.41)	0.91
>25 and ≤ 29	1049	123 (12%)	70 (57%)	926 (88%)	513 (55%)	1.03	(0.71,1.49)	0.88
> 29	585	30 (5%)	19 (63%)	555 (95%)	296 (53%)	1.79	(0.81,3.96)	0.15

*23 days had missing hematocrit and are not included in the rows below.

**Comparing RBC transfusion to no RBC transfusion

The interaction between the three hemtocrit categories and RBC transfusion was not significant (p-value = 0.60).

FIGURES

Figure 1. Relationship between morning platelet count and patient-days with bleeding outcomes. A) Unadjusted patient-day percentages with Grade 2A+ bleeding, with 95% confidence intervals. B) Odds ratios for Grade 2A+ bleeding compared to the reference category of ≥81,000/μL, with 95% confidence intervals, taking into account within-person correlation. The 16 degree-of-freedom test for any association between morning platelet count category and Grade 2A+ bleeding has p<0.001. C) Unadjusted patient-days percentages with Grade 3+ bleeding, with 95% confidence intervals. D) Odds ratios for Grade 3+ bleeding compared to the reference category of ≥81,000/μL, with 95% confidence intervals, taking into account within-person correlation. The 16 degree-of-freedom test for any association between morning platelet count category and Grade 3+ bleeding has p=0.85.

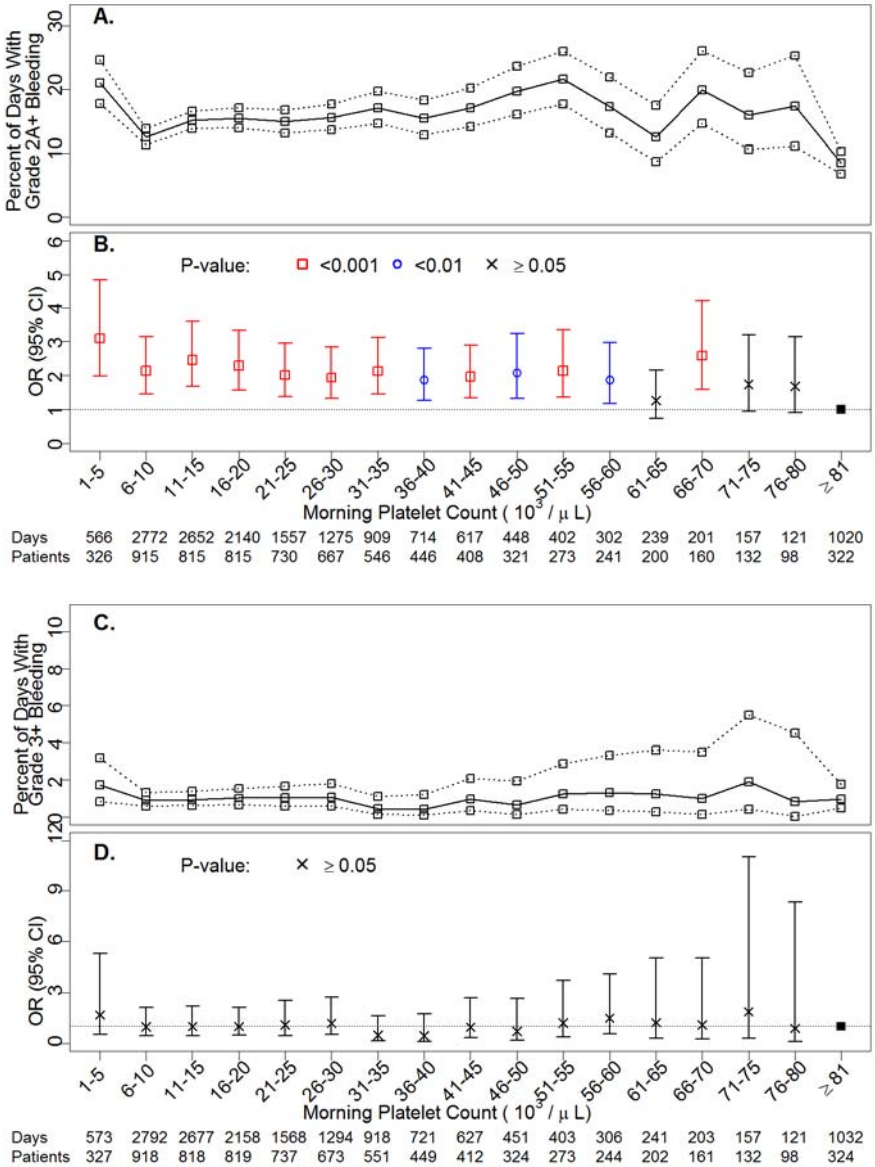


Figure 2. Association between morning platelet count and Grade 2A+ bleeding by stratum. A) Unadjusted percentage of patient-days with Grade 2A+ bleeding, by stratum. B-D) Odds ratios and 95% confidence intervals, taking into account the within patient correlation, comparing morning platelet count categories to the reference category of ≥81,000/μL, for the ALLO (B), AUTO (C), and CHEMO (D) strata respectively.

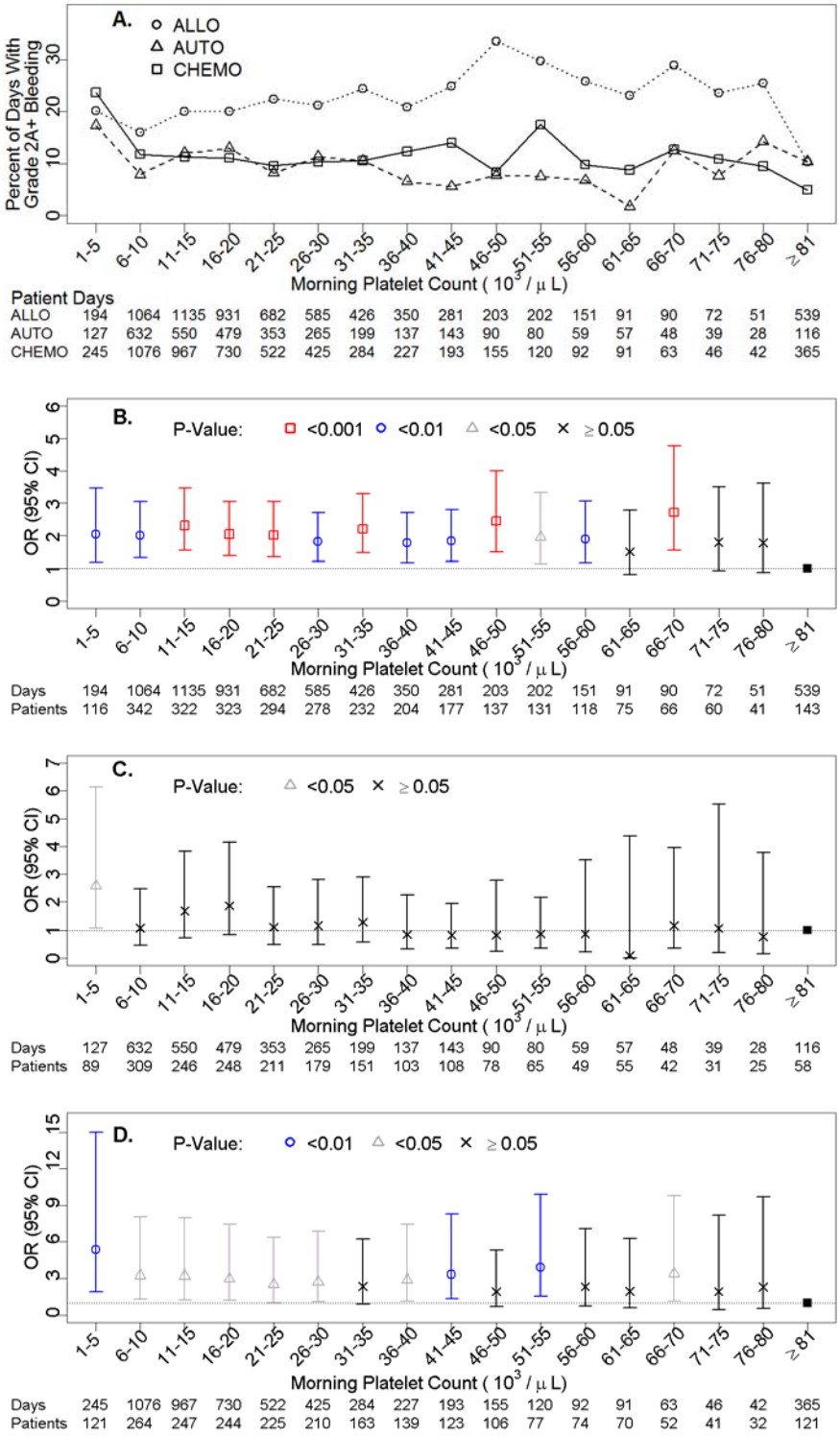


Figure 3. Relationship between morning hematocrit and percentage of patient-days with bleeding outcomes. A) Unadjusted patient-days percentages with Grade 2A+ bleeding , with 95% confidence intervals. B) Odds ratios for Grade 2A+ bleeding compared to the reference category of hematocrit > 29%, with 95% confidence intervals, taking into account within-person correlation. The 2 degree-of-freedom test for any association between morning hematocrit category and Grade 2A+ bleeding has p=0.002. C) Unadjusted patient-days percentages with Grade 3+ bleeding , with 95% confidence intervals. D) Odds ratios for Grade 3+ bleeding compared to the reference category of hematocrit > 29%, with 95% confidence intervals, taking into account within-person correlation. The 2 degree-of-freedom test for any association between morning hematocrit category and Grade 3+ bleeding has p<0.001.

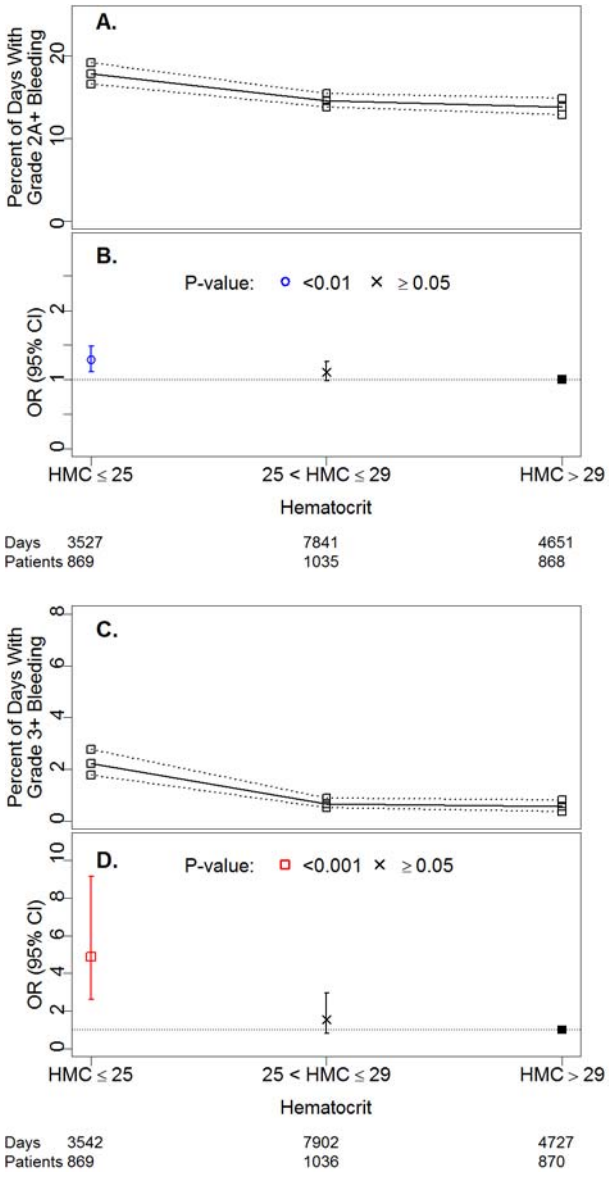


Figure 4. Relationship between bleeding grade and availability of fibrinogen, aPTT, and INR data. The y-axis indicates percent of patient days with laboratory test performed, among all patient days within specified bleeding grade.

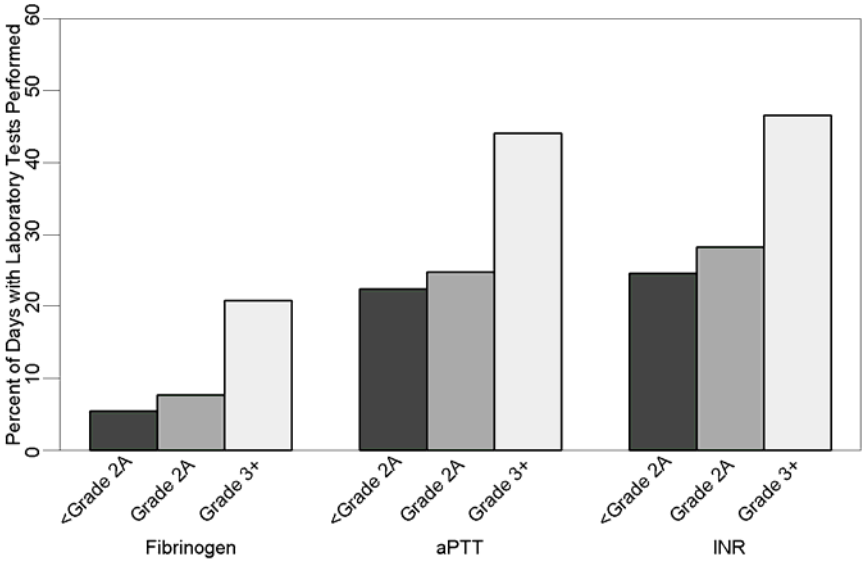


Figure 5. Relationship between aPTT category and percentage of days with bleeding outcomes. A) Unadjusted patient-days percentages with Grade 2A+ bleeding , with 95% confidence intervals. B) Odds ratios for Grade 2A+ bleeding compared to the reference category of aPTT ≤ 30, with 95% confidence intervals, taking into account within-person correlation. The 2 degree-of-freedom test for any association between aPTT category and Grade 2A+ bleeding has p=0.002. C) Unadjusted patient-days percentages with Grade 3+ bleeding , with 95% confidence intervals. D) Odds ratios for Grade 3+ bleeding compared to the reference category of aPTT ≤ 30, with 95% confidence intervals, taking into account within-person correlation. The 2 degree-of-freedom test for any association between aPTT category and Grade 3+ bleeding has p=0.40.

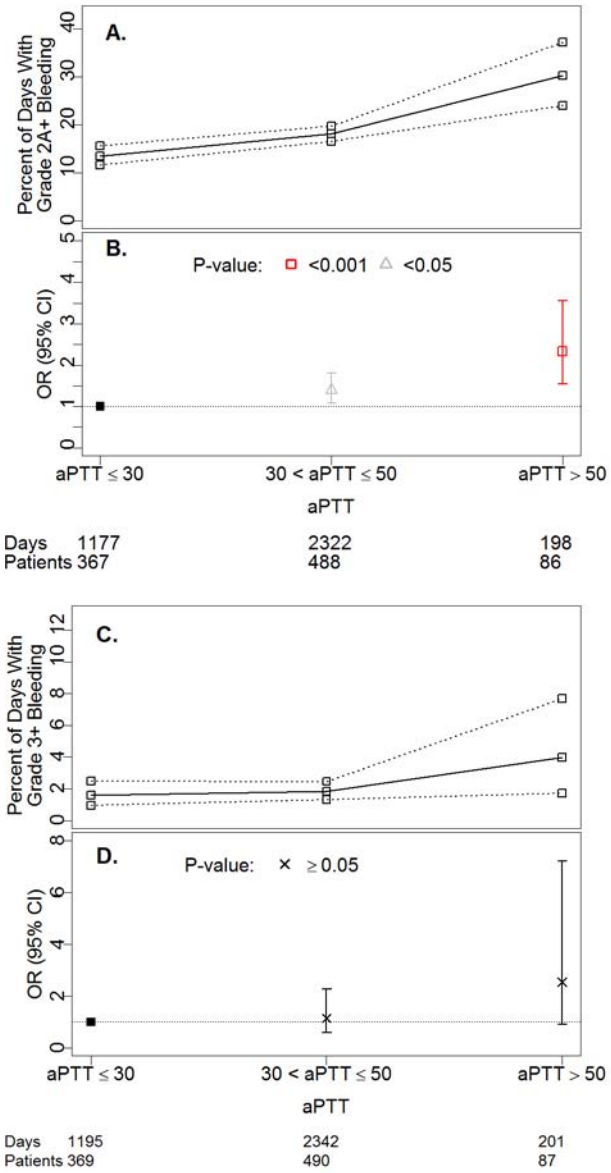
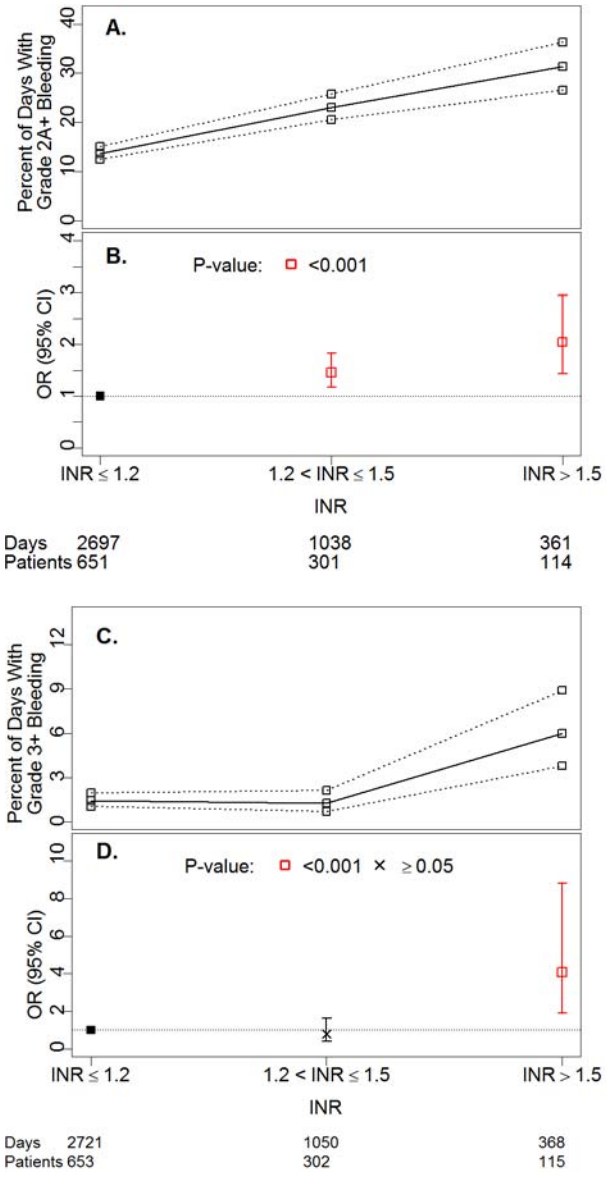


Figure 6.
Relationship between INR category and percentage of days with bleeding outcomes. A) Unadjusted patient-days percentages with Grade 2A+ bleeding , with 95% confidence intervals. B) Odds ratios for Grade 2A+ bleeding compared to the reference category of INR ≤ 1.2, with 95% confidence intervals, taking into account within-person correlation. The 2 degree-of-freedom test for any association between INR category and Grade 2A+ bleeding has p<0.001. C) Unadjusted patient-days percentages with Grade 3+ bleeding , with 95% confidence intervals. D) Odds ratios for Grade 3+ bleeding compared to the reference category of INR ≤ 1.2, with 95% confidence intervals, taking into account within-person correlation. The 2 degree-of-freedom test for any association between INR category and Grade 3+ bleeding has p=0.04.



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Laboratory predictors of bleeding, and effect of platelet and RBC transfusions on bleeding outcomes, in the PLADO Trial

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