



The risk of bleeding in thrombocytopenic patients with acute myeloid leukemia

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Background and Objectives. Patients with acute myeloid leukemia are at risk of bleeding. The risk factors for different severities of bleeding have been poorly studied.

Design and Methods. Data from Rebulla *et al.*¹ were analyzed in an exploratory analysis using multivariate Cox regression analyses for time-to-first bleed with time-dependent covariates reflecting measures of clinical and laboratory variables on the previous day. The relationships of the variables with three bleeding categories were studied: mild bleeding (WHO grades 1 and 2) clinically significant bleeding (grades 2,3 and 4) and severe bleeding (grades 3 and 4).

Results. Bleeding of any severity occurred in 149 (58.4%) of 255 patients. There were 743 days of bleeding over 7335 patient-days of observation. Risk factors for mild bleeding included increased body temperature and decreased platelet count; the risk was decreased with administration of antifungal medication or platelet transfusion on the previous day. Risk factors for clinically significant bleeding included grade 1 bleeding on the previous day, decreased platelet count and elevated body temperature. Decreased platelet count and mild bleeding on the previous day were risk factors for severe bleeding. Higher hemoglobin values were associated with a delay in the time-to-first clinically significant bleed.

Interpretation and Conclusions. These results support clinical guidelines for increasing the platelet transfusion threshold in the presence of fever and support the use of milder bleeding symptoms as an outcome in clinical trials. The suggestion that hemoglobin concentration may be predictive of bleeding risk supports the hypothesis that this may be a valuable intervention in anemic thrombocytopenic patients at high risk of bleeding.

Key words: bleeding, acute leukemia, hemostasis, risk factors.

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Patients with acute leukemia undergoing induction chemotherapy or patients undergoing stem cell transplantation (SCT) procedures have prolonged thrombocytopenia because of the cytotoxic therapies they receive and because of their underlying disorders. The relationship between bleeding and thrombocytopenia has been well described and bleeding is a frequent complication occurring after SCT or induction chemotherapy for acute myeloid leukemia (AML).^{1,2} It is generally accepted that prophylactic or therapeutic platelet transfusion decreases the risk of bleeding in patients with acute leukemia and, to a somewhat lesser extent, following SCT.³⁻⁵ However, despite the administration of prophylactic platelet transfusions, these patients remain at risk of clinically significant hemorrhage. In fact, historical data demonstrate that clinically significant bleeding occurs in approximately 20% to 32% of thrombocytopenic patients with AML (excluding patients with promyelocytic leukemia) and in 34% to 58% of patients undergoing allogeneic SCT.^{1,5-9} In addition to thrombocytopenia, numerous

factors have been suggested to increase the risk of bleeding including fever, sepsis, infection, anticoagulant therapy, medications, coagulation abnormalities, platelet function defects, hyperleukocytosis, anatomic lesions, uremia, hypoalbuminemia, recent bone marrow transplantation, recent hemorrhage, and low hematocrit.¹⁰⁻¹³ In patients with acute leukemia, few studies have been published to shed light on the independent predictive role of these factors on the risk of bleeding and to provide estimates of their impact on risk for different severities of bleeding.

In this study, we had the opportunity to further characterize the relationship between some of these risk factors and bleeding. For the purposes of generating hypotheses, we sought to identify laboratory and clinical variables (including hemoglobin concentration and infection) which are risk factors for mild, clinically significant, and severe bleeding. In addition, we examined the relationship between minor bleeding and more severe bleeding.

Methods

Data from a previously reported study by Rebulli *et al.* were analyzed to identify variables that were predictive of different severities of bleeding and to determine the degree of risk.¹ In this study, published in 1997, two prophylactic platelet transfusion triggers were compared in patients with AML (excluding patients with acute promyelocytic leukemia) receiving the first course of induction chemotherapy.¹ Patients were randomized into two groups in which the standard platelet transfusion trigger ($20 \times 10^9/L$) or the experimental lower platelet transfusion trigger ($10 \times 10^9/L$) was used. The patients' platelet counts were monitored on a daily basis and when a count dropped below the assigned threshold, prophylactic platelet transfusions were given. Bleeding was assessed daily and information about various clinical and laboratory variables was also documented daily. The original study protocol was approved by the Commission for Human Experimentation of the Health Authority of the Region of Lombardy (Italy) and by the ethics committees of the participating institutions. Permission to use this data set for the current analysis was given by the Italian investigators. Additional ethics review was not required for these analyses as all data were anonymized.

Grading of bleeding severity

In the original study by Rebulli *et al.*, bleeding severity was graded according to an eight-point Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) severity grading system as follows: 0, no bleeding; 1, petechiae or mucosal or retinal bleeding that did not require red cell transfusion; 2, melena, hematemesis, hematuria, or hemoptysis; 3, any bleeding that required red cell transfusion; 4, retinal bleeding accompanied by visual impairment; 5, non-fatal cerebral bleeding; 6, fatal cerebral bleeding; 7, fatal non-cerebral bleeding.¹ For the purposes of this analysis, bleeds were classified in terms of severity according to the more widely used World Health Organization (WHO) Severity Grading System: grade 1, petechial bleeding; grade 2, mild blood loss; grade 3, gross blood loss; and grade 4, debilitating blood loss.¹⁴ Therefore, in the WHO grading system, grade 1 is the mildest form of bleeding, characterized by petechiae, mucosal bleeding, or retinal bleeding without vision impairment. Grade 2 bleeds include melena, hematemesis, hematuria, and hemoptysis. Grade 3 bleeds include any bleeds requiring transfusion of red cells and grade 4 bleeds include retinal and cerebral bleeds associated with morbidity and fatal bleeds. Therefore, bleeds classified as grades 5, 6, or 7 in the original Italian database were grouped with WHO grade 4 bleeds. By convention, bleeds of WHO grade 2 severity or greater are considered clinically significant and grade 3 and 4 bleeds are considered to be severe.

Table 1. Variables investigated with respect to their association with bleeding.

Variable	Definition
Antibiotic administration	Administration of antibiotics on the previous day.
Antifibrinolytic medication administration	Administration of antifibrinolytic medications on the previous day.
Antifungal medication administration	Administration of antifungal medications on the previous day.
Antiviral medication administration	Administration of antiviral medications on the previous day.
Corticosteroid administration	Administration of corticosteroid medications on the previous day.
Chemotherapy administration	Administration of chemotherapy on the previous day.
Platelet transfusion administered	The administration of a platelet transfusion for any indication on the previous day.*
RBC transfusion administered indication on the previous day.	The administration of a RBC transfusion for any indication on the previous day.
Platelet transfusion administered during period of observation	The administration of a platelet transfusion on any day prior to the assessment of bleeding during the period of observation.*
Hemoglobin concentration	The hemoglobin concentration (g/L), measured in the morning on the previous day.
Platelet count	The platelet count ($\times 10^9/L$), measured in the morning on the previous day.
Positive blood culture	Positive blood culture drawn on the previous day.
Presence of clinical infection	The clinical diagnosis of infection, as recorded in the original study database.
Body temperature	Body temperature in °C measured in the morning of the previous day.
Bleeding status	Bleeding on previous day. Bleeding was assessed on a daily basis by the physician in charge. Bleeding severity was graded according to the WHO Bleeding Severity Grading System

*Platelet concentrates were prepared by one of three methods: (1) platelet-rich plasma; (2) buffy coat; or (3) apheresis. Each platelet transfusion involved one unit of platelet concentrate obtained from a 450 mL blood donation per 10 kg of body weight or one apheresis concentrate. RBC: red blood cell; WHO: World Health Organization.

Variables considered

Fifteen variables were considered with respect to their association with bleeding on the following day: the administration of antibiotics; antifibrinolytic medications; antifungal medications; antiviral medications; corticosteroids, or chemotherapy; platelet transfusion; red blood cell (RBC) transfusion; any prior platelet transfusion during the period of observation; hemoglobin concentration; platelet count, positive blood culture; presence of clinical infection; body temperature; and bleeding status (Table 1). The presence of clinical infection was determined by the patient's treating physician in the original study and was determined on a daily basis

after consideration of the patient's symptoms, signs, and relevant laboratory results. This information was transcribed from the patient's chart to a standard data collection form by study personnel during the original study. The data were then entered into the study database. The relationship of the predictors with three different types of bleeding were investigated: mild bleeding (grades 1 and 2); clinically significant bleeding (grades 2, 3 and 4); and severe bleeding (grades 3 and 4).

This was an exploratory analysis directed at hypothesis generation and this is why several categories of bleeding severity were used as outcomes. Grade 2 bleeds have traditionally been included in clinically significant outcomes as they are reasonably frequent events. In fact, clinically significant bleeding, historically, has been defined as bleeding of grade 2 or higher severity. Therefore, we felt it was relevant to explore the risk factors for this type of bleeding. However, we were also interested in the predictors of *mild* bleeding (i.e. grades 1 and 2) versus those of more severe bleeding (i.e. grades 3 and 4). Therefore, grade 2 bleeds were included in two of the categories.

Data analysis

Descriptive statistics were computed to characterize the patient population in terms of frequencies and associated percentages, and medians and ranges for continuous variables. The primary analyses were based on Cox regression models for time-to-event data, with time-varying explanatory variables chosen to capture the status of patients over time with respect to a battery of clinical and laboratory variables. Patients were considered at risk of bleeding from the day of admission to hospital to the occurrence of complete remission, resistance to chemotherapy, a platelet count of more than $100,000/m^3$, or death — whichever occurred first. During this period, bleeding status and WHO grade of bleeding were documented on a daily basis, along with the laboratory and clinical variables.

While bleeding data were available over the entire period of risk, we focused on models for the time to the first day with bleeding of each severity due to the difficulty in determining when bleeds resolved. Subjects were removed from the risk set for analyses based on a particular category of bleeding severity upon the occurrence of a more severe bleed. Predictors were either fixed, or based on values for time-varying explanatory variables up to 7 days prior to the day of bleeding assessment. Multivariate models were fit by including all main effects in a Cox regression model and conducting stepwise backwards elimination in which variables not significant at the 5% level were removed from the model one at a time until only significant variables (if any) remained.¹⁵

Some questions were based on specific variables as predictors (e.g., does WHO grade 1 bleeding predict

Table 2. Characteristics of the patients.

Characteristic	N=255
Female sex-N (%)	120 (47.1)
Age (years)	
Median (Q1, Q3)	50 (37, 59)
Minimum; Maximum	16; 74
FAB classification of AML—N (%)	
M0	12 (4.7)
M1	65 (25.5)
M2	65 (25.5)
M4	58 (22.7)
M5	38 (14.9)
M6	2 (0.8)
M7	1 (0.4)
Undefined	14 (5.5)
Platelet transfusion threshold	
$10 \times 10^9/L$ N (%)	135 (52.9)
$20 \times 10^9/L$ N (%)	120 (47.1)
Duration of observation (days)	
Median (Q1, Q3)	28 (23, 33)
Minimum; Maximum	3; 64
Number of platelet transfusion episodes per patient	
Median (Q1, Q3)	7 (4, 10)
Minimum; Maximum	1; 27
Number of red cell transfusion episodes per patient	
Median (Q1, Q3)	6 (4, 9)
Minimum; Maximum	1; 25
Patients with bleeding—N (%)	
Grade ≥ 1	149 (58.4)
Grade ≥ 2	52 (20.4)
Grade ≥ 3	28 (11.0)
Grade 4	5 (2.0)

Q1: first quartile; Q3: third quartile; FAB: French-American-British; AML: acute myeloid leukemia.

WHO grade 2 or greater bleeding) and in these cases models focused on a more narrow set of predictors. To relax the assumption of a linear effect of temperature on bleeding risk, categorical temperature variables were constructed using four temperature ranges: $<37.5^\circ\text{C}$; $37.5\text{--}37.9^\circ\text{C}$; $38.0\text{--}38.4^\circ\text{C}$; and $>38.5^\circ\text{C}$. Descriptive analyses were performed using the SAS System for Windows, Release 8.02 and the time-to-event and recurrent event analyses were performed with S-PLUS Version 6.1 for Windows (Insightful Corporation, Seattle, WA, USA).

Results

The study database included information on 255 patients aged 16 to 74 years with a diagnosis of AML admitted to hospital to undergo induction chemotherapy treatment in 21 centers in Italy. Patients were excluded from the study if they had acute promyelocytic leukemia, secondary AML, if they had received a blood transfusion before the diagnosis of AML, or if they declined to participate. One hundred and thirty-five (52.9%) patients were randomized to receive platelet transfusion at a platelet threshold of $10 \times 10^9/L$ and 120 patients (47.1%) were randomized to a platelet transfu-

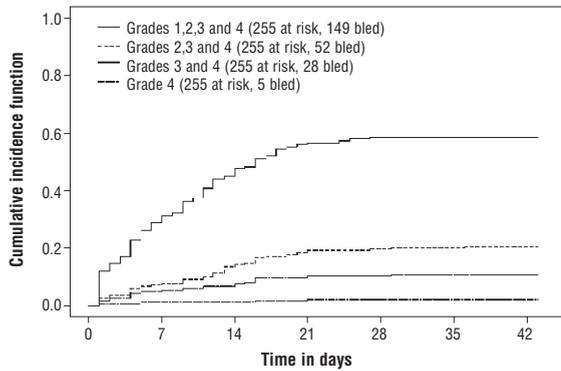


Figure 1. Cumulative incidence functions¹⁶ for the occurrence of at least one bleed of WHO grades 1-4, 2-4, 3-4 and 4.

sion threshold of $20 \times 10^9/L$. The methodology and results of this study have been previously described in detail.¹ The characteristics of the study patients are listed in Table 2. For the analysis performed in this study, patients were considered to be at risk of bleeding from the day of study entry until discharge or death.

Bleeding frequency

Bleeding (grades ≥ 1) occurred in 149 (58.4%) of the 255 patients. There were a total of 743 days of bleeding over 7335 patient-days of observation. The bleeds on these 743 days were of grade I severity on 560 (75.4%) days, of grade 2 severity on 108 (14.5%) days, of grade 3 on 51 (6.9%) days, and of grade 4 on 24 (3.2%) days. These numbers vary slightly from those published in the study by Rebutta *et al.*¹ as we were unable to resolve a discrepancy of a bleeding event in one patient. Figure 1 displays the cumulative incidence functions¹⁶ for the occurrence of at least one bleed of any grade (WHO grade ≥ 1), a clinically significant bleed (WHO grade ≥ 2), a severe bleed (WHO grade ≥ 3), and a first debilitating bleed (WHO grade 4).

Variables predicting bleeding

Mild bleeding (grades 1 and 2)

Six variables were associated with mild bleeding in the multivariate analysis: administration of antifungal medication, corticosteroid administration, a higher platelet count and platelet transfusion decreased the risk of mild bleeding, while the presence of clinical infection and an increase in body temperature increased the risk. After backwards elimination, four variables remained significant in the final model: administration of antifungal medication, body temperature; platelet transfusion administered; and platelet count (Table 3). When controlling for the other variables, if a patient had received a platelet transfusion on the previous day, the risk of

Table 3. Predictors of bleeding: results of multivariate Cox regression analyses with time-dependent predictors following backwards elimination.

Variable**	Bleeding Severity								
	Mild (Grades 1 and 2)			Clinically Significant (Grades 2,3 and 4)			Severe (Grades 3 and 4)		
	RR	95% CI	p value	RR	95% CI	p value	RR	95% CI	p value
Antifungal medication	0.59	0.39-0.90	0.014	-	-	-	-	-	-
Presence of clinical infection	1.98	1.00-3.92	0.05	-	-	-	-	-	-
Body temperature [§]	1.52	1.25-1.85	<0.005	1.87	1.40-2.49	<0.005	-	-	-
Platelet transfusion administered	0.45	0.28-0.72	<0.005	-	-	-	-	-	-
Platelet count*	0.97	0.96-0.98	<0.005	0.96	0.93-0.98	<0.005	0.96	0.93-0.99	0.005

variables refer to the day prior to the assessment of bleeding; [§]all variables are dichotomous with the exception of "platelet count" and "body temperature"; [§]RR describes the risk for every 1°C increase in body temperature; ^{}RR describes the risk for every $1 \times 10^9/L$ increase in platelet count.

mild bleeding was decreased by 55% (RR 0.45; 95% CI (0.28, 0.72); $p < 0.005$) and for every $1 \times 10^9/L$ increase in platelet count there was a 3% reduction in the risk of mild bleeding on the next day (RR 0.97; 95% CI (0.96, 0.98); $p < 0.005$). The administration of antifungal medications resulted in a decrease of the risk of mild bleeding on the next day by 41% (RR 0.59; 95% CI (0.39, 0.90); $p = 0.014$). The presence of an elevated body temperature increased the risk of mild bleeding by 52% (RR 1.52; 95% CI (1.25, 1.85); $p < 0.005$). The presence of clinical infection increased the risk of mild bleeding on the next day by 98%, however, this reached only borderline statistical significance (RR 1.98; 95% CI (1.00, 3.92); $p = 0.05$).

Clinically significant bleeding (grades 2, 3 and 4)

In the multivariate analysis, an increase in body temperature increased the risk of clinically significant bleeding and an increased platelet count decreased the risk. Antibiotic use was found to decrease the risk of clinically significant bleeding but with borderline statistical significance. When backwards elimination was performed, body temperature and platelet count remained significant in the final model (Table 3). When controlling for body temperature and platelet threshold group, we found that for every $1 \times 10^9/L$ increase in the platelet count, there was a 4% reduction in the risk of bleeding on the next day (RR 0.96; 95% CI (0.93, 0.98); $p < 0.005$). Similarly, when controlling for the platelet count, the presence of an elevated body temperature was associat-

Table 4. Results of Cox regression analyses with time-dependent categorical temperature variables.

Temperature Range (°C)*	Bleeding Severity					
	Clinically Significant (Grades 2,3 and 4)			Severe (Grades 3 and 4)		
	RR	95% CI	p value	RR	95% CI	p value
< 37.5 ^a	-	-	-	-	-	-
37.5-37.9	2.02	0.88-4.62	0.096	0.98	0.23-4.27	0.98
38.0-38.4	2.43	1.00-5.90	0.039	2.61	0.87-7.86	0.079
>38.5	3.95	1.90-8.20	0.0001	1.62	0.44-5.91	0.40

*variables refer to the day prior to the assessment of bleeding; ^areference range.

ed with an 87% increase in the risk of clinically significant bleeding on the next day (RR 1.87; 95% CI (1.40, 2.49); $p < 0.005$).

Severe bleeding (grades 3 and 4)

In the multivariate analysis, the administration of antifungal medication was found to increase the risk of severe bleeding. The presence of clinical infection also tended to increase the risk of severe bleeding, but this did not reach statistical significance. An increased platelet count tended to decrease the risk of severe bleeding, but this also did not reach statistical significance. When backwards elimination was performed, only platelet count remained significant in the final model (RR 0.96; 95% CI (0.93, 0.99); $p = 0.005$) (Table 3). When controlling for the effects of the other variables, for every unit increase in the platelet count, there was a 4% reduction in the risk of severe bleeding on the next day.

Minor bleeding predicting more severe bleeding

The majority of severe bleeds (grades 3 and 4) were preceded by bleeds of lesser severity. The presence of grade 1 bleeding on the previous day was associated with a 2.6 times increased risk of clinically significant bleeding (grades 2, 3 and 4) (RR 2.55; 95% CI (1.18, 5.49); $p = 0.017$). When looking at the risk of severe bleeding, the presence of mild bleeding (grades 1 and 2) on the previous day was associated with a 3.1 times increased risk of severe bleeding (grades 3 and 4) bleeding (RR 3.05; 95% CI (1.17, 7.95); $p = 0.023$). Furthermore, the presence of grade 1 bleeding, although not reaching statistical significance, was associated with a 2.8 times increased risk of severe bleeding (grades 3 or 4) on the following day (RR 2.83, 95% CI (0.95-8.40), $p = 0.06$). Grade 2 bleeding alone was not predictive of severe bleeding (grades 3 and 4) on the next day (RR 2.86; 95% CI (0.37, 21.93); $p = 0.31$).

Body temperature and bleeding

The risk of bleeding was assessed according to category of body temperature relative to the reference inter-

val of less than 37.5°C (i.e. normal). For each of the other temperature ranges (37.5-37.9°C; 38.0-38.4°C; and administration $\geq 38.5^\circ\text{C}$), the relative rate and 95% confidence interval was determined for the different bleeding severities (Table 4). For mild bleeding, the data were too sparse to provide meaningful estimates of relative risk. For clinically significant bleeding (grades 2, 3 and 4), the risk of bleeding increased as the body temperature increased. The risk of bleeding increased significantly when the patient's body temperature was between 38°C and 38.4°C (RR 2.43; 95% CI (1.00, 5.90); $p = 0.039$) and greater than or equal to 38.5°C (RR 3.95; 95% CI (1.90, 8.20); $p = 0.0001$) when compared to when the body temperature was less than 37.5°C. For severe bleeding (grades 3 and 4), the risk of bleeding did not increase significantly with higher body temperature, possibly because this was a less frequent outcome.

Hemoglobin concentration predicting bleeding

Based on a Cox regression model in which daily hemoglobin values (g/L) were treated as a time-dependent covariate, we found that higher hemoglobin values were associated with a delay in the time of the first clinically significant bleed (grades 2, 3 and 4). Specifically, a 10 g/L increase in hemoglobin was associated with a relative risk of clinically significant bleeding of 0.78 (RR 0.78; 95% CI (0.61, 1.00); $p = 0.048$). Hemoglobin concentration was not predictive of severe bleeding (grades 3 and 4) (RR 0.84; 95% CI (0.61, 1.17); $p = 0.31$).

Infection and bleeding

In the Cox regression models, elevated body temperature was found to increase the risk of mild and clinically significant bleeding. In a univariate model, when patients were stratified according to platelet threshold assignment, the presence of clinical infection increased the risk of clinically significant bleeding (grades 2, 3 and 4) on the next day by 3.35 times (RR 3.35, 95% CI (1.60, 7.00), $p < 0.005$). When the converse analysis was performed, it was found that there was no statistically significant effect of bleeding (mild or clinically significant) on infection status the following day (RR 1.48; 95% CI (0.76, 2.89); $p = 0.24$ and RR 0.96; 95% CI (0.35, 5.93); $p = 0.77$).

Discussion

This study demonstrated that, in the thrombocytopenic patients studied, an increase in the platelet count was associated with a decrease in the risk of all severities of bleeding (mild, clinically significant, and severe). This finding is not entirely consistent with that of previous studies. The association of bleeding with platelet count has been well documented in the literature. Gaydos *et al.* were the first to document this in

patients with acute leukemia in 1962.² They demonstrated that there was a linear relationship between bleeding and platelet count. Slichter and Harker quantified the relationship between bleeding and platelet count by measuring stool blood loss and concluded that at platelet counts less than $5 \times 10^9/L$, stool blood loss was markedly elevated; at platelet counts between 5 and $10 \times 10^9/L$ it was slightly increased, and at platelet counts greater than $10 \times 10^9/L$, stool blood loss was no different from that of normal subjects.¹⁷ We have also previously estimated the risk of bleeding based on platelet counts and found an eight times greater risk when counts were below $5 \times 10^9/L$ and a two-fold increase in risk when platelet counts ranged from 5 to $15 \times 10^9/L$ compared to counts in the range of 20 to $29 \times 10^9/L$.¹⁶ More recent studies have looked at the relationship of the platelet count and bleeding in thrombocytopenic patients receiving prophylactic platelet transfusions. A retrospective review of 2942 thrombocytopenic oncology patients using multivariate analysis demonstrated no relationship between morning platelet count or lowest platelet count of the day and bleeding.¹⁰ A second study involving 64 thrombocytopenic patients with a diagnosis of leukemia, lymphoma or bone marrow transplant did demonstrate an association between platelet count and minor bleeding; however, there was less of an association between platelet count and major bleeding.¹¹ In contrast to these studies, our results demonstrate an association between platelet count and all severities of bleeding (mild, clinically significant, and severe). The reasons for these differences are not clear but may be due to the fact that the data in our study were collected prospectively in a randomized controlled trial which included severely thrombocytopenic patients.

The administration of antifungal medication was found to be associated with a decrease in the risk of mild bleeding, but not clinically significant or severe bleeding. This may represent an effect specific to the mechanism of mild or petechial bleeding (i.e. the preservation of endothelial integrity). However, it may also simply be due to the lack of power to detect predictors of clinically significant or severe bleeding because of smaller numbers of events used for this analysis. One of the medications included in the *antifungal* category was amphotericin B. The decrease in the risk of mild bleeding with the administration of antifungal medications is somewhat surprising as amphotericin B has been demonstrated to have a detrimental effect on platelet membrane glycoprotein resulting in decreased platelet recovery and survival following transfusion.¹⁸⁻²⁴ Therefore, one might have hypothesized that we would have seen an increased risk of mild bleeding in patients being administered antifungal medications. A previous study documented that the administration of amphotericin B was a significant predictor of major bleeding in a multivariate analysis but the association of ampho-

tericin B with mild bleeding was not investigated.²⁵ This association warrants further study.

An elevated body temperature and/or the clinical diagnosis of infection on the previous day were found to be associated with an increased risk of all types of bleeding (mild, clinically significant and severe). We found that the presence of clinical infection was a statistically significant predictor of bleeding when all patients were considered, with the risk of clinically significant bleeding increased by 3.35 times on the following day. Slichter *et al.* demonstrated that the presence of fever was significantly associated with an increased risk of refractoriness to platelet infusions.²⁴ Similarly, Goldberg *et al.* showed that there was a statistically poorer incremental response to platelet transfusions in febrile versus afebrile patients with gynecologic cancer treated with chemotherapy with platelet counts less than $30 \times 10^9/L$.²⁶ However, it should be noted that, unlike in our study, elevated body temperature was not associated with increased minor or major bleeding episodes in the group of patients with platelet counts less than $30 \times 10^9/L$.²⁶ Furthermore, fever and bacteremia were not demonstrated to be associated with an increased risk of bleeding in a large retrospective study in thrombocytopenic oncology patients.¹⁰

Guidelines for the transfusion of platelet products frequently include the provision that a higher platelet transfusion threshold should be used for patients with fever or sepsis.^{27,28} However, the evidence for this recommendation, until now, has been based on expert opinion and observational studies demonstrating an association between infection and poor platelet count increment following platelet transfusion, and older studies with results that are potentially confounded by the prevalent use of acetylsalicylic acid as an antipyretic.^{24,26,29-31} To our knowledge, our study is the first to demonstrate that the presence of infection is an independent risk factor for bleeding. Furthermore, in the past, there has been some uncertainty about whether infection increases the risk of bleeding or whether bleeding increases the risk of infection (or both).³² We found that the presence of bleeding was not predictive of infection on the following day.

The majority of severe bleeds were preceded by bleeds of lesser severity. Even very minor bleeding, such as petechiae, was found to be associated with an increased risk of more severe bleeding. Patients experiencing a grade 1 bleed (petechiae) were 2.5 times more likely to have a clinically significant bleed on the following day. Furthermore, patients experiencing milder forms of bleeding (grades 1 and 2) were three times more likely to have a severe bleed the following day. These data provide support for the hypothesis that, mechanistically, mild bleeding is associated with the risk of severe bleeding. This is an important finding for several reasons. Firstly, it provides support for the use of

mild bleeding as a study outcome measure. Many clinicians feel that the only bleeding that is truly of consequence is bleeding of grade 3 or 4 in severity. Because this type of bleeding occurs relatively infrequently, a clinical trial using severe bleeding as its primary outcome would require a very large sample size. However, if grade 1 and 2 bleeds are confirmed to be predictive of more severe bleeding, they may be able to be used as clinically relevant surrogate outcomes. This could allow the required sample size of studies to be decreased because as an outcome increases in frequency, associations can be detected with a smaller sample size. Secondly, this finding provides theoretical support for therapies that decrease the amount of clinically *insignificant* or nuisance bleeding. This finding suggests that these therapies may actually play a role in decreasing the frequency of clinically significant bleeding. Furthermore, because our results suggest that bleeding of grades 1 and 2 increases the risk of grade 3 and 4 bleeding, it may be hypothesized that, mechanistically, these types of bleeding are related.

Higher hemoglobin values were associated with a decrease in the risk of a clinically significant bleed. A 10 g/L increase in hemoglobin concentration decreased the risk of clinically significant bleeding by 22% on the following day. These data support our hypothesis that a higher hemoglobin level may be associated with less bleeding in thrombocytopenic patients and are consistent with experimental evidence demonstrating that hematocrit may play a role in determining bleeding risk in thrombocytopenic subjects. Using a thrombocytopenic rabbit model, Blajchman *et al.* demonstrated that the bleeding time was inversely correlated with the hematocrit.³³ This study showed that increasing red cell mass by transfusions partially corrected the bleeding time in anemic, thrombocytopenic animals. Similar effects have been demonstrated in man in uremic and thrombocytopenic subjects and patients undergoing cardiopulmonary bypass surgery.³⁴⁻⁴⁰ This is an interesting finding and warrants additional study. To this end, we have recently completed a pilot randomized controlled study investigating the effect of hemoglobin concentration on bleeding events in patients with acute leukemia.⁴¹

The limitations of this study should be acknowledged. Firstly, this study was an exploratory data analy-

sis; therefore, it is possible that it lacked power to detect significant associations that may have actually existed. Secondly, our analyses were limited to data available in the original database; therefore, we were unable to perform analyses looking at all possible risk factors for bleeding. Thirdly, this study represents an analysis of data collected and published in the late 1990s; therefore, there is a chance that some of the findings would be different if more recently collected data were used. Finally, although identified *a priori*, many tests of significance were performed in this analysis, so it is possible that even statistically significant associations seen may have been due to chance. Replication of this sort of data acquisition and analyses will either substantiate or refute these findings and, ultimately, significant findings should be evaluated prospectively, ideally with a randomized controlled trial. A major strength of this study is that the analysis was performed using the database collected as part of a large rigorously performed clinical trial with careful daily collection of a wide spectrum of clinical and laboratory measures including a detailed daily assessment of bleeding. This rich source of data facilitated the use of time-varying covariates in analyses directed at modeling the time to the first bleed of a particular severity. By using time-varying covariates, one is best able to identify and quantify the effect of dynamic risk factors and the reported relative risks represent estimates of effects on risk of bleeds within the next 24 hours.

In conclusion, these results support current clinical guidelines for increasing the platelet transfusion threshold in the presence of fever. These results also provide support for the use of milder bleeding symptoms as an outcome measure in clinical trials. Finally, the suggestion that hemoglobin concentration may be predictive of bleeding risk supports the hypothesis that this may be a valuable intervention in anemic thrombocytopenic patients at high risk of bleeding.

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