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The Netherlands

# Bleeding in hemato-oncology patients: beyond the platelet paradigm

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**Citation**
Cornelissen, L. L. (2022, June 22). *Bleeding in hemato-oncology patients: beyond the platelet paradigm*. Retrieved from https://hdl.handle.net/1887/3421434

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## Beyond the platelet paradigm

Loes Cornelissen

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Cover / layout: Wendy Schoneveld (www.wenzid.nl)
Print: ProefschriftMaken (www.proefschriftmaken.nl)
ISBN: 978-94-6423-848-8

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Financial support for printing of this thesis was kindly provided by Sanquin.

# Bleeding in hemato-oncology patients

Beyond the platelet paradigm

**Proefschrift**

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof. dr. ir. H. Bijl
volgens besluit van het college voor promoties
te verdedigen op woensdag 22 juni 2022
klokke 11.15 uur

door

**Loes Laura Cornelissen**

Geboren te Zevenaar in 1984

| | |
|---|---|
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| **Copromotor** | Dr. C. Caram Deelder |
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# Chapter 1

## General introduction and outline of the thesis

Content partly published: NED TIJDSCHR HEMATOL 2020;17:276-82

*'…Admitted to the hematology ward last night, was Ms. X, a thirty-year-old woman, presenting with petechia, hematomas, and fever, which are caused by a newly diagnosed acute myeloid leukemia. She received antibiotics and needs to be prepared for intensive chemotherapy. This morning, a blood cell count showed a platelet count of $30x10^9$/L and a Hb of 4.5mmol/L. Last night, transfusion thresholds have not been discussed yet, they need to be decided…'*
*'… Hereby we write to inform you about Mr. Y, a 46-year-old patient, who was admitted to our hospital for an allogeneic stem cell transplantation with myeloablative conditioning, to treat his acute lymphatic leukemia. The stem cell transplantation was complicated by prolonged neutropenia and fever, for which broad spectrum antibiotics were prescribed. Currently, there is an ongoing BK-virus associated hemorrhagic cystitis for which multiple transfusions, saline bladder irrigation, and pain medication have been given. Mr. Y was released home with a urinary tract catheter and has a follow up appointment at the urologist. For hemostatic support, which has been troublesome, follow up at the outpatient clinic is planned…'*
*'…I would like to ask a question about Mr. Z. He is 72 years old and is admitted to our hematology ward to treat his relapsed follicular lymphoma. Two hours ago, his nose started oozing blood a bit, not that much, but it has been quite long now. It is bothering the patient. He already had a platelet transfusion today because he had a platelet count of $8x10^9$/L. My colleague said that two days ago, he did not respond that well to his platelet transfusion. Do I need to do something else right now…?'*

## Introduction

At the morning medical rounds, in discharge letters, in calls about admitted patients during the night shifts: bleeding is often encountered as a complication of hemato-oncological disease or treatment. Indeed, although these patients routinely receive prophylactic platelet transfusions, still bleeding is frequent. Many factors like patient characteristics, disease status, and treatment factors have been proposed to influence the occurrence of bleeding. In this introduction, the rationale behind the currently applied bleeding prevention strategy is described, as well as the evidence on which it is based.

**Prophylactic platelet transfusions in hemato-oncology patients**

To prevent clinically relevant bleeding, hemato-oncological patients who have a transient bone marrow failure receive prophylactic platelet transfusions during episodes of deep thrombocytopenia.

Already in the 1960's, it was clear that platelet transfusions reduced the number of severe bleedings. A study reporting causes of death in acute leukemia, including patients from 1954 to 1963, showed that the number of patients who had a bleeding when they died from acute leukemia declined from 67% to 37% when platelet transfusions became available to treat active bleeding. The number of bleedings that were registered to be the cause of death dropped from 20% to 14%.(1) Furthermore, small randomized trials showed the effect of prophylactic platelet transfusions,(2-4) which eventually lead to platelet prophylaxis (i.e. in the absence of bleeding) to become the standard of care in hemato-oncology patients since the nineteen seventies.(5) Based on larger studies, investigating platelet doses and platelet count triggers,(6, 7) transfusion guidelines advise to prescribe prophylactic platelet transfusions to patients with a transient, deep thrombocytopenia at a threshold of $10x10^9$/L.(8-12)

Despite the shown benefits of this prophylactic transfusion policy, bleeding still occurs. However, the incidence reported in different studies varies dramatically, from 19% to 89%.(13-21) This variability is at least partly due to the different subpopulations of hemato-oncology patients that were included in the studies, different monitoring methods to detect and register bleeding, different follow-up periods, and the different bleeding scores that were applied. Even between studies that use comparable bleeding scores, such as alterations of the World Health Organization (WHO) bleeding score,(22) incidences still vary. For WHO bleeding score with grades 2, 3, and 4 bleedings, incidences have been reported to range between 19% and 70%.(14, 16)

Besides the fact that clearly not all bleeding is prevented by the current prophylactic platelet transfusion strategy, there is also a strong indication that some patients have a very low risk of bleeding. In such patients, platelet transfusions are likely not useful and lead to unnecessary health care costs and labor, while being a potential cause of side effects.

Due to the prophylactic policy currently in practice, the hematology patients now consume approximately two-thirds of all platelet transfusions.(23) Of these, around 75% are described to be transfused prophylactically.(23, 24) In a country like the Netherlands, which produces approximately 52,000 units of platelets per year, it can be estimated that 35,000 will be transfused to hematology patients, and around 27,000 of those will be given prophylactically.(25) Consequently, because of the high transfusion rates, hemato-oncology patients are at high risk of being exposed to platelet mediated transfusion reactions, like transfusion mediated infections (occurring in approximately 0.001% of platelet transfusions), or allergic reactions (occurring in approximately 0.3% of platelet transfusions).(26, 27) Also, anti-HLA antibodies are frequently encountered in up to 43% of hemato-oncology patients. These antibodies can lead to platelet transfusion refractoriness, which has been described in 5-15% of patients who are on chronic platelet transfusion support.(28)

**Prophylactic platelet transfusions versus therapeutic platelet transfusions**

To better assess the amount of overtreatment by prophylactic platelet transfusion strategies, it is important to compare this with the situation in which platelet transfusions are only administered in case of active bleeding events; the so-called therapeutic strategy. Two randomized controlled trials (RCT's) compared these two strategies in adult hemato-oncology patients who were admitted for chemotherapy or stem cell transplantations.(13, 14) Both studies showed a benefit for the prophylactic platelet transfusion strategy to prevent combined WHO grades 2, 3, and 4 bleeding outcomes in 30 days. Stanworth et al. reported an incidence of WHO grades 2, 3, and 4 bleeding of 43% in patients in the prophylactic transfusion arm, versus 50% of these bleedings in the therapeutic-transfusions-only arm.(13) In this study, the reported incidence of serious bleedings (of WHO grade 3 and 4) was below 2%; with no discernable difference between the treatment groups. The observed benefit of prophylactic platelet transfusions was clear in patients receiving intensive chemotherapy or allogeneic stem cell transplantation (SCT). In contrast, patients who were admitted for autologous SCT hardly benefitted from prophylactic platelet transfusions.(29) As a result, and provided that close monitoring of bleeding is feasible, some guidelines now recommend a therapeutic transfusion strategy for patients undergoing autologous SCT. (9, 10)

The RCT of Wandt et al. showed that 19% of the patients with prophylactic platelet transfusions suffered from WHO grades 2, 3, and 4 bleeding, while with therapeutic transfusions this incidence increased to 42%.(14) In this study, a difference was also observed for WHO grade 3 and 4 bleedings. However, many grades 3 and 4 bleedings were intracerebral. Since the study used different diagnostic policies towards intracranial bleedings for the different randomization arms, this difference could be caused or aggravated by detection bias.

**Clinically relevant bleeding**

Currently, most studies use the WHO bleeding score, with grade 2, 3 and 4 bleedings as the primary outcome.(22) Over the years, several research groups have modified these grades, to reduce variation in assessment.(30) Important to realize in this respect, is that the majority of reported bleedings are WHO grade 2 bleedings. Not all of these are of direct importance for clinical care or for the patients' well-being. Other bleeding scores with more emphasis on the clinically relevance of bleeding are available (figure 1). The International Society on Thrombosis and Haemostasis (ISTH) bleeding scale, for example, distinguishes clinically relevant non-major and major bleedings from not-clinically-relevant bleeding.(31, 32) Likewise, the Bleeding Severity Measurement Scale (BSMS), which is especially designed for patients with thrombocytopenia induced by chemotherapy, separates bleeding which is clinically relevant (named clinically significant) from non-significant bleeding, i.e. that are expected not to have a real impact on the patient or the required intensity of patient care.(33) Prophylactic platelet transfusions should aim specifically at the prevention of bleeding that has a relevant impact on patients or patient care. Ideally, this strategy should therefore also be validated using only clinically relevant bleeding as the primary outcome. Unfortunately, due to the much higher number of patients that would be required in such a study, no RCT's have used this outcome yet.

**Hemato-oncology patients with a persistent severe thrombocytopenia**

While most research attention goes to those hemato-oncological patients who, due to the effects of acute disease or intensive chemotherapy, have the deepest cytopenias and associated morbidities, these conditions are usually transient. There is also a hemato-oncological population with chronic bone marrow failure and hence persistent severe thrombocytopenia. High intensity chemotherapy and other conditions that are associated with bleeding, like inflammation and infections, will be present less frequently in such patients. Thus, these factors can not readily be used to predict bleeding in this population. Whereas in the short term a lower bleeding risk is expected in this group, the chronic thrombocytopenia is eventually likely to induce a high cumulative incidence of bleeding. So far, there are no published RCT's investigating the effect of prophylactic platelet transfusions in this subpopulation with long-lasting thrombocytopenia. Moreover, even observationally registered incidences of bleeding are hardly described in the literature. Interestingly, an observational study in patients with myelodysplastic syndromes reported high cumulative incidences of overall and WHO grade 3 and 4 bleeding of respectively 83% and 14%, in a median time of 27 weeks.(34) In this study, prophylactic platelet transfusions, tranexamic acid, or both did not seem to lower the incidences of WHO grade 3 and 4 bleedings. This study however was small, with non-randomized treatments, making the results likely liable to confounding by indication.(34)

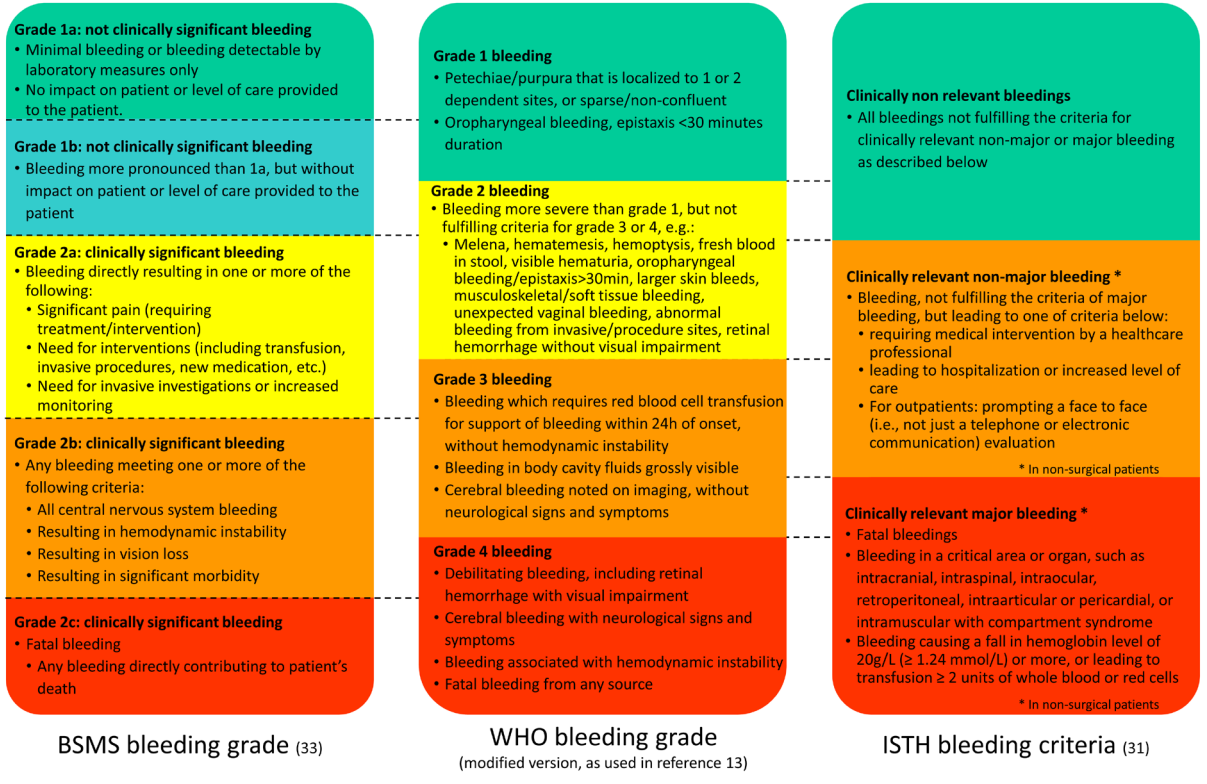


**Figure 1. Comparison of bleeding scores**
The WHO bleeding grade, which has been mostly used in research so far, does not provide a clear difference between clinically significant/relevant bleeding and non-significant/relevant bleedings, as do the BSMS and ISTH criteria. WHO grade 2 bleedings are only partly classified as clinically relevant, namely if there is a clear impact on the patient or medical care. The size of the boxes are not representative of the incidence of bleedings, but illustrate the overlap in BSMS and ISTH criteria with the WHO grading system. Abbreviations: BSMS = Bleeding Severity Measurement Scale, WHO = World Health Organisation, ISTH = International Society on Thrombosis and Haemostasis.

While some guidelines indicate that more liberal transfusion thresholds, or even a therapeutic platelet transfusion strategy, might be applied in these patients, evidence for such advice is insufficient. Overall, there is no consensus on the best strategy for bleeding prevention in patients with persistent severe thrombocytopenia. (9-12)

**Risk factors for bleeding**

In conclusion, there seems to be a general benefit of prophylactic platelet transfusions to prevent bleeding in transient severe thrombocytopenia. However, both even better bleeding prevention than currently achieved, and a reduction in unnecessary use of platelet transfusions clearly require the personalization of supportive transfusion strategies. Understanding of risk factors is indispensable to identify patients with a high, low, or virtually absent risk of bleeding and might enable adapting the platelet transfusion strategy accordingly.

So far, platelet counts are used to guide platelet transfusions. Platelet counts, however, correlate poorly with bleeding. Only at platelet counts of $5x10^9$/L or lower, a substantial increase in bleeding events is seen, where remarkably at counts from $6x10^9$/L to $80x10^9$/L bleeding frequencies remain more or less stable.(16, 35) Indeed, in trials that investigated platelet transfusion triggers, no benefit was observed in raising thresholds from $10x10^9$/L, to $20x10^9$/L, or $30x10^9$/L.(7) Moreover, increasing the platelet dose per transfusion also failed to lower the remaining bleeding incidence.(6) This suggests that, additionally to platelet count, other factors modulate the bleeding risk in hemato-oncology patients. Meanwhile, platelet transfusions are unable to overcome all mechanisms leading to bleeding.

Several clinical factors have been reported to be associated with an increased risk of bleeding in hemato-oncology patients, like sex (with a higher risk for women),(36) and diseases with a poor prognosis.(20) Furthermore, associations were shown between fever, infection, or sepsis and the occurrence of bleeding.(36-38) Results from animal studies also support the association between bleeding and infection which is more evident in case of thrombocytopenia.(39, 40) Furthermore, conditions known for their effect on hemostasis (like uremia or use of anticoagulants), are also associated with bleeding in hemato-oncological patients.(41, 42) Other conditions associated with bleeding, like usage of penicillin or medication against invasive mold infections, graft versus host disease, splenomegaly and bleeding in the preceding days are mechanistically less clear.(9) Moreover, the clinical relevance of most of these associations is uncertain because they are derived from (small) observational datasets or post-hoc analyses, or were absent in other datasets.

Notwithstanding the low level of evidence, some guidelines advise to consider increasing the platelet transfusion threshold in patients with additional risk factors. (9, 10) In clinical practice, the absence of high quality evidence often leads to heterogeneity

in the adherence to such guidelines and thus in the use of different thresholds.(43) As discussed, studies investigating different transfusion triggers, did not show a reduction in the number of bleedings with the use of a higher threshold.(7) However, in those studies patients with additional potential risk factors for bleeding were never studied separately. Therefore, it remains unclear if high-risk patients may benefit from the use of higher platelet triggers.

Invasive procedures like lumbar punctures or insertion of central venous catheters are evidently risk factors for bleeding. Yet, even for these procedures there is no strong evidence for the benefit of higher platelet transfusion thresholds.(44, 45) Still awaiting the results of an ongoing RCT study on this topic,(46) the Dutch 2019 transfusion guidelines, advises to prescribe prophylactic platelet transfusions at higher triggers in case of invasive procedures, as do other international guidelines.(8-12) Similarly, without strong evidence, but based on expert opinion, increased thresholds are also advised for patients with a transient thrombocytopenia who use therapeutic anticoagulation or platelet aggregation inhibitors, who cannot stop such medication.(8)

Overall, although there are many risk factors described that are likely to influence bleeding risk, the precise quantification of this increased risk is still lacking. Further, the effects of applying alternative bleeding preventive strategies, if such risk factors are present, are not well understood either.

**Biomarkers**

Clinical conditions that modulate the bleeding risk will only lead to bleeding if they negatively affect hemostasis. Therefore, next to platelet counts, additional biomarkers for hemostasis (e.g. biomarkers that reflect platelet function, coagulation, fibrinolysis or endothelial function) can also be of importance to predict the bleeding risk.

In this respect, a decreased platelet function or activation state has been suggested to have predictive value.(18, 47, 48) A reduced p-selectin expression and a diminished (*ex vivo*) platelet response to standard platelet stimulation have both been observed in patients who developed bleeding. These results, however, could equally well imply that either circulating platelets have diminished functionality, or that they have a reduced 're-activation' in vitro because of high functional *in vivo* platelet activation.

Also, some endothelial markers have been described to be associated with bleeding. For example, in leukemia patients an association between bleeding and high values of syndecan-1, which as part of the glycocalyx is indicative of endothelial damage, is described.(49) Likewise, urine albumin excretion, known to be a biomarker of renal vascular wall damage, was found to be associated with bleeding in hemato-oncology patients.(50)

Associations between coagulation-markers and coagulation assays and bleeding are not consistent.(35, 51) However, studies investigating these (and other) potentially

important biomarkers are often small, and a direct comparison between studies is complicated by sampling at different stages of treatment of the patient. Therefore, no biomarkers have consistently been established to predict the bleeding risk in hemato-oncology patients.

**Alternatives for prophylactic platelet transfusions**

To further reduce the amount of bleeding events in hemato-oncology patients, alternatives for or additions to prophylactic platelet transfusions such as TPO-mimetics or antifibrinolytic agents, like tranexamic acid, have been studied.(52) However, again, studies are insufficient to conclude if the bleeding incidence can be relevantly influenced by these strategies.(53, 54) Recently, a trial was completed investigating tranexamic acid, as an adjunctive anti-bleeding therapy. Although the complete trial results are awaited, first results did not report a benefit in intensively treated hemato-oncological patients.(55) Autologous SCT patients have been reported to have less benefit of prophylactic platelet transfusions.(29) An ongoing RCT in this patient group will determine if tranexamic acid can be used as an alternative strategy to prevent bleeding, instead of platelet transfusions. (56)

## Aim and outline of the thesis

With the current prophylactic platelet transfusions, bleeding is still a commonly occurring problem in hemato-oncology patients, and information on risk factors that can guide prediction of bleeding is insufficient. The aim of this thesis is to provide more insight into bleeding in hemato-oncology patients, by investigating risk factors that are needed for prediction, and by describing current clinical practice of preventive strategies.

First, we aimed to investigate the current clinical practice of bleeding prevention. For patients who are intensively treated, and for whom the deep thrombocytopenia is expected to be transient, guidelines provide clear advises for general transfusion thresholds, and sometimes suggestions for patients with (suspected) additional bleeding risks. However, the absence of clear evidence of altered thresholds in patients with additional risk factors leads to variability in care. While this variability was reported before for patients with deep, transient thrombocytopenia, for patients who suffer from persistent deep thrombocytopenia, due to chronic bone marrow failure, optimal bleeding prevention has not been investigated, leading to a lack of clear advice in guidelines. In **chapter 2**, we report on a Dutch survey which assessed both usage of prophylactic platelet transfusions and tranexamic acid to prevent bleeding in hematological outpatients who suffer from persistent severe thrombocytopenia. Besides the current clinical practice, we describe which clinical conditions guide clinical decision

making for the preventive therapy.

Of all bleeding events, intracranial hemorrhage is one of the most feared. As with other bleeding complications, the association with platelet counts and intracranial hemorrhage has already been studied. However, besides platelet counts at the time of hemorrhage, the course of the preceding platelet counts, including the lowest 'through' counts in preceding days and the percentages of time exposed to such low platelet counts have not been investigated for intracranial hemorrhage. These platelet count parameters were hence studied as possible risk factors for intracranial hemorrhage in a case-control study which is reported in **chapter 3**. Furthermore, in this chapter, the number of platelet transfusions needed to maintain the target platelet thresholds was studied for its association with intracranial hemorrhage.

Since the etiology of bleeding is multifactorial, there are many other clinical factors that may predict the occurrence of intracranial hemorrhage in acute leukemia patients. In **chapter 4**, in the same case-control population as described in chapter 3, we focus on the predictive power of one likely risk factor, namely the presence of cardiovascular risk factors, which likely lead to chronic vascular damage. The association of such factors with intracranial hemorrhage might be of importance, because of the concurrent risk factors for diminished vascular integrity in this patient population, for example thrombocytopenia and inflammation.

Improving bleeding prevention in hemato-oncology patients, can likely be improved by validation of risk factors that can be used in a prediction model to identify patients who indeed have a very high bleeding risk. For the purpose of identifying such risk factors, we performed a post-hoc analysis in the dataset of the randomized controlled TOPPS trial, and investigated if baseline parameters – suggested to be associated with bleeding risk – indeed could be used to predict the actual occurrence or absence of bleeding that was observed in the study cohort. Furthermore, we performed a heterogeneity of treatment effects analysis, with the goal to explore if patients with different bleeding risks at baseline benefitted differently from a prophylactic platelet transfusion strategy. Results of this study are described in **chapter 5**.

Although bleeding in hemato-oncological patients has been studied quite often, there is still much unknown about contributing risk factors. Consequently, well-functioning and validated models to predict bleeding are lacking. Both for etiologic research into risk factors for bleeding in hemato-oncology patients, as for adequate risk prediction, large datasets of sufficient variables to enable extensive adjusting and/or modeling will be necessary. Ideally, these datasets will include time varying clinical variables. Additionally, biomarkers for hemostasis or endothelial function might add to adequate prediction. In **chapter 6**, we present the study design for the ongoing BITE (Bleeding In Thrombocytopenia Explained) study. This study will not only assess the overall incidence of bleeding in hemato-oncology patients, but also in subgroups.

Furthermore, a case control study nested within the study population will enable us to further investigate risk factors for bleeding. Sampling of biomarkers will be performed in the last phase of the BITE study. After identification of clinical and laboratory risk factors, both baseline and time-varying data of this study can contribute to the adequate prediction of bleeding risk of hemato-oncology patients.

In **chapter 7** we discuss the main findings of this thesis, as well as our view on the implications for future research.

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# Chapter 2

## Platelet transfusion and tranexamic acid to prevent bleeding in outpatients with a hematological disease: a Dutch nationwide survey

Eur J Haematol. 2021 Mar;106(3):362-370. doi: 10.1111/ejh.13555

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## Abstract

**Objectives**

There is scarce evidence about the effectiveness of anti-bleeding measures in hematological outpatients experiencing persistent severe thrombocytopenia. We aim to describe clinical practice and clinicians' considerations on the administration of prophylactic platelet transfusions and tranexamic acid (TXA) to outpatients with acute leukemia, myelodysplastic syndrome (MDS), or aplastic anemia (AA) in the Netherlands.

**Methods**

We conducted an online survey among members of the Dutch Society for Hematology.

**Results**

The survey was filled out by 73 respondents. Prophylactic platelet transfusions are widely used in acute leukemia and MDS outpatients receiving disease-modifying treatments (87%-98% of respondents). TXA is predominantly prescribed in case of bleeding (tendency) (71%-88% of respondents). Conditions potentially increasing bleeding risks highly variably influence clinicians' decision-making on anti-bleeding regimens, which includes a wide range in adhered platelet thresholds.

**Conclusion**

Considering that both the contribution of prophylactic platelet transfusions as well as TXA to limit bleeding is insufficiently evidence-based, there is an urgent need for trials on optimal anti-bleeding strategies in this outpatient population, which should encompass efficacy, logistic, financial and quality of life aspects.

## Introduction

Thrombocytopenia due to bone marrow disease and/or myelotoxic treatments is a common phenomenon in hematological patients. In order to prevent clinically relevant bleeding, prophylactic platelet transfusions (i.e. indicated by a platelet count threshold, in the absence of bleeding) are administered.[1, 2] Indeed, randomized controlled trials demonstrated reduced bleeding incidences with such a strategy in hospitalized patients undergoing intensive chemotherapy and/or allogeneic stem cell transplantations.[3, 4] Nevertheless, clinically relevant bleeding is not eliminated and alternative anti-bleeding strategies are nowadays explored, including alternative treatments and the identification of reliable bleeding predictors.[5, 6]

Next to this intensively treated patient population, a subgroup of hematological outpatients suffers from persistent severe thrombocytopenia due to e.g. refractory bone marrow disease, inducing chronic bone marrow failure. Actual bleeding risks for this specific outpatient population are unknown, but, one may argue those to be relatively low compared to the intensively treated hospitalized patients. Conversely, due to the chronic state of their low platelet counts, a large fraction of this population may eventually experience significant bleeding. One Canadian registry for patients with myelodysplastic syndrome (MDS) and secondary acute myeloid leukemia (AML) indeed reported bleeding in 83% of patients during a median follow-up period of 27 weeks, with 12% of patients experiencing WHO grade 3 or 4 bleeding.[7, 8] However, the attributive effect of platelet transfusion in this outpatient setting is unknown, although a few small observational studies suggested safety, logistical, and financial advantages of a stringent platelet transfusion policy.[7, 9] One randomized trial, which could have gained important insights into the efficacy of prophylactic transfusions in outpatients was unfortunately terminated early because of poor recruitment.[10] Therefore, so far high quality evidence on any potential benefits weighted against adverse risks of a prophylactic versus therapeutic platelet transfusion regimens in this outpatient population is lacking.

Consequently, current guidelines are based on expert opinion and mainly advise to only transfuse the thrombocytopenic (out)patient population suffering from chronic bone marrow failure on a therapeutic rather than on a prophylactic base.[11-13] Other guidelines suggest to consider an adjusted platelet count threshold,[14] while the recently updated Dutch transfusion guideline in this respect lacks any recommendations.[15]
In addition to platelet transfusions, preventative anti-bleeding measures may also include the use of the anti-fibrinolytic drug tranexamic acid (TXA).[11] Compared to platelet transfusions, TXA has the advantage of oral administration, thereby overcoming the necessity of intramural care. Outside the hematological setting, the use of TXA has proven to be beneficial in therapeutic settings, reducing blood loss and limiting

morbidity and mortality during e.g. massive trauma, surgery and obstetric bleeding. Evidence to justify its use for hematological thrombocytopenic patients is scarce and inconclusive.[16] Remarkably, the aforementioned Canadian MDS registry study did not find differences in grade 3-4 bleeding frequencies among patients treated with TXA versus TXA and/or prophylactic platelet transfusions versus neither of those, although confounding by indication should be considered.[7] Hopefully several ongoing large-scaled randomized studies in hospitalized patients will clarify the possible prophylactic role of TXA, with or without additional platelets.[5, 17]

However, the present lack of knowledge is likely to result in a high variability of practices on how best to prevent bleeding in hemato-oncologic outpatients.

To assess this, we performed nationwide survey among hematology clinicians across the Netherlands regarding the extent of use, and considerations on indications of platelet transfusions and TXA in hematological outpatients suffering from persistent severe thrombocytopenia due to underlying bone marrow disease.

## Methods

A nationwide web-based survey of hematology clinicians was conducted in the Netherlands between October 2019 and February 2020.

The questionnaire was accessible via a weblink and distributed via email by the Dutch Society for Hematology. Members comprise the large majority of registered hematologists in the Netherlands as well as a proportion of hematology residents and physician assistants. All are involved in treatment decisions on bleeding prevention in the Netherlands, either completely independent or following consultation of a senior hematologist. Reminders were sent out via the newsletter of the society and via personal communication by members of the benign working party of the society to colleagues in their region. Prior to distribution, the survey was piloted among the study team and three other hematologists to assess content and time required for survey completion.

Study data were collected in a web-based database (Castor) and securely stored at the Leiden University Medical Center.

The survey (translation available via the supplementary material) focused specifically on acute leukemia, myelodysplastic syndrome (MDS), and aplastic anemia (AA) outpatients. Since we expected that the disease stage, and appurtenant treatment, might influence the chosen prophylactic bleeding policies, we specified several patient groups. With regard to acute leukemia and MDS, questions were subdivided based on whether patients were 1. in between or shortly after curatively-intended induction chemotherapy courses; 2. receiving hypomethylating agents with a palliative intention;

3. ineligible for any disease-modifying treatment. Questions on AA involved all patients outside the context of a hematopoietic allogeneic stem cell transplantation. Specific domains of the questionnaire involved: 1. clinician practices' demographics; 2. use of a prophylactic platelet transfusion policy and its thresholds; 3. clinical conditions determining the use of a prophylactic platelet transfusion policy; 4. prophylactic use of TXA; 5. clinical conditions determining the use of TXA; 6. clinicians' estimations on bleeding risks with a prophylactic versus therapeutic platelet transfusion policy.
The survey used the following definitions: prophylactic platelet transfusions i.e. transfusions prescribed based on a certain platelet count threshold which may differ per patient or physician; therapeutic platelet transfusions i.e. transfusions prescribed in case of (clinically relevant) bleeding or preceding an intervention; clinically relevant bleeding i.e bleeding events that lead to (additional) medical care, e.g. visit to the emergency department or additional outpatient clinic visit on short term, therapeutic transfusions, admission to the hospital, additional diagnostics or treatments. Any tendency to bleeding referred to minor, clinically non-relevant bleeding e.g. petechiae.

Due to the descriptive nature of our survey, no formal statistics were performed but results are presented descriptively.

## Results

Of the 562 members contacted, 73 (13%) responded at least to one domain (table 1). Of these 73 respondents, 55% completed the entire questionnaire. The majority of respondents were hematologists (81%), working in hospitals which perform both allogeneic and autologous stem cell transplantations (45%, i.e. academic hospitals), with a median working experience of 10.5 years. Respondents represented 38 out of 89 (43%) Dutch hospitals.

A minority of respondents worked at hospitals that do not treat some of the patient categories covered by this survey (table 1). In those instances, these respondents were excluded from these particular calculations.

**Use of prophylactic anti-bleeding therapies**

Figure 1 describes numbers and percentages of respondents who routinely use prophylactic platelet transfusions or TXA per patient category. Almost all actively treated MDS and acute leukemia outpatients are offered prophylactic platelet transfusions (87-98%), while this is only considered for the minority of patients ineligible for or refractory to any disease modifying treatment (35% and 34%). Similarly, the vast majority of aplastic anemia patients receive prophylactic platelet transfusions (82%). Oppositely, TXA is hardly routinely prescribed in any of these patient populations

(0-7%), but is generally regarded as supportive care in situations of clinically relevant bleeding or bleeding tendency (71%- 88%). Here, TXA is mostly used as an additive to prophylactic platelet transfusions in patients receiving any type of treatment (74% to 100%), while in the palliative setting without any disease modifying treatment, TXA is also chosen as solitary regimen (MDS 47% and acute leukemia 44%, supplementary table 1).

**Table 1.** Characteristics of respondents

| | Total n=73 | |
|---|---|---|
| **Function**[†] | | |
| Hematologist | | 59 (81%) |
| Resident hematology | | 4 (6%) |
| Other[‡] | | 10 (14%) |
| **Years of working experience in hematology**[§] | **10.5** | **(5-19)** |
| **Echelon classification of hospital**[¶] | | |
| Level A | | 33 (45%) |
| Level B | | 7 (10%) |
| Level C- HIC | | 6 (8%) |
| Level C- SCT | | 6 (8%) |
| Level C- HIC + C-SCT | | 8 (11%) |
| Level D | | 9 (12%) |
| Unknown | | 4 (6%) |
| **Outpatient population that is treated per respondent**[#] | | |
| MDS with chemotherapy | | 60 (82%) |
| MDS with hypomethylating agents | | 69 (95%) |
| MDS without disease-modifying treatment | | 68 (93%) |
| Leukemia with chemotherapy | | 58 (80%) |
| Leukemia with hypomethylating agents | | 68 (93%) |
| Leukemia without disease-modifying treatment | | 71 (97%) |
| Aplastic anemia | | 51 (70%) |

† Values are numbers (percentage of total of respondents)
‡ Physician assistants (n=7), pediatric hematologist (n=1), resident not in training for hematologist (n=1), oncologist with hematology care (n=1)
§ Median (IQR), 72 participants responded
¶ Level A hospitals are allowed to perform allogenic and autologous stem cell transplantations (SCT)
Level B hospitals are allowed to perform autologous SCT
Level C-HIC hospitals deliver intensive hematological care, for example acute leukemia treatment
Level C-SCT hospitals deliver post-autologous stem cell transplantation care
Level D hospitals deliver non-intensive hematological care, i.e. treatment that is not expected to induce intense and long-lasting pancytopenia
# Values are numbers (percentage of total of respondents) of those who treat the specific patient population at their clinical practice

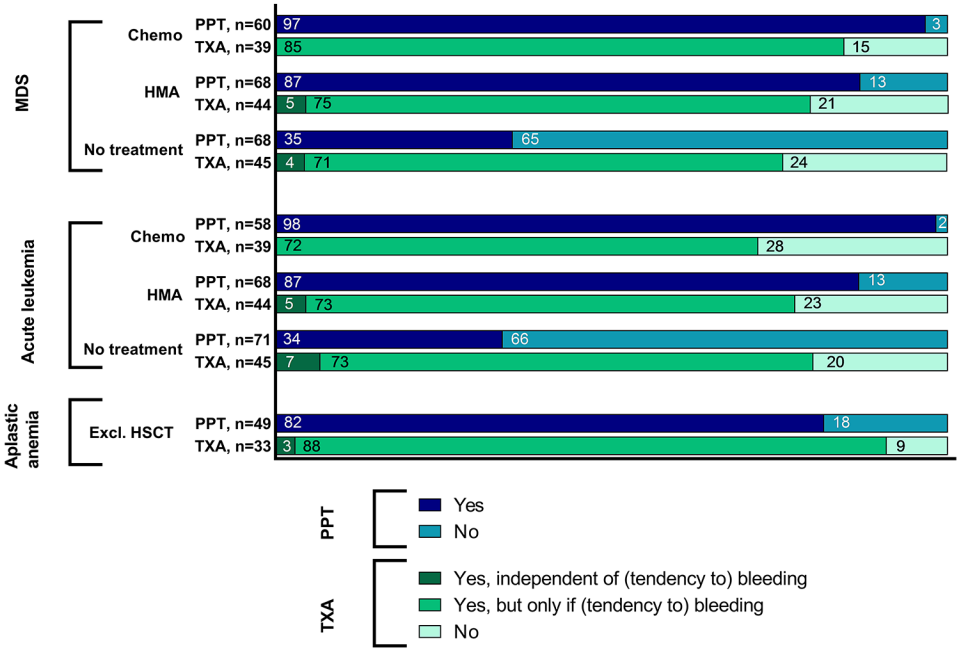


**Figure 1. Prophylactic anti-bleeding options considered per diagnosis and treatment modality**
Values in bars indicate percentages of respondents. Absolute numbers of respondents per question are presented at the left side of the bar. Chemo: outpatients in between or shortly after intensive chemotherapy courses. HMA: outpatients treated with hypomethylating agents, e.g. azacitidine or decitabine. No treatment: outpatients not receiving any disease modifying treatment i.e. refractory disease, treatment ineligible, palliative setting. Aplastic anemia excl. HSCT: outpatients excluding those in work-up for or having received an allogeneic hematopoietic stem cell transplantation. MDS= myelodysplastic syndrome, PPT= prophylactic platelet transfusion, TXA= tranexamic acid. Data represents question 1 and 6a of survey, see supplementary material.

## Clinical conditions modifying prophylactic anti-bleeding treatment

Several clinically related conditions may modulate anti-bleeding preventative measures. The most likely ones were assessed in this survey (Figure 2, supplementary table 2).

Figure 2 illustrates the strong heterogeneity in how clinicians value certain clinical conditions as determinants for anti-bleeding strategies. In general, recent clinically relevant bleeding (<three months), and continuous use of platelet aggregation inhibitors or therapeutically dosages of anticoagulant medication are valued most important, especially for the regimen of prophylactic platelet transfusions. In addition, clinicians are quite reluctant to start TXA in patients with a medical history of cerebral or coronary ischemic events.

Furthermore, presence of fever, red blood cell transfusion dependency and low hematocrit levels are considered as important clinical factors when deciding to give prophylactically platelet transfusions (25%-43%). Such conditions are considered hardly relevant for TXA decision making (supplementary table 2).

## Platelet thresholds

In general, a platelet threshold of ≤10x10$^9$/L is routinely applied for all acute leukemia, MDS and AA outpatients (Figure 3, panel A; 77-100%). Though, when clinical conditions that potentially increase bleeding risks are present, a wide range of thresholds between 10x10$^9$/L up to 50x10$^9$/L is applied (Figure 3, panel B). In case of use of platelet aggregation inhibitors (PAI) or therapeutic anticoagulants, over 90% of respondents increased standard platelet transfusion thresholds above 10 x10$^9$/L, the majority to 20 x10$^9$/L to 30x10$^9$/L.

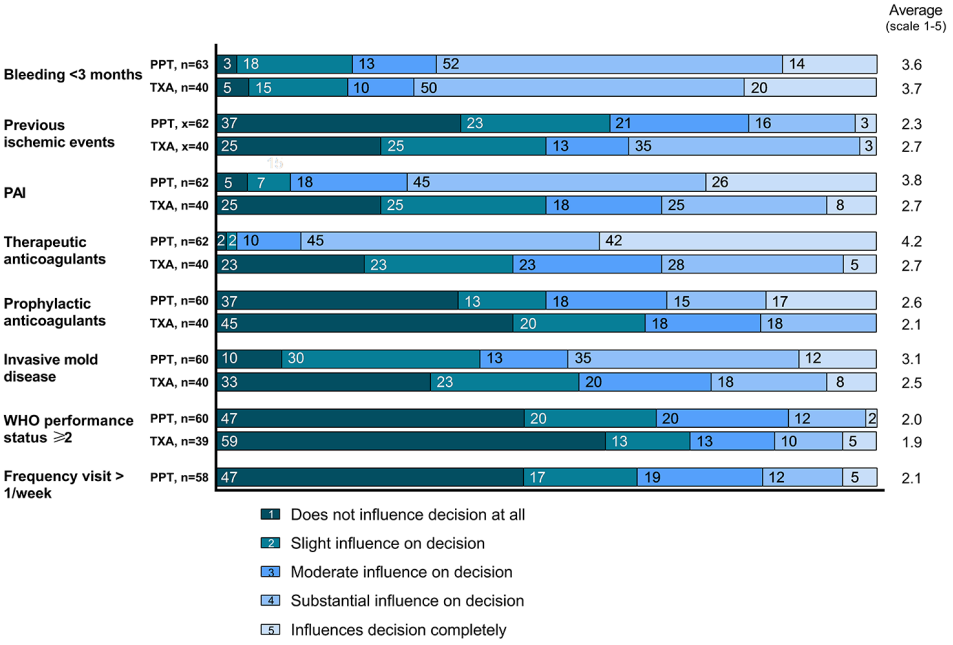


**Figure 2. Clinical conditions considered in decision-making on prophylactic anti-bleeding treatments**
Values in bars indicate percentages of respondents. Absolute numbers of respondents per question are presented at the left side of the bar. The average score per clinical condition is reported at the right side of the bar (minimum score 1, maximum score 5). Bleeding <3 months: clinically relevant bleedings in the past three months. Previous ischemic events: medical history of cardiac or cerebral ischemic event. PAI: the need or wish to continue platelet aggregation inhibitors. Therapeutic anticoagulants: the need or wish to continue therapeutic dosage of low molecular weight heparin, vitamin K antagonist or direct oral anticoagulant. Prophylactic anticoagulants: the need or wish to continue prophylactic dosage of low molecular weight heparin Invasive mold disease: presence of cerebral or pulmonary invasive mold disease. WHO = World Health Organization, performance status of 2: ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours. Frequency visit > 1/week: need to visit the outpatient clinic with a frequency of more than once weekly – only surveyed for platelet transfusions, not for TXA. Data represents question 3 and 6c of survey, see supplementary material.

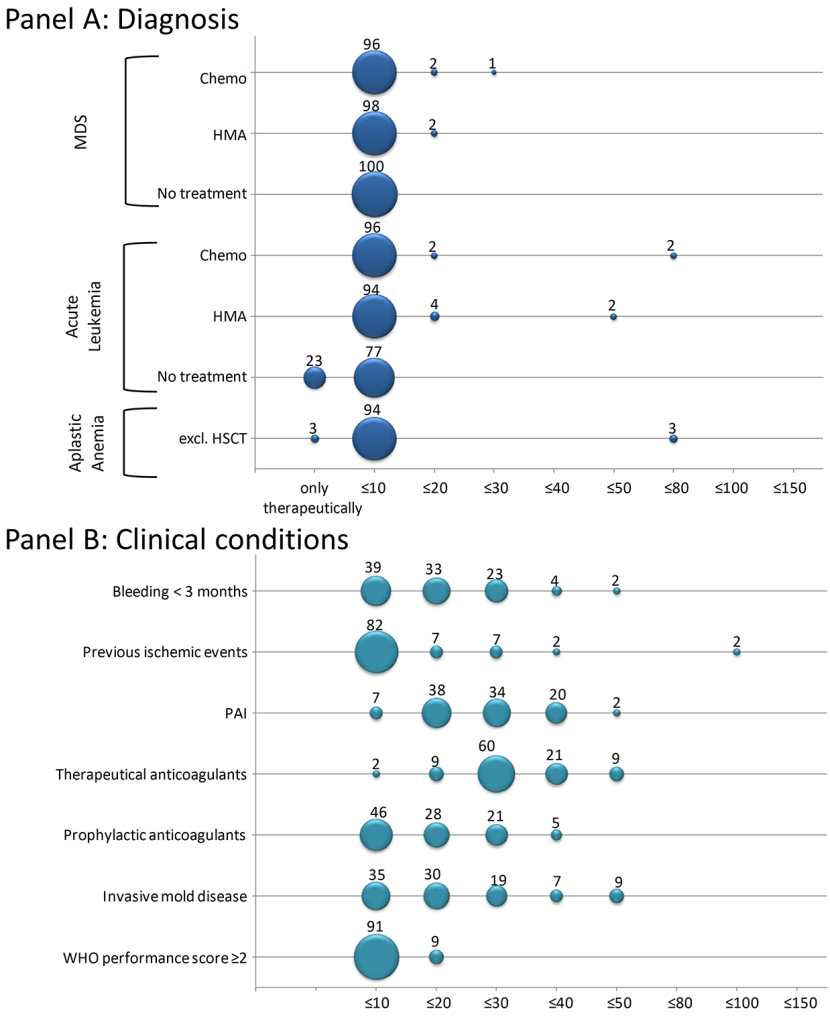


**Figure 3. Applied platelet count thresholds**
The size of and numbers in the bubbles indicate percentages of respondents routinely adhering to a specific platelet threshold. **Panel A**: platelet thresholds per patient category. Chemo: outpatients in between or shortly after intensive chemotherapy courses. HMA: outpatients treated with hypomethylating agents, e.g. azacitidine or decitabine. No treatment: outpatients not receiving any disease modifying treatment i.e. refractory disease, treatment ineligible, palliative setting. Aplastic anemia excl. HSCT: outpatients excluding those in work-up for or having received an allogeneic hematopoietic stem cell transplantation. MDS= myelodysplastic syndrome. **Panel B**: platelet thresholds specified per clinical condition. Bleeding <3 months: clinically relevant bleedings in the past three months. Previous ischemic events: medical history of cardiac or cerebral ischemic event. PAI: the need or wish to continue platelet aggregation inhibitors. Therapeutic anticoagulants: the need or wish to continue therapeutic dosage of low molecular weight heparin, vitamin K antagonist or direct oral anticoagulant. Prophylactic anticoagulants: the need or wish to continue prophylactic dosage of low molecular weight heparin. Invasive mold disease: presence of cerebral or pulmonary invasive mold disease. WHO = World Health Organization, performance status of 2: ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours. Data represents question 2 and 4 of survey, see supplementary material.

**Estimated bleeding risks**

Figure 4 illustrates estimated six months' incidences of clinically relevant bleeding under a prophylactic versus therapeutic-only platelet transfusion strategy. The vast majority of clinicians estimate the likelihood of a bleeding event under a prophylactic regimen to be low, i.e. <10% over six months' time. Switching to a therapeutic-only regimen (panel B) is expected to increase the risk of bleeding according to most clinicians. However, estimates on the magnitude of this increase again are widely variably, with some estimating even bleeding risks over 50%.

## Discussion

This nationwide survey among hematology clinicians identified a heterogenous practice of and considerations on the use of prophylactic platelet transfusions and TXA among acute leukemia, MDS and AA outpatients in the Netherlands.

First, our results indicate the stage of the disease to be an important determinant of prophylactic anti-bleeding strategies. Hence, prophylactic platelet transfusions are widely applied in patients receiving disease-modifying treatment, and far less in patients without active treatment options. Oppositely, TXA, although orally available and cheap, is seldom applied on a prophylactic base. This wide use of a prophylactic platelet transfusion strategy may not come as a surprise, since the 2011 version of the Dutch transfusion guideline recommended so for all thrombocytopenic patients originating from an acquired bone marrow failure.[18] This guideline was recently updated, now restricting this advice to patients with a transient rather than chronic bone marrow failure.[15] Importantly, these advices are extrapolated from studies performed in intensively treated (in)patients. Indeed, it is completely unknown whether the observed protective anti-bleeding results of platelet transfusions similarly apply to outpatient settings where mucosal-damage and extensive inflammation are uncommon clinical conditions.[11, 19] Yet, with benefits per platelet transfusion to potentially be less, adverse effects of longer term platelet transfusions are not abandoned, including a cumulative risk of transfusion reactions, [20] financial costs, and logistic challenges for the patient and the hospital. The few studies performed so far indeed questioned the effectiveness and net benefit of prophylactic platelet transfusions in the setting of persistent thrombocytopenia, although the size and design of these studies warrants firm conclusions.[7, 9] Despite the fact that some international guidelines have taken these arguments into account and nuanced advices to a therapeutic-only transfusion strategy for patients with chronic bone marrow failure[11-13], our survey illustrates a general reluctance to a therapeutic-only transfusion strategy for hematological outpatients, as clinicians believe such a strategy to substantially increase bleeding risks.

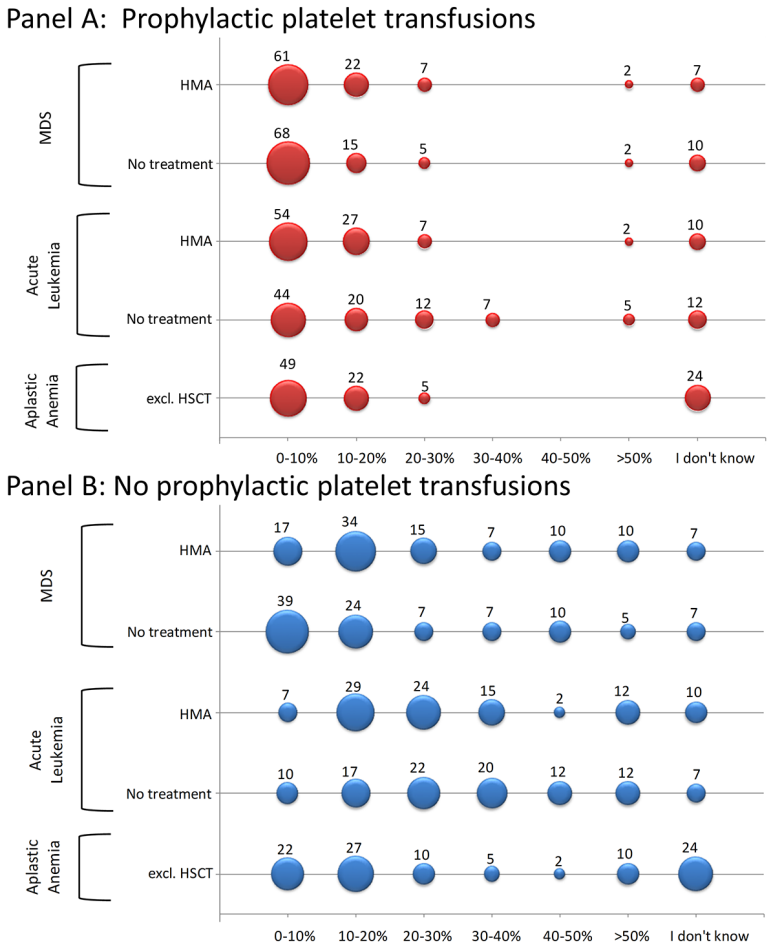


**Figure 4. Estimated 6-month cumulative incidence of clinically relevant bleeding**
The size of and numbers in the bubbles indicate percentages of respondents per patient category. **Panel A:** estimated 6 months' bleeding incidence with prophylactic platelet transfusion. **Panel B:** estimated 6 months' bleeding incidence with therapeutic-only platelet transfusions. HMA: outpatients treated with hypomethylating agents, e.g. azacitidine or decitabine. No treatment: outpatients not receiving any disease modifying treatment i.e. refractory disease, treatment ineligible, palliative setting. Aplastic anemia excl. HSCT: outpatients excluding those in work-up for or having received an allogeneic hematopoietic stem cell transplantation. MDS= myelodysplastic syndrome. Data represents question 8 of survey, see supplementary material.

Second, our survey illustrates that several clinical conditions modulate the decision to initiate preventive anti-bleeding strategies, especially with regard to prophylactic platelet transfusion strategy. Remarkably, in situations believed to be associated with increased bleeding risks, a wide range of platelet thresholds is applied. Again, this seems to reflect an extrapolation of evidence on additional bleeding risk factors

available from intensively treated hospitalized patients.[11, 12, 18] However, such evidence is lacking for hematological outpatients with chronic bone marrow failure.

Some limitations of this survey need to be taken into consideration. The survey was sent out to all Dutch hematological clinicians, thereby aiming for a representative overview of clinical practices in the Netherlands. Despite our efforts, the response (13%) was moderate and overrepresented by clinicians working in academic hospitals (45%). This may have biased our outcomes to policies mainly applied within the academic setting. On the other hand, hematologists working in the field of clinical transfusion medicine completed this survey (verified by personal communication). While they are responsible for transfusion policies across their hospital and geographic region, their responses increase the validity of our results.

By having the survey spread via the Dutch Society for Hematology, we were able to send our survey request to the majority of our intended population. Unfortunately, due to privacy regulations, provision of a personalized weblinks and thereby filling out individual sections of the questionnaire at different time points was not possible. This probably explains why only 55% completed the entire survey including the final part on TXA use. However, as the use of TXA and the likelihood of a responder to complete the survey are unrelated, it seems unlikely that this biased results on TXA.

Further, one may argue whether opinions on prophylactic platelet transfusion indications also reflect underlying practical considerations. Although our survey did not verify any existence of such considerations, absence of constraints in infrastructural resources of both the Dutch blood supply organization as well as hospitals' outpatient departments should at all times enable facilitation of platelet transfusions whenever deemed indicated. We thus reckon capacity issues not to have skewed our results to a specific prophylactic strategy.

Finally, this survey was only sent out in the Netherlands. The objectified heterogeneity of practices likely relate to the absence of advices in the Dutch nationwide transfusion guideline on how to manage persistent severe thrombocytopenia in chronic bone marrow failure.[15] In contrast, some international guidelines specifically suggest against prophylactic platelet transfusions,[11-13] or to adjust thresholds.[14] None of these guidelines specifically comment on use of TXA in the absence of bleeding. Consequently, it seems likely that practices differ per country.

In conclusion, in the Netherlands, prophylactic platelet transfusions in contrast to TXA use are highly integrated in routine care to hematological outpatients suffering from persistent severe thrombocytopenia, despite the lack of any evidence in this clinical setting. Clinical practice is furthermore characterised by a large heterogeneity in decision reasoning and its outcomes with regard to clinical conditions generally assumed to increase bleeding risks.

The results of this survey underline the current gap in knowledge on bleeding and preventive strategies in hematological patients with chronic bone marrow failure. Further research should focus on (cumulative) bleeding incidences and bleeding predictors in this specific patient population. Second, there is a need to set up a large-scaled comparative RCT on the effectiveness, safety and patients' burdens of various anti-bleeding strategies for these patients. Finally, these outcomes would need to be incorporated into existing guidelines.



### Summary statements

1. What is the new aspect of your work?
   It is currently unknown how to best prevent bleedings in acquired persistent severe thrombocytopenia, this survey provides insight in current clinical practices of anti-bleeding strategies among hematological outpatients in the Netherlands.
2. What is the central finding of your work?
   Currently applied preventive anti-bleeding strategies for patients with acquired persistent thrombocytopenia lack uniformity; platelet transfusions are the mainstay of prophylactic strategies in this setting, but there is a large inter-physician variability in decisions made on indications and agents used, both being strongly but heterogeneously influenced by various clinical conditions.
3. What is (or could be) the specific clinical relevance of your work?
   These results underline the current gap in knowledge, and emphasize the need for further research, including a RCT on the effectiveness, safety and patients' burdens of various anti-bleeding strategies, ultimately aiming to improve supportive care in this specific stage of disease.

**Author contributions**

LLC designed the study, performed the research, analyzed the data and wrote the manuscript. CCD and RTM designed tables and figures and revised the manuscript. JJZ and DE designed the study and revised the manuscript.

**Acknowledgements**

We thank the members of the Dutch Blood Transfusion Related Research Consortium, especially Johanna G. van der Bom, for their valuable input in the design of this study, and the benign working group of the Dutch Society for Hematology for their contributing efforts in reminder communication. This study was sponsored via an internal grant from Sanquin (grant number PPOC 16–26, www. sanquin. nl)

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# Supplementary material

**Supplementary table 1.** Use of TXA†

| Diagnosis and treatment | TXA **instead** of prophylactical platelet transfusions | TXA **in addition to** prophylactical platelet transfusions | Other‡ |
|---|---|---|---|
| **MDS** | | | |
| Intensive chemotherapy treatment § | 0/32 (0%) | 31/32 (97%) | 1/32 (3%) |
| Hypomethylating agents ¶ | 5/35 (14%) | 26/35 (74%) | 4/35 (11%) |
| No disease modifying treatment# | 16/34 (47%) | 15/34 (44%) | 3/34 (9%) |
| **Acute leukemia (myeloid or lymphoid)** | | | |
| Intensive chemotherapy treatment § | 0/28 (0%) | 28/28 (100%) | 0/28 (0%) |
| Hypomethylating agents¶ | 4/34 (12%) | 28/34 (82%) | 2/34 (6%) |
| No disease modifying treatment# | 16/36 (44%) | 17/36 (47%) | 3/36 (8%) |
| **Aplastic anemia** + | 4/28 (14%) | 23/28 (82%) | 1/28 (4%) |

†Values are numbers (percentage of total of respondents). Respondents who do not treat the specific patient population at their clinical practice were not taken into account in calculations. Denominators (numbers of responders per question) differ per subgroup, since not all respondents completed all questions.
‡ Other: policy variable and dependent of e.g. respondents who prescribe TXA sometimes instead of, and sometimes in addition to prophylactical platelet transfusions, based on specific clinical characteristics such as disease severity, severity (former) bleedings, treatment response etc.
§ Outpatients in between or shortly after intensive chemotherapy courses; hydroxycarbamide or hypomethylating agents excluded
¶ Outpatients who are treated with hypomethylating agents, e.g. azacitidine or decitabine
# Refractory disease or treatment ineligible, palliative setting
+ Excluding patients in work-up for, or having received an allogeneic hematopoietic stem cell transplantation.
Data represents question 6b of survey, see translation survey.

**Supplementary table 2.** Other clinical variables considered in decision-making on prophylactic anti-bleeding treatments†

| Clinical variable | Prophylactic platelet transfusions n=53 | Prophylactic TXA n=40 |
|---|---|---|
| Hematocrit level | 30% | 15% |
| Leukocyte count | 2% | 3% |
| RBC transfusion dependency | 25% | 8% |
| CRP | 4% | 5% |
| Fever ≥38.5°C | 43% | 13% |
| Chronic or recurrent infections | 4% | 5% |
| Albumin | 0% | 0% |
| Kidney function / urea | 17% | 13% |
| Fibrinogen level | 17% | 25% |
| Liver enzymes / liver function | 15% | 13% |

†Values are percentages of respondents.
RBC= red blood cell
Data represents question 5 and 7 of survey, see translation survey.

## English translation of survey (original in Dutch)

**Definitions**

**Prophylactic platelet transfusions**: transfusions that are prescribed based on a certain platelet count threshold. The applied threshold can differ per patient or physician.

**Therapeutic platelet transfusions**: transfusions that are prescribed in case of (clinically relevant) bleeding or preceding an intervention.

**Clinically relevant bleeding**: bleeding events that lead to (additional) medical care, e.g. visit to the emergency department or additional outpatient clinic visit on short term, therapeutic transfusions, admission to the hospital, additional diagnostics or treatments.

**General questions respondents**

Name of hospital: *(text field)*

Profession: *(hematologist, resident hematology, other)*

Years of working experience within hematology: *(numeric text field)*

## Survey questions

**Question 1**

Which hematological outpatients suffering from a disease-related severe thrombocytopenia do you in general administer prophylactic platelet transfusions? *Please choose one answer per diagnosis/situation.*



| Hematology outpatient populations | Answer options | | |
|---|---|---|---|
| MDS patients receiving (or recently receiving) intensive chemotherapy (excluding hydroxycarbamide or hypomethylating agents) | Yes | No | Situation does not apply to my clinical practice |
| MDS patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | Yes | No | Situation does not apply to my clinical practice |
| MDS patients without any disease modifying treatment | Yes | No | Situation does not apply to my clinical practice |
| Acute leukemia patients receiving (or recently receiving) intensive chemotherapy (excluding hydroxycarbamide or hypomethylating agents) | Yes | No | Situation does not apply to my clinical practice |
| Acute leukemia patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | Yes | No | Situation does not apply to my clinical practice |
| Acute leukemia patients without any disease modifying treatment (palliative setting) | Yes | No | Situation does not apply to my clinical practice |
| Aplastic anemia patients (with or without recent ATGAM and/or cyclosporin treatment, but excluding patients prior to or following allogeneic stem cell transplantation) | Yes | No | Situation does not apply to my clinical practice |

**Question 2**

Only to be answered if the corresponding item in question 1 was answered with 'yes'.

At what platelet count threshold do you in general administer prophylactic platelet transfusions to these hematological outpatients, given the absence of use of anticoagulants and platelet inhibitors? *Please, only select one answer per diagnosis/situation.*

| Hematology outpatient populations | Answer options | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MDS patients receiving (or recently receiving) intensive chemotherapy (excluding hydroxycarbamide or hypomethylating agents) | Only therapeutic transfusions (independent of platelet count) | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| MDS patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | Only therapeutic transfusions (independent of platelet count) | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| MDS patients without any disease modifying treatment | Only therapeutic transfusions (independent of platelet count) | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Acute leukemia patients receiving (or recently receiving) intensive chemotherapy (excluding hydroxycarbamide or hypomethylating agents) | Only therapeutic transfusions (independent of platelet count) | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Acute leukemia patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | Only therapeutic transfusions (independent of platelet count) | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Acute leukemia patients without any disease modifying treatment (palliative setting) | Only therapeutic transfusions (independent of platelet count) | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Aplastic anemia patients (with or without recent ATGAM and/or cyclosporin treatment, but excluding patients prior to or following allogeneic stem cell transplantation) | Only therapeutic transfusions (independent of platelet count) | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |

**Question 3**

To what extent do the following conditions influence your decision to initiate prophylactic platelet transfusions?

*1: No influence*
*2: Some influence*
*3: Moderate influence*
*4: Substantial influence*
*5: Complete influence*

| Clinical condition | Answer options | | | | |
|---|---|---|---|---|---|
| Clinically relevant bleeding during the past 3 months | 1 | 2 | 3 | 4 | 5 |
| Prior cerebral or cardiac ischemic event | 1 | 2 | 3 | 4 | 5 |
| Need or wish to continue platelet aggregation inhibitors | 1 | 2 | 3 | 4 | 5 |
| Need or wish to continue therapeutic (doses of) anticoagulants (LMWH, Vitamin K antagonist or DOAC) | 1 | 2 | 3 | 4 | 5 |
| Need or wish to continue prophylactic dose of LMWH | 1 | 2 | 3 | 4 | 5 |
| Angio-invasive mold infection (pulmonary or cerebral) | 1 | 2 | 3 | 4 | 5 |
| WHO performance score ≥2 *(definition: ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours)* | 1 | 2 | 3 | 4 | 5 |
| Outpatient clinic visits > 1 per week | 1 | 2 | 3 | 4 | 5 |

Abbreviations: LMWH = low molecular weight heparin, DOAC = direct oral anticoagulants

**Question 4**

In the presence of the following clinical condition: at what platelet count threshold do you, regardless of the specific hematological diagnosis, in general administer prophylactic platelet transfusions?

| Clinical condition | Answer options | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Clinically relevant bleeding during the past 3 months | No influence on platelet transfusion threshold | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Prior cerebral or cardiac ischemic event | No influence on platelet transfusion threshold | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Need or wish to continue platelet aggregation inhibitors | No influence on platelet transfusion threshold | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Need or wish to continue therapeutic (doses of) anticoagulants (LMWH, Vitamin K antagonist or DOAC) | No influence on platelet transfusion threshold | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Need or wish to continue prophylactic dose of LMWH | No influence on platelet transfusion threshold | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Angio-invasive mold infection (pulmonary or cerebral) | No influence on platelet transfusion threshold | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| WHO performance score ≥2 *(definition: ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours)* | No influence on platelet transfusion threshold | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |
| Outpatient clinic visits > 1 per week | No influence on platelet transfusion threshold | ≤10 | ≤20 | ≤30 | ≤40 | ≤50 | ≤80 | ≤100 | ≤150 |

Abbreviations: LMWH = low molecular weight heparin, DOAC = direct oral anticoagulants, WHO = World Health Organization

**Question 5**

Which other clinical parameters do you take into account when deciding on a prophylactic platelet transfusion regimen for hematological outpatients?
*Tick all that apply.*

- ☐ Albumin
- ☐ Hematocrit
- ☐ Liver enzymes / liver function
- ☐ CRP
- ☐ Leukocyte count
- ☐ Platelet count
- ☐ Chronic red blood cell transfusion dependency
- ☐ Fever i.e. temperature ≥38.5°C
- ☐ Fibrinogen
- ☐ Kidney function/urea
- ☐ Chronic or recurrent infections
- ☐ Other, namely.....

**Question 6a**

Whom of these outpatients suffering from a disease-related severe thrombocytopenia would you prescribe tranexamic acid?
*Please choose one answer per diagnosis/situation.*

| Hematology outpatient populations | Answer options | | | |
|---|---|---|---|---|
| MDS patients receiving (or recently receiving) intensive chemotherapy (excluding hydroxycarbamide or hypomethylating agents) | Yes, independently of presence of (clinically relevant) bleeding or bleeding tendency | Yes, but only in case of presence of (clinically relevant) bleeding or bleeding tendency | No | Situation is not applicable to my clinical practice |
| MDS patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | Yes, independently of presence of (clinically relevant) bleeding or bleeding tendency | Yes, but only in case of presence of (clinically relevant) bleeding or bleeding tendency | No | Situation is not applicable to my clinical practice |
| MDS patients without any disease modifying treatment | Yes, independently of presence of (clinically relevant) bleeding or bleeding tendency | Yes, but only in case of presence of (clinically relevant) bleeding or bleeding tendency | No | Situation is not applicable to my clinical practice |
| Acute leukemia patients receiving (or recently receiving) intensive chemotherapy (excluding hydroxycarbamide or hypomethylating agents) | Yes, independently of presence of (clinically relevant) bleeding or bleeding tendency | Yes, but only in case of presence of (clinically relevant) bleeding or bleeding tendency | No | Situation is not applicable to my clinical practice |
| Acute leukemia patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | Yes, independently of presence of (clinically relevant) bleeding or bleeding tendency | Yes, but only in case of presence of (clinically relevant) bleeding or bleeding tendency | No | Situation is not applicable to my clinical practice |
| Acute leukemia patients without any disease modifying treatment (palliative setting) | Yes, independently of presence of (clinically relevant) bleeding or bleeding tendency | Yes, but only in case of presence of (clinically relevant) bleeding or bleeding tendency | No | Situation is not applicable to my clinical practice |
| Aplastic anemia patients (with or without recent ATGAM and/or cyclosporin treatment, but excluding patients prior to or following allogeneic stem cell transplantation) | Yes, independently of presence of (clinically relevant) bleeding or bleeding tendency | Yes, but only in case of presence of (clinically relevant) bleeding or bleeding tendency | No | Situation is not applicable to my clinical practice |

**Question 6b**

Only to be answered if the corresponding item in question 6a was answered with 'yes'.

Tranexamic acid is started as follows:

- o Instead of prophylactic platelet transfusions
- o In addition to prophylactic platelet transfusions
- o Other, namely.....

**Question 6c**

If question 6a was not answered with "Yes" in any scenario, question 6c is not shown

To what extent do the following conditions influence your decision to initiate tranexamic acid?

*1: No influence*
*2: Some influence*
*3: Moderate influence*
*4: Substantial influence*
*5: Complete influence*

| Clinical condition | Answer options | | | | |
|---|---|---|---|---|---|
| Clinically relevant bleeding during the past 3 months | 1 | 2 | 3 | 4 | 5 |
| Prior cerebral or cardiac ischemic event | 1 | 2 | 3 | 4 | 5 |
| Need or wish to continue platelet aggregation inhibitors | 1 | 2 | 3 | 4 | 5 |
| Need or wish to continue therapeutic (doses of) anticoagulants (LMWH, Vitamin K antagonist or DOAC) | 1 | 2 | 3 | 4 | 5 |
| Need or wish to continue prophylactic dose of LMWH | 1 | 2 | 3 | 4 | 5 |
| Angio-invasive mold infection (pulmonary or cerebral) | 1 | 2 | 3 | 4 | 5 |
| WHO performance score ≥2 *(definition: ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours)* | 1 | 2 | 3 | 4 | 5 |

Abbreviations: LMWH = low molecular weight heparin, DOAC = direct oral anticoagulants

**Question 7**

Which other clinical parameters do you take into account when deciding on prescribing tranexamic acid to hematological outpatients?
*Tick all that apply.*

- ☐ Albumin
- ☐ Hematocrit
- ☐ Liver enzymes / liver function
- ☐ CRP
- ☐ Leukocyte count
- ☐ Platelet count
- ☐ red blood cell transfusion dependency
- ☐ Fever i.e. temperature ≥38.5°C
- ☐ Fibrinogen
- ☐ Kidney function/urea
- ☐ Chronic or recurrent infections
- ☐ Other, namely.....

**Question 8a**

What is your estimation on the 6-month risk for the occurrence of a clinically relevant bleeding in the setting of a disease-related severe thrombocytopenia and outpatient treatment if NO prophylactic platelet transfusions are administered?

| Hematology outpatient populations | Answer options | | | | | | |
|---|---|---|---|---|---|---|---|
| MDS patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |
| MDS patients without any disease modifying treatment | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |
| Acute leukemia patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |
| Acute leukemia patients without any disease modifying treatment (palliative setting) | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |
| Aplastic anemia patients (with or without recent ATGAM and/or cyclosporin treatment, but excluding patients prior to or following allogeneic stem cell transplantation) | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |

**Question 8b**

What is your estimation on the 6-month risk for the occurrence of a clinically relevant bleeding in the setting of a disease-related severe thrombocytopenia and outpatient treatment if prophylactic platelet transfusions are administered?

| Hematology outpatient populations | Answer options | | | | | | |
|---|---|---|---|---|---|---|---|
| MDS patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |
| MDS patients without any disease modifying treatment | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |
| Acute leukemia patients receiving hypomethylating agents (e.g. azacitidine, decitabine) | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |
| Acute leukemia patients without any disease modifying treatment (palliative setting) | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |
| Aplastic anemia patients (with or without recent ATGAM and/or cyclosporin treatment, but excluding patients prior to or following allogeneic stem cell transplantation) | 0-10% per 6 months | 10-20% per 6 months | 20-30% per 6 months | 30-40% per 6 months | 40-50% per 6 months | >50% per 6 months | Don't know |


3

# Chapter 3

## Thrombocytopenia and the effect of platelet transfusions on the occurrence of intracranial hemorrhage in patients with acute leukemia – a nested case-control study

Ann Hematol. 2021 Jan;100(1):261-271. doi: 10.1007/s00277-020-04298-7

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## Summary

We designed a study to describe the incidence of intracranial hemorrhage according to severity and duration of thrombocytopenia, and to quantify the associations of platelet transfusions with intracranial hemorrhage in patients with acute leukemia.

In this case-control study nested in a cohort of 859 leukemia patients cases (n=17) were patients diagnosed with intracranial hemorrhage who were matched with control patients (n=55). We documented platelet counts and transfusions for seven days before the intracranial hemorrhage in cases, and in a "matched" week for control patients. Three measures of platelet count exposure were assessed in four potentially important time periods before hemorrhage.

Among these leukemia patients we observed a cumulative incidence of intracranial hemorrhage of 3.5%. Low platelet counts were, especially in the three to seven days preceding intracranial hemorrhage, associated with the incidence of intracranial hemorrhage, although with wide confidence intervals. Platelet transfusions during the week preceding the hemorrhage were associated with higher incidences of intracranial hemorrhage; rate ratios (95% confidence interval) for one or two platelet transfusions, and for more than two transfusions compared to none were 4.04 (0.73 to 22.27) and 8.91 (1.53 to 51.73) respectively.

Thus, among acute leukemia patients, the risk of intracranial hemorrhage was higher among patients with low platelet counts and after receiving more platelet transfusions. Especially the latter is likely due to clinical factors leading to increased transfusion needs.

## Introduction

Patients with acute leukemia frequently suffer from bleeding events [1], of which intracranial hemorrhage (ICH) is one of the most serious [2-5]. Reported incidences of (symptomatic) intracranial hemorrhage vary between 2.8% up to 6.1% [2, 5, 6], and fatal intracranial hemorrhages explain more than 50 percent of fatal bleedings among acute leukemia patients [7].

Acute leukemia patients may develop intracranial hemorrhage due to various causes. Besides risk factors that also play a role in the general population, like age, hypertension, male sex and ethnicity [8-10], leukemia or cancer specific risk factors have been established. Among others, these are graft versus host disease, hyperleukocytosis and thrombocytopenia [11-14]. Of these, low platelet count is generally considered one of the most important risk factors for bleeding in hemato-oncological patients. It is, however, not conclusively established if, and at what platelet counts, the risk of intracranial hemorrhage increases in this patient population [2, 5-7, 12, 15, 16]. Moreover, prolonged exposure to low platelet counts (≤ $10x10^9$/L) may be associated with even higher bleeding risks [17, 18]. We hypothesized that longer periods with low platelet counts as well as lower (through) platelet counts can both determine an increasing risk on intracranial hemorrhage. If these time and trough measures are stronger associated with bleeding risk, this could have implications for future treatment strategies.

To prevent bleeding, hemato-oncology patients with low platelet counts are generally treated with prophylactic platelet transfusions [19-21]. The trigger to transfuse is commonly set at a platelet count of $10x10^9$/L [22-24]. Prophylactic platelet transfusions reduced the risk of bleedings in patients with a World Health Organization (WHO) score of ≥2 [25] from 50% to 43% [26], with most benefit for patients with acute myeloid leukemia or intensive chemotherapy treatment [16, 17, 27]. However, the large majority of bleeds is thus not prevented despite platelet transfusions. This raises questions about the causes of bleeding both when patients are treated with prophylactic platelet transfusions and also when they are not. Interestingly, recent high-level evidence suggestst that among neonates and among patients with hemorrahgic stroke, both prophylactic and therapeutic platelet transfusions may increase the risk of bleeding and/or mortality and morbidity [28, 29].

How exactly the depth and length of thrombocytopenia and the given platelet transfusions interact and modulate the risk of critical bleeding like intracranial hemorrhage is presently unknown.

Therefore, the objective of this exploratory study was to describe the association of platelet counts assessed in several time periods and severities with the incidence of intracranial hemorrhage in acute leukemia patients. Also, we wanted to examine the association between platelet transfusions and the incidence of intracranial hemorrhage.

## Methods

### Case identification and control selection

We performed a matched case-control study nested in a cohort of patients with acute promyelocytic leukemia, acute myeloid leukemia, acute lymphoblastic leukemia, or myelodysplastic syndrome in four hospitals in the Netherlands. Patients with intracranial hemorrhage were identified via an algorithm based on electronically available health care data [30]. Charts were reviewed to confirm the diagnosis and type of hemorrhage. All patients with confirmed intracranial hemorrhage were potential case patients for our study. Potential cases were excluded if no clinical data was retrievable, the date of bleeding was unclear, it was not the first intracranial hemorrhage, the diagnosis was unclear or unconfirmed, or if there were no eligible control matches possible.

For each case a minimum of one to a maximum of four control patients were selected from the same cohort, based on availability. The amount of four controls was chosen to ensure optimal power [31]. Controls were matched to case patients according to hospital, diagnosis, and indication for admission. For diagnosis, matching was performed on both the disease, as well as disease status (first diagnosis versus relapsed disease). Control patients with MDS could be matched to a patient with AML if the patient was treated according to an AML protocol, suggesting progression to AML. Matching was performed for several reasons. First, matching allows for correction of risk factors for bleeding that might be difficult to correct for in unmatched analysis. Second, matching on hospital was performed to correct for confounders that cannot easily be measured, for example differences in local treatment protocols.

### Implicated time periods and data collection

We studied exposures (thrombocytopenia/platelet transfusions) during the week preceding the event of intracranial hemorrhage and defined four potentially implicated time periods within that week: one, three, five and seven days preceding the hemorrhage. Date of bleeding (called “index date”) was defined according to the date of cerebral imaging as well as the date of neurological symptoms or consultation from a neurologist. Figure 1 illustrates the “implicated” periods for control patients; namely the week that coincided with the implicated period of the matched case patient on their timeline since the start of treatment if the patient was currently admitted for chemotherapy or stem cell transplantation. If the admission indication was a complication of former therapy or disease, the implicated period was counted from the first day of the current admission.

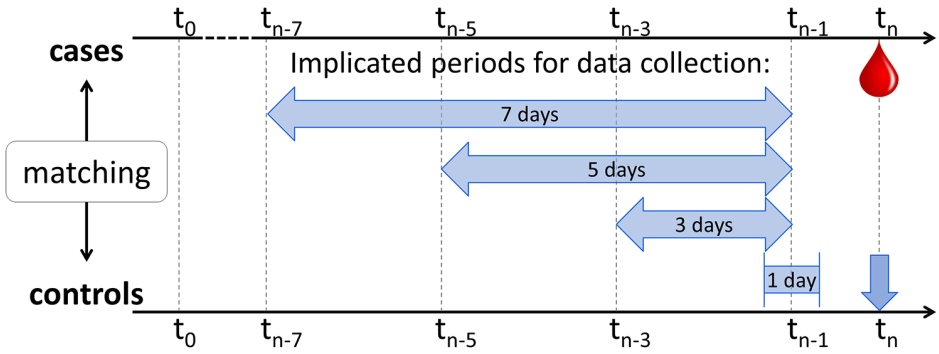


**Figure 1. Design: implicated time periods**
$t_0$ = first day of treatment if indication for admission was chemotherapy or stem cell transplantation, or day of admission if indication for admission was a complication of former treatment or disease. $t_n$ = index day: day of intracranial hemorrhage for cases, and corresponding day for controls. Matching was performed for hospital, diagnosis and indication for admission

We gathered laboratory data, transfusion data, and clinical variables of all cases and controls from the medical files (see supplementary material).

**Definitions of exposure categories for thrombocytopenia**

Three different measures of platelet count were defined to take into account both severity and duration of thrombocytopenia in the potential association between platelet count and intracranial hemorrhage (figure 2). These three measures were all assessed for each implicated time period.

First, the presence of one or more nadir platelet counts of ≤ $10x10^9$/L for each implicated period was assessed. As we were studying a seven-day period, a patient with at least one platelet count ≤ $10x10^9$/L could have between one and seven low platelet counts, for the 5-day period the value varied between one and five, etc.

Second, the presence of one or more nadir platelet counts of ≤ $20x10^9$/L for each implicated period was investigated.

Third, we calculated the percentage of hours with a platelet count ≤20 $x10^9$/L. All platelet counts measured were put on a time line. Any change in platelet counts, between two measured platelet counts, was assumed to be linear. Between actual platelet count measurements, for each hour the expected platelet count was interpolated. The number of hours this expected or measured platelet count was below ≤20 $x10^9$/L was expressed as a percentage of the total time in hours the patient was followed in all implicated time periods.

We intended to study percentage of days ≤ $10x10^9$/L, but too few patients had several days with platelet count ≤ $10x10^9$/L.

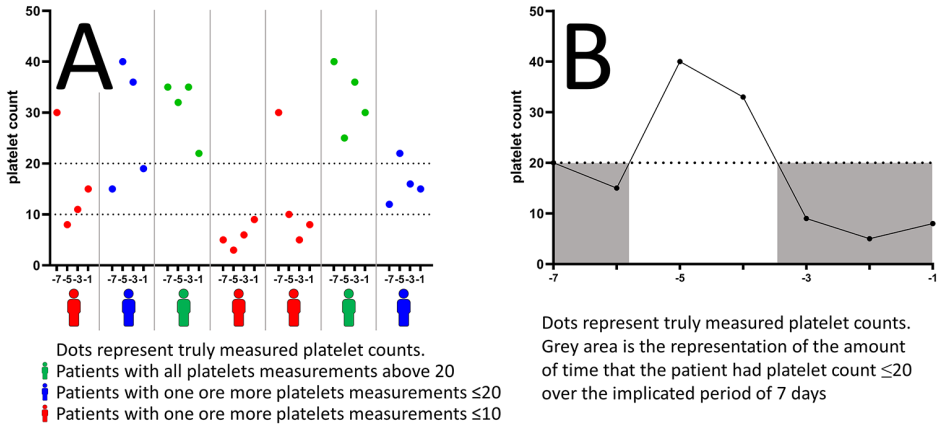


**Figure 2. Defined measures of platelet count**
Example data, for explanation of the used platelet count measures that are defined. All measures are obtained for all three predefined implicated time periods, but only the seven-day period is graphically presented.
Panel A: platelet measure of at least one platelet count ≤10x10$^9$/L or ≤20x10$^9$/L. Dots represent platelet counts at different days. Persons in red have at least one platelet count below the threshold of ≤10x10$^9$/L. Persons in blue have at least one platelet count within ≤10x10$^9$/L and ≤20x10$^9$/L and persons in green have no platelet counts below both thresholds. For the platelet measure of at least one platelet count ≤10x10$^9$/L persons in red were analyzed as 'yes', and for the platelet measure of at least one platelet count within ≤20x10$^9$/L persons in red and blue were analyzed as 'yes'.
Panel B: platelet measure of the percentage of hours with a (expected) platelet count ≤20x10$^9$/L. In the graph, dots represent truly measured platelet counts and the grey areas are the implicated time periods with such a (expected) platelet count. For every patient a timeline was made of all present platelet counts per implicated time period. We assumed a linear relation between the platelet count within 2 consecutive measurements, lines were therefore interpolated. For every hour between the first and the last measurement of platelet count, the expected measured platelet count was calculated. The percentage of hours with a (expected) platelet count ≤20x10$^9$/L was calculated afterwards. The reason we chose for hours ≤20x10$^9$/L, instead of the more clinical used trigger of hours below ≤10x10$^9$/L, was that we anticipated that the percentage of hours below ≤10x10$^9$/L would be very small, since this is a transfusion indication. Thus, it would lead to non-positivity.

### Measures of platelet transfusion

To provide an estimate of the association between platelet transfusions and the occurrence of intracranial hemorrhage we categorized the number of platelet transfusions (no transfusions, 1-2 transfusions, >2 transfusions) per period. These categories were selected since for intensively treated patients 1-2 platelet transfusions a week were expected to be a normal amount. Also, we explored the sum of platelet transfusions as a continuous variable per implicated period.

### Statistical analyses

We used conditional logistic regression models, which adjusts for matching variables, to assess the associations of the different measures of thrombocytopenia with the incidence of intracranial hemorrhage. In the adjusted analyses, we adjusted for one

potential confounding variable at the time. Since incidence-density sampling was used for selection of controls, odds ratios were interpreted as incidence rate ratios (RR) [32]. Because patients admitted for other indications then chemotherapy or SCT were more likely to have higher platelet counts, a post-hoc sensitivity analysis was performed excluding patients who were admitted for another reason than chemotherapy or SCT.

Also, for both categorical and continuous measures of platelet transfusions, conditional logistic regression was performed to assess the association between platelet transfusion and intracranial hemorrhage. This was adjusted for the different measurements of platelet counts that were defined.

Clinical factors can confound the association with intracranial hemorrhage. This was assessed via multivariable conditional logistic regression. The models combined one defined measure of platelet count or platelet transfusion with one clinical variable at a time.

Given that our sample size is small, the analyses are exploratory.



**Ethical considerations**

The medical ethical committee of the LUMC waived the need for informed consent (see section Declarations).

## Results

**Characteristics of the study population**

We identified 30 patients who had suffered an intracranial hemorrhage within the cohort of 859 patients with leukemia (cumulative incidence 3.5%). Thirteen patients had to be excluded for the predefined reasons presented in figure 3. Eventually, 72 patients (17 cases and 55 controls) were analyzed in the case-control study.

Distribution of values of matching variables and general characteristics across case and control patients are presented in table 1. In the case patients, acute myeloid leukemia was the most frequent diagnosis (65%) and with 77% the most frequent indication for admission was remission induction chemotherapy. Relapsed disease occurred in 29% of case patients, others had a first diagnosis. Due to frequency matching, a direct comparison between these percentages with percentages of matched, selected controls is not appropriate. The type of intracranial hemorrhage was most often intracerebral or subdural (both 35%). One patient suffered a subarachnoid hemorrhage (6%). Of the combined bleedings (24%) three patients had an intracerebral and subarachnoid hemorrhage, and one patient suffered from intracerebral and subdural hemorrhage. Two patients were prescribed tranexamic acid in the 7-day implicated period; one case patient and one control.

In total, 482 platelet count tests were performed for all cases and controls in the implicated seven day periods; of these, 56 (11.6%) were ≤10x10$^9$/L (from a total of 27 of 72 included patients) and 138 (28.6%) were ≤20x10$^9$/L (from a total of 43 of 72 included patients). Numbers of cases and controls with low platelets counts per implicated periods are given in table 2.

The median number of platelet transfusions per implicated period are presented in table 3. For the seven-day period, cases had a median of three transfusions (range 0 to 12) and controls a median of one transfusion (range 0 to 9). Other platelet product characteristics are presented in the supplementary material (table S1). In total, case patients received 95 platelet transfusions, and control patients 107. Besides a higher total percentage of irradiated platelet products in the case patients (51.6% versus 38.3% in control patients), platelet product characteristics did not differ relevantly between cases and controls.

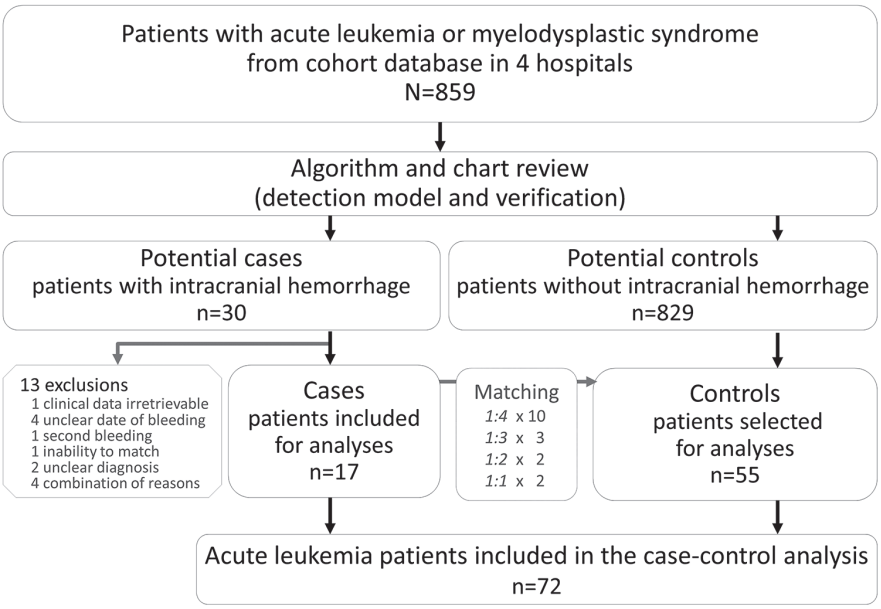


**Figure 3. Flow chart**
Inclusion period differed per hospital: hospital A June 2011 until March 2017, hospital B January 2010 until December 2015, hospital C January 2010 until December 2015, hospital D Jan 2013 until December 2015. Reasons for exclusion of 13 cases are specified. Unclear date of bleeding was encountered for example when a patient entered the hospital with non-acute neurological symptoms and intracranial hemorrhage was found on the day of admission. One patient excluded for unclear diagnosis never had a pathology result before death, one patient was initially diagnosed as acute leukemia but later classified as lymphoma. One patient had a second intracranial hemorrhage, which already altered transfusion policies. For one case with a double diagnosis of leukemia and intracerebral lymphoma no eligible match was found. Finally, four patients had a combination of above reasons. If more than four eligible controls were identified, controls closest to the case in calendar time were selected

**Table 1.** Characteristics of the study population*

| Matching variables | Cases n=17 | Controls n=55 | Total n =72 |
|---|---|---|---|
| **Diagnosis** | | | |
| ALL | 5 (29) | 19 (35) | 24 (33) |
| AML/MDS | 11 (65) | 35 (64) | 46 (64) |
| APL | 1 (6) | 1 (2) | 2 (3) |
| **First diagnosis or recurrent disease** | | | |
| First diagnosis | 12 (71) | 46 (84) | 58 (81) |
| Relapsed disease | 5 (29) | 9 (16) | 14 (19) |
| **Treatment phase** | | | |
| Remission induction | 13 (77) | 47 (86) | 60 (83) |
| Consolidation therapy | 1 (6) | 1 (2) | 2 (3) |
| Allogeneic SCT | 1 (6) | 1 (2) | 2 (3) |
| Other | 2 (12) | 6 (11) | 8 (11) |
| **Non-matching variables** | | | |
| **Sex** | | | |
| female | 8 (47) | 21 (38) | 29 (40) |
| male | 9 (53) | 34 (62) | 43 (60) |
| **Age§** | **65 (52 to 70)** | **57 (42 to 68)** | **58 (43 to 68.5)** |
| **Death†** | 8 (47) | 5 (9) | 13 (18) |

* Values are numbers (percentage of total) unless specified differently
§ Age in years, median (IQR)
† mortality not specific to bleeding (all-cause mortality)
Since controls are matched to cases, numbers presented for controls are dependent on control selection and therefore cannot be compared to numbers presented for cases.
ALL: acute lymphoid leukemia, AML: Acute myeloid leukemia, MDS: myelodysplastic syndrome, APL: acute promyelocytic leukemia, SCT: stem cell transplantation



### Platelet count and the incidence of intracranial hemorrhage

To assess the impact of thrombocytopenia on intracranial hemorrhage for the four implicated time periods, we correlated our three defined measures of platelet count with the incidence of intracranial hemorrhage (table 2).

When thrombocytopenia was defined as one or more count ≤10 x$10^9$/L, we observed that during the three, five and seven-day periods, the incidence of intracranial hemorrhage was higher after occurrence of such low platelet counts. For one or more count ≤20 x$10^9$/L the association was present in all implicated periods. However, the confidence intervals are mostly very wide, compatible with the possibility of the true association showing both higher and lower incidences. When we assessed the association between the occurrence of one or more platelet count below 10x$10^9$/L and intracranial hemorrhage in the seven-day period, we found an incidence rate ratio (RR)

of 1.79 (95% confidence interval (CI) 0.50 to 6.39). In case of one or more platelet counts below 20x10$^9$/L during the seven-day period, the RR was 4.21 (CI 0.83 to 21.26), meaning that patients with at least one platelet count below 20x10$^9$/L most likely had a 4.21 higher rate of intracranial hemorrhage compared to patients with no platelet counts below 20x10$^9$/L. For all the other time periods, RR's and CI's are given in table 2.

Low platelet counts often lead to transfusion, meaning that the occurrence of low trough levels as assessed above do not take the precise time of deep thrombocytopenia into account. To assess the impact of the amount of time with thrombocytopenia, we

**Table 2.** Presence of low platelet count measures in case control population and rate ratios for associations between three different measures for low platelet count and the incidence of intracranial hemorrhage during the next one, three, five and seven days among patients with acute leukemia

| Platelet count | Implicated time period | Cases n=17‡ | Controls n=55‡ | RR (95% CI) |
|---|---|---|---|---|
| **One or more platelet counts ≤10 x10$^9$/L** | | | | |
| | 1 day | 1 (6%) | 5 (9%) | 0.67 (0.06 to 7.00) |
| | 3 days | 6 (35%) | 12 (22%) | 1.94 (0.44 to 8.56) |
| | 5 days | 7 (41%) | 17 (31%) | 1.66 (0.41 to 6.79) |
| | 7 days | 8 (47%) | 19 (35%) | 1.79 (0.50 to 6.39) |
| **One or more platelet counts ≤20 x10$^9$/L** | | | | |
| | 1 day | 8 (47%) | 13 (24%) | 3.64 (0.91 to 14.58) |
| | 3 days | 10 (59%) | 22 (40%) | 2.33 (0.63 to 8.62) |
| | 5 days | 13 (76%) | 27 (49%) | 5.47 (1.08 to 27.75) |
| | 7 days | 13 (76%) | 30 (55%) | 4.21 (0.83 to 21.26) |
| **Percentage of hours platelet count ≤20 x10$^9$/L** | | | | |
| | 1 day | 6% (0% to 78%) | 0% (0% to 100%) | 1.01 (0.21 to 4.88) |
| | 3 days | 38% (0% to 51%) | 0% (0% to 69%) | 0.86 (0.16 to 4.47) |
| | 5 days | 28% (3% to 36%) | 0% (0% to 43%) | 1.90 (0.34 to 10.79) |
| | 7 days | 22% (2% to 33%) | 4% (0% to 41%) | 1.86 (0.30 to 11.57) |

‡ for **One or more platelet counts ≤10 x10$^9$/L or ≤20 x10$^9$/L** the numbers represent the number of distinct cases or controls with platelet count measurements below 10 and 20 and the percentage according to the total of cases or controls. For **Percentage of hours platelet count ≤20 x10$^9$/L** the numbers represent the median and interquartile range. **One or more platelet counts ≤10 x10$^9$/**L: measure that describes the presence of at least one platelet count ≤10 x10$^9$/L within every defined implicated time period. **One or more platelet counts ≤20 x10$^9$/**L: measure that describes the presence of at least one platelet count ≤20 x10$^9$/L within every defined implicated time period. **Percentage of hours platelet count ≤20 x10$^9$/L**: the percentage of the number of hours that platelet count was ≤20 x10$^9$/L from the total number of hours between the first and last measurement of platelet count in each implicated time period. To calculate the number of hours with a platelet count ≤20 x10$^9$/L, a linear trend within two actual measurements was assumed and for every hour the expected platelet count was interpolated. The percentage of hours with a platelet count ≤20 x10$^9$/L is a measure that describes duration of thrombocytopenia. Presented RR's are for a person with 100% of hours ≤20 x10$^9$/L, compared to 0% of hours. In the seven day period, for a patient with 25% of hours with a platelet count ≤20 x10$^{9L}$/, the RR would be $1.86^{0.25}$= 1.17, for a patient with 50% of hours with a platelet count ≤20 x10$^9$/L the RR would be $1.86^{0.50}$= 1.36, for 75% of hours ≤20 x10$^9$/L it would be $1.86^{0.75}$= 1.59, etc.

next assessed the association between the percentage of hours with a platelet count ≤20x10$^9$/L with the occurrence of intracranial hemorrhage. Since platelet count was not determined every hour, the percentage of hours with a platelet count ≤20x10$^9$/L was calculated after interpolation of truly measured platelet counts leading to an estimated measure per hour (see figure 2). For the seven-day period, patients with 100% of hours at a platelet count ≤20x10$^9$/L had a 1.86 (CI 0.30 to 11.57) higher rate of intracranial hemorrhage (reference 0%). This is the RR for 100% of the hours; for smaller percentages of hours this RR can be calculated. For example, for a patient with 25% of hours at a platelet count ≤20 x109L/, the RR would be $1.86^{0.25}$= 1.17. RR's for the other implicated periods for all three measures of platelet count are shown in table 2.



Most studies investigating bleeding risk in hemato-oncology patients take only patients receiving active treatment into account, not also patients who are admitted for treatment or disease related complications. We did include the latter patient population, and to see if this affected our results, a post-hoc sensitivity analysis excluding patients with other indications for admission then chemotherapy or stem cell transplantation was performed. This did not relevantly change the RR's for platelet count in intracranial hemorrhage (supplementary material: table S2).

Since there are potential confounding clinical factors that can influence the association of platelet count with intracranial hemorrhage, as predefined additional analysis we corrected all analysis above for these variables that were collected from the electronic patient files. Table S3 (online supplementary material) presents this corrected RRs for the association of the differently defined measures of platelet count with the incidence of intracranial hemorrhage. Overall, results did not differ relevantly and/or consistently over the time periods.

**Platelet transfusions and the incidence of intracranial hemorrhage**

Our findings indicated that the incidence of intracranial hemorrhage was higher in patients who had received platelet transfusions (table 3). The RR's for 1-2 platelet transfusions compared with 0 were between 2.16 (CI 0.37 to 12.55) and 4.04 (CI 0.73 to 22.27) for the different implicated periods. The latter, for example, is the RR for the seven-day implicated period, indicating that the most likely incidence of intracranial hemorrhage for a patient who received 1 or 2 transfusions was 4.04 higher compared with a patient without platelet transfusions. For patients who received > 2 platelet transfusions, RR's differed between 8.12 (CI 0.80 to 82.20) and 13.11 (CI 1.91 to 90.03) for the different implicated periods, so the incidence of intracranial hemorrhage was up to 13.11 times as high in patients who received more than two transfusions compared with none.

Platelet transfusions are given in case of low platelet counts, therefore they might be seen as a surrogate marker for thrombocytopenia. To assess if associations between

**Table 3.** Crude and adjusted rate ratios for the association between platelet transfusions and the incidence of intracranial hemorrhage among patients with acute leukemia

| | Number of platelet transfusions* | | Platelet transfusions | RR (95% CI) | |
|---|---|---|---|---|---|
| Implicated time period | Cases (n=17) | Controls (n=55) | Category | Crude | Adjusted for one or more platelet counts ≤10 x10$^9$/L |
| 1 day | 0 (0 to 2 ) | 0 (0 to 2 ) | 0 | Ref | Ref |
| | | | 1 to 2 | 3.86 (1.08 to 13.79) | 4.50 (1.20 to 16.90) |
| | | | >2 | - | - |
| 3 days | 2 (0 to 4) | 0 (0 to 5) | 0 | Ref | Ref |
| | | | 1 to 2 | 2.32 (0.60 to 9.01) | 2.36 (0.54 to 10.40) |
| | | | >2 | 8.12 (0.80 to 82.2) | 8.27 (0.73 to 93.51) |
| 5 days | 3 (0 to 9) | 1 (0 to 7) | 0 | Ref | Ref |
| | | | 1 to 2 | 2.16 (0.37 to 12.55) | 2.21 (0.32 to 15.23) |
| | | | >2 | 13.11 (1.91 to 90.03) | 13.36 (1.78 to 100.28) |
| 7 days | 3 (0 to 12) | 1 (0 to 9) | 0 | Ref | Ref |
| | | | 1 to 2 | 4.04 (0.73 to 22.27) | 4.09 (0.70 to 23.85) |
| | | | >2 | 8.91 (1.53 to 51.73) | 9.02 (1.47 to 55.49) |

* Platelet transfusions, median (range): number of platelet transfusions received by case and control patients per implicated period

platelet transfusion and intracranial hemorrhage were also independent of platelet counts, we adjusted for our defined measures of platelet count. The risk of increasing numbers of platelet transfusions on intracranial hemorrhage mostly stayed stable or increased in case of one or more platelet counts ≤10x10$^9$/L and percentage of hours with a platelet count ≤20x10$^9$/L. RR's decreased in case of one or more platelet counts ≤20x10$^9$/L, but the direction of the effect stayed the same (one or more platelet counts ≤10x10$^9$/L: see table 3, other measures of platelet count: see supplementary material, table S4). As an additional explorative and predefined analysis, we assessed if the association was similar when looking at the number of transfused platelets on a continuous scale, instead of the categorical scale. For all investigated implicated periods, the incidence rates of intracranial hemorrhage were higher with increasing number of units of transfused platelets (see supplementary material: table S5). The RR's ranged between 1.48 (CI 1.06 to 2.07) and 2.46 (CI 1.02 to 5.91) within the periods. These RR's are for one additional transfusion and increase rapidly if more transfusions are given. To illustrate, the crude RR for the seven-day period of 1.48 was for one additional platelet transfusion, if a patient had 2 platelet transfusions the rate ratio would be $1.48^2$=2.19, for 3 transfusions $1.48^3$=3.24 etc.

Finally, since we expected that clinical conditions might influence the found associations, we also explored if the RRs for the association between platelet transfusion

and intracranial hemorrhage was affected by potential confounders (see online supplementary material: table S6). Adjustment for some clinical variables did decrease or increase the incidence rate ratio in a potentially relevant manner, which showed consistent directions within implicated periods. This means that the variables fever, presence of a trauma like a fall or procedure, presence of non-intracranial bleedings and usage of antiplatelet or anticoagulant medication, were potentially relevant confounding variables based on our data.



## Discussion

In this case-control study among leukemia patients, we observed that one or more platelet counts below thresholds of both $10x10^9$/L and $20x10^9$/L, and an increasing percentage of hours below $20x10^9$/L were associated with intracranial hemorrhage, especially when low platelet counts occurred more than one day before the event of the hemorrhage. However, the estimates of these associations lacked precision. Platelet transfusions were also associated with the occurrence of subsequent intracranial hemorrhage; these estimates of association were likewise imprecise.

The point estimates of the association between all the defined measures of low platelet counts and the incidence of intracranial hemorrhage show a clear trend of higher incidences of intracranial hemorrhage when platelet counts are low. The most likely rate ratios are especially increased if platelet counts were low at three, five or seven days before the hemorrhage. In contrast, no increased incidence is seen in the period of one day before hemorrhage for two out of our three defined measures of platelet count. Although almost all point estimates go in the same direction, and an increased incidence of intracranial hemorrhage when platelet counts are low is thus most likely, the confidence intervals are wide, due to low numbers of patients. This means that the true effect size could lay in a wide range of values, from strongly harmful to even protective.

Quantitative evidence on the association between platelet counts and the occurrence of intracranial hemorrhage among patients with leukemia is scarce. Some reports focused on fatal intracranial hemorrhage [2, 5-7, 15]. One study did find an association between thrombocytopenia and the occurrence of intracranial hemorrhage in a subgroup of post allogeneic stem cell transplantation patients.[12] Two RCT's investigated the effect of prophylactic platelet transfusions on the occurrence of bleeding. Therapeutically treated patients had lower platelet counts compared to prophylactically transfused patients. One RCT did not find a difference in occurrence of grade 3 and 4 bleedings (including intracranial hemorrhage) [26], while the other did see more intracerebral hemorrhage in the therapeutically transfused group [16]. However,

the latter RCT had a different CT scan policy for both study arms, which likely reduced the number of confirmed intracranial hemorrhage in the control arm.

Moreover, most studies describe associations of bleeding with platelet counts of only one day, or do not clarify fully which platelet counts are taken into consideration for the analysis. However, it has also been suggested that there may be a longer lag time before low platelet counts can lead to bleeding [18]. Our results suggest that potentially a prolonged thrombocytopenia (three to seven days) is leading to more intracranial hemorrhages. Our study is as far as we know the first to define several implicated periods and several measures of platelet count, to investigate the association between both time and trough of low platelet counts and intracranial hemorrhage.

Platelet counts are not surprisingly strongly related with platelet transfusions in this patient population. Low platelet counts lead to transfusions, and transfusions affect future platelet counts. Since in this study we also saw an association between platelet transfusions and intracranial hemorrhage, ideally you would like to adjust for the potential confounding effect of platelet transfusions. However, this is extremely difficult, even if one would have a large dataset, given that platelet counts and platelet transfusions are so strongly interdependent, and that multiple platelet counts, and transfusions would need to be considered (see online supplementary material: figure S1). In our small sample size, such corrections are impossible.

In the present study, also platelet transfusions were associated with an increased incidence of intracranial hemorrhage, especially when > 2 transfusions were given in an implicated period.

Since low platelet counts are often the reason for platelet transfusion, we aimed to correct for the defined measures of platelet count. Due to the fact that patients often had multiple transfusions and multiple platelet count determinations, a reliable and complete correction is again not possible in our dataset. Nevertheless, by adding the different defined measures of platelet count into the model, we see that this did not influence the observed association between platelet transfusion and intracranial hemorrhage in our study. Therefore, we infer that it seems plausible that the need for platelet transfusions, or platelet transfusions itself in the circumstances where they are frequently needed, might increase the incidence of intracranial hemorrhage, and that this is at least partly independent of platelet counts. However, other clinical factors that lead to an increasing need for platelet transfusions, for example conditions leading to increased platelet consumption, are very likely responsible for the latter observed association with intracranial hemorrhage. To investigate the impact of such potential confounding clinical conditions we corrected for them by adding relevant clinical factors in the regression model. Indeed, we identified anticoagulation/antiplatelet therapy and other (non-intracranial) bleeding events as possible confounders. These were also previously suggested to increase bleeding risk in haemato-oncology patients [22]. For

causal interpretation, an extensive multivariable model in an individual patient data meta-analysis of studies like ours would be essential allowing adjustment for all confounding. Besides confounding, the observed association between platelet transfusions and intracranial hemorrhage may also be due to relative functional defects of the transfused platelets. Platelet concentrates are known to develop storage lesions, which can lead to reduced platelet quality [33, 34]. Moreover, one could argue that the transfusions contribute to intracranial hemorrhage by other mechanisms. Platelets do not only act in primary hemostasis, but also have immunomodulatory functions. Inflammation is likely to influence bleeding risks, especially in thrombocytopenic conditions [17, 35-39]. The idea that platelet transfusions lead to adverse outcomes, is indeed reported by two RCT's, both showing adverse effects on morbidity and mortality in very different patients populations, namely patients with an hemorrhagic cerebral vascular accident while using antiplatelet agents and thrombocytopenic neonates [28, 29]. The mechanisms behind these findings, however, are unclear. Finally, the observed associations could also be due to chance.



**Strengths and limitations**

A strength of this study is the matching of case and control patients on diagnosis and treatment. This allowed adjustment for these important known risk factors for this rare, but feared, bleeding complication.

Also, matching on hospital was performed to correct for confounders that are not easily quantified, like differences in local treatment policies. Additionally, we matched case and control patients on time after starting treatment or after the admission date. During admission, a leukemia patient is likely exposed to different platelet counts and other clinical risk factors, mostly determined by the exposure to intensive cytoreductive treatment. By matching case and control patients on time after therapy/admission, we minimized confounding by direct treatment effects.

Another asset of the study is the completeness of information for our main variables, namely platelet counts and platelet transfusions. A strong feature of the study is that we examined multiple measures of platelet counts during a week before the intracranial hemorrhage. With these different measures we could explore various possible influences of thrombocytopenia, like trough level and duration, on the incidence of intracranial hemorrhage during one, three, five and seven days before the hemorrhage. To the best of our knowledge, this has not been performed in other studies.

Finally, our study may be a novel framework which enables taking time-aspects, and thrombocytopenia severity into account. Our nested case-control study, that to our knowledge was not applied before, allowed exploration of effects of time and severity, via defining various implicated time periods for multiple measures of the

exposure on the outcome intracranial hemorrhage.

Our study has also some limitations. First, our sample size was too small to assess some potentially interesting and relevant measures of platelet count. Since patients are transfused as soon as platelet counts drop below 10 x$10^9$/L, the time below this value could not be sufficiently assessed. Even with a larger study population, the frequent transfusions would likely still minimize the amount of time ≤ 10 x$10^9$/L. Therefore, although this cut-off point it is the most widely used transfusion trigger, we could not assess the effect of time below 10 x$10^9$/L on the occurrence of intracranial hemorrhage.

Furthermore, as discussed earlier, due to the small sample size we could only correct for one variable at the time. Therefore, by the lack of multivariate analysis residual confounding remains. While we aimed to assess causality, although proving causality is never possible [40, 41], all results have to be interpreted as hypothesis generating only. Confirmation in larger studies will be necessary, although challenging due to rarity of intracranial hemorrhage. In addition, biological mechanisms should be investigated.

Also, we may have missed patients that acutely died due to severe intracranial hemorrhage, leading to potential bias. These patients remain undetected in the applied algorithm due to the absence of laboratory or additional diagnostics. The number of these missed patients is likely to be very limited. So, a relevant change of the findings is not to be expected, except for inducing a lower incidence of intracranial hemorrhage. Finally, given the retrospective nature of collecting data, it was not always possible to distinguish if platelet transfusions were truly prophylactic. Transfusion triggers were often not recorded clearly, and might have been higher than 10x$10^9$/L in case of an assumed higher bleeding risk.[22-24] Possibly also some therapeutic transfusions might have been included, if they were actually given for a unrecorded (probably minor) bleeding event. Patients who already need therapeutically platelet transfusions have proven to be more prone to bleeding, and thereby are likely to also have a higher risk for intracranial bleeding.

## Conclusion

In summary, we quantified the association between low platelet counts and the incidence of intracranial hemorrhage in leukemia patients. Longer periods of thrombocytopenia were associated with a higher risk.

The number of administered platelet transfusions was also associated with intracranial hemorrhage. Incidences especially increased for patients receiving >2 platelet transfusions. Nonetheless, this study cannot imply any causality between the

platelet transfusion and intracranial hemorrhage. More likely, our findings suggest that there is an association between platelet transfusion and other clinical risk factors that lead to an increased transfusion need. Indeed, this observed association should not lead to withholding prophylactic platelet transfusions. Future research needs to establish whether and when platelet transfusions or other possible preventive measures provide protection against intracranial hemorrhage among patients with leukemia or not.



## Declarations

### Compliance with Ethical Standards

This observational study used only routinely reported data. Data was collected by researchers who are trained medical doctors. There were no additional risks for the patients and all data was pseudonymized before analyses. The medical ethical committee of the LUMC judged the study not to meet the criteria for the Medical Research Involving Human Subjects Act (Dutch: Wet Medisch Wetenschappelijk Onderzoek met Mensen). Furthermore, the majority of patients were expected to be deceased. Obtaining an informed consent (from patients or relatives) was thought to be a larger burden and invasion of privacy than performing the study, with the available data, without informing the patients or their relatives. The need for informed consent was therefore waived by the ethical committee.

### Funding

The research was funded by an internal grant from Sanquin (grant number: PPOC-12-028 PRG).

**Conflicts of interest**

JLK receives a research subsidy from TerumoBCT. JK is scientific cofounder and shareholder of Gadeta and is inventor on multiple patents on gdTCRs, gdTCR ligands and isolation strategies. JK receives research support from Gadeta, Miltenyi Biotech and Novartis. JJZ is in the scientific advisory council of Novartis / Amgen / Sanofi and received a speakers fee, he also received a speakers fee from Vifor Pharma. The other authors declare no disclosures.

**Authors' contributions**

L.L.C. designed and performed research, analyzed and interpreted data, wrote the paper; A.L.K. designed and performed research, drafted and revised the paper; C.C-D. analyzed the data, designed the figures, drafted and revised the paper; R.A.M. designed research, interpreted data, drafted and revised the paper; P.A.B. drafted and revised the paper; K.M.K.V. drafted and revised the paper, E.A.M.B. drafted and revised the paper; J.L.H.K. designed research, drafted and revised the paper; J.J.Z. interpreted data, drafted and revised the paper; J.G.B. designed research, supervised statistical analysis, interpreted data, drafted and revised the paper.

**Availability of data and code**

Data and codes are accessible by the data management team. On request, after approval of the last author and if a legal data sharing agreement is arranged, data and / or codes might be transferred, without any identifying information of subjects.

**Acknowledgements**

We thank the medical ethical committee of the LUMC for their assessment and approval of the study (IRB number G16.067). We thank Peter Kemper for co-arranging the data retrieval from the hospitals. Furthermore, we thank the Scientific Committee of the Sanquin/LUMC Jon J van Rood Center for Clinical Transfusion Medicine for the advice and improvement of the statistical analysis plan, as well as the final paper.

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## Supplementary material

**Explanation data collection**

Laboratory and transfusion data were extracted from medical records electronically. Laboratory data consisted of all measured platelet counts, leukocyte counts and hemoglobin levels. Transfusion data consisted of all given platelet transfusions and red blood cell transfusions. Platelet products that were used were standard 5-donor buffy coat derived concentrates that were leuko-depleted. If there was a clinical indication, the product could be ABO matched, HLA matched or radiated.



Clinical data of the included case and control patients were collected by manual chart review. Data were entered using a secure online Case Report Form. All variables that could change over time were collected for every separate day in the week preceding the index date. Variables collected were:

- General characteristics: age, WHO performance score at admission, BMI, intoxications (alcohol and smoking), ABO blood group, hospital
- Bleeding characteristics (cases only): bleeding description, interventions after bleeding, date of clinically relevant bleeding (= index day, NB: controls also have a corresponding index day registered)
- Data about diagnosis and indication for admission: Diagnosis in groups, as well as described in conclusions, disease activity at index day (active disease, partial remission, complete remission), disease status (new, relapse, transformation etc.), indication for admission (e.g. remission induction chemotherapy, consolidation therapy, allogeneic stem cell transplantation (SCT), etc., all including description), transplantation details, date of start of treatment/admission
- Comorbidities: description of present comorbidities, need for usage of antihypertensive medication/ cholesterol-lowering medication/medication for diabetes mellitus/medication for ischemic heart disease, bleeding events reported in medical history before diagnosis, presence of graft versus host disease in index period
- Medication in 10 days before index day: e.g. anti-coagulant medication, antiplatelet medication, anti-infectious medication, chemotherapy, immuno-suppressive medication, etc.
- Infection data in 7 days before index day: highest temperature per day (in case temperature was higher than 38 degrees Celsius), presence or suspicion of infection, results of cultures and PCR's, active infection treatment, radiology results, infection in conclusion or differential diagnosis, etc.
- Non-intracranial bleedings in 7 days before index day: presence described in medical records, and if so, description and WHO bleeding grade.

- Transfusion data in 7 days before index day: Triggers for platelet and erythrocyte transfusions, prophylactic or therapeutic transfusions, number of transfused products, platelet refractoriness described
- Other possible risk factors in 7 days before index day: presence of trauma or intervention (including lumbar punctures), vomiting

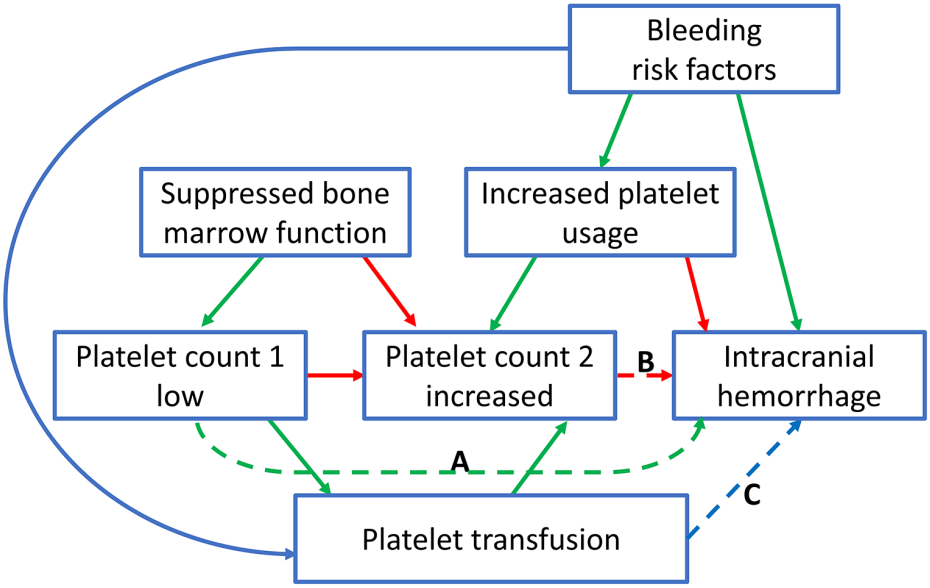


**Figure S1. Interdependence of platelet count and platelet transfusion**
Platelet count and platelet transfusion are clearly interdependent, making adequate correction for each other very hard.
Dotted lines represent the studied associations. Green arrows are (likely) inducing effects, while red arrows are likely inhibitors.
The **A** and **B** represent the studied associations between low platelet count(s) and intracranial hemorrhage.
Ad **A**. Low platelet counts before transfusions, are lower because of suppressed marrow function and by usage of platelets e.g. in hemostasis. The low platelet counts however increase (green arrow A) the risk of intracranial hemorrhage. Due to platelet count triggers the low platelet count 1 leads to platelet transfusions, which increases the platelet counts after the transfusion(s).
Ad **B**. The deeper the through of the low platelet counts the more the effect of transfusions on platelet count increase is mitigated. However, more increased post transfusion platelet counts inhibit intracranial hemorrhage.
The **C** represents the observed positive association between platelet transfusion and intracranial hemorrhage. However, this association is not easily to evaluate apart from the association A between low platelet counts and hemorrhage, since low platelet counts increases the number of transfusions. Also, the number of platelet transfusions is dependent on transfusion triggers, that can be adjusted based on bleeding risk factors: the association of platelet transfusion with intracranial hemorrhage might also be based on bleeding risk factors leading to increased transfusion needs/increased triggers.

**Table S1.** Distribution of platelet product characteristics

| Platelet product characteristics | Cases n=17 95 platelet transfusions | Controls n=55 107 platelet transfusions | Total n =72 202 platelet transfusions |
|---|---|---|---|
| **Irradiation*** | | | |
| Yes | 49 (51.6%) | 41 (38.3%) | 90 (44.6%) |
| No | 46 (48.4%) | 66 (61.7%) | 112 (55.5%) |
| **Number of donors*** | | | |
| Apheresis – single donor | 3 (3.2%) | 9 (8.4%) | 12 (5.9%) |
| Pooled – five donors | 92 (96.8%) | 98 (91.6%) | 190 (94.1%) |
| **Hyperconcentrated*** | | | |
| Yes | 13 (13.7%) | 11 (10.3%) | 24 (11.9%) |
| No | 82 (86.3%) | 96 (89.7%) | 178 (88.1%) |
| **Storage medium*** | | | |
| Plasma | 73 (76.8%) | 90 (84.1%) | 163 (80.7%) |
| Pas-C | 22 (23.2%) | 17 (15.9%) | 39 (19.3%) |
| **Storage time (days)§** | 4.3 (1.3) | 4.6 (1.6) | 4.4 (1.5) |

* Results are number (percentage)
§ Results are mean (standard deviation)
Individual patients could have received a combination of products for all categories.

**Table S2.** Crude rate ratios (RR) for platelet count on intracranial hemorrhage in total population of patients with all indications for admission, and in subgroup of patients who are admitted for chemotherapy or stem cell transplantation

| Platelet count | Index period | RR (95% CI) | |
|---|---|---|---|
| | | Total Population n=72 | Subgroup n=64 |
| **Percentage of hours platelet count ≤20 x10$^9$/L** | | | |
| | 1 day | 1.01 (0.21 to 4.88) | 0.79 (0.15 to 4.16) |
| | 3 days | 0.86 (0.16 to 4.47) | 0.71 (0.13 to 3.98) |
| | 5 days | 1.90 (0.34 to 10.79) | 1.72 (0.30 to 10.00) |
| | 7 days | 1.86 (0.30 to 11.57) | 1.66 (0.26 to 10.54) |
| **One or more platelet counts ≤20 x10$^9$/L** | | | |
| | 1 day | 3.64 (0.91 to 14.58) | 3.11 (0.75 to 12.90) |
| | 3 days | 2.33 (0.63 to 8.62) | 1.92 (0.50 to 7.45) |
| | 5 days | 5.47 (1.08 to 27.75) | 4.66 (0.88 to 24.54) |
| | 7 days | 4.21 (0.83 to 21.26) | 3.46 (0.66 to 18.10) |
| **One or more platelet counts ≤10 x10$^9$/L** | | | |
| | 1 day | 0.67 (0.06 to 7.00) | 0.67 (0.06 to 7.00) |
| | 3 days | 1.94 (0.44 to 8.56) | 1.94 (0.44 to 8.56) |
| | 5 days | 1.66 (0.41 to 6.79) | 1.66 (0.41 to 6.79) |
| | 7 days | 1.79 (0.50 to 6.39) | 1.79 (0.50 to 6.39) |

Post hoc sensitivity analysis, exclusion of all patients admitted for another indication than chemotherapy or stem cell therapy.
Total population cases n=17, controls n=55. Subgroup analysis cases n=15, controls n=49.

**Table S4.** Crude and adjusted rate ratios for the association between platelet transfusions and the incidence of intracranial hemorrhage among patients with acute leukemia

| | | | RR (95% CI) | | | |
|---|---|---|---|---|---|---|
| **Implicated time period** | **Category** | **n** | **Crude** | **adjusted for one or more platelet count ≤10 x10⁹/L** | **adjusted for one or more platelet count ≤20 x10⁹/L** | **adjusted for % of hours platelet count ≤20 x10⁹/L** |
| **Platelet transfusions** | | | | | | |
| 1 day | 0 | 52 | Ref | Ref | Ref | Ref |
| | 1 to 2 | 20 | 3.86 (1.08 to 13.79) | 4.50 (1.20 to 16.90) | 2.67 (0.63 to 11.33) | 2.32 (0.59 to 9.17) |
| | >2 | 0 | - | - | | - |
| 3 days | 0 | 39 | Ref | Ref | Ref | Ref |
| | 1 to 2 | 23 | 2.32 (0.60 to 9.01) | 2.36 (0.54 to 10.40) | 2.04 (0.43 to 9.59) | 2.94 (0.69 to 12.55) |
| | >2 | 10 | 8.12 (0.80 to 82.2) | 8.27 (0.73 to 93.51) | 6.96 (0.59 to 82.31) | 9.74 (0.92 to 103.04) |
| 5 days | 0 | 30 | Ref | Ref | Ref | Ref |
| | 1 to 2 | 24 | 2.16 (0.37 to 12.55) | 2.21 (0.32 to 15.23) | 1.41 (0.20 to 9.98) | 2.14 (0.35 to 13.08) |
| | >2 | 18 | 13.11 (1.91 to 90.03) | 13.36 (1.78 to 100.28) | 7.45 (0.88 to 63.07) | 12.99 (1.81 to 93.49) |
| 7 days | 0 | 23 | Ref | Ref | Ref | Ref |
| | 1 to 2 | 26 | 4.04 (0.73 to 22.27) | 4.09 (0.70 to 23.85) | 3.01 (0.44 to 20.48 | 4.06 (0.71 to 23.18) |
| | >2 | 23 | 8.91 (1.53 to 51.73) | 9.02 (1.47 to 55.49) | 6.50 (0.93 to 45.42) | 8.94 (1.49 to 53.69) |

**Table S5. Crude and adjusted rate ratios*, for the association between platelet transfusions (on a continuous scale) and the incidence of intracranial hemorrhage among patients with acute leukemia**

| Implicated time period | Crude | adjusted for one or more platelet counts ≤10 x$10^9$/L | adjusted for one or more platelet counts ≤20 x$10^9$/L | adjusted for % of hours PLT ≤20 x$10^9$/L |
|---|---|---|---|---|
| 1 day | 2.46 (1.02 to 5.91) | 2.61 (1.07 to 6.36) | 1.99 (0.78 to 5.07) | 1.80 (0.75 to 4.34) |
| 3 days | 1.92 (1.10 to 3.36) | 1.91 (1.08 to 3.40) | 1.90 (1.01 to 3.58) | 2.01 (1.18 to 3.62) |
| 5 days | 1.60 (1.08 to 2.37) | 1.58 (1.06 to 2.36) | 1.46 (0.99 to 2.15) | 1.58 (1.07 to 2.34) |
| 7 days | 1.48 (1.06 to 2.07) | 1.47 (1.05 to 2.05) | 1.40 (1.01 to 1.95) | 1.47 (1.06 to 2.05) |

*Rate ratios are to be interpreted as continuous, thus for example one platelet transfusion has a crude RR of $1.48^1$ for the seven-day period, two transfusions $1.48^2$, three transfusions $1.48^3$ and so on.

Table S3 and S6 can be viewed online, at the supplementary material section of doi: 10.1007/s00277-020-04298-7 *(Ann Hematol. 2021 Jan;100(1):261-271)*


4

# Chapter 4

## Association between cardiovascular risk factors and intracranial hemorrhage in patients with acute leukemia

Eur J Haematol. 2022 Apr;108(4):310-318. doi: 10.1111/ejh.13737

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## Abstract

**Background**

Intracranial hemorrhage is seen more frequently in acute leukemia patients compared to the general population. Besides leukemia related risk factors, also risk factors that are present in the general population might contribute to hemorrhagic complications in leukemia patients. Of those, cardiovascular risk factors leading to chronic vascular damage could modulate the occurrence of intracranial hemorrhage in these patients, as during their disease and treatment acute endothelial damage occurs due to factors like thrombocytopenia and inflammation.

**Objectives**

Our aim was to explore if cardiovascular risk factors can predict intracranial hemorrhage in acute leukemia patients.

**Methods**

In a case control study nested in a cohort of acute leukemia patients, including 17 cases with intracranial hemorrhage and 55 matched control patients without intracranial hemorrhage, data on cardiovascular risk factors was collected for all patients. Analyses were performed via conditional logistic regression.

**Results**

Pre-existing hypertension and ischemic heart disease in the medical history were associated with intracranial hemorrhage, with an incidence rate ratio of 12.9 (95% confidence interval (CI) 1.5 to 109.2) and 12.1 (95% CI 1.3 to110.7), respectively.

**Conclusion**

Both pre-existing hypertension and ischemic heart disease seem to be strong predictors of an increased risk for intracranial hemorrhage in leukemia patients.

## Introduction

Intracranial hemorrhage comprises intracerebral hemorrhage, subdural hemorrhage, epidural hemorrhage and subarachnoid hemorrhage.(1) In the general population, several risk factors have been described to be associated with the incidence of intracranial hemorrhage. These include male sex, higher age, African-American or Asian ethnicity, and trauma.(1-8) In addition, cardiovascular risk factors affecting the vascular wall, including hypertension, diabetes mellitus, smoking, and hypercholesterolemia have an effect on the risk of intracranial hemorrhage.(2, 4, 9, 10) More specifically, these cardiovascular risk factors are associated with intracerebral hemorrhage, while the other types of intracranial hemorrhage are more strongly associated with traumata or vascular malformations.(1)

Patients with acute leukemia have an increased risk for hemorrhage, including intracranial hemorrhage. Incidences of intracranial hemorrhage, during admission or follow-up in the outpatient clinic, are reported between 2.8% and 6.1%.(11-14) This incidence is much higher than what is observed in the general population, in which the incidence of intracerebral hemorrhage has been reported as 2.46/10,000 person-years.(15)

Specifically for acute promyelocytic leukemia (APL), a subtype of leukemia that is notorious for serious bleeding, it has been described that especially in the microvasculature of the brain, high annexin-2 and t-PA levels of the APL-cells contribute to intracranial hemorrhage.(16, 17) However, the biological mechanism underlying the high intracranial hemorrhage occurrence in the complete population of acute leukemia patients is not completely understood. Yet, in addition to disease and treatment associated thrombocytopenia, endothelial damage is known to be associated with an increased bleeding risk. The latter is likely to be a common phenomenon in this population, due to thrombocytopenia, inflammation, leukocytosis, graft versus host disease, and other disease and treatment related risk factors.(18-20) Therefore, all these factors could contribute to the observed increased risk of bleeding.(14, 21-26)

On top of these leukemia and treatment associated risk factors, other factors may also contribute to the occurrence of intracranial hemorrhage. Risk factors associated with chronic vascular damage in the general population can of course also be present in leukemia patients. Given the acute damage to their vessel walls, from which acute leukemia patients invariably suffer, the additional presence of pre-existing chronic damage to the vessel wall could act synergistically and be a relevant predictor of intracranial hemorrhage.

Therefore, we aimed to explore and estimate the predictive value of a history of hypertension, diabetes mellitus, hypercholesterolemia, ischemic heart disease, overweight, obesity and smoking with the occurrence of intracranial hemorrhage among patients with acute leukemia.

## Methods

### Case identification and control matching

To assess the association of cardiovascular risk factors and intracranial hemorrhage, we performed a matched case control analysis, in an existing nested case control population of acute leukemia patients. As previously described, we used an automated algorithm to identify potential cases of intracranial hemorrhage, from a database with routinely collected clinical data, extracted from the electronic patient records of a cohort of patients with acute leukemia or myelodysplastic syndrome (MDS).(26, 27) Seventeen identified cases could be included for case control analysis and were matched to one to four control patients (Figure 1). Matching was performed on several likely major risk factors for bleeding, which in an unmatched population could mask true associations of other predictors: diagnosis (acute promyelocytic leukemia (APL); other acute myeloid leukemia (AML) or MDS; acute lymphoid leukemia (ALL)); indication for admission (induction chemotherapy; consolidation chemotherapy; allogeneic stem cell transplantation (SCT); other indications for admission); disease status (first diagnosis; relapsed disease) and time from start of treatment to the day of bleeding. Matching was performed according to incidence density sampling (i.e. matched on time, expressed as days since admission or start of chemotherapy), so the odds ratio would directly estimate the incidence rate ratio.(28)

The medical charts of patients selected as potential controls were checked for the absence of intracranial hemorrhage until the date matched to the bleeding date of the case patient (i.e. the index date(26)). Case patients could be selected as control patient for other case patients, if the date of intracranial hemorrhage was later than the matched date of bleeding for that case. Per case, we selected up to four control patients, based on availability. In total, 55 controls were selected. The total case control population therefore contained 72 patients (Figure 1). For all these patients, more extensive data than was already available from the database of electronic patient records was obtained via chart review and added to the existing dataset.

### Variable definition

Information on hypertension, diabetes mellitus, dyslipidemia and ischemic heart disease was collected from the electronic patient record. Pre-existing hypertension was defined as any hypertension severe or persisting enough to lead to current use of antihypertensive medication, or registered medication use in medical history. Similarly, for diabetes mellitus and hypercholesterolemia evidence for any kind of glucose and cholesterol lowering medication current or in history was used. Mild diabetes or hypercholesterolemia, for example leading to lifestyle advices without the need for medication, were not included. Ischemic heart disease was defined as any prior diagnosis of ischemic heart disease.

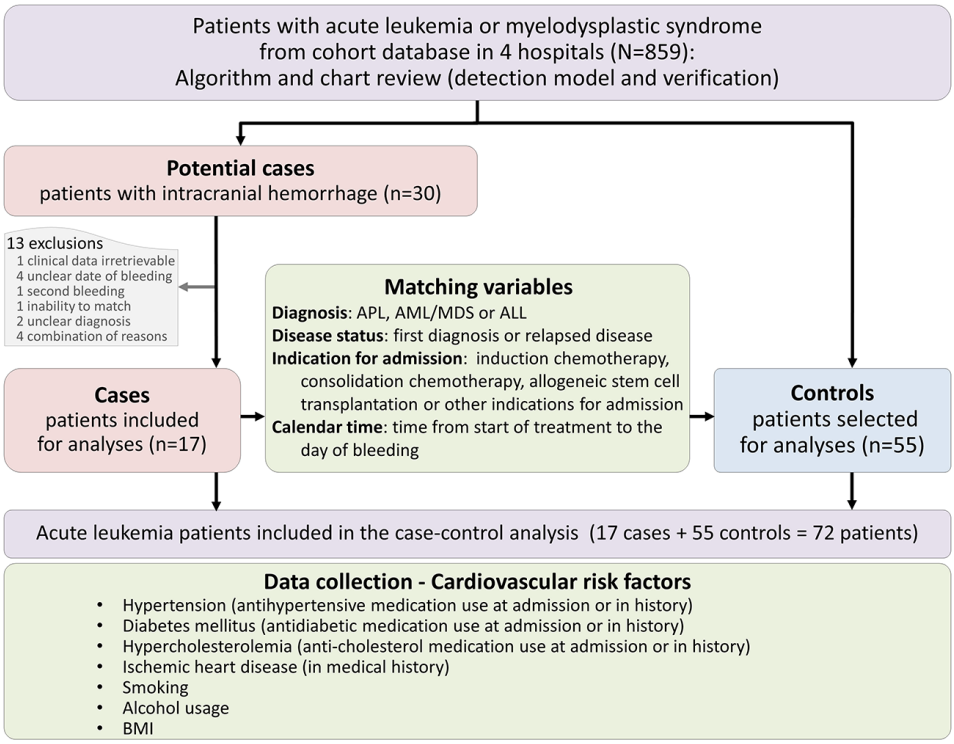


**Figure 1. Flowchart**
Excluded cases did not differ substantially from included cases (see supplementary material table S1), although, as reason for exclusion, part had unclear diagnoses and more of the excluded patients were admitted for other reasons then disease modifying treatment. Inclusion period differed per hospital: hospital A June 2011 until March 2017, hospital B January 2010 until December 2015, hospital C January 2010 until December 2015, hospital D Jan 2013 until December 2015.

To collect information on alcohol use (yes/no) and smoking (current smoker, past smoker, never smoked or unknown) the doctor's notes in the charts were reviewed. Finally, the body mass index (BMI) on admission was also obtained from the electronic patient records, and categorized as normal, overweight (BMI 25-30) and obesity (BMI>30).(29) Missing data during the chart review was recorded in the category 'unknown' for the variables smoking, alcohol use and BMI.

In addition to the matching criteria, other variables collected to describe the study population were sex, age in years, all-cause mortality during admission, platelet count and use of anticoagulant medication or platelet aggregation inhibitors. Since the two latter can differ in time, we defined an index date for both cases and controls.(26) The index date was the date of bleeding for case patients and a time matched date for control patients. For platelet count we both registered the platelet count on the day before the index date, as well as the lowest platelet count in a week preceding the

index date. We defined the use of therapeutically dosed anticoagulant medication or platelet aggregation inhibitors as at least one dosage of medication in the ten days preceding the index date.

**Prophylactic platelet transfusion policies**

All hospitals applied to the at that time available nationwide guideline for platelet prophylaxis,(30) and platelet transfusions were administered at platelet counts below $10x10^9$/L. Higher platelet count thresholds could be applied when it was deemed necessary, but were not protocolized. Altered thresholds were not always noted in the medical records.

**Statistical analysis**

Because matching procedures create spurious associations of variables that are directly or indirectly associated with the matching variables, we performed matched analyses to remove these false associations.(31) Univariate matched conditional logistic regression models were used for each potential predictor. To analyze associations of cardiovascular risk factors with intracerebral hemorrhage, as a subtype of intracranial hemorrhage, we performed prespecified subgroup analyses for only the cases with such a bleeding focus. Here, we included both patients who had a solitary intracerebral hemorrhage, as well as patients who, based on the radiology reports, had an intracerebral hemorrhage combined with another location of intracranial hemorrhage. Although we already matched on diagnosis, as a post-hoc analysis, we performed a subgroup analysis based on diagnosis to explore the influence of the underlying disease on the results.

**Ethical considerations**

The medical ethical committee of the Leiden University Medical Center (LUMC) approved the study and waived the need for informed consent, for retrospective data collection, as did the other participating hospitals. The statistical analyses plan was approved, prior to analyses, by the Scientific Committee of the Department of Clinical Epidemiology of the LUMC, consisting of epidemiologists and statisticians.

## Results

**Description of the case control study population**

The characteristics of the studied population are presented in Table 1. Since the case control ratio differed based on availability of eligible control patients, numbers and percentages are descriptive only and cannot be directly compared to those of case

**Table 1.** Characteristics of the study population

| Matched variables* | Cases n=17 | Controls n=55 |
|---|---|---|
| **Diagnosis** (n, %) | | |
| ALL | 5 (29%) | 19 (35%) |
| AML/MDS | 11 (65%) | 35 (64%) |
| APL | 1 (6%) | 1 (2%) |
| **First diagnosis or recurrent disease** (n, %) | | |
| First diagnosis | 12 (71%) | 46 (84%) |
| Relapsed disease | 5 (29%) | 9 (16%) |
| **Treatment phase** (n, %) | | |
| Remission induction | 13 (76%) | 47 (86%) |
| Consolidation therapy | 1 (6%) | 1 (2%) |
| Allogeneic SCT | 1 (6%) | 1 (2%) |
| Other | 2 (12%) | 6 (11%) |
| **Non-matched variables** | | |
| **Sex** (n, %) | | |
| female | 8 (47%) | 21 (38%) |
| male | 9 (53%) | 34 (62%) |
| **Age** † (median, IQR) | 65 (52 to 70) | 57 (42 to 68) |
| **Platelet count** (x$10^9$/L, median, IQR) | | |
| Day before index date ‡ | 21 (14 to 42) | 30 (16 to 71) |
| Lowest value in a week § | 11 (7 to 17) | 15 (9 to 55) |
| **Anti-coagulation and/or Platelet aggregation inhibitors** ¶ (n, %) | 5 (29%) | 5 (9%) |
| **Death**# (n, %) | 8 (47%) | 5 (9%) |

Values are numbers (percentage of total) unless otherwise specified.
* Since controls are matched to cases, numbers for matched variables presented for controls are dependent on control selection and therefore cannot be compared to numbers presented for cases. So, the observed similarities or differences between cases and controls, for these variables, is artificially induced by the number of eligible controls that were present, and cannot be interpreted in any other way.
† Age in years, median (IQR)
‡ lowest platelet count on the day before the index date (i.e. the date of bleeding for cases and a matched date for control patients)
§ lowest platelet count per patient, in the seven days before the index date
¶ At least one dose of platelet aggregation inhibitors or one therapeutic dose of anti-coagulant medication in an implicated period of 10 days before the index date. No patients were on double platelet aggregation inhibitors and/or anti-coagulation.
# all-cause mortality
Abbreviations: ALL: acute lymphoid leukemia, AML: Acute myeloid leukemia, MDS: myelodysplastic syndrome, APL: acute promyelocytic leukemia, SCT: stem cell transplantation, PAI: platelet aggregation inhibitors

patients. One AML case was matched to a control patient that was diagnosed with MDS, but treated as an AML.

For most baseline characteristics that were not matched, there were no relevant differences between case and control patients. All-cause mortality was substantially

higher in case patients; 47% deceased during admission, while this was 9% for the control patients. Also, cases more often use anti-coagulant medication or platelet aggregation inhibitors (29% in cases versus 9% in control patients).

**Cardiovascular risk factors**

The incidence rate ratio's (RR) and 95% confidence intervals (CI) for all cardiovascular risk factors are presented in Table 2. For hypertension, the rate ratio for intracranial hemorrhage was 12.9 (95% CI 1.5 to 109.2), indicating that patients on antihypertensive medication have a 12.9 times higher rate of intracranial hemorrhage compared to the patients who did not use or were never registered to use antihypertensive medication in their history. Additionally, ischemic heart disease was found to be associated with intracranial hemorrhage (rate ratio of 12.1; 95% CI 1.3 to 110.7).

The risk factors diabetes mellitus type 2 (no patients suffered from type 1), hypercholesterolemia, smoking, and alcohol use also showed a positive association with intracranial hemorrhage. However, the wide confidence intervals precluded any firm conclusions about these factors. Finally, overweight and obesity, with a rate ratio approaching unity, were not associated with intracranial hemorrhage.

**Subgroup analyses**

In the general population cardiovascular risk factors are particularly associated with the intracerebral subgroup of intracranial hemorrhages. To assess if this was also the case in our patients, we performed predefined subgroup analyses selecting patients with intracerebral hemorrhage. Ten case patients and their 27 matched controls could be included in these analyses. Of these, one case patient had a combined intracerebral and subdural hemorrhage, three case patients had a combined intracerebral and subarachnoid hemorrhage and six case patients only had an intracerebral hemorrhage focus. The results for these subgroup analyses are presented in Table 2. While variability of estimates increases, due to the decreased sample size, overall direction of associations remains, suggesting little to no difference for this subgroup, compared to the whole study population. The risk factors hypercholesterolemia and ischemic heart disease could not be estimated in the subgroup analyses due to non-positivity (i.e., some (sub)categories did not contain patients due to the reduced sample size).

In table S2, we present the RR for hypertension and ischemic heart disease per subgroup of diagnosis. Due to small numbers per subgroup not all RR's could be calculated, but the direction of the effect is similar in AML/MDS patients, and exclusion of patients with APL did not influence the results.

**Table 2.** RR's for cardiovascular risk factors on intracranial hemorrhage

| Cardiovascular risk factors | Main analysis cases with intracranial hemorrhage (any type) and matched controls (complete case control population) N=72 | | | Subgroup analysis only cases with intracerebral hemorrhage and matched controls N= 37 | | |
|---|---|---|---|---|---|---|
| | Cases | Controls | RR (95% CI) | Cases | Controls | RR (95% CI) |
| **Hypertension** | | | | | | |
| No | 9 (53%) | 45 (82%) | reference | 6 (60%) | 23 (85%) | reference |
| Yes | 8 (47%) | 10 (18%) | 12.9 (1.5 to 109.2) | 4 (40%) | 4 (15%) | 6.7 (0.7 to 64.1) |
| **Diabetes** | | | | | | |
| No | 15 (88%) | 52 (95%) | reference | 9 (90%) | 25 (93%) | ref |
| Yes | 2 (12%) | 3 (5%) | 2.7 (0.4 to 20.5) | 1 (10%) | 2 (7%) | 1.7 (0.1 to 30.8) |
| **Hypercholesterolemia** | | | | | | |
| No | 16 (94%) | 52 (95%) | reference | 10(100%) | 27(100%) | reference |
| Yes | 1 (6%) | 3 (5%) | 1.4 (0.1 to 16.4) | 0(0%) | 0(0%) - | † |
| **Ischemic heart disease** | | | | | | |
| No | 13 (76%) | 53 (96%) | reference | 8 (80%) | 27 (100%) | reference |
| Yes | 4 (24%) | 2 (4%) | 12.1 (1.3 to 110.7) | 2 (20%) | 0 (0%) - | † |
| **Smoking** | | | | | | |
| Never | 4 (24%) | 26 (47%) | reference | 3 (30%) | 14 (52%) | reference |
| Yes, currently or past | 8 (47%) | 16 (29%) | 3.5 (0.8 to 14.5) | 6 (60%) | 6 (22%) | 4.7 (0.7 to 32.2) |
| Unknown | 5 (29%) | 13 (24%) | 2.8 (0.6 to 13.0) | 1 (10%) | 7 (26%) | 0.9 (0.1 to 10.6) |
| **Alcohol use** | | | | | | |
| No | 3 (18%) | 16 (29%) | reference | 2 (20%) | 10 (37%) | reference |
| Yes | 9 (53%) | 22 (40%) | 2.0 (0.5 to 8.6) | 6 (60%) | 6 (22%) | 4.0 (0.6 to 25.5) |
| Unknown | 5 (29%) | 17 (31%) | 1.3 (0.3 to 6.0) | 2 (20%) | 11 (41%) | 0.4 (0.0 to 5.2) |
| **BMI** | | | | | | |
| <25 | 8 (47%) | 24 (44%) | reference | 6 (60%) | 15 (56%) | reference |
| 25-30 | 4 (24%) | 19 (35%) | 0.9 (0.2 to 4.3) | 1 (10%) | 6 (22%) | 0.6 (0.1 to 7.7) |
| >30 | 2 (12%) | 9 (17%) | 1.0 (0.2 to 6.4) | 1 (10%) | 3 (11%) | 2.3 (0.1 to 51.5) |
| Unknown | 3 (18%) | 3 (5%) | 7.2 (0.6 to 89.2) | 2 (20%) | 3 (11%) | 4.6 (0.2 to 94.2) |

Values represent the number of patients (%).
† no RR provided due to non-positivity
Hypertension: defined by need for antihypertensive medication at admission or in the medical history; Diabetes mellitus: defined as need for anti-diabetic medication in the medical history (so mild diabetes without need for medication is not included); High cholesterol levels: defined as need for cholesterol lowering agents at admission or in the medical history; Ischemic heart disease: defined as presence in medical history; Smoking: categorial variable divided in: yes (smoking currently or in past), no (registered as never smoked), unknown; Alcohol: categorical variable divided in: uses alcohol at all, never uses alcohol, unknown; BMI: body mass index score that was available at the day closest to the index date. <25 is a normal weight, 25-30 is overweight and >30 is obesity.

## Discussion

In this nested, matched case control study, we observed that in patients with acute leukemia, pre-existent hypertension and a history of ischemic heart disease were both strongly associated with an increased risk of intracranial hemorrhage.

Although the study has a small sample size, and therefore the precision of the magnitude of the association is suboptimal, a strength of our study is the matched case control study design with up to four controls per case patients. This design ensured optimal exploration of the included population, with a maximum of the potential power for intracranial hemorrhage as important bleeding outcome.(32) A limitation of our study is that, due to the chosen study objective and the small sample size, the observed associations cannot be explained as causality. The aim of our current study was to explore predictive values. However, it would also be of interest to study these associations etiologically, thus with correction for confounders. Yet, this would require a much larger dataset. Another limitation is that, given the matched case control design, predictive values, sensitivity and specificity of the cardiovascular risk factors could not be generated. For this purpose, a cohort study design would be necessary.

With a prevalence of approximately 31% in adults, (33) hypertension is highly prevalent in the general population, but even more so in patients with intracranial hemorrhage. It has been reported that 64% to 76% of patients with intracerebral hemorrhage and 38-42% of patients with other subtypes of intracranial hemorrhages were already diagnosed with hypertension prior to the intracranial hemorrhage.(34-36) This association could be explained by accumulating degenerative changes to the small vessels, resulting in an increased risk of ruptures of the small arterioles.(37, 38)

In our acute leukemia population, the risk of pre-existing hypertension, with a rate ratio of 12.90, shows to be substantially higher as compared to the general population. A meta-analysis of case control studies reported an overall odds ratio of 3.77 for the association between hypertension and the incidence of intracerebral hemorrhage in the general population.(2) However, a direct comparison of the meta-analyses data with our own results is not warranted for two reasons. First, the aforementioned meta-analysis included studies that analyzed only intracerebral hemorrhage while acute as well as pre-existing hypertension with and without need for medication were pooled. (2) The latter is important because hypertension might, next to indirectly via induction of chronic vascular changes, also be a direct acute cause of bleeding as well. Instead, we intended to analyze pre-existing hypertension only, defined as the need for medication at some point in medical history, but included all types of intracranial hemorrhage. Although it is likely that the increased risk is due to the specific leukemia population that was studied, these differences in bleeding outcomes and definitions of hypertension exposures may explain part of the difference in the magnitude of the

observed association between the former meta-analysis and our results. We did not include blood pressures during admission, since they may be affected by many factors that are only present during admission, and therefore may not reflect the level of pre-existing blood pressures. Second, given the wide confidence interval, the possibility that our result is overestimated also needs to be considered. However, given the high maximum likelihood estimation, it is most likely that the association of hypertension with intracranial hemorrhage in leukemia patients, is indeed higher as compared to the general population.

We also observed a similarly likely high association between ischemic heart disease in the medical history and intracranial hemorrhage. Indeed, patients with ischemic heart disease are known to have vascular damage of the coronaries, and are more likely to also have vascular damage elsewhere, like peripheral artery disease or cerebral vascular disease.(39) However, since five out of six patients who suffered ischemic heart disease in our population also (previously) used antihypertensive medication, our results for ischemic heart disease might also reflect the association of hypertension with intracranial hemorrhage.



The clear association of intracranial hemorrhage with cardiovascular risk factors like hypertension and ischemic cardiac disease that we observed, even in our small sample size study, was as we hypothesized. Whereas hematologists mostly focus on direct and mostly temporary leukemia specific risk factors, like biomarkers of hemostasis and coagulation, and clinical risk factors, this study demonstrates that also chronic pre-existing risk factors likely contribute to the bleeding risk. Leukemia and/or its treatment associated thrombocytopenia not only compromises platelet dependent high flow system hemostasis, but also vascular wall integrity. The latter can also be aggravated by the administered therapy, inflammation or concurrent infections and thus multiplicate the cardiovascular risk.(18, 19, 40-42) While the precise contribution of all these factors in the observed rate ratios of course needs further research, both long standing hypertension and ischemic cardiac disease could in our opinion be viewed as a proxy for general pre-existing arterial damage.(37-39) This pre-existing arterial damage, together with the acute risk factors for vascular damage and bleeding that is specific for (treated) leukemia, could logically add to the observed high risk in leukemia for intracranial hemorrhage. Hence, it can explain the association of intracranial hemorrhage with cardiovascular risk factors. Although our current study only demonstrates the predictive power of these two risk factors, it would also be of interest to investigate causal associations. This would, however, only be possible in a larger dataset, with sufficient power for multivariate adjusted analyses. Besides giving better clues for causality, larger datasets could in the future also lead to multivariate prediction models that include both the relevant chronic risk factors like hypertension, and transient leukemia associated risk factors or biomarkers of hemostasis and coagulation.

For the other CVD-associated conditions (diabetes mellitus, hypercholesterolemia, smoking, alcohol use), except for overweight or obesity, we also observed positive associations. Although in line with our further findings and hypothesis, our small sample size led to wide confidence intervals and hamper solid conclusions about the effect size and direction of the associations.

In the current study, we were unable to investigate if the predictive effect is different for patients with long term use of antihypertensive medication, or patients who need high dosages of these drugs. For example, we were unable to divide hypertension in groups of patients who had (a substantial period) of adequate hypertension control with medication, or patients who were still hypertensive while using antihypertensive medication. Since time of exposure to hypertension (with inadequate control) can contribute to the amount of vascular damage that is expected, this would be of interest to study in future. For the other risk factors, it would be also of interest to be able to divide into risk factors that are well controlled versus inadequate controlled for.

In addition, we investigated intoxications. For alcohol, although a less classical risk factor, not only the total amount but also the pattern of drinking has been described as cardiovascular risk factor.(43, 44) Although details on alcohol use were only available for 31 patients, high alcohol intake did not seem to differ between the cases and controls, with respectively one case (11%) on average drinking > 14 units of alcohol each week, compared to three (14%) of the control patients. A dose and time dependent relation is also known for smoking and the risk of cardiovascular disease.(45) Again, for only eight of our patients an estimation of the pack-years was available. Although for three cases the median package years was 35 (IQR 20 to 35) and for 5 controls the median was 25 (IQR 10-34), these data are insufficient for a corroborating conclusion.

We observed that patients with an intracranial hemorrhage died more often during admission compared to patients without intracranial hemorrhage. Although it has been described that intracranial hemorrhage in leukemia patients leads to a high mortality rate,(12) based on our data we cannot distinguish which patients died directly due to intracranial hemorrhage, and which patients had other causes of death.

While we currently focused on intracranial hemorrhage, being one of the most feared and serious bleeding events, patients with acute leukemia are also at risk for bleedings in other organ systems. It might be hypothesized that cardiovascular risk factors, besides increasing the incidence of intracranial hemorrhage, are also associated with other clinically relevant bleeding events. Future research should investigate this hypothesis.

## Conclusions

In conclusion, pre-existing hypertension and a history of ischemic heart disease seem strongly associated with intracranial hemorrhage in acute leukemia patients. Although we only studied their potential predictive power on intracranial hemorrhage, and we cannot claim any causal relations, the observed associations are in line with the known causality between cardiovascular risk factors and vascular damage.

If confirmed in larger data sets, with more precise estimates, these cardiovascular risk factors may eventually be used to identify leukemia patients with an increased risk for intracranial hemorrhage. The goal is to prevent this complication in these patients. Therefore, in patients with an increased risk, additional or altered bleeding preventive strategies should be studied, for example the effect of higher platelet transfusion thresholds or additional hemostatic medication. Also, the effect of stricter regulation of hypertension in leukemia patients should be investigated.



### Summary statements

1. What is the new aspect of your work?
   While most studies investigating intracranial hemorrhage in leukemia focus on leukemia specific characteristics, we investigate the predictive value of cardiovascular risk factors to see if to what extend these predictors in the general population also apply to the population with acute leukemia.
2. What is the central finding of your work?
   A history of hypertension and/or ischemic heart disease seem strong predictors of intracranial hemorrhage in acute leukemia patients; the magnitude of this effect is likely higher compared to the general population.
3. What is (or could be) the specific clinical relevance of your work?
   Especially pre-existing hypertension may help to identify leukemia patients with a high risk of intracranial hemorrhage.

**Conflict of interest statement**

JK works at an institution that received a fee by Miltenyi, Novartis, Gadeta. JLK works at an institution that received a research grant from Terumo BCT. The other authors declare no conflicts of interest.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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# Supplementary material

**Table S1.** Characteristics of included versus excluded cases for main analysis

| Matched variables | Included cases n=17 | Excluded cases n=13 |
|---|---|---|
| **Diagnosis** (n, %) | | |
| ALL | 5 (29%) | 2 (15%) |
| AML/MDS | 11 (65%) | 6 (46%) |
| APL | 1 (6%) | 2 (15%) |
| Unclear / unmatchable diagnosis | 0 (0%) | 3 (23%) |
| **First diagnosis or recurrent disease** (n, %) | | |
| First diagnosis | 12 (71%) | 7 (54%) |
| Relapsed disease | 5 (29%) | 2 (15%) |
| Unclear | 0 (0%) | 2 (15%) |
| **Treatment phase** (n, %) | | |
| Remission induction | 13 (76%) | 7 (54%) |
| Consolidation therapy | 1 (6%) | 0 (0%) |
| Allogeneic SCT | 1 (6%) | 0 (0%) |
| Other indications or untreated | 2 (12%) | 6 (46%) |

**Table S2.** RR's for a history of hypertension and ischemic heart disease on intracranial hemorrhage specified per diagnosis

| APL | | Total | Cases | Controls | RR (95% CI) |
|---|---|---|---|---|---|
| Hypertension | No | 2 (100%) | 1 (100%) | 1 (100%) | reference |
| | Yes | 0 | 0 | 0 | * |
| Ischemic heart disease | No | 2 (100%) | 1 (100%) | 1 (100%) | reference |
| | Yes | 0 | 0 | 0 | * |
| **AML/MDS** | | **Total** | **Cases** | **Controls** | **RR (95% CI)** |
| Hypertension | No | 31 (67%) | 5 (45%) | 26 (74%) | reference |
| | Yes | 15 (33%) | 6 (55%) | 9 (26%) | 8.3 (0.9 to 73.9) |
| Ischemic heart disease | No | 42 (91%) | 9 (82%) | 33 (94%) | reference |
| | Yes | 4 (9%) | 2 (18%) | 2 (6%) | 4.7 (0.41-54.4) |
| **ALL** | | **Total** | **Cases** | **Controls** | **RR (95% CI)** |
| Hypertension | No | 21 (88%) | 3 (60%) | 18 (95%) | reference |
| | Yes | 3 (12%) | 2 (40%) | 1 (5%) | * |
| Ischemic heart disease | No | 22 (92%) | 3 (60%) | 19 (100%) | reference |
| | Yes | 2 (8%) | 2 (40%) | 0 | * |
| **ALL or AML/MDS** | | **Total** | **Cases** | **Controls** | **RR (95% CI)** |
| Hypertension | No | 52 (74%) | 8 (50%) | 44 (81%) | reference |
| | Yes | 18 (26%) | 8 (50%) | 10 (19%) | 12.9 (1.5 to 109.2) |
| Ischemic heart disease | No | 64 (91%) | 12 (75%) | 52 (96%) | reference |
| | Yes | 6 (9%) | 4 (25%) | 2 (4%) | 12.1 (1.3 to 110.7) |
| **All diagnosis combined** | | **Total** | **Cases** | **Controls** | **RR (95% CI)** |
| Hypertension | No | 54 (75%) | 9 (53%) | 45 (82%) | reference |
| | Yes | 18 (25%) | 8 (47%) | 10 (18%) | 12.9 (1.5 to 109.2) |
| Ischemic heart disease | No | 66 (92%) | 13 (76%) | 53 (96%) | reference |
| | Yes | 6 (8%) | 4 (24%) | 2 (4%) | 12.1 (1.3 to 110.7) |

* Due to non-positivity or small group numbers, no relative risk could be calculated


5

# Chapter 5

## Expected individual benefit of prophylactic platelet transfusions in hemato-oncology patients based on bleeding risks

Transfusion. 2021;61:2578–87. https://doi.org/10.1111/trf.16587

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## Abstract

**Background**

Prophylactic platelet transfusions prevent bleeding in hemato-oncology patients, but it is unclear how any benefit varies between patients. Our aim was to assess if patients with different baseline risks for bleeding benefit differently from a prophylactic platelet transfusion strategy.

**Study design / methods**

Using data from the randomized controlled TOPPS trial (Trial of Platelet Prophylaxis), we developed a prediction model for World Health Organization grade 2, 3 and 4 bleeding risk (defined as at least one bleeding episode in 30 days) and grouped patients in four risk-quartiles based on this predicted baseline risk. Predictors in the model were baseline platelet count, age, diagnosis, disease modifying treatment, disease status, previous stem cell transplantation and the randomization arm.

**Results**

The model had a c-statistic of 0.58 (95% Confidence Interval (CI) 0.54 to 0.64). There was little variation in predicted risks (quartiles 46%, 47%, and 51%), but prophylactic platelet transfusions gave a risk reduction in all risk quartiles. The absolute risk difference (ARD) was 3.4% (CI -12.2 to 18.9) in the lowest risk quartile (quartile 1), 7.4% (95% CI -8.4 to 23.3) in quartile 2, 6.8% (95% CI -9.1 to 22.9) in quartile 3 and 12.8% (CI -3.1 to 28.7) in the highest risk quartile (quartile 4).

**Conclusion**

In our study, generally accepted bleeding risk predictors had limited predictive power (expressed by the low c-statistic), and, given the wide confidence intervals of predicted ARD, could not aid in identifying subgroups of patients who might benefit more (or less) from prophylactic platelet transfusion.

## Introduction

Patients with hematological malignancies often develop thrombocytopenia as a direct consequence of their disease and/or treatment regime. Thrombocytopenia is weakly associated with bleeding, varying from skin bleeds to major bleeding in organs, among others cerebral hemorrhage.[1]

Current guidelines recommend to administer prophylactic platelet transfusions to patients with hemato-oncological disorders at a platelet count threshold of <10x10$^9$/L to prevent bleeding.[2-6] Guidelines also recommend to consider giving prophylactic transfusions at higher platelet count thresholds if patients have an expected higher bleeding risk, or to withhold prophylactic transfusions if the bleeding risk is relatively low, for example in autologous stem cell recipients.[2,3] The quantification of bleeding risks, however, is not standardized, resulting in considerable variation in transfusion strategies in clinical practice.[2-7]

In the randomized controlled TOPPS trial (Trial of Platelet Prophylaxis), it was found that prophylactic platelet transfusions reduce bleedings with a World Health Organization (WHO) bleeding grade of 2, 3 or 4, compared to no-prophylactic platelet transfusions (i.e. therapeutic).[8,9] What is more, this beneficial effect differed between subgroups of patients with the least effect for patients receiving autologous stem cell transplantation (SCT).[10] Other clinical variables, like fever and sex, also seemed to influence the effect of prophylactic platelet transfusion on bleeding in this trial.[11]

Overall, there remains limited quantitative evidence on how prophylactic platelet transfusions reduce the bleeding risk differently in patients with likely divers a priori bleeding risks. Where trial results give a quantification of the effect of transfusion strategies for the 'average' patient in the trial population, in practice this 'average' patient does not exist. Average effects from a trial do not necessarily apply to individual patients, in whom the actual treatment effect may differ (heterogeneity of treatment effect).[12,13] Traditionally, heterogeneity of treatment effects is investigated by comparing subgroups of patients based on a single variable. However, combining multiple patient characteristics might enable a better personalized prediction of the effect of prophylactic platelet transfusions. For example, one can imagine that a female patient with acute leukemia who has a platelet count of 45x10$^9$/L before treatment receiving intensive cytoreductive chemotherapy will benefit more from a prophylactic platelet transfusion strategy than a male who receives an autologous SCT to treat lymphoma with a platelet count of 155x10$^9$/L at the day of admission. All these, and other, clinical factors when combined can contribute to a bleeding risk, and patients with different bleeding risks may benefit differently from platelet transfusions. To know this at the start of an intensive treatment regime, such as a SCT or chemotherapy, could potentially lead to more personalized prophylactic platelet transfusion strategies.

We therefore aimed to quantify effects of a prophylactic platelet transfusion strategy compared with a therapeutic platelet transfusion strategy on the occurrence of WHO grade 2, 3 or 4 bleeding stratified by predicted baseline bleeding risks of patients with hemato-oncological diseases.

## Methods

For this study, we used the data of the TOPPS trial. The design was described previously.[8,14] In short, 600 hemato-oncological patients were randomized in a prophylactic arm receiving platelet transfusions based on a threshold of $10x10^9$/L, and a therapeutic (or no-prophylaxis) arm receiving platelet transfusions in case of active bleeding. The primary outcome was the occurrence of WHO grade 2, 3 or 4 bleedings. The dataset for the analysis consisted of all 598 patients who were also included in the analysis of the TOPPS trial, of whom 47% (279 patients) developed at least one WHO grade 2, 3 or 4 bleeding during 30-day follow-up. Since we used previously collected data of one of the largest datasets for this subject, and larger trials are not likely performed in the future, no formal sample size calculation was performed for this post-hoc analysis of RCT data.

**Predictors of bleeding risk**

We developed a model to predict the risk of WHO grade 2, 3 or 4 bleeding within 30 days after randomization. To improve the stability of this model, we selected a limited number of baseline characteristics for inclusion in the model. The selection was made based on i) prior research that showed associations between the variables and the outcome, ii) the completeness of the data, iii) expert opinion. Selected variables were age at randomization, platelet count on day of randomization, sex, diagnosis (acute leukemia versus other), disease modifying treatment (chemotherapy/allogeneic SCT versus autologous SCT), disease status (new diagnosis versus relapsed disease), the presence of a SCT in medical history, and the randomization arm.[8,11,15-18] The randomization arm was added because ignoring treatments that affect the outcome in the prediction model can lead to an inaccurate predicted probability.[15,19] Thus, adding the randomization arm improves the prediction of the treatment effect in a heterogeneity of treatment effect analysis. Although proof of interactions cannot be obtained with the present sample size, based on clinical reasoning, interaction terms were included for the likely deemed interactions between prophylactic platelet transfusions and diagnosis, as well as for prophylaxis and treatment.

**Missing data**

Missing values were imputed. Given the low numbers of missing values (in total six subjects had one missing variable, Table 1), we imputed the modal value for missing categorical values. For the continuous variable platelet count, the subsequent value within three days of the randomization date of the same patient was used. If the value was unknown for these days, we imputed the median observed value of the other patients. To check robustness of the findings we performed sensitivity analyses in the subjects without missing values (n=592).

**Development of bleeding risk prediction model**

We developed a logistic regression model to predict the risk of WHO grade 2, 3 or 4 bleeding within 30 days after randomization.[20] To correct for optimism, we performed 'shrinkage' of all regression coefficients using penalized Ridge regression. The goal of this is to attempt to create a model that is better applicable to external datasets. Shrinkage in this respect diminishes the effect of all variables, which are likely overoptimistic in the original dataset.[21] The linearity assumption was visually checked for continuous variables; no quadratic terms or splines were deemed necessary.

After development of the model, we calculated the individual predicted 30-day risk of bleeding. For this step, to calculate the risk in absence of prophylactic transfusions for the complete population, we assumed a therapeutic platelet transfusion strategy for all patients, irrespective of their actual treatment allocation. This was necessary to be able to compare the risk with and without prophylaxis for the heterogeneity of treatment effect analysis described below, and enabled usage of the complete dataset for more power. All steps below were executed for a model without shrinkage (binary logistic regression) and for the penalized model (Ridge regression). Below, the results of the penalized model are presented; results for the crude model are presented in the supplementary material.

**Model predictive performance**

Performance of the model was expressed via the discriminative ability of the model (c-statistic), and as a visualization of the comparison between the predicted probability against the observed risk of bleeding (calibration plot).

**Heterogeneity of treatment effect analysis**

To assess the heterogeneity of treatment effects, patients were stratified in four quartiles by their predicted baseline risk. Within the quartiles, we examined heterogeneity of the effect of prophylactic versus therapeutic transfusions by estimating the odds ratio (OR) and the absolute risk difference (ARD) with 95% confidence intervals (95% CI) between the predicted number of bleedings with and without prophylactic transfu-

sions. These confidence intervals are to be considered as a measure of precision only. They were not used for formal statistical testing, given the application of Ridge penalization, and the exploratory nature of this study.

## Results

The baseline characteristics of participants in the TOPPS-trial are presented in Table 1. A minority of patients was diagnosed with acute leukemia (19%) and most patients received an autologous SCT (70%). Relapsed disease occurred in approximately 1/3 of patients, and 8% had a bone marrow transplantation in the past. 65% of patients were men, the median age was 58 years and the median platelet count at day of inclusion

**Table 1.** Baseline characteristics of randomized patients comparing characteristics for patients based on the occurrence of WHO grade 2, 3 or 4 bleeding

| | Total cohort (n=598) | No WHO grade 2, 3 or 4 bleeding (n=319) | WHO grade 2, 3 or 4 bleeding (n=279) | p-value¥ |
|---|---|---|---|---|
| **Age at inclusion (years)†** | 58 (49 - 63) | 57 (49 - 63) | 59 (51 - 64) | 0.1044 |
| **Platelet count day inclusion ($x10^9$/L)†** | 41 (30 - 50) | 41 (31 - 51) | 40 (29 - 50) | 0.3391 |
| **Male sex (%)** | 387 (65%) | 223 (70%) | 164 (59%) | 0.005 |
| **Diagnosis (%)** | | | | 0.421 |
| Lymphoma/myeloma/other | 482 (81%) | 261 (82%) | 221 (79%) | |
| Acute leukemia | 116 (19%) | 58 (18%) | 58 (21%) | |
| **Disease modifying treatment (%)** | | | | 0.726 |
| Autologous SCT | 420 (70%) | 226 (71%) | 194 (70%) | |
| Chemotherapy/allogeneic SCT | 178 (30%) | 93 (29%) | 85 (30%) | |
| **Disease status (%)** | | | | 0.407 |
| New diagnosis | 397 (66%) | 207 (65%) | 190 (68%) | |
| Relapsed disease | 201 (34%) | 112 (35%) | 89 (32%) | |
| **Stem cell transplantation in history (%)** | 45 (8%) | 26 (8%) | 19 (7%) | 0.535 |
| **Randomization arm (%)** | | | | 0.070 |
| Therapeutic arm | 300 (50%) | 149 (47%) | 151 (54%) | |
| Prophylactic arm | 298 (50%) | 170 (53%) | 128 (46%) | |

In total, 6 values were missing and imputed (one value per patient): platelet count was imputed for 3 patients, disease status for 1 patient and SCT in history for 2 patients.
† Median (interquartile range); ¥ p-value refers to Kruskal-Wallis equality-of-populations rank test when median is reported and Pearson's chi-squared for equality of proportions
Abbreviations: WHO= World Health Organization

**Table 2.** Multivariable analysis for primary outcome of WHO grade 2, 3 or 4 bleeding: odds ratios and 95% confidence intervals (CI), for both the crude model as the model after Ridge penalization.

| | Crude model OR (95% CI) | Odds ratio penalized model* |
|---|---|---|
| Age at inclusion | 1.01 (0.99 to 1.02) | 1.00 (0.99 to 1.02) |
| Platelet count on day inclusion | 1.00 (0.99 to 1.01) | 1.00 (0.99 to 1.01) |
| Female sex (ref = male) | 1.65 (1.17 to 2.33) | 1.27 (0.90 to 1.80) |
| Diagnosis acute leukemia (ref=lymphoma/myeloma/other) | 0.92 (0.37 to 2.31) | 1.00 (0.40 to 2.49) |
| Disease modifying treatment chemotherapy or allogeneic SCT (ref=Autologous SCT) | 0.74 (0.34 to 1.61) | 0.96 (0.44 to 2.09) |
| Disease status- relapsed disease (ref= new diagnosis) | 0.96 (0.66 to 1.38) | 0.96 (0.67 to 1.39) |
| SCT in history (ref = no ) | 0.82 (0.42 to 1.60) | 0.92 (0.47 to 1.80) |
| Randomization arm (ref = therapeutic) | 0.93 (0.63 to 1.38) | 0.81 (0.55 to 1.19) |
| Interaction term randomization arm and diagnosis | 1.45 (0.40 to 5.20) | 1.22 (0.34 to 4.40) |
| Interaction term randomization arm and disease modifying treatment | 1.72 (0.57 to 5.19) | 1.16 (0.38 to 3.49) |

*Ridge penalization method, confidence intervals are only to be interpreted as an indication of precision, not as a statistical test
Abbreviations: ref = reference category, WHO = Word Health Organization, SCT=stem cell transplantation



was 41x10$^9$/L. Women had more bleeding events (55%, compared to 42% of men) and as reported earlier,[8] the incidence of WHO bleeding grade 2, 3 or 4 was higher in the therapeutic arm (50%) compared with the prophylactic arm (43%). Results from table 1 were not used for variable selection for our prediction model (variable selection was pre-specified), but are only descriptive.

Table 2 shows the odds ratio (OR) for WHO grade 2, 3 or 4 bleeding for all selected variables in the multivariable model, with accompanying 95% CI's. After correcting for optimism via penalization, the point estimates of most variables were approximating an OR of 1. The complete model with intercept and all regression coefficients is presented in the supplementary material, as is the crude model before penalization.

The c-statistic of the model after penalization and internal validation was 0.58 (95% CI 0.54 to 0.63), indicating that when two random patients with different bleeding outcomes are chosen, in 58% the predicted bleeding risk was lower in the patient without bleeding compared to the patient with a bleeding event.[22] The calibration plot of our model is presented in Figure 1, the slope of the plot was 2.04 (0.76 to 3.32) with an intercept of -0.06 (-0.22 to 0.10). A good calibration would have a slope approximating 1. However, due to shrinkage of the prediction model, predicted probabilities were shrunken towards the group average and consequently the model appears to be underfitted (i.e. calibration slope >1) as is expected after penalization.

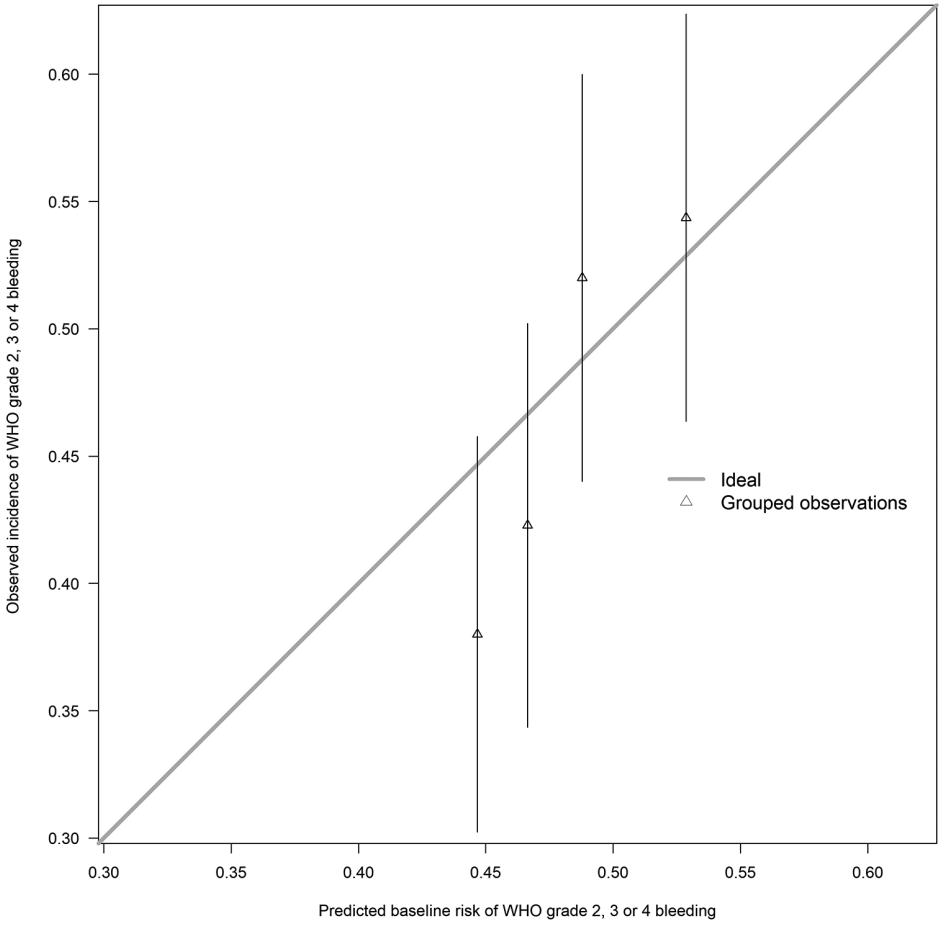


**Figure 1**
The triangles in this calibration plot of the predictions of WHO grade 2, 3 and 4 bleedings indicate the predicted probabilities and observed frequencies for all four risk quartiles (based on assumption of a therapeutic transfusion strategy). The diagonal line represents ideal calibration, when observed and predicted probabilities are identical. The calibration slope is 2.04 (0.76 to 3.32) with an intercept of -0.06 (-0.22 to 0.10). The c-statistic is 0.58 (0.53 to 0.62).
Abbreviations: WHO = Word Health Organization

Figure 2 shows the distribution of predicted baseline risk; all risks varied between 41% and 55%. Based on quartiles, four bleeding risk groups were defined: <46% (risk quartile 1), 46-47% (risk quartile 2), 47-51% (risk quartile 3) and >51% (risk quartile 4).

Figure 3 presents incidence rates of WHO grade 2, 3 or 4 bleedings, the OR's and risk differences when comparing the prophylactic strategy versus the therapeutic-only strategy for all patients. In all quartiles of baseline risk, the observed incidence of bleeding was higher if patients received therapeutic platelet transfusions (panel A). In panel B the

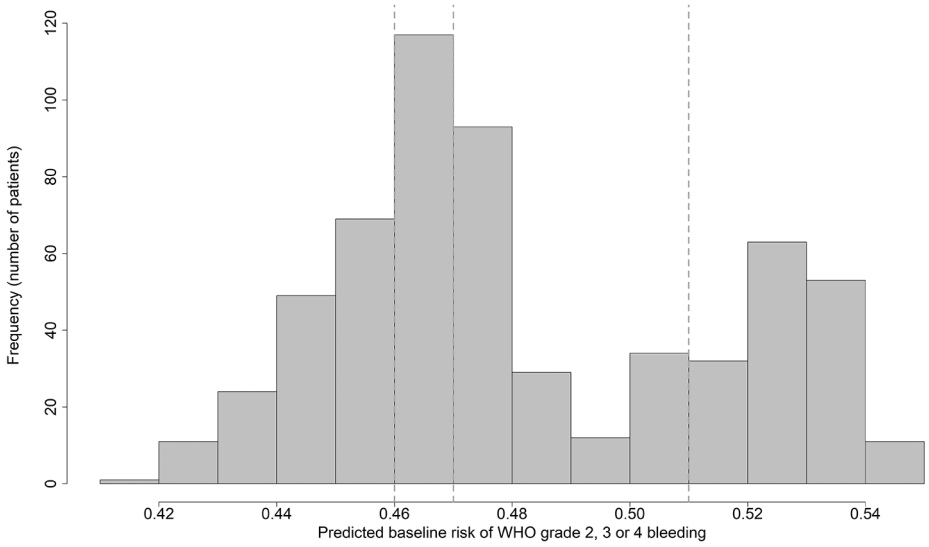


**Figure 2**
Predicted absolute risk of WHO grade 2, 3 or 4 bleeding (based on assumption of a therapeutic transfusion strategy) is represented as the absolute risk of outcome on the x-axis and the frequency of each absolute risk category (0.41-0.42, 0.42-0.43, etc.) in the trial population on the y-axis. The dotted lines represent the cut-off for the four quartiles of predicted risk on bleeding.
Abbreviations: WHO = Word Health Organization



OR's per quartile are displayed along with the overall odds ratio of the trial. For all quartiles, the OR is < 1, indicating a general benefit of prophylactic transfusions. The first risk quartile has an OR closer to 1, namely 0.87 (95% CI 0.45 to 1.68) compared to the overall OR (overall OR 0.74, 95% CI 0.54 to 1.03). In the fourth risk quartile the OR is more extreme compared to the overall OR, namely 0.59 (95% CI 0.31 to 1.14). The absolute risk difference (ARD, panel C) hence was most pronounced in the highest bleeding risk quartile (12.8%, 95% CI -3.1 to 28.7). This could indicate that patients in the highest risk quartile might benefit most from the prophylactic platelet transfusions, but given the wide confidence intervals this conclusion cannot be drawn on these current data. The ARDs in the other risk quartiles were 3.4% (95% CI -12.2 to 18.9), 7.4% (95% CI -8.4 to 23.3), and 6.8% (95% CI -9.1 to 22.9) respectively for risk quartiles 1, 2 and 3.

As a sensitivity analysis, we performed a complete case analysis using information about the 592 subjects with complete information. Results were comparable to those of the analysis of all 598 subjects (see supplementary material).

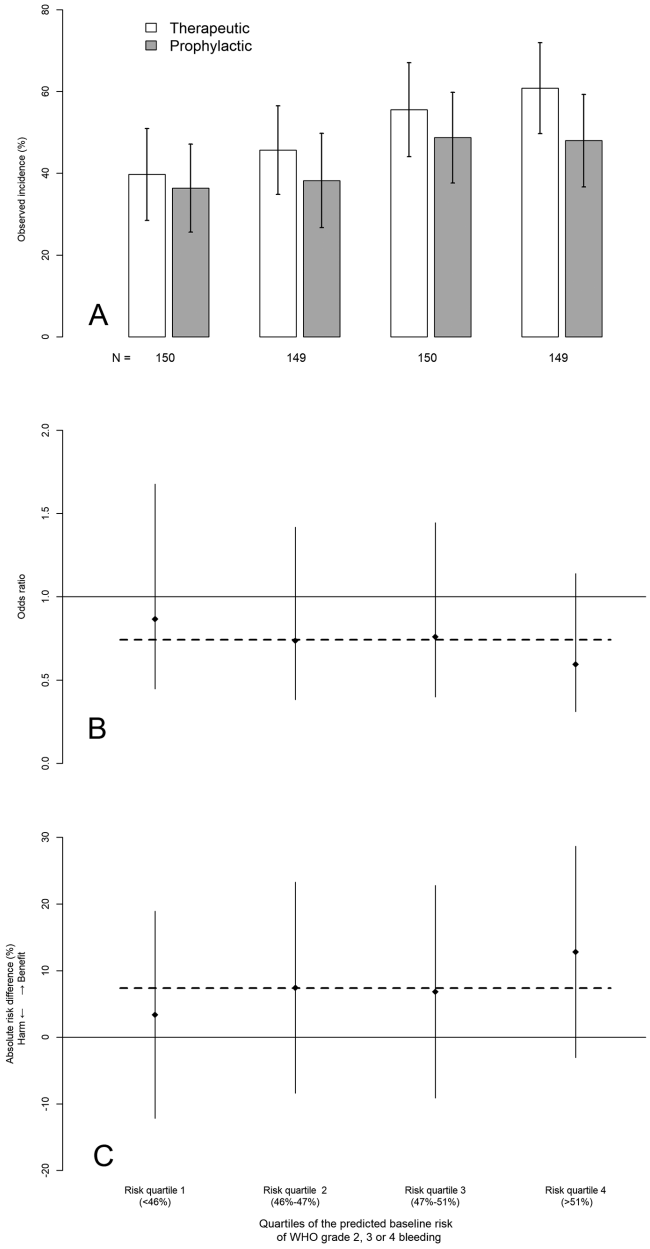


**Figure 3**
Event rates (panel A), odds ratios (panel B) and absolute risk differences (panel C) are presented for all four risk quartiles, comparing a prophylactically and therapeutically platelet transfusion strategy with respect to WHO grade 2, 3 or 4 bleeding. Vertical lines represent 95% confidence intervals, horizontal dotted lines represent overall trial results. A positive absolute risk reduction represents the risk decrease for a prophylactic platelet transfusion strategy as compared to a therapeutic platelet transfusion strategy.
Abbreviations: WHO = Word Health Organization

## Discussion

In this post-hoc analysis of the TOPPS trial, we aimed to assess if patients with different baseline risks for WHO grade 2, 3 or 4 bleeding might benefit differently from prophylactic platelet transfusions. We found that a combination of generally accepted predictors of bleeding risk did not have much predictive power, as indicated by the low c-statistic and the small variation in risks across the risk quartiles. Although the absolute risk difference was most substantial in patients with the highest baseline bleeding risks, these differences were not statistically significant. Based on these analyses we cannot at baseline identify subgroups of patients who benefit more or less than the average effect found in the TOPPS trial.

Originally, we expected that a combination of variables could predict bleeding risk accurately, and that patients with higher bleeding risk would show a larger benefit of prophylactic transfusion. This hypothesis was based on analyses suggesting that several baseline characteristics are associated with the outcome of bleeding in hemato-oncological patients in single variable subgroup analysis.[2,10,11] From this analysis also a limited benefit for prophylactic platelet transfusions was shown for patients receiving an autologous SCT as compared to those patients receiving chemotherapy or an allogeneic SCT.[10,23,24].



However, our combined analysis of the earlier suggested baseline risk factors for bleeding in our study, was not strongly related with bleeding. Looking at e.g. platelet count, we included the baseline value since our aim was to predict bleeding at baseline. We hypothesized that a 'low platelet count at baseline' might be predictive of 'low platelet counts during admission', the latter known to be associated with bleeding risk.[8,11,23,25] More specific, both disease modifying treatments and diagnosis in the context of all other baseline risk factors, did not relevantly influence the predicted bleeding risk. Our bleeding risk prediction model therefore has a poor discriminative ability.[26] This is reflected in the low c-statistic, but also in the small range of predicted risks, namely between 41% and 55%. There are several possible explanations for this lack of predictive power reported in our analysis.

First, a potential explanation for the poor discriminative ability of the model is that our baseline characteristics contained mostly dichotomized variables. Incorporating more continuous baseline variables leads to more variation in predicted risks, but besides age and platelet count, no other continuous variables at baseline were selected beforehand to be likely predictors.

A second possible explanation may be that the sample size was not sufficient to capture the differences to actually identify the nuances in predictive values.

Thirdly, an important explanation could be that, although the included variables were shown to be associated with bleeding in isolation, bleeding is obviously influenced

by additional factors than baseline demographics alone. Instead, bleeding risk might be much better predicted by combining the baseline characteristics with characteristics that vary during treatment. Examples of such time-varying variables, which were not included in our model, are nadirs and averages of low platelet counts during admission, transfusion yields, but also a range of clinical factors such as concurrent infections and mucositis. In addition to clinical variables, biomarkers of platelet function, coagulation or endothelial function that reflect hemostasis could add to the predictive performance of the model. Such biomarkers could be baseline values as well (either inherited or acquired), or time varying during treatment. Thus, further research of time-varying variations should preferably also focus on biomarkers for hemostasis, as potential predictors for bleeding in our patients.[27-30]

However, adding such dynamic characteristics was not part of the present research question while time-dependent modelling likely needs even larger data sets than even that of the TOPPS trial. When such datasets become available in the future, the predictive performance and the clinical applicability of such time-varying bleeding prediction models, that require more frequent re-evaluation of bleeding risk compared to a baseline bleeding risk model, needs to be shown.

In our model, all patients regardless of the predicted bleeding risk benefited from the prophylactic transfusions. The absolute risk differences varied between 3.4% for quartile 1 (patients with the lowest predicted risk) and 12.8% for patients in quartile 4. Although beforehand a larger benefit in the highest risk groups was expected, with the small range of predicted bleeding risk and the wide confidence intervals and based on the included baseline characteristics solely, we cannot conclude that the benefit for patients truly differs between the risk quartiles. Our findings, despite of the limitations of our risk prediction model, can be of importance for clinicians to realize that in our study of almost 600 participants even a combination of baseline risk factors could not distinguish between subgroups with different prophylaxis effects. Of course if in future better bleeding risk discrimination becomes possible, the benefit of prophylactic platelet transfusions needs to be differentially assessed again.

There are some additional limitations that should be considered in our analysis. Firstly, in both our current as well as the original subgroup analysis of the TOPPS RCT – as in any study –, unmeasured confounding of the subgroup effect is possible, meaning that an observed subgroup effect cannot be causally attributed to the subgroup.[31] The odds ratios we present in Table 2 only serve a prediction purpose, and should not be mistaken as evidence for a real causal (in this regard a weak protective) effect of the variable on the risk of bleeding. In that regard, it is also important to clarify that in a shrunken prediction model, the wide confidence interval of the variable 'randomization arm' as presented in table 2 does not mean that the original results of the TOPPS trial should be viewed differently.

A further intrinsic limitation of our study is that our predictive model was developed and tested in the same dataset. Although necessary because qualitative good and large datasets are not easily available, this can lead to an over-optimistic model.[32,33] We tried to minimize this 'overfitting' by applying Ridge penalization. This technique shrinks the regression coefficients towards zero, which aims to result in a more reliable model when applied to other datasets. This strategy to (partly) correct the optimism of the model, comes at the cost of having predicted risks that are too close to the group average risk. Indeed, there was overfitting of the data in the original logistic regression model, and substantial shrinkage was needed. Earlier studies suggest that the more shrinkage is needed, the harder it will be to estimate the amount of shrinkage that is required.[21] What is more, Ridge regression confidence intervals do not have their usual interpretation and are solely reported to show the spreading of the results. All in all, similar as the crude model, the predictive performance of the penalized model remained poor (respectively 0.59, 95% CI 0.55 to 0.64 and 0.58, 95% CI 0.53 to 0.62). Therefore, we conclude that independent of additional penalization, baseline risk factors are suboptimal for predicting relevant bleeding.

The decision to divide patients in quartiles based on their predicted risk was made since such a risk categorization is described in literature before.[20] Looking at more than four groups, moreover, is likely increasingly impractical for clinical practice. Furthermore, more numerous categories would negatively affect the power of analyses leading to probably no additional information from such.

Strengths of this study are that this study is the first to investigate if the beneficial effect of prophylactic platelet transfusions in hemato-oncological patients differs in patients with varying baseline bleeding risks, the latter based on a combination of readily available patient characteristics. Also, a strong suit of our analysis is that instead of a subgroup analysis based on a single variable, we considered many characteristics that likely influence each other, which can lead a more accurate prediction of personalized treatment effects.[12,13] This allows for a more comprehensive evaluation of bleeding risk prediction in this population. In addition, with this technique, besides the odds ratio, we were able to estimate absolute risk differences, which is described to be of greater clinical relevance compared to a relative scale.[12] Another strength is the fact that we predefined all included variables and analysis, instead of statistical selection procedures, to avoid overfitting.[34] Lastly, a major asset of our study is that it is performed in a high quality RCT dataset. Indeed, with 598 patients the TOPPs study is one of the largest studies investigating platelet prophylaxis in this patient population.[8]

In summary, baseline risk factors have low discriminative ability to predict bleeding. With the limitations of the poor prediction of our model leading to uncertainty of our conclusions, patients in all risk groups seemed to benefit from a prophylactic platelet transfusion strategy. While patients in a higher risk group seem to benefit more, we

could not provide statistical evidence for this. Future models that incorporate dynamic (time-dependent) clinical characteristics and biomarkers of hemostasis and endothelial disruption may support better prediction of bleeding, and influence the expected individual benefit for patients with different bleeding risk in time. However, so far and based on this study, we are unable to identify patients with more or less benefit of prophylaxis. Therefore prophylactic platelet transfusions should remain a standard practice for most hemato-oncological patients who receive intensive therapy, although recognizing that many patients continue to experience bleeding events despite prophylaxis.

**Acknowledgements**

We would like to thank Cara Hudson (from NHSBT Clinical Trials Unit) for assisting with the data management. Also, we would like to thank all the centers that participated in the TOPPS trial.

**Funding**

The main trial was funded by the National Health Service Blood and Transplant Research and Development Committee and the Australian Red Cross Blood Service.



**Conflict of interest**

JJZ is in a scientific advisory council of Sanofi and received a speaker's fee. LLC, CCD, SFF-G, RHHG, SJS, and JGvdB have disclosed no conflicts of interest.

**Ethics and approval**

The original study protocol was approved by independent ethics committees in the United Kingdom and Australia. The current statistical analysis plan was approved by the science committee of the department of Clinical Epidemiology of the Leiden University Medical Center, in Leiden, the Netherlands. The main trial was registered on Controlled-Trials.com number, ISRCTN08758735.

**Authors contributions**

LLC designed the study, interpreted the results, and wrote the manuscript; CCD designed the study, interpreted results, analyzed the data and revised the manuscript; SFFG advised on the study design and revised the manuscript; RHHG advised on study design/statistical procedures, reviewed the statistical procedures and revised the manuscript; SS designed the TOPPS study and revised the manuscript; JJZ designed the study, interpreted results and revised the manuscript; JGB designed the study, interpreted results and revised the manuscript.

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## Supplementary material

### Complete models

### Crude model

Logit(P (WHO bleeding grade 2, 3 or 4)) = -0.5824† + (0.0081 * age of inclusion) + (0.0031 * platelet count on day of inclusion) + (0.5022 * sex) + (-0.0794 * diagnosis) + (-0.0708 * randomisation arm‡) + (0.3703 * diagnosis * randomisation arm) + (0.5429 * disease modifying treatment * randomisation arm)

### Penalized model

Logit(P (WHO bleeding grade 2, 3 or 4)) = -0.2631† + (0.0035 * age of inclusion) + (0.0013 * platelet count on day of inclusion) + (0.2398 * sex) + (-0.0025 * diagnosis) + (-0.0417 * disease modifying treatment) + (-0.0398 * disease status) + (-0.0788 * SCT in history) + (-0.2138 * randomisation arm‡) + (0.2014*diagnosis * randomisation arm) + (0.1466 * disease modifying treatment * randomisation arm)

### Add the following numbers in formula

| | |
|---|---|
| Age of inclusion: | Age in years |
| Platelet count on day of inclusion: | Platelet count, ...x$10^9$/L |
| Sex: | Female =1, Male =0 |
| Diagnosis: | Acute leukemia =1, Lymphoma, Myeloma or Other =0 |
| Disease modifying treatment: | Chemotherapy or allogeneic SCT =1, Autologous SCT =0 |
| Disease status: | Relapsed disease =1, New diagnosis =0 |
| SCT in history: | Yes =1, No =0 |
| Randomization arm‡: | Prophylactic =1, Therapeutic =0 |

†The intercept of the models represents the risk for patients who would have the value zero for all variables in the model, even for age and platelet count. It therefore is not applicable for any individual patient but could be seen as a baseline risk to which can be altered in both directions based on the true values of the other variables.
‡ The randomization arm was added because ignoring treatments that affect the outcome in the prediction model can lead to an inaccurate predicted probability.(1) Since the original TOPPS paper found that the therapeutic transfusion arm was on average inferior to the prophylactic transfusion arm, the predicted risk of bleeding could be lower than the 'true' risk when not taking the randomization arm into account.
Abbreviations: WHO = Word Health Organization, SCT = stem cell transplantation

1. Groenwold RH, Moons KG, Pajouheshnia R, Altman DG, Collins GS, Debray TP, et al. Explicit inclusion of treatment in prognostic modeling was recommended in observational and randomized settings. J Clin Epidemiol. 2016;78:90-100.

## Sensitivity Analyses

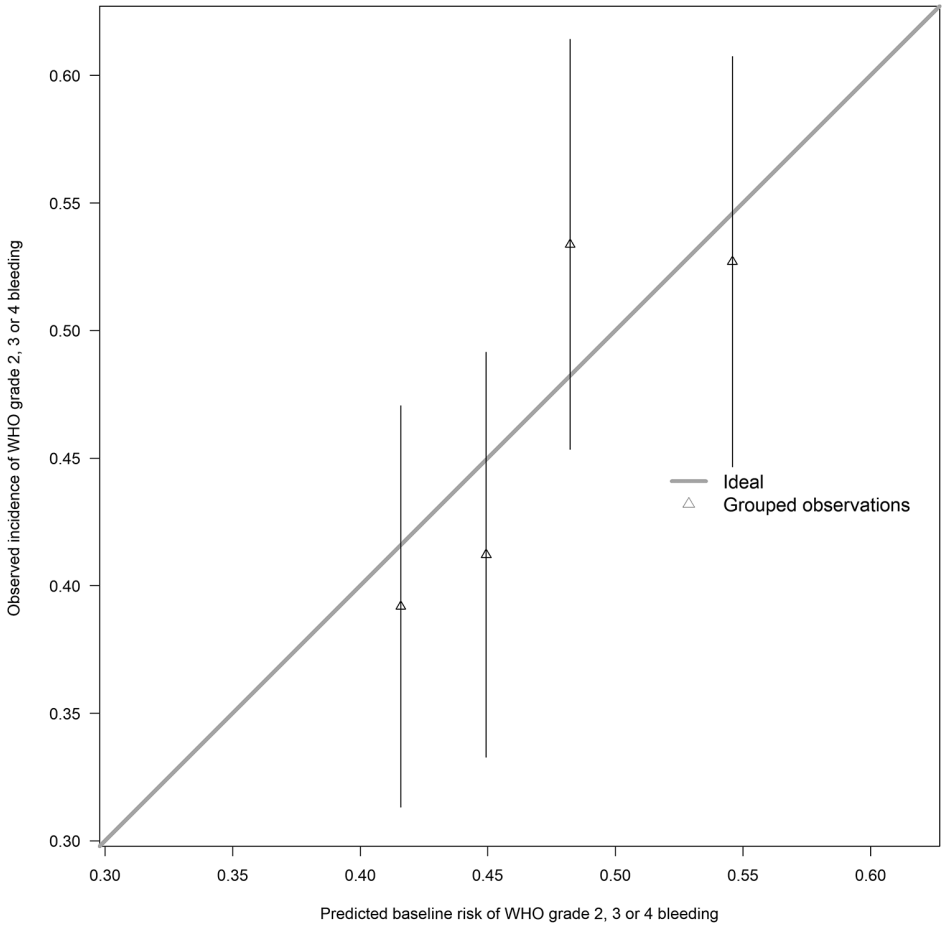


**Figure S1. Calibration plot of predictions of WHO grade 2, 3 or 4 bleeding in complete case analysis (n=592)**

Validity of predictions of WHO grade 2, 3 or 4 bleeding in complete case analysis. The triangles indicate the predicted probabilities and observed frequencies for all four risk quartiles (based on assumption of a therapeutic transfusion strategy). The diagonal line represents ideal calibration, when observed and predicted probabilities are identical. The calibration slope is 1.25 (0.43 to 2.06) with an intercept of -0.03 (-0.19 to 0.13). The c-statistic is 0.57 (0.53 to 0.62).

Figure S1 is comparable to figure 1, meaning that imputing baseline values when missing (n=6) did not influence our results in a relevant matter.

Abbreviations: WHO = Word Health Organization

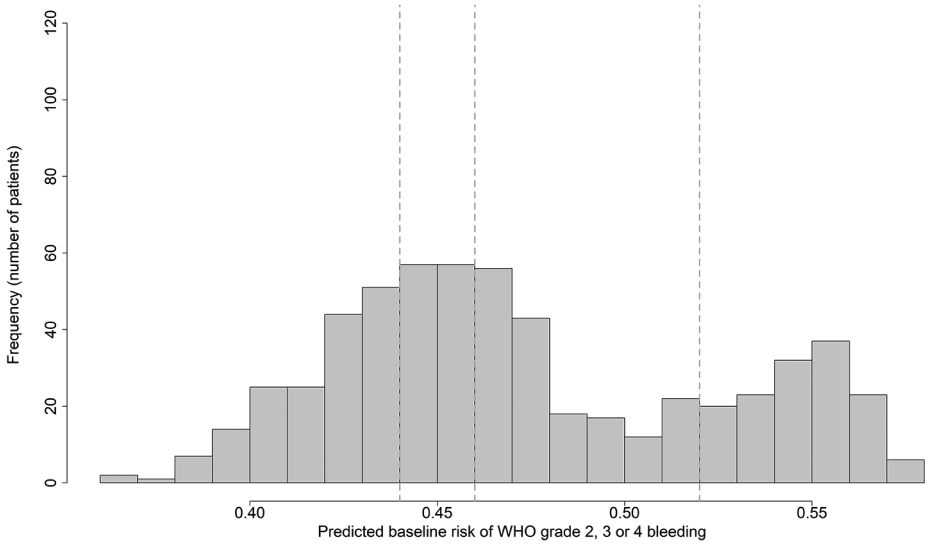


**Figure S2. Histogram of predicted absolute risk of WHO grade 2, 3 or 4 bleeding in complete case analysis (n=592)**

Predicted absolute risk of WHO grade 2, 3 or 4 bleeding in complete case analysis (based on assumption of a therapeutic transfusion strategy) is represented as the absolute risk of outcome on the x-axis and the frequency of each absolute risk category (0.36-0.37, 0.37-0.38 etc.) in the trial population on the y-axis. The dotted lines represent the cut-off for the four quartiles of predicted risk on bleeding.

Figure S2 is comparable to figure 2, meaning that imputing baseline values when missing (n=6) did not influence our results in a relevant matter.

Abbreviations: WHO = Word Health Organization

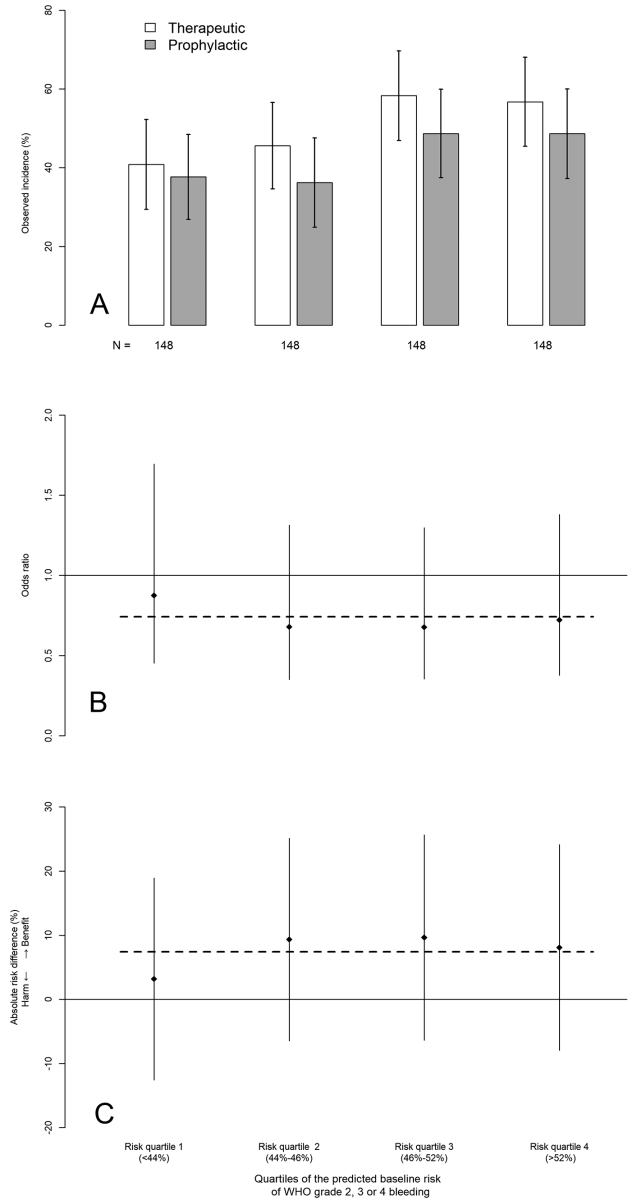


**Figure S3. Observed risks, odds ratios and absolute risk differences between a prophylactically and therapeutically platelet transfusion strategy with respect to WHO grade 2, 3 or 4 bleeding in complete case analysis (n=592).**

Event rates (panel A), odds ratios (panel B) and absolute risk differences (panel C) for the complete case analysis are presented for all four risk categories, vertical lines represent 95% confidence intervals, horizontal dotted lines represent overall trial results. A positive absolute risk reduction represents the risk decrease for a prophylactic platelet transfusion strategy as compared to a therapeutic platelet transfusion strategy. Figure S3 is comparable to figure 3, meaning that imputing baseline values when missing (n=6) did not influence our results in a relevant matter.

Abbreviations: WHO = Word Health Organization

## Results crude model

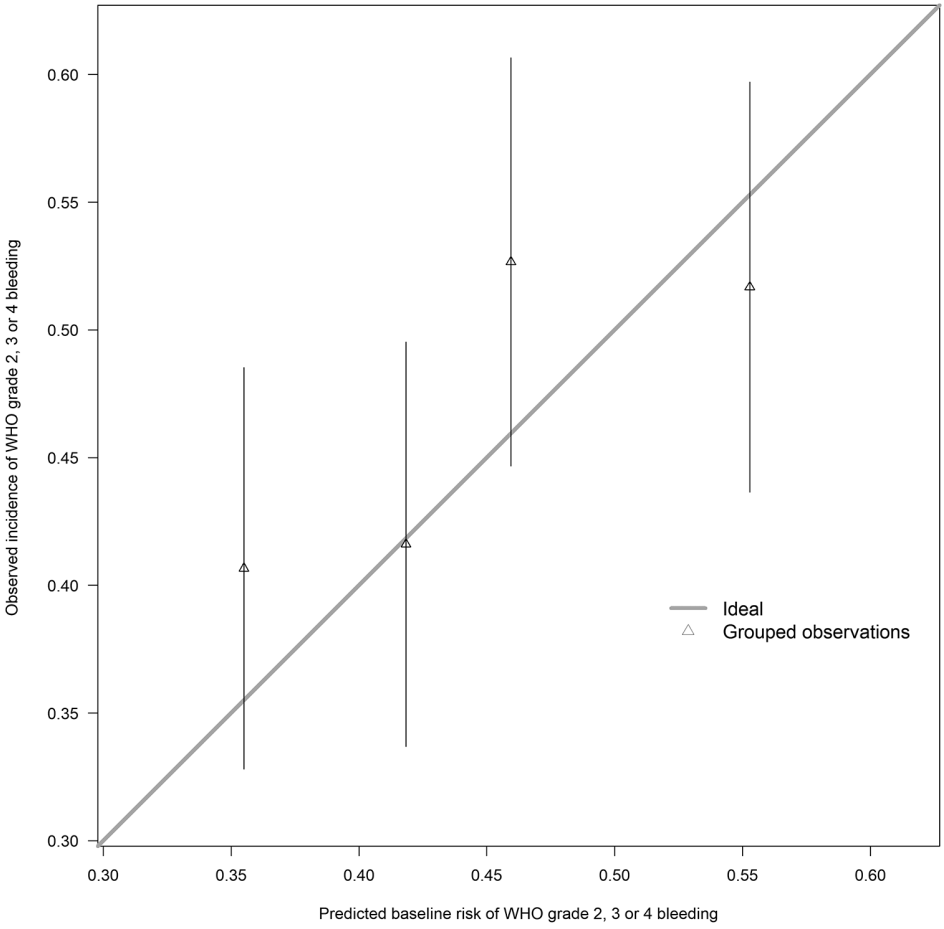


**Figure S4. Calibration plot of predictions of WHO grade 2, 3 or 4 bleeding of crude prediction model**
Validity of predictions of WHO grade 2, 3 or 4 bleeding when applying a crude prediction model, i.e. without correction for optimism. The triangles indicate the predicted probabilities and observed frequencies for all four risk groups (based on assumption of a therapeutic transfusion strategy). The diagonal line represents ideal calibration, when observed and predicted probabilities are identical. The calibration slope is 0.69 (0.16 to 1.22) with an intercept of 0.08 (-0.08 to 0.25). The c-statistic is 0.56 (0.52 to 0.61).
Abbreviations: WHO = Word Health Organization

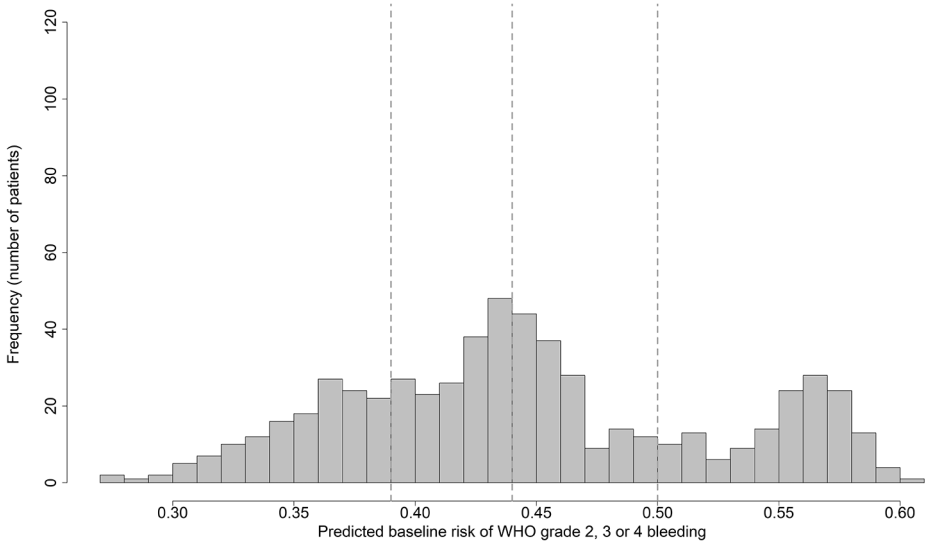


**Figure S5. Histogram of predicted absolute risk of WHO grade 2, 3 or 4 bleeding of crude prediction model**

Predicted absolute risk of WHO grade 2, 3 or 4 bleeding based on the crude prediction model without correction for optimism (based on assumption of a therapeutic transfusion strategy) is represented as the absolute risk of outcome on the x-axis and the frequency of each absolute risk category (0.27-0.28, 0.28-0.29 etc.) in the trial population on the y-axis. The dotted lines represent the cut-off for the four quartiles of predicted risk on bleeding.

Abbreviations: WHO = Word Health Organization

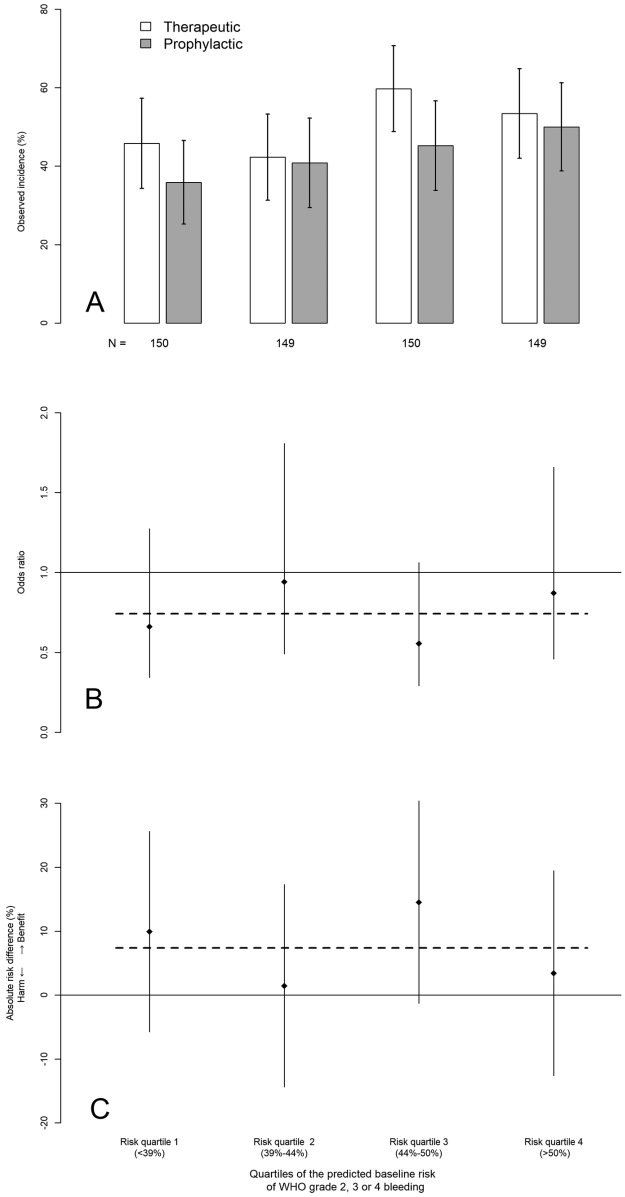


**Figure S6. Observed risks, odds ratios and absolute risk differences between a prophylactically and therapeutically platelet transfusion strategy with respect to WHO grade 2, 3 or 4 bleeding for crude prediction model**

Event rates (panel A), odds ratios (panel B) and absolute risk differences (panel C) based on the crude prediction model, without correction for optimism, are presented for all four risk categories, vertical lines represent 95% confidence intervals, horizontal dotted lines represent overall trial results. A positive absolute risk reduction represents the risk decrease for a prophylactic platelet transfusion strategy as compared to a therapeutic platelet transfusion strategy.

Abbreviations: WHO = Word Health Organization

# Chapter 6

## Risk factors for bleeding in haemato-oncology patients - a nested case control study: The BITE study protocol

*(Bleeding In Thrombocytopenia Explained)*

BMJ Open. 2020 Jun 30;10(6):e034710. doi: 10.1136/bmjopen-2019-034710

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## Abstract

**Introduction**

Haemato-oncological patients often receive platelet count driven prophylactic platelet transfusions to prevent bleeding. However, many prophylactically transfused patients still bleed. More knowledge on risk factors for bleeding is therefore needed. This will enable identification of bleeding risk profiles on which future transfusion policy can be optimised. The present BITE (Bleeding In Thrombocytopenia Explained) study aims to identify clinical conditions and biomarkers that are associated with clinically relevant bleeding events.

**Methods and analysis**

A matched case-control study nested in a cohort of haemato-oncological patients in the Netherlands. We collect a limited number of variables from all eligible patients, who together form the source population. These patients are followed for the occurrence of clinically relevant bleeding. Consenting patients of the source population form the cohort. Cases from the cohort are frequency matched to selected control patients for the nested case-control study. Of both case and control patients more detailed clinical data is collected.

**Study Population**

Adult haemato-oncological patients, who are admitted for intensive chemotherapeutic treatment or stem cell transplantation, or who received such treatments in the past and are readmitted for disease or treatment related adverse events.

**Statistical analysis**

Bleeding incidences will be calculated for the total source population, as well as for different subgroups. The association between potential risk factors and the occurrence of bleeding will be analysed using conditional logistic regression, to account for matching of case and control patients.

**Ethics and dissemination**

The study was approved by the Medical Research Ethics Committee Leiden Den Haag and Delft, and the Radboudumc Committee on Research Involving Human Subjects. Approval in seven other centres is foreseen. Patients will be asked for written informed consent and data is coded before analyses, according to Dutch privacy law. Results will be published in peer reviewed journals.

**Study registration**

Dutch web portal Toetsing Online (NL62499.058.17), Clinicaltrials.gov (NCT03505086).

## Introduction

To prevent clinically relevant bleeding events, haemato-oncology patients usually receive prophylactic platelet transfusions, mostly on a platelet count trigger of $10x10^9$/L.[1] Although platelet counts seem to be poorly related with the occurrence of bleeding,[2] patients treated with trigger-based prophylactic platelet transfusions in randomised controlled trials experience less bleeding as compared to patients with therapeutic transfusions, i.e. triggered by bleeding symptoms.[2 3] In one trial, the incidence of bleeding was 50% in patients without prophylactic transfusions, compared with 43% in patients who did receive prophylactic transfusions.[2] Hence, these data show that the present prophylactic transfusion strategy, is largely ineffective because it does not prevent bleeding in a significant percentage of patients. On the other hand, half of the patients seem overtreated because this percentage shows no bleeding symptoms without transfusions.

Personalisation of platelet transfusion strategies could improve patient care in the haemato-oncological population. Additional to platelet counts, also other factors have been implicated to influence bleeding risk in haemato-oncology patients, like disease stage, disease type, type of treatment (chemotherapy and allogeneic stem cell transplantation (SCT) versus autologous SCT), fever or presence of infection, Graft versus Host Disease (GvHD), splenomegaly, need for RBC transfusion, and the presence of uraemia.[2 4-7] Finally, intrinsic factors of the patient are likely to be of influence, like an increased bleeding or thrombotic tendency. Knowledge on these, and other additional risk factors is so far not sufficient to change the currently applied prophylactic transfusion strategy into a more personalised transfusion strategy.

The mechanisms explaining these risk factors, are likely changes in the haemostatic system. So, additional to platelet counts, we hypothesize it is also important to gain insight in biomarkers characterizing platelet function, vascular integrity, the plasmatic coagulation and fibrinolytic system and their relation to the bleeding tendency in these patients.

The BITE study investigates the role of potential risk factors on clinically relevant bleeding in haemato-oncology patients who have or have had a thrombocytopenic period. Most research investigating prophylactically platelet transfusions has been performed in patients during their treatment (i.e. chemotherapy, SCT). Following such treatments, readmission for disease or treatment related complications, however, is quite common. We therefore also investigate bleeding incidence and bleeding risk factors in patients readmitted after receiving intensive therapy in the past.

Additionally, the BITE study will study actual haemostatic biomarkers for platelet, vascular and coagulation dysfunction,[8-12] that are likely influenced by these clinical risk factors. Therefore, the BITE study will, in a next phase, also incorporate blood and urine

sampling in a subpopulation of patients during their admission. Such biomarkers could possibly be used to identify high risk patients and even better predict bleeding, and thereby add to a more personalized prophylactic regimen. Also, these biomarkers could lead to a better understanding of the potential causal mechanisms for bleeding.

## Study objectives

**Primary objective**

1. To describe and quantify the contribution of potential risk factors to clinically relevant bleeding in haemato-oncological patients, who have or have had a thrombocytopenic period.

**Secondary objectives**

1. To quantify the incidence of bleeding in hospitalized haemato-oncological patients, and for subgroups based on their diagnosis and indication for admission.
2. To identify other haemostatic biomarkers besides platelet counts as predictors of bleeding, and as potential mechanistic explanations of any associations between clinical risk factors and bleeding.
3. To compare WHO and ISTH bleeding score grades for any associations with the studied risk factors
4. To develop a risk factor-based prediction score for bleeding, as basis for personalized prevention of bleeding.
5. To quantify the association between evident pre-existing bleeding tendencies and bleeding during haemato-oncological disease.

## Methods and analysis

**Study design**

The BITE study is a multicentre matched case-control study nested in a cohort of adult haemato-oncological patients in the Netherlands from 2018 to 2023. Nine Dutch hospitals have agreed to participate (5 university medical centres and 4 large regional community hospitals). With 5 of 8 university centres in the Netherlands we estimate to have about 25% of all Dutch haemato-oncologic patients in our source population. Dutch transfusion guidelines ensure reasonable standardization on prophylactic platelet transfusion strategies and additional support. However, by stratification of case and control patients per centre any variations in transfusion strategies between centres are expected to be largely controlled for. The hospital names are given in the supplemental material.

The study has a two-step approach (figure 1). First, in all participating hospitals all patients admitted to the haematology ward are screened for eligibility by a trained member of the local study team. If eligible, patients are part of the total source population. For all patients in the source population information about diagnosis and indication for admission is recorded, as well as the occurrence of a clinically relevant bleeding during admission (also see study population, data collection and supplemental material). Patients in the source population are asked informed consent for eventual participation in the cohort for the nested case control study. Consenting patients form the cohort population and are marked as BITE study participants in the local certified electronic patient systems, e.g. HiX or EPIC.

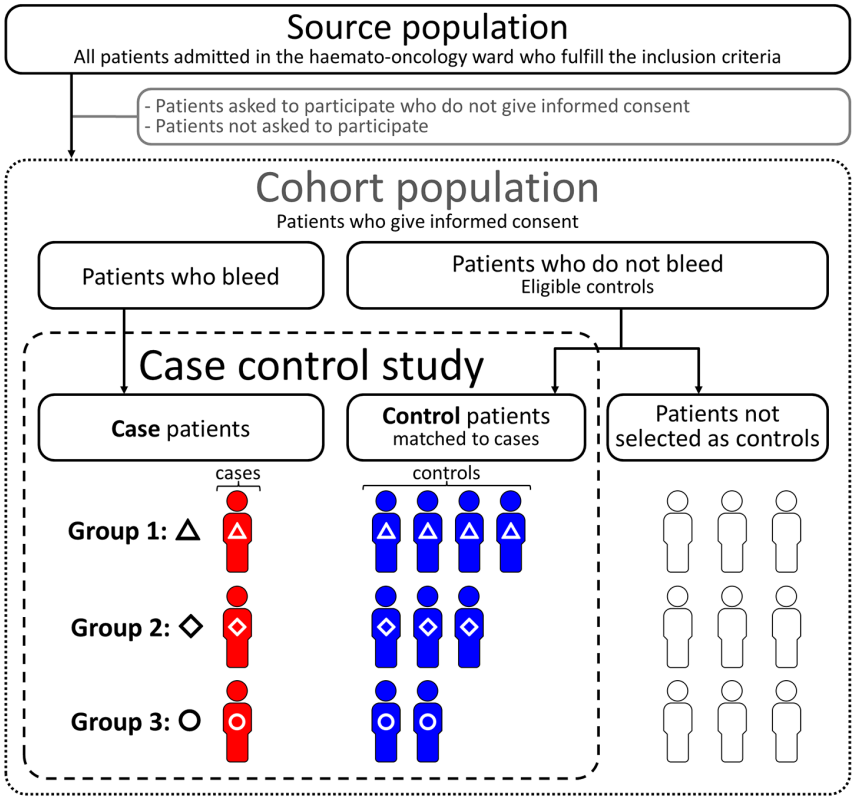


**Figure 1. Source population, cohort population and case control study**
The source population consists of all patients fulfilling the eligibility criteria. The source population will be used for calculation of incidence rates. For this purpose, minimal data is collected. The cohort population consists of all consenting patients in the source population. Case identification and control selection is performed from the cohort population. The case control study is performed with consenting patients who have clinically relevant bleeding during admission (cases) and one to four matched controls per case. This population will be used for estimating rate ratios for different potential risk factors and the occurrence of clinically relevant bleeding and developing a prediction score. For these purposes, extensive data collection is performed.

Second, within this cohort we perform a nested matched case control study. Case patients are those with a clinically relevant bleeding. Control patients are sampled from the cohort.

**Definition clinically relevant bleeding**

A uniform and practical scoring of bleeding severity is of great importance. The WHO score for bleeding is often used.[13] WHO grade 3 and 4 bleedings are mostly clinically relevant, and for example can lead to red cell transfusions or haemodynamic instability. On the other hand, grade 1 bleedings, like petechiae, are not directly harmful. The WHO grade 2 score comprises a large variety of bleeding events of which some certainly have clinical relevance.

Another scoring system, the 'International Society on Thrombosis and Haemostasis' (ISTH) score explicitly discerns clinically relevant major and non-major bleedings. Here, a bleeding is defined as major if it is fatal or symptomatic in a critical organ, when it induces a haemoglobin (Hb) drop of at least 1.24 mmol/L (2 g/dL) or when it leads to two or more red blood cell transfusions.[14] Non-major bleeding according to the ISTH criteria is only defined as clinically relevant if additional medical evaluation or intervention is required.[15] In the WHO criteria the latter are for a large part categorized as grade 2 bleedings, but are there not discerned for their clinical relevance.

In this study, we define clinically relevant bleeding as all clinical relevant bleeding according to ISTH criteria (i.e. major and non-major).[14 15] Hence, case patients are all patients with bleedings requiring substantial additional medical intervention. According to the WHO scoring system,[13] this includes grade 3 and 4 bleedings, as well as all grade 2 bleedings leading to additional medical care. Both bleeding grade scores are registered.

**Study Population**

***Eligibility criteria for the source population***

- Patients of ≥ 18 years who are admitted with a haemato-oncological disease (including myelodysplastic syndrome with intensive treatment and aplastic anaemia with intensive treatment) or who are admitted because of disease or treatment related complications for at least one night.
- Who receive chemotherapy or stem cell transplantation (SCT), or have received such intensive therapy (likely to induce the need for prophylactic platelet support) at any time since haematological diagnosis.
- Who (are expected to) have a thrombocytopenic period with platelet counts of < 50 of at least 5 days or have experienced such a thrombocytopenic period in the past because of the treatment mentioned above.

***Recruitment and consent***

All patients in the source population are (if logistically possible) asked for written informed consent via a local research team. Via this written informed consent patients can consent for potential inclusion as case or control patient. Non-consenting patients will be registered to determine the total number of eligible admissions and clinically relevant bleedings, which is needed to calculate incidences. Inclusions started in December 2018, currently two hospitals are including patients. So far, 468 patients were registered in the logs of the source population, of which 318 (68%) were asked for informed consent. The response rate of consent was 75% (239 patients with signed informed consent). Of these, in 32 patients (13%) a clinically relevant bleeding was reported, which is a slightly higher rate compared to the expected bleeding incidences used for sample size calculation.

***Identification and selection of cases and controls***

Treating physicians are asked to report any case of clinically relevant bleeding to the local study team. The study team registers all reported clinically relevant bleedings of the entire source population. If needed physicians are asked for details of bleeding incidents. To minimize underreporting of clinically relevant bleeding, reporting is actively monitored on a weekly basis by the study team. This is done by asking whether clinically relevant bleedings occurred in weekly grand rounds and by personal contact with treating physicians on the ward. Patients with bleeding, if they gave consent, become case patients. The bleeding event is thereafter graded according to both WHO and ISTH scores by a trained member of the study team. Cases of doubt are discussed with the local principal investigator (an experienced haematologist) for confirmation. Control patients are selected from the cohort based on the matching criteria, which are hospital, diagnosis, indication for admission, and time.

Matching is performed to efficiently adjust for diagnosis and treatment.[16] Matching per hospital allows for adjustment of local differences, e.g. in treatment. Additionally, we match cases and controls on days from start therapy or days of admission if the patient is currently not treated (figure 2). The time from admission is of influence on the risk of many exposures, and primarily on the association of the effect of intensive chemotherapy and bleeding. Without matching for time, cases and controls would therefore not be comparable in this exposure time which can lead to incorrect effect measures for other variables as well. A potential control patient is excluded as control if he/she also experienced a clinically relevant bleeding up to the date that corresponds with the index date of the case patient (see also figure 2 and section data collection). If a control patient develops clinically relevant bleeding after the matched index date for the case patient, the control can also be included as a case patient.

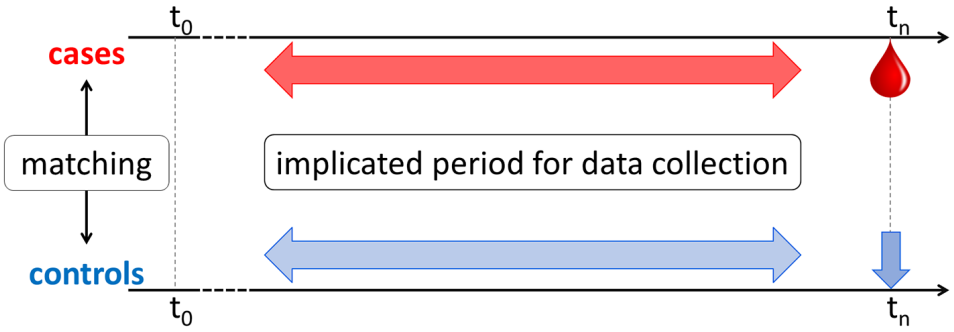


**Figure 2. Graphic explaining the index period used for data collection**
$t_0$ =first day of treatment or day of admission
$t_n$= index day: treatment day or admission day, and bleeding day for cases
Matching was performed for hospital of admission, diagnosis and admission indication

Per case, we match up to a maximum of 4 controls, as this is thought to be the most optimal ratio to estimate risk ratios even when exposure is rare.[17] If more than four eligible control patients are available, we select the ones closest in calendar time to the case. The maximum time span between the bleeding event of the case and the date of admission of the control is one year.

For validation of completeness of case identification, per hospital 100 patients from the cohort will be randomly sampled. In this sample, we will check if the bleeding incidence is as expected and if for 'non-bleeding patients' no unreported clinically relevant bleeding is noted in their clinical records.

***Sample size***

For calculation of the sample size, a power of 80% and confidence interval of 95% (alpha=5%) was used. Based on a 1:4 ratio of cases and controls, inclusion of 1.000 patients (i.e. 200 cases and 800 controls) will give this case control study enough power to detect risk ratio (RR) of 2 or smaller, depending on the prevalence of the risk factor. However, inclusion of four control patients might not be feasible for (all) cases. Even if for example only a 1:2 ratio is reached, the number of cases needed to detect a RR of 2, is only slightly higher, especially when exposure prevalence is relatively high (see figure 3). Therefore, during the entire study we intend to include 200 cases and 400-800 control patients.

A previous study observed an incidence of WHO grade 2 bleedings of 56% and of WHO grade 3-4 bleeding of 7.8%.[18] Based on these incidences, and an expected number of 2.000 admissions with a thrombocytopenic period in participating and future participating hospitals per year, we expect between 75 and 150 patients with clinically

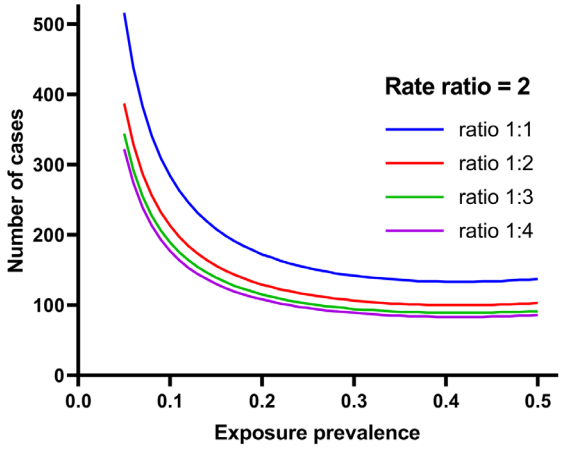


**Figure 3. Study size necessary to detect a relative risk of 2**
Lines indicate the number of cases needed to achieve 80% power to detect a statistically significant difference (i.e. type one error rate smaller than 5%), at different exposure prevalences, if the true relative risk is 2. Ratio indicates the ratio of cases to controls. At a ratio of 1:4 fewer cases will always be needed to achieve the same power, compared to the other rate ratios causing the 1:4 line to be entirely below the 1:1, 1:2 and 1:3 lines. The more controls per case, the fewer cases we need to achieve this power.

relevant bleeding per year. Not all hospitals start enrolling patients at the same time, and some of cases will be missed. Therefore, we estimate to initially include 30-50 cases of clinically relevant bleeding per year, this number will increase when more hospitals enrol.

The biomarker sampling will start in a next phase of the study. Consequently, we will not have samples of all cases and controls and we expect to only have power for hypothesis generating conclusions.



## Data collection

### *Clinical data and bleeding assessment tool*

For all patients in the source population the following information is recorded: diagnosis, indication for admission, age at admission, date of admission and discharge. For cases the date of bleeding is recorded by the local study team.

For the case or control patients, additional clinical and laboratory data are collected. Where possible, data collection is electronic (e.g. transfusion data and laboratory results). Data will be requested and extracted after identification of all case patients and selection of matched controls. The data will be extracted by each hospitals' information technology department (IT). Every hospital involved in the BITE study has a dedicated IT department regularly involved in research. The information is then merged to the BITE study database.

Other variables are extracted from the medical records. This includes among others general characteristics (e.g. sex, age, BMI), infection parameters, relevant co-medication (e.g. anti-coagulation), interventions, trauma or vomiting during admission, comorbidities, and outcome after admission (see supplementary material). In addition, we ask patients to fill out a questionnaire about their bleeding tendency before diagnosis. The questionnaire is a Dutch translation of the validated ISTH self-bleeding assessment tool.[19 20]

Data is not collected for the entire duration of admission. Instead, for every case an implicated period is determined, which is the 7 days preceding bleeding. For controls, the implicated period will be the same 7 days calculated from day of start of chemotherapeutic treatment or from day of admission, if the patient is not admitted for chemotherapy or SCT (figure 2).

The source population data collection is performed daily by the local research team, which registers all eligible patients as soon as possible after admission to the hospital. Additional data of cases and controls is collected from medical records by the researchers and trained study personnel.

***Collection and storage of laboratory samples***

In a subset of hospitals, after initial implementation of the BITE study, laboratory sampling will also be started. Samples are only obtained from patients who are admitted for chemotherapy or stem cell transplantation. During routinely performed blood sampling from venepuncture or from a central line, additional blood samples (10 ml of citrate plasma) will be drawn twice a week for a maximum of 4 weeks, with additional samples directly after admission and in cases also after clinically relevant bleeding. Additionally, urine samples will be collected to investigate microalbuminuria as a marker for endothelial damage and potential predictor of bleeding. Urine samples will be collected after admission and once a week for a maximum of four weeks of admission.

All blood samples will be stored at -70/-80 degrees Celsius until enrolment of new patients is ended. At that point measurements will be planned. Urine samples will be measured within 1 year, since after that levels of albumin may decline. [21]

**Data security**

We document identifiable source population data in a “per-hospital” secured excel file, used as log file. This file is specifically designed for this study and only accessible for the certified local study team authorized to the secured environment in which the log file is safeguarded. For every unique patient, a unique study number is automatically generated. The log file is also used as a key to the study codes at the local hospital. Data is shared with the study team only after removal of all directly identifiable

information. The documents shared at the end of the study period with the data management of the study will only contain study numbers.

Non-directly identifiable data collected from the medical records of cases and controls are coded and transferred to good clinical practice conform CRFs, in a certified secured online system (Castor®, Information Security ISO 27001 – Standards for Information Security Assurance). Access to the Castor CRF page is only possible for registered users who are authorised by a data management team. Data collection is only performed by persons certificated for Good Clinical Practice or the Dutch version (BROK course). Electronically derived data from the electronic patient files will be transferred without identifiable information to a GCP-certified data management team. After data cleaning by the data management team, the data will be made available to the researchers to perform analyses. The data is saved in a secured environment in the Leiden University Medical Centre (LUMC) for a minimum of 15 years.

**Monitoring and quality assurance**

Each participating site of the BITE study will be monitored, with a minimum frequency of once a year. Source data verification is performed for patients randomly sampled from each hospital. Monitoring and auditing is performed according to a monitoring/auditing plan that has been approved by the LUMC.

**Patient and Public involvement**

Patients are involved from the moment that the research team asks for consent. In the design of the studies patients were not involved. For the questionnaire, we asked the first group of patients that were included how they experienced the content and time investment. Since no problems occurred in this respect, we kept the questionnaire in the current format.

**Statistical analysis**

We will calculate incidence rates of bleeding in the total source population and for subgroups of diagnosis and indication for admission. Besides induction, consolidation, types of transplant, "other" indications for admission are described and grouped (e.g. bleeding, granulocytopenic fever, (types or sites of) infections) to allow additional analyses.

Furthermore, we will examine associations of potential risk factors with the incidence of bleeding in the nested case control study by conditional logistic regression, adjusting for matching factors (i.e. diagnosis and treatment) and other confounding factors that will be selected for each exposure variable separately. Because controls are selected via an incidence-density sampling procedure based on time at risk (see also figure 2), the odds ratios will be interpreted as incidence rate ratios with 95% confidence intervals.[22]

Detailed analysis plans will be written and peer-reviewed by an established scientific committee (e.g. Sanquin Research/LUMC) before data is made available for analyses.

**Ethics and dissemination**

The Medical Research Ethics Committee Leiden Den Haag and Delft approved the BITE study, which is conducted according to the principles of the Declaration of Helsinki (last update 2008) and the Dutch Medical Research Involving Human Subjects Act (last update March 2017). Also, the Radboudumc Committee in Research Involving Human Subjects approved enrolment in the Radboudumc. Seven other study sites have signed a research declaration, showing their willingness to participate in enrolment (Erasmus MC, Maastricht UMC, Amsterdam UMC (location VUmc), Meander Medical Center, St. Antonius hospital, Haga teaching hospital and the Máxima MC). In each study centre local procedures to obtain approval from the board of directors and/or ethical committees are followed. We foresee approval in the Erasmus MC and Maastricht UMC in the summer of 2020, and expect to start local procedures for the other hospitals later in 2020 or beginning 2021.

The BITE study is registered at the Dutch web portal Toetsing Online (NL62499.058.17) and at the international web portal clinicaltrials.gov (NCT03505086). Changes in protocol and amendments will be approved by the involved ethical borders and registered before implementation.

Data of consenting patients are coded for privacy reasons, according to the Dutch version of the European General Data Protection Regulation, which is effective from May 2018.

The final publication(s) of the study results will be written by the coordinating investigators and principal investigator. A draft manuscript of each paper is first sent to all co-authors for review and feedback. After revision, the manuscript will be sent to a peer reviewed scientific journal. Authors of the manuscript will include the coordinating investigators, principal investigator, local principal investigators who have included more than five cases and others who have made significant scientific contributions. The results will be published in several papers in peer reviewed journals, based on the different objectives.

### Strengths and limitations of this study

1. The prospective documentation of a cohort of haemato-oncology patients with intensive chemotherapy or stem cell transplantation enables incidence density sampling of incident cases of clinically relevant bleeding and optimally matched control patients.
2. This study design enables the quantification of associations between measured risk factors and major bleeding maximally adjusted for confounding and selection bias.
3. The incidence of clinically relevant bleeding is reliably estimated in a large unselected source population due to weekly communication of the study team with treating physicians.
4. Missing blood samples in a large number of patients may lead to imprecise estimates of the associations between biomarkers and bleeding risk.
5. Some haemato-oncology patients may die before measurements are done, which may lead to selection bias.

**Authors contributions**

LLC devised the study protocol, wrote the draft of the manuscript and reviewed the literature. JJZ and RAM contributed to the conception of the study idea and gave input for the protocol. RAM gave the statistical support. CCD prepared the figures. CCD, JGB, JJZ and RAM critically reviewed the manuscript, as well as the complete study protocol. All authors approved the submitted version.

**Funding statement**

This ongoing clinical study is supported by an internal grant from Sanquin (grant number PPOC 16-26, www.sanquin.nl) and the Ministry of Health, Welfare and Sport of the Netherlands. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests statement**

JJZ reports that the Leiden University Medical Center, his employer, is structurally compensated for his work on blood transfusion medicine by Dutch Blood Supply organisation Sanquin, also during the conduct of the study. The other authors declare no conflicts of interest.

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## Supplementary material

Patient information letters can be found at BMJ Open. 2020 Jun 30;10(6):e034710. doi: 10.1136/bmjopen-2019-034710

**Variables collected for source population**

- Diagnosis: acute myelocytic leukemia, acute lymphatic leukemia, promyelocytic leukemia, chronic myelocytic leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, myelodysplastic syndrome, aplastic anemia
- Indication for admission: remission-induction chemotherapy, consolidation chemotherapy, autologous stem cell transplantation, myeloablative allogeneic stem cell transplantation, non-myeloablative allogeneic stem cell transplantation, other indication
- Additional admission specifications: for example (type of) infection, chemotherapy schedule, etc.
- Date of admission and date of discharge
- Clinically relevant bleeding during admission: yes or no (including date)
- Sex
- Age at date of admission
- Informed consent: yes or no

**Variables collected in cases and control patients**

- General characteristics: year of birth, WHO performance score at admission, BMI, intoxications (alcohol and smoking), ABO rhesus status patient
- Bleeding characteristics (cases only): involved organ system, bleeding description, interventions after bleeding, WHO bleeding score (with distinction of grade 2a: not clinically relevant, and grade 2b: clinically relevant), ISTH bleeding score, date of clinically relevant bleeding (= index day, NB: controls also have a corresponding index day registered)
- Data about diagnosis and indication for admission: Diagnosis in groups, as well as described in conclusions, additional diagnostic testing leading to diagnosis, disease activity at index day (active disease, partial remission, complete remission), disease status (new, relapse, transformation etc.), indication for admission (e.g. remission induction chemotherapy, consolidation therapy, allogeneic SCT, etc, all including description), previous stem cell transplantations in medical history, previous radiation therapy in medical history, transplantation details, Indications for admission to the hematology ward, including complications during admission for previous admissions, etc.

- Comorbidities: description of present comorbidities, need for usage of antihypertensive medication/ cholesterol-lowering medication/medication for diabetes mellitus/medication for ischemic heart disease, bleeding events reported in medical history before diagnosis
- Medication in 10 days before index day: e.g. anti-coagulant medication, antiplatelet medication, anti-infectious medication, chemotherapy, immuno-suppressive medication, etc.
- Infection data in 7 days before index day: highest temperature per day, presence or suspicion of infection, results of cultures and PCR's, active infection treatment, radiology results, infection in conclusion or differential diagnosis, etc.
- Mucositis related data in 7 days before index day: Mentioning of mucositis (or suspicion) in medical record, location (possible) mucositis, WHO grading mucositis, usage of medication for mucositis, need for tube feeding or total parental feeding
- Clinically non-relevant bleedings in 7 days before index day: presence described in medical records, and if so, description and WHO bleeding grade.
- Transfusion data in 7 days before index day: Triggers for platelet and erythrocyte transfusions, prophylactic or therapeutic transfusions, number of transfused products, platelet refractoriness described
- Transplantation related complications in 7 days before index day: which complication (Venous occlusive disease, capillary leak syndrome, engraftment syndrome, HSCT-associated thrombotic microangiopathy, haemorrhagic cystitis, idiopathic pneumonia syndrome, graft versus host disease), in case of graft versus host disease: acute versus chronic, location, severity.

Other possible risk factors in 7 days before index day: presence of trauma or intervention, mobility status patients, vomiting

# Chapter 7

General discussion and future perspectives

## General discussion

Bleeding is a commonly encountered problem in hemato-oncology patients. Despite widely applied prophylactic platelet transfusions that aim to prevent episodes of hemorrhage, bleeding still occurs. It is estimated that between 1.2% and 7.8% of the patients experience severe bleeding during treatment.[1-5] Here, severe bleeding is defined as a bleeding with a World Health Organization (WHO) bleeding score of grade 3 or 4.[6] Bleeding of WHO grade 2 or higher may occur in up to 71% of patients within 30 days of admission for intensive chemotherapy,[3] while up to 89% of all patients experience a bleeding of any grade during their hospital admission.[5]

For more effective prevention, and thus a further reduction in clinically relevant bleeding episodes, more knowledge on bleeding in hemato-oncology patients is needed. First, it is important to understand which factors are contributing to the development of bleeding. This can help to establish targeted prophylactic interventions in the future. Second, adequate prediction of bleeding is essential, to identify patients who might benefit from specific preventive interventions.

The main goal of this thesis was to contribute to these knowledge gaps. We described a part of the current clinical practice in patients with persistent deep thrombocytopenia, a subpopulation that has had little attention in research so far. Furthermore, we identified conditions that are associated with (intracranial) bleeding. Also, we aimed to predict the effect of the most widely applied bleeding prophylaxis, namely prophylactic platelet transfusions, for individual patients.

### Current clinical practice

With the ultimate ambition to be able to prevent clinically relevant bleeding more efficiently in the future, a first step is to identify potential points of improvement by describing current clinical practice. For this, a summary of the general recommendations and considerations for prophylactic platelet transfusions for hemato-oncology patients from established transfusion guidelines is provided in table 1.[7-12]

#### *Platelet prophylaxis in patients with transient thrombocytopenia*

For hospitalized patients, who receive intensive therapies such as high dose chemotherapy or stem cell transplantations (SCT), and for whom the thrombocytopenia is expected to be transient, all guidelines recommend giving prophylactic platelet transfusions at platelet counts of < $10x10^9$/L. However, for specific subgroups or clinical conditions, recommendations in the different guidelines are inconsistent.[13] Stable patients receiving an autologous SCT form a specific group for whom prophylactic platelet transfusions could be withheld, according to two guidelines.[8, 9] This advice is based on secondary analysis of one randomized controlled trial (RCT), which suggest

**Table 1.** Guideline recommendations on prophylactic platelet transfusion in adult hemato-oncology patients

| Country | Publication year | Organization | Transient thrombocytopenia | Altered thresholds | Persistent deep thrombocytopenia |
|---|---|---|---|---|---|
| The Netherlands | 2020 | Federation of Medical Specialists | - Give one standard dose of prophylactic platelet transfusion to patients receiving intensive chemotherapy of allogeneic SCT at a platelet count of > $10x10^9$/L | - Altered thresholds for several invasive procedures are advised<br>- Altered thresholds for patients with a need for platelet aggregation inhibitors or anticoagulant medication are recommended | - Given a lack of evidence no advices on prophylactic transfusions, or alternative bleeding prevention, are given |
| United Kingdom | 2017 | British Committee for Standards in Haematology | - Give one standard dose of prophylactic platelet transfusion to patients receiving intensive chemotherapy of allogeneic SCT at a platelet count of ≥ $10x10^9$/L<br>- Consider not giving prophylaxis to well patients receiving an autologous SCT | - Consider increasing the threshold up to $20x10^9$/L in patients judged to have additional risk factors for bleeding, individual review based<br>- Altered thresholds for several invasive procedures are advised | - Use a no-prophylaxis strategy in asymptomatic patients without or on low intensive therapy<br>- Give prophylaxis to patients who do receive intensive treatment |
| United States | 2015 | AABB | - Give one standard dose of prophylactic platelet transfusion to hospitalized patients with therapy induced thrombocytopenia at a platelet count of ≥ $10x10^9$/L | - Altered thresholds for lumbar puncture and central venous catheter placement are advised<br>- Altered thresholds for non-hospitalized patients can be considered | - Not specifically mentioned; altered thresholds for non-hospitalized patients can be considered |
| United States | 2018 | ASCO | - Give one standard dose of prophylactic platelet transfusion to patients who receive therapy, including allogeneic SCT, at a platelet count of ≥ $10x10^9$/L<br>- A therapeutic only transfusion strategy may be used in experienced centers for patients receiving an autologous SCT | - Higher thresholds may be advisable in patients with signs of hemorrhage, high fever, hyperleukocytosis, rapid fall of platelet count or coagulation abnormalities<br>- Altered thresholds for several invasive procedures are advised | - In absence of active treatment, patients can be observed without prophylactic transfusions, and transfused if bleeding occurs |
| International | 2015 | International Collaboration for Transfusion Medicine Guidelines | - Prophylactic platelet transfusion (low or standard dose) should be given at a platelet count of ≥ $10x10^9$/L (NB this is not specifically mentioned for only transient, but for all patients with hypoproliferative thrombocytopenia) | - Not specifically mentioned | - Not specifically mentioned |
| United Kingdom | 2015 | NICE | - Give one standard dose of prophylactic platelet transfusion | - Altered thresholds for several invasive procedures are considered | - Do not routinely offer prophylactic platelet transfusions to patients with chronic bone marrow failure |

Abbreviations: SCT = stem cell transplantation, AABB = Association for the Advancement of Blood & Biotherapies, ASCO = American Society of Clinical Oncology, NICE = National Institute for Health and Care Excellence

that these patients do not benefit from the transfusions, while patients with intensive chemotherapy or allogeneic SCT do benefit.[14] A meta-analysis of the results of this RCT with the results of one other RCT did not provide conclusive results regarding the lack of benefit in patients with autologous SCT. Accordingly, some of the guidelines do not give specific guidance for patients with autologous SCT.[7, 10-12] It would be of great interest to study the non-benefit in these patients once more. If confirmed, we would infer that the advice to withheld platelet transfusions from patients receiving an autologous SCT should be wider applied in guidelines, and clinical practice.

Likewise, for conditions in which bleeding risk is considered high, different guidelines give conflicting advises, or are unspecific. This reflects the lack of evidence on which risk factors, or combination of risk factors, are most important. Moreover, the effectivity of prophylactic platelet transfusions, let alone altered prophylactic strategies, in patients with various risk factor profiles are unknown. Examples of clinical conditions or circumstances that potentially increase bleeding risk are infections or sepsis, graft versus host disease, and the need for anticoagulant therapy.[15-18] For clinically admitted patients who undergo intensive therapy, there is substantial heterogeneity in transfusion practice, especially in the presence of such expected risk factors.[19]

***Platelet prophylaxis in patients with persistent severe thrombocytopenia***

Next to the intensively treated population with transient thrombocytopenia, a significant number of patients suffer from persistent severe thrombocytopenia. These patients have chronic bone marrow failure and are not eligible for, or are refractory to, curative treatments. Most often, they are outpatients. For this specific, and far less studied, population, incidences of bleeding have only scarcely been described. These outpatients are generally more 'stable', with a relatively low bleeding risk profile in the absence of inflammation and other risk factors that complicate intensive treatments. Hence the momentary incidence of bleeding among these clinically stable, outpatients with persistent severe thrombocytopenia is expected to be relatively low. However, due to the long period of thrombocytopenia, the cumulative bleeding incidence increases with time, likely leading to substantial long term bleeding incidences.

For patients with persistent severe thrombocytopenia, evidence on how best to prevent bleedings is lacking. Based on expert opinion, some international guidelines suggest to consider to withhold part of the prophylactic transfusions in this population (table 1).[8, 9, 11] However, these recommendations are, again, not consistent between the different guidelines.

The clinical practice of bleeding prevention in these outpatients with persistent severe thrombocytopenia in the Netherlands has not been described before, and was explored in **chapter 2.** Platelet prophylaxis appeared widely applied in these patients, especially when recently receiving intensive chemotherapy, or when treated with

hypomethylating agents as anti-cancer therapy. The most applied platelet transfusion threshold is 10x10$^9$/L, conform the guidelines in intensively treated patients. A minority of physicians choose higher thresholds, like a trigger of 20x10$^9$/L, up to sporadically even 80x10$^9$/L. For some subpopulations a therapeutic only transfusion policy is considered by others.

Another group of patients do not receive any disease modifying therapies, like low-dose chemotherapy. These patients are transfused with prophylactic platelet transfusion far less. Mostly, they are frailer and/or have a shorter life expectancy compared to patients who do receive treatment.[20-22] Our survey did not provide insight in the reasoning behind withholding prophylactic platelet transfusions. However, we presume the benefit of prophylaxis is probably weighed smaller as compared to the burden a patient may experience by visiting the hospital frequently. Another reason for less platelet support may be that, in the absence of disease modifying treatment, the bleeding risk is often estimated as low.

***Alternative anti-bleeding strategies***

Besides bleeding prevention via prophylactic platelet transfusions, alternative strategies to avoid (clinically relevant) bleeding are also of interest. Hemostasis is an interplay between platelets, the endothelium, and coagulation and fibrinolysis. Therefore, agents optimizing any of these factors can potentially help in the prevention of bleeding.[23] Agents that have been studied in the hemato-oncological population include thrombopoietin mimetics, platelet poor plasma and desmopressin. Although the data is scarce, so far, no benefits of these measures have been described. [24, 25]

Another potentially effective anti-bleeding strategy is to inhibit fibrinolysis. In this context, tranexamic acid is the most frequently used anti-fibrinolytic agent. It is a synthetic drug, which binds plasminogen and thereby reduces the conversion to plasmin, and consequently decreases fibrin degradation. In several non-hemato-oncological populations, tranexamic acid provides adequate protection against bleeding.[26, 27] Moreover, tranexamic acid has the advantages of being easily administered orally and having relatively little adverse effects. Therefore, it is sometimes used as an alternative or adjunct to transfusions to prevent bleeding in patients with (persistent) thrombocytopenia.[28, 29]

Since there is little known on the extend of usage of tranexamic acid, as well as on the clinical reasoning for prescription, we surveyed the clinical use of tranexamic acid in hematological outpatients. The results were presented in **chapter 2**. In the Netherlands, clinicians hardly ever prescribe tranexamic acid to patients without recent or active clinically non-relevant bleeding. The fact that tranexamic acid is not often given for pure prophylactic purposes in the Netherlands, may not reflect its usage elsewhere. A Canadian observational study of 99 patients with myelodysplastic

syndrome, described the incidence of bleeding for different antibleeding strategies.[28] It was found that 28% of patients received solely prophylactic tranexamic acid, and 39% had both platelet transfusions and tranexamic acid as bleeding prophylaxis. Only 19% received solely platelet prophylaxis and 13% did not receive any prophylaxis. So, in contrary to our results, in this study the majority of patients received tranexamic acid. Intriguingly, this study reported no significantly different number of WHO grade 3 or 4 bleedings between the four patient groups.[28] Yet the efficacy of tranexamic acid remained uncertain, because the study was small and potentially the result of confounding. In other small studies, tranexamic acid has not been shown to be effective to prevent bleeding in the hemato-oncological population.[30] Preliminary results of a large RCT suggest that prophylactic tranexamic acid in adjunct to regular platelet prophylaxis in patients with intensive chemotherapy or SCT does not positively affect the clinical outcomes of patients[31] Similarly it is not clear whether tranexamic acid, as adjunct or substitute, positively affects the prognosis of outpatients with persistent deep thrombocytopenia,. Thus, there remains an important medical need in identifying effective alternative interventions to prevent bleeding.

**Prediction of the effect of prophylactic platelet transfusions on bleeding**

The beneficial effect of prophylactic platelet transfusions at a threshold of $10x10^9$/L on reducing the occurrence bleedings in intensively treated hemato-oncology patients is clearly established.[32] However, bleeding is by far eradicated. Thus, although this strategy is effective for part of the patients, many still bleed. Additionally, other patients would never bleed even in absence of prophylaxis.[1, 2] Therefore, to establish a more efficient use of transfusions, it is important to identify patients that do benefit from prophylactic transfusions, those that might need additional measures, and patients that do not need transfusions at all.

In the literature, by far most attention has gone to the effect of platelet count, and platelet count driven transfusion strategies, on the occurrence of bleeding. However, besides platelet counts, several other patient characteristics and clinical conditions associated with the bleeding risk have been described.[8, 15-17, 33-36] These expected risk factors are also potentially important to identify patients who could benefit more from transfusions. However, a risk prediction model that includes risk factors to predict the effect of prophylactic transfusions is lacking so far.

In **chapter 5**, we present a prediction model based on baseline characteristics of clinical intensively treated patients with hemato-oncological diseases. We included baseline risk factors that have been described to be associated with bleeding before. When combined, their predictive power was low. Furthermore, based on these baseline bleeding risks, no patient subgroups could be identified that clearly benefitted more or less from prophylactic transfusion strategies. Several reasons, all argued in the

discussion of chapter 5, can explain why the selected baseline risk factors together could not predict bleeding more accurately. In our opinion the lack of information on, mostly short term, time-varying clinical conditions, plays a pivotal role. These time-varying conditions, like platelet count, inflammation, or other processes that temporarily impact the hemostatic integrity, are however not available for data analysis in most studies. One would need large numbers of both patients and relevant variables and take timing of the separate conditions and the eventual (absence of) bleeding events into account.

**Intracranial hemorrhage – etiology and prediction**

Of all bleeding complications in patients with leukemia, intracranial hemorrhage is one of the most feared, since it has a strong impact on quality of life and life expectancy.[37-40] In **chapter 3** we demonstrated it is likely that low platelet counts are associated with intracranial hemorrhage. This result may not come as a surprise, since several studies already described an association.[41-43] Mostly, the platelet count at the day before bleeding, or the bleeding day itself, is studied. However, we show that in time periods up to 7 days preceding the bleeding event, this association becomes stronger than the more generally applied association of platelet count one day before bleeding. Also, the percentage of time with low platelet counts is likely associated with intracranial hemorrhage. Platelet transfusions also seem associated with intracranial hemorrhage. Probably, this is due to general conditions that lead to raised transfusion thresholds and hence more transfusions. Higher thresholds are among others applied when anticoagulant medication or platelet aggregation inhibitors is needed, or when other (non-intracranial) bleeding events occur. In other words, intracranial hemorrhage is more likely to be caused by these threshold-increasing clinical conditions, instead of by the ensuing raise in platelet transfusions.

To prevent intracranial hemorrhage, it would be worthwhile to identify patients who are more likely to develop these events. Bleeding is obviously not only influenced by platelet counts, but also by the condition of the vascular wall.[44, 45] Therefore, we focused on cardiovascular risk factors that are likely to compromise the vascular wall chronically. In **chapter 4,** we demonstrate that pre-existing hypertension and a history of ischemic heart disease both are strongly associated with the occurrence of intracranial hemorrhage in patients with acute leukemia. Such predictors are easily obtainable in clinical care. It needs further investigation to confirm if these predictors, as hypothesized, lead to intracranial hemorrhage via the combination of chronic vascular damage and acute vascular effects of chemotherapy and thrombocytopenia. Also, studies on the clinical consequence of alternative preventive strategies in patients with increased risk might eventually lead to improved clinical outcomes.

## Future perspectives

With the studies presented in this thesis, we assessed aspects of bleeding in hemato-oncology patients. First, we described clinical care to prevent bleeding. Second, we investigated clinical risk factors for and predictors of (intracranial) bleeding in hemato-oncology patients. Finally, we studied the effect of risk factors of bleeding on the treatment effect of prophylactic platelet transfusions.

Given the continuing high incidence of bleeding, and the large amounts of prophylactic platelet transfusions administered, it is essential to identify both hematology patients with high, and low bleeding risks. Moreover, for efficient clinical use of transfusions it is important to identify patients who are likely to benefit from platelet prophylaxis. Accordingly, identification of patients who don't need prophylactic transfusions would improve transfusion practice. The harms of platelet transfusions, the burdens, and the considerable costs should be avoided if they serve no benefit. Moreover there is a remaining medical need for more effective bleeding prevention by adjunctive or altered bleeding prevention strategies.

The current standardized and generalized – platelet count threshold based-prophylactic platelet transfusion policy, which is used for a very heterogeneous patient population, is suboptimal. Our studies are steps towards more effective and efficient bleeding prevention, by exploring options beyond platelet counts.

**Clinically relevant bleeding and patient perspectives**

When striving to have a both effective and efficient policy to prevent bleeding, there are several considerations that need to be addressed. As an important first step, one must wonder what we truly aim to prevent when giving platelet prophylaxis. Not all bleeding events lead to substantial burden or harm for patients. Almost all evidence about prophylactic platelet transfusion medicine is based on bleedings classified by the WHO bleeding grade.[6] This score was originally validated for another purpose, namely to score therapy related toxicity instead of primarily reporting of bleeding. While using slightly different variations of the WHO score, most studies focus on WHO 2, 3 and 4 bleedings as a combined main outcome. However, not all of these bleedings may lead to direct danger, alterations of medical treatment, intensified care, transfusions, or invasive procedures. Instead, there are other bleeding scores, namely the ISTH bleeding score and BSMS bleeding score, that try to divide bleedings into clinically relevant versus non-relevant.[46, 47] These scores have so far not often been used in the hemato-oncological population. A recent study (P.F. Ypma, submitted for publication) reports on readjudication of WHO bleeding scores used in a large platelet transfusion RCT in hemato-oncological patients.[48] They describe that 97.1% of the WHO grade 2 bleedings and even 30.4% of WHO grade 3 bleedings were classified as non-

relevant bleeding according to the ISTH bleeding score. This would mean that these bleedings did not lead to increased or altered medical care. For the BSMS scores, even 97.8% of WHO grade 2 bleedings were not judged as clinically significant, for WHO grade 3 bleedings this percentage was 34.8%. These interesting findings emphasize that indeed the combined outcome of WHO bleeding grade 2, 3 and 4 consist of many bleedings that are not of clinical relevance. Although both the ISTH and the BSMS bleeding scores are in need for further evaluation and validation in the particular patients population of thrombocytopenic hemato-oncological patients, these scores might align better with the present medical needs.

An obstacle in studying the outcome of only clinically relevant bleedings is that the incidence is low. One needs large sample sizes in a RCT or cohort study with clinically relevant bleeding as an outcome. For these rare outcomes, case control studies may be preferred as study design to efficiently and realistically study clinically relevant bleeding.[49]

It is increasingly recognized that studies focusing on clinically relevant bleedings, should also include how patients experience the burden of bleeding. At the same time, their opinion on both benefits and inconveniences of preventive strategies needs to be accounted for. Patient centered outcomes are more and more acknowledged as an important end point for clinical studies.[50, 51] Yet all the before mentioned bleeding scores are designed by physicians or expert researchers, and lack patients perspectives. It is the patient who might experience the benefit from transfusions, but also who is at risk of transfusion related complications and burdens. This applies to transfusions given to bridge transient or therapy induced thrombocytopenia, but perhaps even more so for outpatients with persistent thrombocytopenia. For the latter group, the benefits are less known, and the burdens of recurrent and cumulative transfusions are likely higher. In transfusion medicine, so far few studies examined patient perspectives, and none reported on platelet transfusions specifically.[52] Weighing the patients view on prophylactic strategies to prevent bleedings is worthy to receive more clinical and scientific attention.



**Persistent deep thrombocytopenia and prevention of bleeding**

Another subject that needs attention when aiming to optimize efficient and effective anti-bleeding strategies, is persistent deep thrombocytopenia due to chronic bone marrow failure. For these patients, well registered bleeding incidences as well as the evidence for effectiveness of bleeding preventive strategies are almost completely lacking.

The ultimate step to improve both knowledge and treatment policies in this group would be to perform a RCT, comparing various prophylactic platelet transfusion thresholds. Ideally, as studied in intensively treated patients,[1, 2] a prophylactic platelet

transfusion strategy should be compared with only prescribing transfusions in case of active bleeding. Also, it could be of value to include an arm with an alternative prophylactic strategy. Though, such a study may have practical difficulties, like completeness of bleeding registration in outpatients. For adequate registration a patient likely needs to be seen or contacted regularly. Another complicating factor may be the long follow-up time needed when studying an outcome that is not very frequent. Where WHO grade 2, 3 and 4 bleedings are not very rare, clinically relevant bleedings have lower incidences.[48] Nonetheless, it would lead to the best possible evidence on how to prevent bleeding via transfusions in this vulnerable patient population.

An alternative could be an observational study, for example on retrospective data or a prospective cohort or case control population. In both of these study designs, also quality of bleeding registration can influence the study results significantly. Reporting of bleeding is likely less in patients without prophylactic transfusions, since they are not as frequently seen in the hospital. For mild bleedings without clinical relevance this perhaps is not worrisome, since these are not the bleedings we are trying to avoid by platelet transfusions. However, missing relevant bleeding would confound the results. Another important difficulty of an observational study would be that physicians often do not report extensively why they choose a prophylactic strategy for one patient, and not for the other. Likely there will be confounding by indication that will be hard to correct for. All difficulties can be expected to be more challenging in retrospective data, compared to prospective observational data. Prospective observational research has the advantage that physicians can be asked to be aware on how they score and report important data in the medical chart. If performed diligently, this would lead to less confounding. Additionally, also in observational prospective studies a long follow-up time is needed in when studying an outcome that is not very frequent. Since bleeding incidences are not widely described for patients with chronic bone marrow failure, sample size calculations will likely be largely based on estimations or small studies. Therefore, in my opinion, also in this patient population a case control design would be preferable for the outcome of clinically relevant bleeding.

Although perhaps not easy, it is important to study the outpatient population with persistent thrombocytopenia specifically, both for their efficiency and for the patient perspectives. While this is important for many treatments and populations, given the expected chronic use of, and time consuming and invasive nature of platelet prophylaxis, especially for these patients this is of crucial importance.

**Identification of bleeding risk and expected benefits of transfusions**

A crucial step in preventing clinically relevant bleeding is to be able to identify patients with a high bleeding risk, or even patients who are likely to profit from transfusions or not.

Bleeding risk continuously changes. The risk in time likely differs more in intensively treated patients compared to patients with chronic thrombocytopenia. The intensive chemotherapy, or conditions that develop during therapy or admission, like infections, fever and mucositis, influence platelet numbers and function, as well as vascular integrity. So far, although likely, it is neither known how these factors interact, nor how they are influenced by other patient characteristics. Therefore, a model that can incorporate time varying variables with 'fixed' risk factors is needed to accurately predict bleeding risk. Such a model could take along many clinical variables, but biomarkers that represent the pathophysiological effects of the clinical conditions might prove to be most informative.

Biomarkers indeed can serve as predictors, while in the mean time learning us more about the balance between vascular integrity, platelet function, platelet counts, the coagulation system and fibrinolysis. Thereby, biomarker studies can help unravel the complex pathophysiologic pathways of bleeding in this particular patient population. This could be helpful in steps toward alternative, biomarker-based, approaches to prevent bleeding, namely therapies that directly target the pathway mostly involved in the impaired hemostasis.

Although most emphasis so far has been on identifying patients who benefit from bleeding preventive strategies, there are two sides of the medal. We also need tools to identify patients that will not benefit from the transfusions, as is described for patients undergoing autologous SCT.[2, 14] As a biological agent that is being administered, platelet transfusions are not without risk. Acute transfusion reactions are rare, but may lead to substantial burden if they occur.[53, 54] Platelet transfusions furthermore can lead to HLA antibodies, which potentially lead to refractoriness.[55, 56] Therefore, exposure of patients who will likely not benefit of transfusions should be avoided where possible. More evidence, perhaps from observational studies investigating the safety of a non-prophylaxis strategy in low risk patient subgroups, is likely needed before withholding of transfusions will be implemented more consistently in guidelines.



**BITE study**

As described, previous studies investigated the effect of prophylactic platelet transfusions in intensively treated hemato-oncology patients, as well as some risk factors for bleeding. Yet, there is still a need to identify additional risk factors, and confirm previously suggested risk factors. Also, it is of importance to clarify how the several risk factors interact over time, and even more importantly which (combination of) risk factors can serve as a robust prediction model to identify patients that are likely to bleed, or not.

In **chapter 6**, we described an ongoing case control study, that intends to fill some of the described gaps of knowledge: the BITE study (Bleeding In Thrombocytopenia Explained). We gather the clinical data for such prediction models in cases with clinically relevant bleeding, and in control patients. Importantly, in this case control study, besides baseline characteristics also time depending data will be collected. Time dependent variables will be looked into from a period of time preceding clinically relevant bleeding for cases, and in a matched time for controls. In this way, risk factors are aimed to be both identified and quantified, taking into account potential time dependent effects of intensive treatment. Also, a dynamic prediction model can be realized, which will be an important next step in accurate bleeding prediction, and hopefully more personalized preventive strategies in future. Furthermore, for a part of the included patients also biomarkers will be measured, concentrating on platelet and endothelial function.

Subsequently, new studies will be needed to show the efficacy of prophylaxis in patients with different bleeding risks. For patients at high risk despite prophylactic platelet transfusions, alternative or additional treatment should be studied. In case of alternative or additional treatments, it would be preferable to focus on treatments that encounter the biological bleeding mechanisms shown by biomarkers. For patients with low bleeding risk, larger studies need to identify the populations that will also have low bleeding risks in absence of prophylactic therapy. For these patients, this would mean they do not need to be exposed to transfusions they will likely not benefit from, but that can burden or harm them. Also, identification of the population that can do without transfusions is important to reduce health care costs and blood supply demands.

To conclude, within the field of transfusion medicine and hematology, there remains a medical need for improved bleeding preventive strategies for hemato-oncology patients. Identification of risk factors, and prediction models leading to personalized estimates of risks and expected benefits, are of great importance to prevent bleeding more effectively and thereby improve the care for and outcomes of hemato-oncology patients.

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# Chapter 8

Summary
Samenvatting

## Summary

In the hemato-oncological population bleeding events are frequently seen, despite widely applied prophylactic platelet transfusions. Part of these bleedings are clinically relevant, leading to for example extended care, invasive procedures, additional medication or transfusions. They may even lead to serious morbidity or mortality. Therefore, to more effectively prevent especially these relevant bleedings, it is important to understand which factors contribute to their development, and to be able to predict which patients are more likely to develop bleeding, or not. In this thesis, we focused on current clinical practice of bleeding prevention in hemato-oncology patients with persistent deep thrombocytopenia, on risk factors for bleeding, and prediction of bleeding.

In **chapter 2**, we evaluated the current clinical practice of bleeding prevention in a subgroup of hemato-oncology patients, namely outpatient patients with persistent deep thrombocytopenia. Also for this subgroup, prophylactic platelet transfusions are commonly provided in the Netherlands, and conform to the guidelines for patients with transient thrombocytopenia applied mostly beneath a platelet count of $10x10^9$/L. We also showed that when patients are not actively treated for their underlying hematological disease, prophylactic transfusions are far less prescribed. Furthermore, we found many different clinical conditions that determine the decision making on platelet prophylaxis. In this regard, previous bleeding events and the use of platelet aggregation inhibitors or anti-coagulant medication were considered most important. For patients with clinical conditions that likely increase bleeding risk, the chosen platelet transfusion thresholds differed substantially. In addition, we surveyed tranexamic acid usage. We showed that this antifibrinolytic agent is mostly prescribed to patients with active or recent bleeding, but hardly ever as prophylaxis in the absence of bleeding. Our results reflect the lack of knowledge on risk factors for bleeding in this particular patient population, and underline the need for more research of bleeding preventive strategies.

**Chapter 3** and **chapter 4** focus on acute leukemia patients with intracranial hemorrhage. In **chapter 3**, we described how absolute platelet counts and the percentage of time with low platelet counts (exploring time-frames up to seven days) were associated with intracranial hemorrhage. We found that longer periods of thrombocytopenia coincide with a higher risk of intracranial hemorrhage. However, due to a small number of patients with intracranial hemorrhage, we could not substantiate a true effect size, nor correct for confounding factors that influence the association between thrombocytopenia and intracranial hemorrhage. We additionally

showed that patients who need a higher numbers of platelet transfusions also seem to have a higher risk. This was especially the case for more than two platelet transfusions in a five to seven day period preceding the intracranial hemorrhage. This association likely reflects conditions that lead to the greater need for transfusions, and thus no direct causal relation.

In **chapter 4** we investigated the predictive association of pre-existent cardiovascular risk factors with intracranial hemorrhage in leukemia patients. Cardiovascular risk factors are described as risk factors and/or predictors of intracranial, mostly intracerebral, hemorrhage in the general population. However, it was not known if these associations are also equally important for leukemia patients. We showed that especially pre-existent hypertension or a history of ischemic heart disease are strong predictors of intracranial hemorrhage in leukemia patients. Moreover, the predictive power seems higher than is expected in the general population. The possible causality of course needs more research, but we hypothesize that the combination of chronic vascular damage (of which hypertension and ischemic heart disease are surrogates) and the acute endothelial damage and low platelet counts during treatment of acute leukemia, synergize and explain the even stronger association. If confirmed, it is of interest to see of patients with pre-existing cardiovascular risk factors benefit from altered or additional interventions to prevent bleeding.

To prevent bleeding more effectively on one hand, while also avoiding unnecessary platelet transfusions, one should be able to predict who is likely to bleed or not, and hence who will likely benefit from prophylactic platelet transfusions. In **chapter 5** we therefore studied the effect of platelet prophylaxis in groups of patients with different baseline characteristics as possible bleeding predictors. To do so, we first designed a prediction model from several baseline characteristics that in previous studies seemed to associate with bleeding. Yet, this prediction model of combined baseline risk factors, had low predictive power and could not really differentiate between high and low bleeding risk groups. Within the small range of predicted risks, via a heterogeneity of treatment effect analysis, we could conclude that patients with different risk factor distributions all seem to benefit more or less equally from the prophylactic platelet transfusions. However, from clinical practice, and other studies, we know that present practice does prevent bleeding in some patients but not in all. On the other hand, other patients could likely do without prophylactic transfusions and not have any relevant hemorrhage. From our findings, we hypothesize that a model including time varying variables should lead to a more accurate prediction of bleeding. This could potentially also better discriminate which patients do or do not benefit from the platelet prophylaxis. Such a dynamic prediction tool in our opinion is an important step in

improving bleeding prevention for hemato-oncology patients and additionally averting unnecessary use of platelet transfusions.

In **chapter 6** we describe the BITE study protocol, an ongoing case control study by which we eventually aim to describe and quantify potential risk factors of bleeding in hemato-oncology patients, as well as the combined effects of risk factors. The way the data is collected namely allows for dynamic prediction as well; by this a personalized and time-specific bleeding risk can be predicted. Hopefully, this will eventually allow more effective and personalized strategies to prevent bleeding in future, and to avoid those strategies if likely unnecessary.

## Samenvatting

Ondanks dat er veel profylactische trombocytentransfusies (bloedplaatjes-transfusies om een bloeding te voorkomen) worden gegeven aan hemato-oncologische patiënten komen bloedingen nog steeds vaak voor. Een deel van deze bloedingen zijn klinisch relevant. Dat houdt in dat ze leiden tot uitbreiding van zorg, invasieve ingrepen of onderzoeken, veranderingen van medicatie, of tot additionele bloedtransfusies. Klinisch relevante bloedingen kunnen zelfs leiden tot ernstige co-morbiditeit of het overlijden van een patiënt. Om in te toekomst zulke relevante bloedingen effectiever te voorkomen is het van belang beter te weten welke factoren bijdragen aan het ontstaan van de bloeding. Ook is het belangrijk om te kunnen voorspellen welke patiënten een hoog danwel laag bloedingsrisico hebben. In dit proefschrift hebben we gekeken naar drie aspecten omtrent bloedingsrisico en preventieve maatregelen bij hemato-oncologische patiënten. Ten eerste keken we naar de huidige klinische praktijk van bloedingspreventie in een subgroep patiënten met een chronische diepe trombocytopenie (verlaagde waarden van bloedplaatjes). Ten tweede hebben we ons gericht op risicofactoren, en tot slot hebben we gekeken naar het voorspellen van bloedingen.

In **hoofdstuk 2** hebben we de huidige klinische praktijkvoering omtrent bloedingspreventie bekeken in een subgroep hemato-oncologische patiënten, namelijk poliklinische patiënten met een chronische diepe trombocytopenie. Aan deze subgroep worden in Nederland ook vaak profylactische trombocytentransfusies gegeven. Verreweg de meeste artsen houden dan dezelfde streefwaarden aan die in de transfusierichtlijnen staan voor klinische patiënten met een tijdelijke trombocytopenie, namelijk een trombocytengetal van $10x10^9$/L. Middels onze enquête hebben we laten zien dat patiënten die geen actieve behandeling meer krijgen om hun onderliggende hematologische ziekte te bestrijden veel minder vaak profylactische trombocytentransfusies krijgen. Ook hebben we in kaart gebracht dat er veel verschillende gezondheidskarakteristieken meegenomen worden in de weging om wel of geen profylactische transfusies te geven. Hiervan worden eerder doorgemaakte bloedingen en het gebruik van antistollingsmedicatie of trombocytenaggregatie-remmers (medicatie die bloedplaatjes-activatie tegengaan) het meest belangrijk geacht. Indien patiënten inderdaad karakteristieken hebben waarvan verwacht wordt dat het bloedingsrisico omhoog kan gaan, dan worden er zeer uiteenlopende grenzen aangehouden waarbij transfusies worden voorgeschreven. Daarnaast hebben we ook in deze enquête gevraagd in welke situaties tranexaminezuur (medicijn dat afbraak van gevormde bloedstolsels remt) wordt voorgeschreven. Dit zogeheten antifibrinolyticum wordt vooral voorgeschreven indien patiënten een actieve bloeding hebben, maar zelden als profylaxe om bloedingen te voorkomen. Onze resultaten laten zien dat er

nog een kennishiaat is omtrent bloedingspreventie in deze specifieke patiëntpopulatie, en ondersteunen dat er meer onderzoek nodig is naar adequate bloedingspreventie in deze patiënten met een chronische diepe trombocytopenie.

In **hoofdstuk 3** en **hoofdstuk 4** focussen we ons op patiënten met acute leukemie die een hersenbloeding, of intracraniële bloeding, hebben. In **hoofdstuk 3** beschrijven we dat zowel absolute trombocytengetallen, als het percentage van de tijd waarin de patiënt lage trombocytengetallen heeft (verdeeld over tijdsperiodes tot 7 dagen voor een bloeding) geassocieerd waren met intracraniële bloedingen. Langere episodes van trombocytopenie lijken het risico op dit soort bloedingen te vergroten. Echter, onze onderzoekspopulatie was maar klein, hierdoor kunnen we het precieze effect niet goed schatten, en kunnen we niet corrigeren voor 'confounders' (factoren die de associatie tussen trombocytopenie en intracraniële bloedingen kunnen beïnvloeden). We hebben ook laten zien dat patiënten die in deze tijdsepisodes meer trombocytentransfusies nodig hebben, ook een verhoogd bloedingsrisico hebben. Dit gold vooral voor patiënten die meer dan twee trombocytentransfusies kregen in een periode van vijf tot zeven dagen voorafgaand aan de intracraniële bloeding. Deze associatie ontstaat meest waarschijnlijk door de klinische condities die tot trombocytentransfusie-behoefte leiden, en niet door een oorzakelijk direct effect van de transfusie op de bloeding.

In **hoofdstuk 4** hebben we onderzocht of pre-existente cardiovasculaire risicofactoren een predictieve waarde hebben voor het ontstaan van intracraniële bloedingen bij patiënten met acute leukemie. In de algemene populatie zijn cardiovasculaire risicofactoren eerder beschreven als risicofactoren en voorspellers voor intracraniële bloedingen, vooral voor hersenbloedingen. Het was echter niet bekend of deze associaties in gelijke mate belangrijk waren voor patiënten met acute leukemie. We hebben aangetoond dat vooral een voorgeschiedenis van hypertensie of ischemische hartziekten sterke voorspellers lijken voor het ontstaan van intracraniële bloedingen in leukemiepatiënten. Deze voorspellende waarde lijkt groter dan in de algemene populatie. Hoewel de potentiële causaliteit meer onderzoek behoeft, is onze hypothese dat de combinatie van chronische vaatschade (waarvoor hypertensie en ischemische hartziekten surrogaatmarkers kunnen zijn) met acute vaatschade en lage trombocytengetallen gedurende intensieve leukemiebehandelingen elkaar versterken en zo de sterkere associatie kan verklaren. Indien toekomstig onderzoek dit kan bevestigen, is het van belang om te onderzoeken of patiënten met een voorgeschiedenis van hypertensie of ischemische hartziekten voordeel zouden ondervinden van aangepaste of aanvullende interventies om bloedingen te voorkomen.

Om bloedingen beter te voorkomen, en tegelijkertijd onnodige trombocytentransfusies te besparen bij patiënten die ze niet nodig hebben, is het noodzakelijk dat we leren voorspellen wie er waarschijnlijk gaan bloeden, en wie niet. Daarnaast is het van belang om te kunnen voorspellen welke patiënten waarschijnlijk baat gaan hebben van profylactische transfusies. In **hoofdstuk 5** hebben we daarom bestudeerd wat het effect is van trombocytentransfusies in groepen van patiënten met verschillende baseline risicofactoren (risicofactoren die bij de start van een behandeling aanwezig zijn). Om dit te doen hebben we eerst een predictiemodel gemaakt, met daarin opgenomen een groep van dergelijke risicofactoren die in eerdere studies geassocieerd zijn met bloedingen. Doch, dit predictiemodel met gecombineerde baseline risicofactoren had een lage voorspellende waarde en kon daardoor niet goed differentiëren tussen hoge en lage bloedingsrisico's. De voorspelde bloedingsrisico's lieten weinig spreiding zien. Wel konden we middels een 'heterogeneity of treatment effect analysis' (analyse die kijkt naar hoe het effect van een behandeling verschilt voor verschillende patiënten) aantonen dat patiënten met verschillende baseline factoren min of meer evenveel baten hadden van de profylactische trombocytentransfusies. Vanuit klinische ervaring, alsmede uit eerdere studies, weten we echter dat de transfusies bloedingen voorkomen bij sommige, maar niet bij alle patiënten. Ook weten we uit andere studies dat sommige patiënten ook zonder profylaxe geen klinisch relevante bloedingen ontwikkelen. We denken dat een model dat niet enkel baseline risicofactoren meeneemt, maar ook naar factoren die wisselen in de tijd, mogelijk tot een meer accurate predictie van bloedingsrisico's. Dit zou uiteindelijk ook kunnen helpen om een onderscheid te maken welke patiënten wel of niet voordeel ondervinden van profylactische transfusies. Een dergelijk 'dynamisch predictiemodel' is naar onze mening een belangrijke stap en betere preventie van bloedingen in hemato-oncologische patiënten. Tegelijkertijd kan het bijdragen aan het voorkomen van het geven van transfusies aan patiënten die ze niet nodig hebben.

In **hoofdstuk 6** presenteren we het BITE studieprotocol. De BITE studie is een lopende case control studie, waarin we beogen om potentiële risicofactoren voor bloedingen in de hemato-oncologische populatie te beschrijven en kwantificeren. De data wordt verzameld op een manier die dynamische predictie mogelijk maakt. Hierdoor willen we een gepersonaliseerd en tijd specifiek bloedingsrisico voorspellen. Hopelijk kan dit in de toekomst bijdragen aan meer effectieve en geïndividualiseerde strategieën om bloedingen te voorkomen, en aan het tegengaan van profylactische behandelingen die bij sommige patiënten niet nodig zijn.


A

# Appendices

Curriculum Vitae
List of publications
Dankwoord

## Curriculum Vitae

Loes Laura Cornelissen was born on August 24 1984 in Zevenaar, The Netherlands. She graduated from the secondary school (VWO) at the St. Ludger College in Doetinchem in 2002. In 2005, she obtained her Cesar Exercise Therapy diploma at the University of Applied Science in Utrecht. After that, she started her medical training at the Utrecht University, from which she graduated and received her medical degree in 2011. Afterwards she worked as a medical doctor (not in training, Dutch: ANIOS) until 2012, at the department of Internal Medicine in the Diakonessenhuis in Utrecht and Zeist. In 2013 she started her clinical training internal medicine in the Meander Medical Center in Amersfoort, which she continued in the University Medical Center Utrecht in 2016. From 2017 to 2021 she temporarily discontinued her clinical training, to work as a full-time researcher. During these years she worked as a PhD candidate at the department Clinical Transfusion Research of Sanquin Research and the Jon J van Rood Center for Clinical Transfusion Research at Leiden University Medical Center. She coordinated the design and initiation of a prospective study (BITE study) investigating risk factors for bleeding in hematology patients, and performed several analyses in databases regarding this same subject. Under supervision of prof. dr. J.J. Zwaginga, prof. dr. J.G. van der Bom, and dr. C. Caram-Deelder, this has led to this thesis. During these four years, she was additionally trained in clinical epidemiology at the department of Clinical Epidemiology of the LUMC. In 2021, she resumed her medical training and started her hematology specialization at the University Medical Center in Utrecht. Loes lives in Utrecht, together with her partner Gerben de Haan, and their daughter Marthe.

# List of publications

## Publications in this thesis

**Cornelissen LL**, Caram- Deelder C, van der Bom JG, et al. Risk factors for bleeding in haemato- oncology patients—a nested case–control study: The BITE study protocol (Bleeding In Thrombocytopenia Explained). BMJ Open. 2020 Jun 30;10(6):e034710. doi: 10.1136/bmjopen-2019-034710.

**Cornelissen LL**, Kreuger AL, Caram-Deelder C, et al. Thrombocytopenia and the effect of platelet transfusions on the occurrence of intracranial hemorrhage in patients with acute leukemia - a nested case-control study. A Ann Hematol. 2021 Jan;100(1):261-271. doi: 10.1007/s00277-020-04298-7. Epub 2020 Oct 17.

**Cornelissen LL**, Caram-Deelder C, Meier RT, et al. Consortium-Dutch Blood Transfusion Related Research T. Platelet transfusion and tranexamic acid to prevent bleeding in outpatients with a hematological disease: a Dutch nationwide survey. Eur J Haematol. 2021 Mar;106(3):362-370. doi: 10.1111/ejh.13555. Epub 2020 Dec 21.

**Cornelissen LL**, Caram-Deelder C, Fustolo-Gunnink SF, et al. Expected individual benefit of prophylactic platelet transfusions in hemato-oncology patients based on bleeding risks. Transfusion. 2021 Sep;61(9):2578-2587. doi: 10.1111/trf.16587. Epub 2021 Jul 15.

**Cornelissen LL**, Kreuger AL, Caram-Deelder C, et al. Association between cardiovascular risk factors and intracranial hemorrhage in patients with acute leukemia. Eur J Haematol. 2022 Apr;108(4):310-318. doi: 10.1111/ejh.13737. Epub 2022 Jan 18. PMID: 34923665.

## Other publications

Esther van Bladel, Rosa de Jager, Daisy Walter, **Loes Cornelissen**, et al. Platelets of patients with chronic kidney disease demonstrate deficient platelet reactivity in vitro. BMC Nephrol. 2012 Sep 28;13:127. doi: 10.1186/1471-2369-13-127.

**Cornelissen, LL**, Zwaginga JJ, Preventieve trombocytentransfusies bij hemato-oncologische patiënten: van trigger-gestuurd tot een per patiënt geoptimaliseerd toekomstperspectief? NED TIJDSCHR HEMATOL 2020;17:276-82
(English title: Prophylactic platelet transfusions in hemato-oncology patients: from transfusion thresholds to possible future perspectives)

## Dankwoord

In januari 2017 begon ik aan een nieuw avontuur, en pauzeerde ik mijn opleiding tot internist-hematoloog om mijn wetenschappelijke vorming te verdiepen. Zowel hetgeen ik in deze jaren heb mogen leren, als het resultaat dat hier voor u ligt, had ik nooit alleen kunnen bereiken. Ik ben dan ook velen dankbaar die zich hebben ingezet om mij te laten groeien.

Ten eerste mijn promotoren en copromotor. Jaap Jan, als promotor bood jij me vanaf dag één vrijheid in onze projecten, en stuurde me bij waar nodig. Je bent altijd in voor een bevlogen discussie, bedankt daarvoor. Anske, ik prijs me gelukkig dat je als me de laatste jaren als promotor hebt willen begeleiden. Je soms kritische, maar vooral heldere blik hebben het onderzoek naar een hoger plan getild. Camila, from data manager to copromotor: I truly believe every PhD student deserves 'a Camila' on their team. You kept my spirit up, kept eye on the end goals, and functioned as practical life saver, and friend.
Uiteraard bedank ik ook de leden van de promotiecommissie, voor de tijd die ze hebben genomen om dit proefschrift te beoordelen.

De staf van de Klinische Epidemiologie wil ik graag bedanken voor alles wat ik van ze heb mogen leren.
Verder bedank ik alle betrokkenen die zich hebben ingezet om de BITE studie, nu of in de toekomst, in hun ziekenhuis te laten draaien. Met name Dorothea, die voortvarend als ze is soms sneller wilde dan wij konden, bedankt voor de fijne samenwerking. De uitvoering van de BITE studie was niet mogelijk geweest zonder de hulp van een geweldig research team, met name het (voormalig) researchteam van Sanquin Leiden! Inge, met je altijd goede stemming was jouw hulp op de afdeling vaak broodnodig, en vooral heel fijn! Rutger, bedankt voor je hulp bij het opzetten van de BITE studie en voor het kritisch meelezen in de afrondende fase.

Dan zijn er natuurlijk nog alle collega-promovendi die mijn tijd in Leiden verrijkt hebben. Aukje, naast je gezelligheid heb ik veel van je geleerd, ik blij dat ik op jouw werk voort mocht bouwen. Ada, je was een fijne kamergenoot; altijd vrolijk en in voor een cappuccino 'light'. Sufia, mijn cursus-maatje, wie had gedacht dat epidemiologie zo gezellig kon zijn!

Mijn voormalig perifere opleider, Rob, bedank ik graag omdat hij me mee heeft genomen op het pad van de hematologie. Jan Jelrik, mijn opleider interne geneeskunde, bedankt voor de ruimte om enkele jaren aan onderzoek te besteden. Uiteraard ook

veel dank aan mijn huidige opleiders bij de hematologie, Anna en Reinier, voor de mogelijkheid om mij op het specialisme hematologie te mogen storten, en voor de steun in het afrondende traject van mijn promotie. Aan alle hematologen, collega-fellows en andere medewerkers van de hematologie in het UMC Utrecht: ik ben blij dat ik deel mag zijn van jullie team!
Ook alle collega's arts-assistenten die in de jaren zijn gepasseerd wil ik bedanken voor de fijne samenwerking en gezelligheid. Anne, Lotte en Anke, naast super-collega's zijn jullie ook heel goede spelletjes- en reismaatjes!

In de afgelopen jaren van hard werken waren alle kostbare momenten met mijn vrienden een heel fijne uitlaatklep. Ik mag me gelukkig prijzen met veel lieve vrienden en vriendinnen, die ik helaas niet allemaal kan noemen, maar die allemaal een bijzonder plekje in mijn hart hebben.
Kristel, Remy, Bauke, Esmee, Lienke en alle grote en kleine aanhang die daar ondertussen bij komt kijken: jullie vriendschap bestaat al sinds onze middelbare schooltijd en is goud waard! Kris, wij hebben al heel wat avonturen samen meegemaakt, bedankt dat je bij dit avontuur als paranimf naast me wilt staan!
Charine, jij bent een enorme steun geweest de afgelopen jaren, op meerdere fronten. Dank je wel voor je vriendschap, betrokkenheid, en bedankt dat je mijn paranimf wilt zijn!

Natuurlijk heel veel dank aan mijn lieve familie. Pap en mam, jullie hebben me met geduld en liefde altijd de kans hebben gegeven mij te ontwikkelen. Het is een rijkdom te weten dat jullie er altijd voor me zijn. Joost, Nadia, jullie staan altijd voor me klaar, en een bezoek aan jullie, Floris en Cato is altijd fijn. Mijn schoonfamilie wil ik ook bedanken, vanaf dag één heb ik mij welkom gevoeld bij jullie!

Lieve Gerben, jij bent de rust en het overzicht die in mijn hoofd soms ontbreken, en de warmte en veiligheid die ik nodig heb. Met jou iedere avond aan tafel mogen, de natuur in gaan, en het samen om niets kunnen lachen heeft me niet alleen deze promotietijd door gesleept, maar heeft me vooral een gelukkiger mens gemaakt! Lieve Marthe, iedere lach van jou is een dosis geluk, iedere dag met jou is een cadeau. Als geen ander laat jij me beseffen wat echt belangrijk is in het leven. Met jullie aan mijn zijde kan ik dankbaar terugkijken op de afgelopen jaren, en kijk ik vooral uit naar al het moois wat onze toekomst nog brengen gaat ♥. Ik hou van jullie!

Loes Cornelissen