Risk factors for bleeding in haemat-oncology patients—a nested case–control study: The BITE study protocol (Bleeding In Thrombocytopenia Explained)

Loes L Cornelissen, Camila Caram-Deelder, Johanna van der Bom, Rutger A Middelburg, Jaap Jan Zwaginga

ABSTRACT

Introduction Haemat-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 study (Bleeding In Thrombocytopenia Explained) 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 haemat-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 for 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 haemat-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 De 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.

Trial registration number NL62499.058.17. NCT03505086; Pre-results.

Strengths and limitations of this study

The prospective documentation of a cohort of haemat-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.

This study design enables the quantification of associations between measured risk factors and major bleeding maximally adjusted for confounding and selection bias.

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.

Missing blood samples in a large number of patients may lead to imprecise estimates of the associations between biomarkers and bleeding risk.

Some haemat-oncology patients may die before measurements are done, which may lead to selection bias.

INTRODUCTION

To prevent clinically relevant bleeding events, haemat-oncology patients usually receive prophylactic platelet transfusions, mostly on a platelet count trigger of 10×10^9/L. Although platelet counts seem to be poorly related with the occurrence of bleeding, patients treated with trigger-based prophylactic platelet transfusions in randomised controlled trials experience less bleeding as compared with patients with therapeutic transfusions, that is, triggered by bleeding symptoms. In one trial, the incidence of bleeding was 50% in patients without prophylactic transfusions, compared with 43% in patients who did receive prophylactic transfusions. 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 haematopoietic population. Additional to platelet counts, also other factors have been implicated to influence bleeding risk in haematology patients, like disease stage, disease type, type of treatment (chemotherapy and allogeneic stem cell transplantation (SCT) vs autologous SCT), fever or presence of infection, graft-versus-host disease, splenomegaly, need for red blood cell (RBC) transfusion and the presence of uraemia. 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 hypothesise it is also important to gain insight in biomarkers characterising platelet function, vascular integrity, the plasmatic coagulation and fibrinolytic system and their relation to the bleeding tendency in these patients.

The Bleeding In Thrombocytopenia Explained (BITE) study investigates the role of potential risk factors on clinically relevant bleeding in haematology patients who have or have had a thrombocytopenic period. Most research investigating prophylactically platelet transfusions has been performed in patients during their treatment (ie, 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 haematologic biomarkers for platelet, vascular and coagulation dysfunction, 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 personalised 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 haematopoietic patients, who have or have had a thrombocytopenic period.

**Secondary objectives**

1. To quantify the incidence of bleeding in hospitalised haemat-oncological patients, and for subgroups based on their diagnosis and indication for admission.
3. To compare WHO and International Society on Thrombosis and Haemostasis (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 personalised prevention of bleeding.
5. To quantify the association between evident pre-existing bleeding tendencies and bleeding during haemat-oncological disease.

**METHODS AND ANALYSIS**

**Study design**

The BITE study is a multicentre matched case–control study nested in a cohort of adult haemat-oncological patients in the Netherlands from 2018 to 2023. Nine Dutch hospitals have agreed to participate (five university medical centres and four large regional community hospitals). With five of eight university centres in the Netherlands we estimate to have about 25% of all Dutch haemat-oncological patients in our source population. Dutch transfusion guidelines ensure reasonable standardisation 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 (online supplementary 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 online supplementary 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, for example, HiX or EPIC.

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...
Source population
All patients admitted in the haemato-oncology ward who fulfill the inclusion criteria

- Patients asked to participate who do not give informed consent
- Patients not asked to participate

Cohort population
Patients who give informed consent

Patients who bleed

Patients who do not bleed

Eligible controls

Case control study

Case patients

Groups:
1. \( \triangle \)
2. \( \diamond \)
3. \( \bigcirc \)

Control patients matched to cases

Patients not selected as controls

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 are 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.

Used. WHO grades 3 and 4 bleeding are mostly clinically relevant, and for example can lead to red cell transfusions or haemodynamic instability. On the other hand, grade 1 bleeding, 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 ISTH score explicitly discerns clinically relevant major and non-major bleeding. Here, a bleeding is defined as major if it is fatal or symptomatic in a critical organ, when it induces a haemoglobin drop of at least 1.24 mmol/L (20 g/L) or when it leads to two or more RBC transfusions. Non-major bleeding according to the ISTH criteria is only defined as clinically relevant if additional medical evaluation or intervention is required. In the WHO criteria, the latter are for a large part categorised as grade 2 bleeding, 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 (ie, major and non-major). Hence, case patients are


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all patients with bleeding requiring substantial additional medical intervention. According to the WHO scoring system, this includes grade 3 and 4 bleeding, as well as grade 2 bleeding 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 haematological 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 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 (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 bleeding, 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 with 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 bleeding of the entire source population. If needed physicians are asked for details of bleeding incidents. To minimise under-reporting of bleeding, reporting is actively monitored on a weekly basis by the study team. This is done by asking whether clinically relevant bleeding occurred in weekly grand rounds and by personal contact with treating physicians on the ward. Patients with clinically relevant 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. Matching per hospital allows for adjustment of local differences, for example, 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 estimates 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.

Per case, we match up to a maximum of four controls, as this is thought to be the most optimal ratio to estimate risk ratios (RR) even when exposure is rare. 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 1 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 CI of 95% (alpha = 5%) was used. Based on a 1:4 ratio of cases and controls, inclusion of 1000 patients (ie, 200 cases and 800 controls) will give this case control study enough power to detect 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
Based on these incidences, and an expected bleeding of 56% and of WHO grade 3–4 bleeding of 7.8%, we intend to include 200 cases and 400–800 control patients. Especially when exposure prevalence is relatively high, the fewer cases we need to achieve the same power, compared with 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.

**Figure 3** Lines indicate the number of cases needed to achieve 80% power to detect a statistically significant difference (ie, type 1 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 with 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.

A previous study observed an incidence of WHO grade 2 bleeding of 56% and of WHO grade 3–4 bleeding of 7.8%. Based on these incidences, and an expected number of 2000 admissions with a thrombocytopenic period in participating and future participating hospitals per year, we expect between 75 and 150 patients with clinically 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 (eg, 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 hospital's information technology (IT) department. 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 (eg, sex, age, body mass index), infection parameters, relevant comedication (eg, anticoagulation), interventions, trauma or vomiting during admission, comorbidities and outcome after admission (see online 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.

Data are 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 SCT. 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 4 weeks of admission.

All blood samples will be stored at −70°C/−80°C 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.

**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 authorised 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

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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 (GCP) conform Case Report Forms (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 GCP 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 (eg, 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 (ie, 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 ORs will be interpreted as incidence rate ratios with 95% CIs.22 Detailed analysis plans will be written and peer-reviewed by an established scientific committee (eg, 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. 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 coauthors 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.

Contributions LL 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, JGvdB, JJZ and RAM critically reviewed the manuscript, as well as the complete study protocol. All authors approved the submitted version.

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Competing interests 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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REFERENCES
