PLATELET TRANSFUSION

IN PATIENTS UNDERGOING MINOR INVASIVE PROCEDURES

Balancing risks and benefits

EMMA VAN DE WEERDT

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Platelet transfusion in patients undergoing minor invasive procedures
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Faculteit der Geneeskunde

Voor mijn broer en zus

CONTENTS

1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

Emma K. van de Weerdt, Bart J. Biemond
and Alexander P.J. Vlaar

GENERAL INTRODUCTION

Background

Minor invasive procedures are frequently performed in patients for the diagnosis and treatment of their illnesses. Invasive procedures, such as central venous catheters, arterial lines, drains and large bore needles enable the administration of drugs, monitoring patient´s vital parameters and analysis of obtained tissue for diagnostic purposes. These invasive procedures can be complicated by bleeding, especially in patients with impaired hemostasis. Hemostasis depends on a sufficient amount of functioning platelets and the availability of clotting factors. Therefore, patients with a low platelet count (thrombocytopenia) or impaired coagulation have an increased risk of bleeding. Severely thrombocytopenic patients have an increased risk for both spontaneous bleeding and bleeding following invasive procedures. Approximately half of thrombocytopenic patients with hematologic cancers suffer from moderate to severe bleeding.[1,2].[1,2] The combination of impaired hemostasis and an indication for a central venous catheter is frequently present in both patients admitted to the intensive care unit (ICU) and patients with hematological malignancies. Even though the association between thrombocytopenia and bleeding is evident, clear evidence that supports the correction of thrombocytopenia by platelet transfusion prior to invasive procedures is lacking.[3,4] Furthermore, the downsides of transfusion; including transfusion-related acute lung injury (TRALI), transfusion associated cardiac overload (TACO), allergic reactions, allo-immunisation and transfusion related infections have become clear in the past decades. Therefore, platelet transfusion should ideally only be administered to prevent bleeding and should be avoided if unnecessary. This thesis focusses on the risk benefit balance of platelet transfusion prior to minor invasive procedures in critically ill and hematologic patients.

Platelets

Platelets are disc-shaped, anucleotide cells that play a pivotal role in primary phase of coagulation, to maintain hemostasis following vascular injury. Platelets derive from fragmentation of megakaryocytes, after which they remain in the circulation for 8-12 days. Circulating platelets retain in their inactivated state due to continuous inhibition by various mediators. When the vessel wall is injured however, platelets get activated by exposure to the subendothelial matrix. (Figure 1). Activated platelets alter their shape and excrete stimulating factors to attract more platelets. Activated platelets bind to both the injured surface with the help of von Willebrand factor and activate the coagulation system, resulting in the formation of fibrin strains and a firm blood clot.

Platelets are the most abundant cells in the blood, with normal platelet counts varying from 150-400 x 10^9 per liter. Thrombocytopenia is defined as a platelet count below 150 x 10^9 /L and below 50 x 10^9/L for severe thrombocytopenia. A low platelet count may occur due to inadequate platelet production, increased consumption, destruction, or pooling in the spleen.[5-7] In particular, thrombocytopenia is a common finding in hematologic and critically ill patients. Thrombocytopenia is present in 8-67% of patients admitted to the ICU, mostly due to severe infection and/or inflammation resulting in coagulation activation and massive platelet consumption.[8,9] In critically ill patients, platelet counts are reversely related to survival, irrespective of the cause of thrombocytopenia.[9] In patients with hematologic malignancies, thrombocytopenia is usually caused by bone marrow failure, caused by the disease itself or the myeloablative effect of chemotherapy.

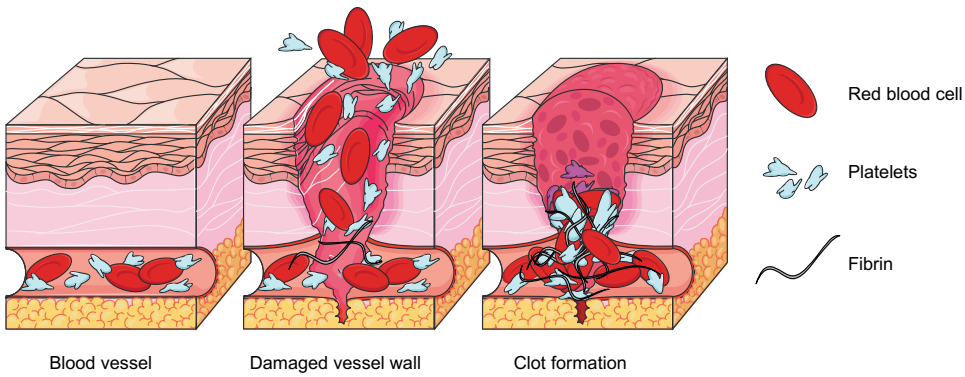

FIGURE 1. Platelets in hemostasis. In the normal situation, platelets are present in the circulation in abundance (left), when endothelium of the vessel wall is damaged (middle), platelets get activated and form a stable plug together with red blood cells and fibrin strands (right).

Platelet concentrates

In the Netherlands almost 250.000 platelet concentrates are used annually (Sanquin Annual report 2017, www.sanquin.nl). Platelet concentrates can be obtained from a single apheresis donor, or by pooling the isolated platelets from multiple whole blood donations. One of the biggest challenges with the isolation and storage of platelets is their fragile nature. During storage, or after centrifugation platelets are known to deteriorate. Unlike other blood products, platelets lose their function and are cleared rapidly from the circulation if they are refrigerated. Therefore, platelets need to be stored at room temperature, in which bacteria can grow more easily. Given this risk of potential bacterial contamination, platelets can only be stored for 5 to 7 days. This makes availability of platelet concentrates a logistical

challenge and the risk of wastage high.[10,11] For clinical research, platelets can be labeled to distinguish transfused platelets from the recipient's own circulating platelets. Labeling of platelets allows evaluation of the effects of donor-, recipient- and platelet storage factors, such as new additive solutions and pathogen-reduction technologies.[12,13] The golden standard for platelet labeling is radiolabeling with radioactive isotopes ^{111}Indium-oxin or ^{51}Chromium. Unfortunately, radiolabeling exposes the recipient to harmful ionization, which makes the method unsuitable in vulnerable patient categories. Moreover, the use of radiolabeled platelets is strictly regulated, which limits its applicability for research purposes. Radiolabeling does not allow tracing multiple populations of platelets concurrently. Also, it is not possible to isolate platelets. Therefore, at the starting point of this thesis, we concluded a non-radioactive alternative to label platelets is desired in order to study the platelet dynamics.

Platelet transfusion triggers

Platelet concentrates can either be transfused prophylactically; i.e. to prevent bleeding, or therapeutically, to stop active bleeding. The appropriate platelet transfusion trigger for various indications is uncertain. Therapeutic platelet transfusions are evidently beneficial in actively bleeding patients with severe thrombocytopenia. However, the majority of platelet transfusions are given prophylactically, for which the benefit to patients is more difficult to assess and remains unclear.[14-16] It is known that prophylactic platelet transfusion fails to eliminate the risk of bleeding and a significant number of patients bleed despite prophylaxis. In clinical practice, minimal platelet count triggers are used to administer platelet transfusions for various indications. Several large studies have studied the minimal safe platelet count in non-bleeding patients. In non-bleeding patients, a platelet transfusion trigger of $10x10^9$ per liter is considered optimal.[1] However, data to determine platelet transfusion triggers for various invasive procedures are limited and predominantly observational in nature. Therefore, recommendations in clinical guidelines are usually based on expert opinion and on low to very low-quality evidence.[17,18] Therefore, the optimal platelet transfusion threshold for various invasive procedures remains unclear.

Risks of transfusion

Platelet transfusion is not without risks to the recipient (Table 1). It is important to keep in mind that transfused cells and plasma proteins are foreign substances that evoke an immunological response in the recipient, which may lead to a range of adverse reactions. These reactions vary from mild immunologic reactions, such as urticaria and post-transfusion fever, to severe reactions, such as post-transfusion hemolysis or respiratory distress.

In its most severe form, transfusion may induce acute respiratory distress within six hours after the transfusion, which is known as *transfusion-related acute lung injury* (TRALI). TRALI is mainly caused by a strong immunological response to transfused human leukocyte antigen (HLA) in a patient that has previously been exposed to this foreign antigen, due to pregnancy or previous transfusions. TRALI is a life-threatening condition, with up to 91% of TRALI patients requiring invasive ventilation and a reported mortality of 17-45%.[19-21]

Another life-threatening complication of transfusion is *transfusion-associated cardiac overload* (TACO).[22-28] TACO is a potentially life-threatening condition in which pulmonary edema develops due to circulatory volume overload in response to transfusion.

The risk of transmittable diseases by transfusion, such as HIV and hepatitis, extremely low due to donor screening. Critically ill and patients with hematologic malignancies are at higher risk of transfusion related morbidity and mortality.[22,29] Fewer transfusions will lead to fewer transfusion related side effects. Whether a restrictive transfusion policy in thrombocytopenic patients undergoing invasive procedures outweighs the bleeding risk is to be determined.

TABLE 1. Risk of platelet transfusion

Adverse event	Incidence
Febrile reaction	7%
Allergic reaction	2%
TRALI	0.08-15.1%[20,22]
Bacterial contamination of PC	0.23-0.32%[30,16]
Bacterial sepsis	0.001%

Central venous catheters

Central venous catheter (CVC) placement is one of the most commonly performed invasive procedures, performed in 8% of all hospitalized patients, in particular in critically ill patients and patients with hematologic malignancies.[31] The catheter provides venous access in a large vessel in either in the neck region (subclavian vein or jugular vein) or groin region (femoral vein). A CVC enables blood sampling, hemodynamic monitoring, hemodialysis and the administration of medication, fluids and parenteral nutrition. Placement of the catheter is complicated by mild bleeding in 3-30% of patients, depending on the physician's experience, insertion site, the use of ultrasound and the coagulation status of the patient.[32-35] Patients in need of a CVC, frequently suffer from hemostatic disorders, including

thrombocytopenia. Physicians are often hesitant to insert a CVC in these coagulopathic patients, due to the perceived increased risk of bleeding. However, major bleeding after CVC placement is rare.[36] Classical coagulation tests, including PT, INR and platelet count are poor predictors of bleeding complications after CVC placement.[3,4,37] Current guidelines are contradicting.[17,38-40] Data from observational studies suggest prophylactic platelet transfusion for patients that undergo elective central venous catheter placement do not need prophylactic platelet transfusion with a platelet count above 20 x 10.[34,41] However, the Dutch guideline states the platelet count should be corrected if below 50×10^9 per liter.[38] Therefore, there is a strong need for prospective studies to evaluate the safety of a more restrictive prophylactic platelet transfusion policy in this category of patients.

Complications of CVC placement

Although pivotal for treatment, CVC-placement may cause serious complications, including pneumothorax, incorrect position, hemothorax and catheter related bloodstream infection.[42-45] The introduction of ultrasound guidance for central venous catheter placement has been a major improvement.[46] Real-time ultrasonographic guidance had led to a reduction of the number of unsuccessful placements, puncture attempts, hematomas, inadvertent arterial punctures and the time to correct placement.[35,46-49] Traditionally a postprocedural chest X-ray is obtained to confirm correct catheter tip position and rule out complications. At the starting point of this thesis we were interested in whether ultrasonography could be used as an alternative for chest X-ray after CVC placement.

OUTLINE OF THIS THESIS

The starting point of this thesis is the balance between prophylactic transfusions to prevent bleeding, and withholding unnecessary transfusions to reduce transfusion related side effects and risks. There is a paucity of evidence to support the current practice, in which transfusion is giving prophylactically. This dissertation focusses on the risk benefit balance of platelet transfusion prior to invasive procedures in critically ill and hematologic patients.

In **Chapter 2** we systematically reviewed the literature on the risk of central venous catheter insertion in patients with severe coagulopathy.

In **Chapter 3** we conducted a nationwide mixed vignette and questionnaire survey to investigate the current practice of Dutch physicians concerning prophylactic platelet transfusion prior to central venous catheter placement.

In **Chapter 4** we described the study protocol of the PACER trial. This is a prospective, randomized controlled trial investigating whether omitting platelet transfusion prior to central venous cannulation is non-inferior compared to no platelet transfusion on relevant bleeding complications in critically ill and hematologic patients with platelets counts between 10 and 50×10^9 per liter.

In **Chapter 5** we systematically reviewed and discussed the limitations of the different bleeding scores used to assess bleeding in clinical trials in thrombocytopenic patients.

In **Chapter 6** we developed and validated a novel method for the in vivo labelling of transfused platelets by the use of the vitamin biotin, as an alternative label for radioactive labelling.

In **Chapter 7** we investigated whether donor characteristics as donor sex or age influence the incidence of TRALI in a secondary analysis on two cohorts of TRALI patients.

In **Chapter 8** we systematically examined the utility of ultrasound instead of chest radiography for catheter tip confirmation and detection of complications after central venous catheter placement.

This thesis is summarized and discussed in **Chapter 9**.

In **Hoofdstuk 10** wordt deze thesis samengevat en bediscussieerd voor niet-ingewijden.

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2

CENTRAL VENOUS CATHETER PLACEMENT IN COAGULOPATHIC PATIENTS: RISK FACTORS AND INCIDENCE OF BLEEDING COMPLICATIONS

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SUMMARY

Background. Central Venous Catheters (CVC) are frequently inserted in patients with coagulation disorders. It is unclear whether pre-procedural correction of hemostasis is beneficial. We determined the incidence of bleeding complications after CVC placement in patients with severe coagulopathy and identified potential risk factors for bleeding.

Methods. MEDLINE and the COCHRANE LIBRARY were systematically searched through November 2015. In order to be included, articles must have reported on hemorrhagic complications with specification of abnormal coagulation testing results. Severe coagulopathy was defined as a reduced platelet count of ≤ 50 x 109 /L, and/ or an elevated international normalized ratio of ≥ 1.5 and/ or partial thromboplastin time of ≥ 45 seconds.

Results. One RCT and 22 observational studies were included. 15.258 catheter insertions were included, of which 3625 in patients with severe coagulopathy. Prior to 2464 CVC-placements, coagulopathy was not corrected. The bleeding incidence varied from 0% to 32%. The severity of coagulopathy did not predict the bleeding risk. No study demonstrated a beneficial effect of prophylactic administration of platelets or FFP to prevent bleeding complications. Retrospective observational studies suggest no pre-procedural correction is required up to a platelet count of 20 x 109 /L, and an INR of 3.

Conclusion. The incidence of major bleeding complications after CVC placement is low, even in coagulopathic patients. Based on a systematic research of literature, strong evidence supporting correction of hemostatic defects prior to CVC insertion, is lacking. However, well powered, RCTs are necessary to determine both the minimal platelet count, and maximal INR and aPTT that is safe prior CVC insertion.

INTRODUCTION

Central venous catheter (CVC) placement is a common procedure to enable blood sampling, central venous pressure measurements and administering medication, chemotherapy or total parental nutrition.[1-4] CVCs are frequently inserted in patients at risk for coagulation disorders, including thrombocytopenia and prolonged bleeding time (PT).[5] Prophylactic correction of coagulopathy prior to CVC placement is associated with the prevention of bleeding complications. However, the use of platelet transfusion in case of thrombocytopenia, and Fresh Frozen Plasma (FFP) in case of a prolonged bleeding time (PT), is potentially harmful. Transfusion products are associated with transfusion-related acute lung injury, transfusion associated cardiac overload, allergic reactions, allo-immunisation and transfusion related infections.[6-13] Conclusive evidence of optimal platelet count and pro-thrombin time prior to CVC placement is lacking.[14] However, current national and international guidelines support correction of thrombocytopenia up to a platelet count of 50 x 109/L and an INR > 1.5 prior to central venous catheter (CVC) placement.[15-17] The aim of this systematic review is to provide an overview of the current evidence on risk factors and incidence of bleeding complications after CVC placement in patients with severe coagulopathy.

MATERIALS AND METHODS

Literature search

MEDLINE was searched, from January 1, 1980, through November 1, 2015 by using the following medical subject heading (MeSH) terms: complications, blood transfusion, blood plasma, fresh frozen plasma, platelets, central venous catheterization, central line, coagulopathy and thrombocytopenia. The following text words were used: CVL and CVC. To identify observational studies, the MeSH terms case-control study and retrospective study were added. The Cochrane Library (2013), which contains the CENTRAL Database of Controlled Trials, the Database of Abstracts of Review Effectiveness, and the Cochrane Database of Systematic Reviews, was also searched.

In addition, the related articles feature of PubMed, which identifies related articles by using a hierarchical search engine that is not solely based on MeSH headings, was used. This search was completed with articles selected by two of the authors (A.V. and B.V.). Although the search was also carried out for language citations not written in English, the resulting article review involved English-language publications only. The search strategy is detailed in Appendix 1.

Abstract review

After all citations based on our search strategy were identified, two of the authors (A.V. and B.B.) independently reviewed each abstract to assess their eligibility. Eligible studies reported on CVC placement in patients with severe coagulopathy. In order to be included, studies had to report bleeding complications for a (sub)group of participants with at least one of the following characteristics: a reduced platelet count of ≤ 50 x 109 /L, an elevated international normalized ratio of ≥ 1.5 and / or partial thromboplastin time of ≥ 45 seconds prior to central venous catheter placement. Exclusion criteria were: 1. Study type: case reports; 2. No full text available; 4. Language: not available in English. 5. Oxford CEBM.

If an abstract was deemed eligible, E.W. and A.V. independently reviewed the respective article, if available, to confirm that it met the inclusion criteria. The two reviewers either had to reach consensus, or use a fourth reviewer (J.B.), to resolve any discrepancies.

Data extraction

Data from the studies to describe study methods, study location, years of data collection, patient's inclusion and exclusion criteria, patient characteristics, study findings, laboratory values and transfusion policy were extracted using a predefined data collection form.. When available, we abstracted data on patients with and without correction of coagulopathy prior to CVC placement. Bleeding complications were categorized as major or minor bleeding as reported by the article. Definitions of bleeding complications per study were extracted and reported.

Article review process

One author reviewed each article (E.W.). Author and journal names were disclosed to the reviewer. All data were extracted by E.W., and verified for accuracy by the second reviewer (A.V.).

Quantitative pooling and presentation of findings

Because of qualitative heterogeneity of the studies and patient heterogeneity, results were not mathematically pooled. Since most studies lacked a comparison group, it was not possible to calculate risk differences. We assessed the risk of bias using the following criteria: i. Whether patient selection occurred randomly or consecutively versus other; ii. Whether investigators excluded non-prespecified patient groups; iii. Explicitness of bleeding criteria; iv. Correction of hemostasis by transfusion products standardized versus left to the discretion of the physician; v. A protocol for CVC placement; vi. Primary data collection versus data chart reports. The results of the assessment are shown in Appendix 2. Risk of bias.

RESULTS

The searches of Medline and the Cochrane library yielded 302 unique articles for screening; 4 additional records were identified through other sources. After removal of pediatric studies, 269 articles were screened on title. Subsequently, 79 articles were screened on abstract-level using the inclusion and exclusion criteria. 31 full-text articles were assessed for eligibility. A total of 23 articles of which 22 observational studies and one RCT were reviewed for bleeding complications. All studies reported on patients with a platelet count below 50 x 109 /L, an INR > 1.5 and or an aPTT > 45 seconds.

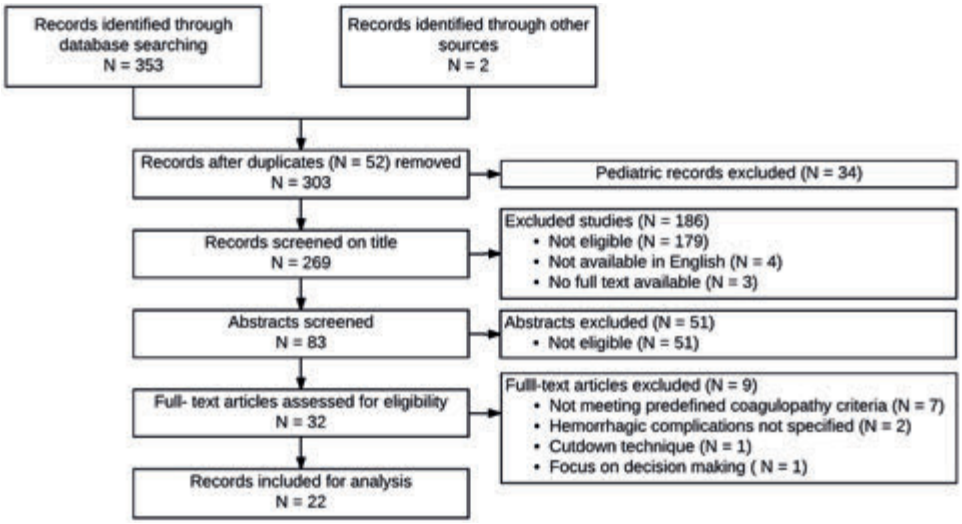

FIGURE 1. Flow diagram depicting study selection

Included studies

In the 22 included studies, a total of 13,280 catheter placements were performed, of which 3,509 cannulations took place in patients with severe coagulopathy. Prior to 950 CVC placements, coagulopathy was corrected with blood donor platelets or plasma. In 2,559 cannulations, coagulopathy was still present during cannulation. We retrieved only one randomized trial. This was an open-label endpoint blinded trial comparing prophylactic use of fresh-frozen plasma (FFP) to no correction of an elevated INR in intensive care patients undergoing an invasive procedure.[18] We included 13 prospective cohort studies and 8 retrospective series. In the prospective studies, coagulopathy was not corrected in six studies, corrected in all patients in one study, corrected at various fixed threshold in

two studies (Table 1), corrected at physician's discretion in two studies, and corrected at physician's discretion in five studies. For the retrospective cohort studies, coagulopathy was corrected at a fixed threshold of 20 x 109 /L in one study, corrected at physician's discretion in six studies and not corrected in one study. Characteristics of studies, patients, procedure and coagulopathy are displayed in Table 1.

The primary focus of the articles included bleeding complications, infectious complications, mechanical complications, technical success rates, method of insertion, site of insertion, the benefit of FFP transfusion and the benefit of platelet transfusion. Endpoints were technical insertion success rates or various catheter related complications, such as mechanical complications, bleeding, thrombosis, and infection.

Study quality

The level of quality of the studies was low. In 7 out of 8 studies reporting major bleeding events, patient and laboratory characteristics were incompletely reported. Data was retrospectively collected by patient charts in nine studies. In five studies, patients that received preprocedural transfusion were excluded. The only RCT included lacked reliability due to the inclusion of only 81 instead of the intended 400 patients.[18]

Coagulopathy. The definition of coagulopathy and its concomitant laboratory thresholds varied across studies. The distribution of abnormal coagulation values and the preprocedural correction of coagulopathy are displayed in Table 1. Thrombocytopenia contributed for the majority of coagulation disorders. Six studies combined the various coagulation test abnormalities to one group.[19-24]. Abnormal coagulation parameters were combined to groups with different terms such as: "severe abnormalities", "high risk of bleeding", "coagulopathy" or "moderate to severe hemostatic disorders" Three studies did not report separate coagulation parameters and also did not specify the number of patients with more than one abnormal laboratory value. To avoid counting patients with multiple hemostatic effect more than once, only the largest group was used.

Bleeding complications. Definitions of both minor and major bleeding varied widely across studies (Table 2). Major bleeding complications were categorized as major as reported by the article. We identified eight studies that reported a total of 13 major bleeding complications (Table 3). Eight major bleeding complications were observed in patients with severe coagulopathy, and five major bleeding complications were reported in patients with mild- tot absent coagulopathy. Only one study provided detailed values of classical coagulation parameters.

TABLE 1. Overview of studies

Study	Design, country	Population	Period	CVCs in patients with abnormal test results (N)	Correction of hemostasis	Additional patient data and procedural details
Fisher, 1999 [25]	Single center, prospective observational study, UK	Liver disease, liver transplantation	1996 - 1997	PLT 10-50 (N)= 146 INR ≥1.5 (N)= 580	FFP and platelets were not routinely given for correction of coagulopathy and were normally only given in other interventional procedures. FFP was given in 5 cases, platelets in 12 cases, prior to cannulation.	Total cohort: 658 CVCs in 283 patients, Landmark method, 18-gauge inducer needle, 53% SCV, 47% IJV; CVCs placed by attending clinician, usually registrar. The number of patients with a combination of platelet count <10 and INR > 1.5 not reported in the article.
Haas, 2010 [26]	Single center retrospective cohort study, USA	All CVC indications, except ICU	2001 - 2008	Total (N) = 745 PLT ≤ 50 (N)= 428 INR ≥1.5 (N)= 361	After exclusion of intercurrent blood product transfusion, 626 CVCs placed in 567 patients with PLT ≤ 50 and / or INR ≥1.5.	Total cohort: 3170 CVCs in 2514 patients, (1783 males, 56%), mean age 54 years; US guidance, 21-gauge inducer needle; IJV 96%, FV1%, EJV 1%; CVCs placed by interventional radiologist physicians.
Kander, 2013 [21]	Single center retrospective cohort study, Sweden	All patients with an indication for a CVC	2009 - 2010	Coagulopathy: (N) = 283 *PLT <50 = 79* *APTT >45 sec = 168* *PT >1.8 = 36*	70 patients received prophylactic platelet concentrate, 14 patients received plasma transfusion	Total cohort: 1737 CVCs in 1444 patients; 49% US-guidance; IJV 68%, EJV 8%, SCV 15%, FV 1%, Placed by anesthesiology residents and specialists; Inducer needle size not reported.
Mey, 2002 [27]	Single center, prospective cohort study, Germany	Hematology-oncology	1994 -1998	PLT ≤ 50 (N)= 116	Thrombocytopenic patients had a platelet count of <20, and received platelet concentrate.	Total cohort: 490 CVCs in 490 patients, (287 male, 59%) mean age 57 years; US-guidance, 14-G needle; 95% IJV.
Müller, 2015 [18]	Multicenter randomized open – label trial, Netherlands	Intensive Care patients	2010 -2013	INR ≥1.5 (N)= 58	Patients with an INR of 1.5-3 were randomized to receiving FFP transfusion (N =29)	Total cohort: 76 invasive procedures, of which 58 CVCs, both US-guided and landmark technique; Proceduralist, site of placement and inducer needle size not reported.
Mumtaz, 2000 [22]	Single center retrospective cohort study, USA	All patients with an indication for a CVC	1997 - 1999	Coagulopathy: (N) = 88 *Definition: INR >1.3, PTT 37 sec, or PLT< 150.*	In 242/333 patients, disorders of hemostasis were corrected prior to CVC placement.	Total cohort: 2010 CVCs in 1825 patients, of which 333 in patients with any disorder of hemostasis; 96% SV, 4% IJV; Proceduralist was mostly a surgical intern supervised by an attending; Use of Ultrasound and inducer needle size not reported.

TABLE 1. Continued

Study	Design, country	Population	Period	CVCs in patients with abnormal test results (N)	Correction of hemostasis	Additional patient data and procedural details
Nosari, 2008 [28]	Single center, prospective cohort study, Italy	Oncohematologic patients	2003 - 2004	PLT ≤ 50 (N)= 77	All 36 patients with a platelet count <30 received prophylactic platelet concentrate.	Total cohort: 388 CVCs in 279 patients (148 males, 53%), mean age 50; IJV 86%, EJV 3%, SCV 1%, FV 9%; Proceduralist, inducer needle size and the use of ultrasound not reported.
Ong, 2012 [29]	Single center, prospective cohort study, USA	TTP	2008 -2010	PLT ≤ 50 (N)= 10	2/11 patients received prophylactic platelet concentrate.	Total cohort: 11 CVCs in 11 patients, (5 males, 45%), median age 38 years; Site of placement, use of US-guidance, inducer needle size and proceduralists not reported.
Oguzkurt, 2005 [23]	Single center, prospective cohort study, Turkey	Hemodialysis patients	2002 -2004	Coagulopathy: (N) = 61 *Definition: PLT ≤ 50, aPTT, and/or INR ≥1.5*	No correction of hemostasis prior to CVC.	Total cohort: 220 CVC in 172 patients (93 males, 54%), mean age 56 years; US-guidance, 18-gauge inducer needle; 100% IJV; Experience of proceduralists not reported.
Ray, 1997 [30]	Single center, Prospective outcomes study, USA	All patients with an indication for a CVC	1995 -1996	PLT ≤ 50 (N)= 37	All patients received platelet transfusion during implementation.	Total cohort: 87 CVCs in 105 patients (60 males, 57%) mean age 50; landmark method, 21-Gauge needle; Placed by interventional radiologists.
Rizvi, 2000 [31]	Single center, prospective cohort study, USA	TTP-HUS	1996 -1999	PLT ≤ 50 (N)= 49	7/18 catheter insertions that had took place in patients with a platelet count of $<20x10^9$ received prophylactic platelet concentrate.	Total cohort: 92 CVCs in 68 patients (20 males, 30%), mean age 50; 71% IJV/SCV, 29% FV; No standard protocol for CVC-placement;
Singh, 2015 [32]	Single center, prospective cohort study, India	Liver disease	2011 -2012	PLT ≤ 50 (N)= 49	No correction of hemostasis prior to CVC.	Total cohort: 699 CVCs in 421 patients (238 males, 57%), mean age 42; US-guided, IJV 100%; Elective setting by trained anesthesiologists; Inducer needle size not reported.
Tercan, 2008 [33]	Single center, prospective observational study, Turkey	Patients with bleeding disorders	2002 -2006	Total (N) = 133 PLT ≤ 50 (N)= 38 INR ≥1.5 (N) =61 PTT (N)= 8	No correction of hemostasis prior to CVC.	Total cohort: 133 CVCs in 119 patients (51 males, 49%), age range 18-95 years; US guidance, 18-G inducer needle; IJV 97%, SCV 1.5%, FV 1.5%; Experienced interventional radiologists placed the CVC.

TABLE 1. Continued

Study	Design, country	Population	Period	CVCs in patients with abnormal test results (N)	Correction of hemostasis	Additional patient data and procedural details
Tomoyose, [34]	Single center, retrospective, observational, Japan	Thrombocytopenic patients with hematological malignancies	2007 - 2009	PLT ≤ 50 (N)= 67	42/67 thrombocytopenic patients received prophylactic platelet concentrate.	Total cohort: 108 CVCs in 72 patients (38 males, 52%) 44 CVCs US-guided, 49 CVCs landmark, 22-G inducer needle; All central veins; The decision of platelet transfusion was left to the discretion of the individual physicians.
Vinson, 2014 [24]	Multicenter retrospective cohort study, USA	Sepsis	2010 - 2012	Moderate to severe: (N) = 300 *Definition: INR ≥2, and/or platelet <50*	20 patients received transfusion: FFP N = 17, Platelets N =3. Inducer needle size not reported.	Total cohort: 936 CVCs in 936 patients, (535 males, 57%), mean age 68 years; Both US-guided and Landmark method; IJV 86%, SCV 13%, FV 1.4%. Proceduralists were predominately attendings (84%).
Weigand, 2009 [35]	Multi center open prospective trial, Germany	Intensive Care & hematologic patients	2005 -2007	Total (N) = 58 PLT ≤ 50 (N)= 19 INR ≥1.5 (N)= 39	No correction of hemostasis prior to CVC.	196 CVCs in 196 patients, (132 males, 68%), mean age 62 years; US-guidance, 18-G inducer needle; IJV 88%, FV 10%, SCV 2%; Number of previous insertions per physician 2-29.
Zeidler, 2011 [36]	Single center, retrospective cohort study, Switserland	Acute leukemia patients	2001 - 2007	PLT ≤ 50 (N)= 234 INR ≥1.5 (N)= 59	All patients with a platelet count below 20 (N=2) received platelet transfusion, between 20-50 it was left to the physician's discretion. 138/234 thrombocytopenic patients received transfusion.	Total cohort: 604 CVCs in 193 patients, (114 males, 59%), median age 49 years; SVC 85%, IJV 15%; Experienced staff from the anesthesiology or intensive care unit. Inducer needle size not reported.

CVC= Central Venous catheter; US = Ultrasound; N = number of procedures; IJV = internal jugular vein; EJV = external jugular vein; SCV = subclavian vein; FV = femoral vein.
PLT = Platelet count x10^9/L; INR = international normalized ratio ;PTT = activated partial thromboplastin time in seconds.

Overall bleeding prevalence in the included studies varied from 0% to 32%. Notably, for the study reporting a 32% bleeding prevalence in the presence of coagulopathy, the overall bleeding incidence was not different for the patients without coagulopathy (p = 0.768)[36]

Platelet count

No randomized controlled trial evaluating the effect of prophylactic platelet transfusion was found. 20 studies reported patients or a subgroup of patients with a platelet count below 50 x 109 /L. These single-arm studies in patients with thrombocytopenia without a comparison group showed a bleeding incidence that varied from 0 to 32%.

Thrombocytopenia was not corrected prior to cannulation in seven studies. In this uncorrected group with a platelet count below 50 x 109 /L, no major bleeding events occurred. In three of these studies without prophylactic platelet transfusion thrombocytopenia was found to be a risk factor for local hematoma and oozing.[25,35,37] One of these found no association between thrombocytopenia and minor bleeding complications.[33]

In eleven studies a small group of patients received platelet transfusion. The decision for prophylactic platelet transfusion was left to the discretion of the physician. No major hemorrhagic events occurred in the patients with a platelet count below 50 x 109 /L. Two studies found thrombocytopenia to be a risk factor for local hematoma and oozing.[22,36] All these hematomas were self-limiting and oozing could be managed by prolonged manual compression or bandage changes in most cases. Mumtaz reported oversewing of the catheter site as an effective solution in case of prolonged bleeding.[22] One study by Kander found no association between thrombocytopenia and minor bleeding complications.[21] Zeidler showed no association between platelet count and bleeding risk, down to a platelet count of 20 x109 /L.[36] Of note, all physicians were experienced and CVC placement was ultrasound guided.

In three studies all patients with a low platelet count received prophylactic platelet concentrate, based on a threshold, varying from 10 x 109 /L to 50 x 109 /L. All patients had a platelet count of < 30 x109 and received prophylactic platelet transfusion in one study[28]. Another observational study compared three groups of patients based on platelet count; namely < 50 x 10 9 /L (N=37), 50-100 x 109 /L (N=35) and > 100 x 109 /L (N=33). There were no major bleeding complications.[38] Notably, patients in the first group received a transfusion of 1 unit of platelets during CVC placement.

TABLE 2. Definitions of bleeding and relevant results

Study	Major bleeding, definition, patients (N)	Minor bleeding, Definition, patients (N)	Relevant results
Barrera, 1996 [39]	Bleeding from the site requiring blood transfusion or mediastinal hematomas.	Oozing of blood and/ or hematomas <5 cm at the CVC site not requiring therapy. Total N=23, (19 in patient with a normal coagulation pattern, 4 in patients with an elevated PT and PTT)	The mean platelet count associated with minor bleeding was 15 ± 5 (6-20) pretransfusion, and 25 ± 16 (2-59) posttransfusion. For the group without complications platelet count was and was 15 (3-20) pretransfusion and 23 ± 13 (2-73) posttransfusion.
Carino, 2012 [40]	Clinically evident bleeding: documented hematoma at the insertion site, line-related blood transfusion, any need for an intervention beyond local manual pressure, or any new radiographic opacity in the hemithorax. N = 1 (table 3)	No specific definition, N not reported.	The overall occurrence of bleeding was 0.3% (95% confidence interval, 0%-2%). No bleeding occurred in patients without prophylactic plasma, (0/73), one case of bleeding occurred in the group of patients that had received plasma (1/27) No benefit of prophylactic plasma was observed. (P=0.6)
De Loughery 1996, [19]	No specific definition. N = 2 (table 3)	Oozing of blood, manageable with dressing changes. N =10	By combining coagulation test, a group at high risk for bleeding could be identified.
Della Vigna, 2009 [20]	Major bleeding. N = 0	Oozing, requiring compression. N =1	45 patients with high risk of bleeding did not experience any bleeding complication.
Doerfler, 1996 [37]	Intrathoracic bleeding seen on X-ray or unexpected decrease in HCT. N = 0	Bleeding that requires direct pressure for 10-20 minutes. 5 patients had bleeding from the skin, 2 small periosteal hematomas. N =7 (6.5%)	Platelet count was the only risk factor statistically associated with minor bleeding. The platelet count associated with this risk in this series was less than 38,000/mL (in all but one case the number was 25,000/mL).
Fisher, 1999 [25]	Any hemodynamically significant hemorrhage. N =1 (table 3)	Superficial oozing or hematoma. PPT ratio ≥ 2 or more = 4/80 (5 %), PTT ≤2 = 6/146 (4 %)	Low platelet independent risk factor for persistent oozing. High INR ($p< 0.01$) and low platelets ($p< 0.05$) were independent risk factors for hematoma formation, whilst regional anticoagulation with heparin ($p< 0.01$) and low platelets ($p < 0.05$) were independent risk factors for persistent oozing.
Haas, 2010 [26]	Bleeding defined according to the Society of Interventional Radiology Technology Assessment Committee reporting standards. [41] N = 3 (0.095%): oozing at catheter exit site requiring treatment, hemothorax, and hematoma.	Minor oozing at the exit site not requiring any intervention other than brief manual compression was not considered a complication.	No bleeding complications in coagulopathic patients. Image-guided placement of TCVCs in patients with a platelet count between 25,000/ dL and 50,000/dL and/or INR between 1.5 and 2 is safe when performed by an experienced physician.

TABLE 2. Continued

Study	Major bleeding, definition, patients (N)	Minor bleeding, Definition, patients (N)	Relevant results
Kander, 2013 [21]	WHO grade 3 or 4 bleeding. N = 0	Bleeding requiring prolonged compression at the insertion site: grade 2 bleeding. N =16 (0.9%)	Fisher's exact test revealed that coagulation independent variables alone yielded no significant differences as risk factor for bleeding observations (896 missing observations)($P = 0.47$).
Mey, 2002 [42]	No specific definition. N = 0	Local hematomas in 4.3% of patients, 10.2% in thrombocytopenic patients.	Complications were significantly more frequent in thrombocytopenic patients (P=0.02), primarily due to an increased incidence of local hematomas.
Müller, 2015 [18]	According to HEME score. [43] N = 0	Prolonged bleeding at the site of insertion or increase in size of subcutaneous hematoma. N not reported for CVC group.	Preprocedural omission of FFP was not associated with increased occurrence of bleeding (relative risk, 1.17; 95% confidence interval [CI], 0.62-2.19; p = 0.78).
Mumtaz, 2000 [22]	Significant bleeding: when an intervention other than digital pressure was necessary to secure hemostasis. N = 4 (table 3)	Occurrence of minor bleeding was not recorded.	A platelet count <50 x 10^9 was the only significant predictor of bleeding complication in patients with abnormal hemostasis.
Nosari, 2008 [28]	No specific definition. No hemothorax. N = 0	Hemorrhage and/ or hematoma, without major sequelae. N = 5	All patients with hemorrhage and/or hematomas were severely thrombocytopenic (5/36, 13.8%) and received transfusion prior to placement.
Oguzkurt, 2004 [23]	No specific definition. N = 0	Oozing of blood N = 3 (1.4%) Small hematoma N = 1 (0.4)	Oozing of blood was seen only in patients with disorders of hemostasis.
Ong, 2012 [29]	Significant bleeding, HCT after line placement. N = 0	Oozing N = 0	No relevant postprocedural oozing in 8 patients with a platelet count of 8-34 x 10^9/L.
Ray, 1997 [38]	Bleeding complications that necessitate intervention. N=0	No specific definition, N not reported.	No bleeding complications.
Rizvi, 2000 [31]	Criteria similar to Ziselman. [44] N = 2 (table 3)	Complications that did not meet the criteria by Ziselman. Minor hemorrhage at the insertion site. N = 2	11 CVCs were inserted when the platelet count was <20 x 10^9/L, without platelet transfusions and without bleeding complications.

TABLE 2. Continued

Study	Major bleeding, definition, patients (N)	Minor bleeding, Definition, patients (N)	Relevant results
Tercan 2008 [33]	Major complication like hemothorax. N = 0	Oozing of blood N = 5 (3.8%) Small hematoma N =2 (1.5%)	There were significant association between high INR and development of hematoma ($p < 0.05$). Platelet count and aPTT were not associated with bleeding complications ($p > 0.05$).
Tomoyose [34]	No hemothorax N = 0	Subcutaneous hematomas 0.0% in US group 7.8% in landmark group	Platelet transfusion rate in thrombocytopenic patients having a platelet count of >20 x 10^9/L was 28.6% (2/7) in the US group and 100.0% in the landmark group (p= 0.002)
Singh, 2015 [32]	A vascular complication for which active medical intervention required like blood transfusion, chest drain insertion. N = 0	Hematoma: development of swelling of >2 cm, at site of skin puncture: 10%. Persistence of ooze even after 15 min of digital compression or requiring change in dressing for >3 times in 24 hours: 13%. Both hematoma and ooze: 4.7%.	Hematoma: INR >3: 12%; INR <3: 10%; PLT <3: 10%; PLT >30 x 10^9/L: 12%. Ooze: INR >3: 12%; INR <3: 13%; PLT <30: 23%; PLT >30 x 10^9/L: 11%. Platelet count (<30) (OR 2.29) was an independent risk factor for oozing.
Vinson, 2014 [24]	Major hemorrhage or minor hemorrhage with procedural intervention. Mild coagulopathy group N =6 Moderate-to-severe coagulopathy group N = 3	Mild coagulopathy group N = 17 Moderate-to-severe coagulopathy group N = 12,	The difference in the incidence of hemorrhagic complications between mild and moderate-to-severe laboratory abnormalities was not significant (P = .32), nor was the difference in the incidence of major hemorrhage combined with minor hemorrhage requiring procedural intervention (P = 1.0). 8 patients with minor bleeding received a post-hemorrhagic intervention.
Weigand, 2009 [35]	A drop of hemoglobin of >1.5 g/dl within 24-36 hours after catheter placement. One patient required a suture. N = 3	Subcutaneaous hematoma and/ or strong bleeding at the puncture site. N = 5	RR for bleeding compared to the study population without hemostatic disorders is not significant for platelets <50, N=19, RR 0.28, P-value 0.25, INR >1.5, N = 39, RR 0.86, P-value 0.90; combined <platelets and INR> 1.5, N= 7, RR 0.695, P-value 0.94. The study did not report the laboratory values of the patients with the major complication.
Zeidler, 2011 [36]	WHO grade 3 to 4 bleeding. N = 0	WHO grade 1, grade 2 bleeding requiring prolonged local compression. Incidence: 32%, with 96% grade 1, 4% grade 2.	Bleeding incidence was 33% in patients with laboratory hemostatic dysfunction, versus 32 without (p =0.768). Only patients with a platelet count of less than 20 x 10^9/L were at higher risk for bleeding both before transfusion OR 2.88 (p = 0.015) and after preprocedural platelet transfusions OR 2.84 (p=0.006), compared to patients with platelet counts of 100 x 10^9 or more.

INR

Eight studies reported patients or a subgroup of patients with an INR ≥ 1.5. One randomized controlled trial evaluated the effect of prophylactic transfusion of Fresh Frozen Plasma on bleeding complications in patients with an INR exceeding 1.5.[18] Prior to 58 CVC placements, patients were randomly assigned to receive FFP (N = 29 in both treatment arms). Preprocedural omission of FFP was not associated with increased occurrence of bleeding (relative risk, 1.17; 95% confidence interval [CI], 0.62-2.19; p = 0.78).

Seven observational studies, of which six were prospective, reported a (sub)group of patients with an INR of ≥ 1.5. Two studies found a significant association between high INR and development of hematoma.[25,33]. Two studies found no association between INR and bleeding.[22,45] One study compared a bleeding and a non-bleeding group, defined as a significant decline in hemoglobin. No baseline difference in INR was found between the bleeding and non-bleeding group (1.39 vs 1.33; p = 0.363).[35] One retrospective study included 100 patients with an INR between 1 and 5.[35] The use of FFP was left to the physicians' discretion. No bleeding occurred in patients without prophylactic plasma, (0/73), one case of bleeding occurred in a patient with an INR of 3.9, who had received plasma (1/27) No benefit of prophylactic plasma was observed. (P = 0.6).

PTT. Four studies reported placement of CVC in patients or a subgroup of patients with a PTT of > 45 seconds in the absence of correction. No association between prolonged PTT and major or minor bleeding was found.

Other risk factors

Various procedural factors were associated with bleeding complications. The influence of catheter site showed conflicting results, varying from no effect, to favoring the Internal Jugular site or Subclavian site.[25,36,39,45] More than one needle pass into the vein was found to be associated with bleeding in three studies,[25,32,39] Other mechanical factors associated with bleeding complications included failed access at the initial site, failure to pass any guidewire and documented arterial puncture.[24,25] Several studies reported no association of mechanical factors on bleeding.[22,23,33,45,46]

A variety of patient characteristics were associated with hemorrhagic complications. One study found more bleeding complications in medical patients compared to surgical or trauma patients.[19] The presence of ascites was also shown to be an independent risk for for postprocedural bleeding.[32] In one study fibrinogen levels were significantly lower in the bleeding group (4.1 g / dL; range 1.0-14.4 g/ dL) compared to the nonbleeding group (4.8 g / dL; range 0.7-14.7 g / dL) in one study.[36]

DISCUSSION

Our goal was to identify the incidence and risk factors of bleeding complications after central venous catheterization in patients with severe hemostatic defects. The 23 included studies showed that major bleeding complications are rare in patients with thrombocytopenia and/or prolonged bleeding time. However, the exact values of platelet count, INR, and PTT in which central venous catheterization can safely be performed remain unclear.

A major shortcoming of current literature on bleeding complications after CVC placement in presence of coagulopathy is study design. Except for one, all studies were observational cohort studies. The rationale for correction of coagulopathy was often incompletely reported.

Furthermore, the definition of bleeding complications varied widely across studies. The reporting of minor bleeding complications varied from no reporting to the reporting of hematomas, prolonged oozing and small interventions such as the placement of a suture, bandage dressing or prolonged manual compression. The lack of consistency in definitions of bleeding complications is a serious problem. A uniform definition should be used in studies investigating bleeding complications. Furthermore, outcomes should be scored on clinical relevant bleeding, as one could question whether a minor bleeding complication is clinically relevant.

Abnormal coagulation parameters are more frequently present in patients with bleeding complications compared to patients without bleeding complications after CVC placement. However, the severity of coagulopathy does not correspond with the bleeding risk. In various invasive procedures, there is insufficient evidence that coagulation test results predict bleeding.[14,47]. DeLoughery (1996) stated that a group at high risk for bleeding could be identified. Unfortunately, the predictive value of the combination of coagulation abnormalities could not be proven. Other authors found no difference in the incidence of hemorrhagic complications for independent coagulation variables alone.[21,24]. The association between severe thrombocytopenia and minor bleeding complications is consistent for the included studies, as seven studies reported evidence supporting this result, while only two studies found no association.

TABLE 3. Major Bleeding complications

Study	PLT	INR	PTT	CVC	Correction	Complication, comments
Carino [40]	118	3.9	NR	IJV	Patient received 5 U of FFP preprocedure.	Postprocedure a large hematoma was noted, and both local compression and an additional 5 U of FFP were used to stop the bleeding. Technical difficulties with placing the line and multiple cannulation attempts were documented and ultimately placement of the line failed.
DeLougery [19]	*"Multiple hemostatic defects"*		NR		Not specified	CVC placement during trauma resuscitation, developed a hemothorax that required chest tube drainage.
DeLoughery [19]	*"Mild hemostatic defects"*		NR		Not specified	Woman developed a hemothorax.
Doerfler [45]	6	NR	NR	SCV	No correction	Postprocedural transfusion of 5 units of PC. One patient with Kaposi's sarcoma of the skin required 1 h of direct pressure to stop the bleeding from the skin.
Fisher [25]	68	<1.5	NR	SCV	Patient received massive blood transfusion for variceal hemorrhage	Hemothorax after accidental subclavian artery puncture in a patient that received prostacyclin therapy for hemofiltration. The hemothorax caused respiratory embarrassment and required evacuation at thoracotomy, but the patient died of multiorgan failure 2 days later.
Rizvi [31]	83	NR	NR	SCV/IJV	Not specified	Death, 28-year-old woman, hemorrhage related in part to systemic lupus erythematosus with recurrent pleuritic and pericarditis that had been threated continually with glucorticoids for 9 years.
Rizvi [31]	75	NR	NR	SCV/IJV	Not specified	Major hemorrhage, that prevented Plasma Exchange treatment.
Tercan [33]	NR	4	NR	IJV	No correction	The catheter had to be removed because of oozing around the catheter could not be stopped, every attempt to stop bleeding, including manual compression and pressure dressing and three units of FFP failed. Manual compression after removal of the catheter stopped the bleeding.
Vinson [24]	207	1.4	NR	SCV	Not specified	Hemothorax in a critically ill ED patient who had a refractory septic shock.
Mumtaz [22]	12	1.2	24	NR	No correction	In a patient with multiple myeloma, manual compression was not sufficient to stop bleeding, hemostasis was obtained by placing a string suture around the catheter entry site.

TABLE 3. Continued

Study	PLT	INR	PTT	CVC	Correction	Complication, comments
Mumtaz [22]	31	1.5	34	NR	No correction	In a patient with septic shock, manual compression was not sufficient to stop bleeding, hemostasis was obtained by placing a string suture around the catheter entry site.
Mumtaz [22]	46	1.1	42	NR	No correction	In a patient with renal failure, manual compression was not sufficient, hemostasis was obtained by placing a string suture around the catheter entry site.
Mumtaz [22]	154	1.1	35	NR	Abnormal hemostasis corrected, type and amount of units not specified.	In a patient with multiple myeloma, abnormal hemostatis was corrected before cannulation. Manual compression was not sufficient to stop bleeding, hemostasis was obtained by placing a string suture around the catheter entry site.

CVC= Central Venous catheter; US = Ultrasound; IJV = internal jugular vein; EJV = external jugular vein; SCV = subclavian vein; FV = femoral vein.
PLT = Platelet count x10^9/L; INR = international normalized ratio ;PTT = activated partial thromboplastin time in seconds; FFP: Fresh Frozen Plasma; PC: Platelet concentrate

The correction of hemostatic defects prior to central venous catheter placement remains a matter of debate. The introduction of ultrasound guidance for CVC placement led to a decrease in the number of puncture attempts and complication rates.[48-52] Current national and international guidelines are based on the landmark technique and subsequently still support correction of thrombocytopenia up to a platelet count of 50 x 109/L and an INR > 1.5 prior to central venous catheter (CVC) placement.[15-17] Various authors advised to transfuse platelets below a platelet count of 20 x 109.[34,36,39] However, there is no profound evidence to support this threshold. The minimum platelet count in which central cannulation can safely be performed without prophylactic platelet transfusion might even be a platelet count of less than 20 x 109. Several studies revealed no significant difference in the outcome of bleeding complications in patients with precatheter correction of hemostasis.[18,22]

Two studies showed no positive effect of prophylactic FFP administration on bleeding complications in patients with prolonged PT.[18,40] In one observational cohort study patients which received FFP had a higher baseline INR.[40] This may have reduced the positive effect of prophylactic FFP. This study had a high risk of selection bias. FFP transfusion was left to the discretion of the operator and no clear rationale for FFP could be found in a substantial amount of patients. The mere preprocedural measurement of PT was a risk factor for FFP transfusion. The other study was a moderate to high quality randomized controlled trial that lacked statistical power due to low inclusion rate.[18]

Hematoma prevalence is also shown to be associated with insertion technique. In an observational study, 1584 placements by landmark method were compared to 2367 catheter placements after introduction of ultrasound guidance. The incidence of hematoma decreased from 8.2% in the landmark group to 1.6% after introduction of ultrasound guidance.[53] Our study included studies with both insertion methods.

Our review comes with a number of limitations. The retrospective and/or observational nature of most of the included studies is a disadvantage. Due to the observational design, selection of patients might have occurred. In a study by Mey (2003), puncture teams in which both the sonographer and the puncturing physician were experienced, more complications occurred compared to teams in which only the sonographer was experienced.[27] This was explained as a result of bias, that made experienced physicians more likely to perform the procedure in clinical situations that made puncture difficult. Important gaps in methodological reporting and the acknowledgement of bias and confounders has previously been described for clinical PLT transfusion studies.[54] Therefore, the low occurrence of bleeding in severely coagulopathic patients could be confounded

by more precocious measures before cannulation in profound coagulopathic patients; such as placement by more experienced staff members or selective use of ultrasound for high-risk patients.

The positive effect of ultrasound-guidance and the effect of experience with the procedure on complication rates have previously been demonstrated.[49,55-57] Hence, the risk of bleeding after central venous catheterization is suggested multifactorial and composed of procedural, patient and physician characteristics. The number of attempts, inadvertent arterial puncture, vein size, vein lesion, patient compliance, obesity and hyperinflation have been associated with bleeding risk.[27,58] Echo-guidance can be used to identify the vessel, or to advance the needle under real-time ultrasound guidance.[59] The use of real-time ultrasound and experience with the procedure reduce the complication rate.[49,55-57] Real-time ultrasound guidance was not yet present in all included studies, which may render bias between studies. Therefore, it is difficult to assess the sole effect of coagulopathy in current observational cohort studies.

In conclusion, a systematic review of the published literature demonstrates that major bleeding after central venous catheterization is rare in severe coagulopathy. The severity of coagulopathy does not correspond with the bleeding risk. Well-powered randomized controlled trials are necessary to determine the minimal platelet count, and maximal INR and PTT that are safe prior to CVC insertion. In these trials, the definition of bleeding complications should be uniform, the physicians involved should be experienced, and standard use of ultrasound should be compulsory.

APPENDIX 1

Search strategy

- We searched allowing any study design, except for case report. Controlled trials, retrospective or prospective cohort study, case-control study, case series. Bleeding complications related to CVC placement had to be reported as an outcome. The subgroup of patients with coagulopathy should be defined, and the bleeding complications should be reported for this subgroup.
- We only included studies in adults. In order not to miss any relevant publication, no filter was used and pediatric records were excluded manually.
- Patients must have received a central venous catheter.
- We performed four searches, with the search terms as described below. An initial search was performed on 01-01-2016, after which the selection of eligible articles took place. The search was repeated on 01-11-2016, to include recent articles.
- The first three searches were performed in MEDLINE using PUBMED with the search terms as described below. This resulted in a total of 338 hits, 302 after removal of duplicates. A search in the Cochrane library added 16 titles, of which 15 were unique.
- 34 pediatric studies were excluded.
- 3 studies were excluded for not being available in the English language.
- 79 abstract were checked for suitability using a predefined form. Of all the 79 articles that were screened on abstract level, we performed a "related articles" search. Relevant articles were checked for duplicates, after which four articles were identified for abstract review.
- Of the 83 abstract screened, 32 full-text articles were carefully read to obtain relevant information.
- A total of 23 articles were included in the current review.

The specific search terms were as follows:

- In MEDLINE: ("Catheterization, Central Venous"[Mesh] OR "Central Catheterization"[tiab] OR "Central Catheterizations"[tiab] OR "Central Venous Catheterization"[tiab] OR "Central Venous Catheterizations"[tiab] OR CVC[tiab] OR CVL[tiab] OR CVCs[tiab] OR "Central Vein Catheterization"[tiab] OR "Central Vein Catheterizations"[tiab]) AND ("Platelet Count"[Mesh] OR "Platelet Count"[tiab] OR "Platelet Counts"[tiab] OR "Platelet Number"[tiab] OR "Platelet Numbers"[tiab] OR "Blood Platelet Disorders"[Mesh] OR "Blood Platelet Disorders"[tiab] OR "Blood Platelet Disorder"[tiab] OR Thrombocytopenia[tiab] OR "Platelet Storage Pool Deficiency"[tiab]) AND ("Hemorrhage"[Mesh] OR "Hemorrhage"[tiab] OR bleeding*[tiab] OR Hematom*[tiab] OR Hemothorax[tiab] OR Haematom*[tiab])

- In MEDLINE: ("Catheterization, Central Venous"[Mesh] OR "Central Catheterization"[tiab] OR "Central Catheterizations"[tiab] OR "Central Venous Catheterization"[tiab] OR "Central Venous Catheterizations"[tiab] OR CVC[tiab] OR CVL[tiab] OR CVCs[tiab] OR "Central Vein Catheterization"[tiab] OR "Central Vein Catheterizations"[tiab]) AND ("Platelet Transfusion"[Mesh] OR "Platelet Transfusion"[tiab] OR "Platelet Transfusions"[tiab] OR "PLT transfusions"[tiab] OR "PLT transfusion"[tiab] OR "fresh frozen plasma"[tiab]) AND ("Hemorrhage"[Mesh] OR "Hemorrhage"[tiab] OR bleeding*[tiab] OR Hematom*[tiab] OR Hemothorax[tiab] OR Haematom*[tiab])

- In MEDLINE: ("Catheterization, Central Venous"[Mesh] OR "Central Catheterization"[tiab] OR "Central Catheterizations"[tiab] OR "Central Venous Catheterization"[tiab] OR "Central Venous Catheterizations"[tiab] OR CVC[tiab] OR CVL[tiab] OR CVCs[tiab] OR "Central Vein Catheterization"[tiab] OR "Central Vein Catheterizations"[tiab]) AND ("Hemorrhage"[Mesh] OR "Hemorrhage"[tiab] OR bleeding*[tiab] OR Hematom*[tiab] OR Hemothorax[tiab] OR Haematom*[tiab]) AND (complication*[ti] OR "Risk Factors"[Mesh] OR "risk factor"[ti])

- In the COCHRANE library: (Central Venous Catheterization* OR Central Catheterization* OR Central Vein Catheterization* OR CVC OR CVL OR CVCs) AND (Hemorrhage OR bleeding* OR Hematom* OR Hemothorax OR Haematom*) AND (complication*)

APPENDIX 2. Risk of bias

Study	Consecutive or Random	No Exclusions	Explicit Bleeding Criteria	Correction of hemostasis standardized	CVC placement standardized	Primary data collection	Criteria met	Oxford CEBM level of evidence [60]
Barrera, 1996 [39]	✓	✓	✓	✗	✗	✓	4	3b
Carino, 2012 [40]	✓	✓	✓	✗	✗	✗	3	4
DeLoughery, 1996 [19]	✓	✗	✓	✗	✗	✗	2	4
Della Vigna, 2009 [20]	✓	✓	✗	✓	✓	✗	4	3b
Doerfler, 1996 [37]	✓	✓	✓	✓	✓	✓	6	2c
Fisher, 1999 [25]	✓	✗	✓	✓	✓	✓	5	2c
Haas, 2010 [26]	✗	✗	✓	✗	✓	✗	2	4
Kander, 2013 [21]	✓	✗	✓	✓	✗	✗	3	4
Mey, 2002 [27]	✓	✗	✗	✓	✓	✓	4	3b
Müller, 2015 [18]	✓	✓	✓	✓	✓	✓	6	2b
Mumtaz, 2000 [22]	✓	✓	✓	✗	✓	✗	4	3b
Nosari, 2008 [28]	✓	✓	✗	✓	✓	✓	5	2c

APPENDIX 2. Continued

Study	Consecutive or Random	No Exclusions	Explicit Bleeding Criteria	Correction of hemostasis standardized	CVC placement standardized	Primary data collection	Criteria met	Oxford CEBM level of evidence [60]
Ong, 2012 [29]	✓	✓	✗	✗	✗	✓	3	4
Oguzkurt, 2005 [23]	✓	✗	✗	✓	✓	✓	4	3b
Ray, 1997 [30]	✗	✗	✗	✓	✓	✓	3	4
Rizvi, 2000 [31]	✓	✗	✓	✗	✗	✓	3	4
Singh, 2015 [32]	✓	✗	✓	✗	✓	✗	3	4
Tercan, 2008 [33]	✓	✗	✓	✓	✓	✓	5	2c
Tomoyose [34]	✓	✓	✗	✗	✓	✗	3	4
Vinson, 2014 [24]	✓	✓	✓	✗	✗	✗	3	4
Weigand, 2009 [35]	✓	✓	✓	✗	✗	✓	4	2c
Zeidler, 2011 [36]	✓	✓	✓	✗	✓	✗	4	2c

We assessed the risk of bias using the following criteria: i. patient selection random or consecutive versus other; ii. Whether investigators excluded non-prespecified patient groups; iii. Explicitness of bleeding criteria; iv. correction of hemostasis by transfusion products standardized versus left to the discretion of the physician; v. a protocol for CVC placement; vi. Primary data collection versus data chart reports.

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3

THE PRACTICE OF PLATELET TRANSFUSION PRIOR TO CENTRAL VENOUS CATHETERIZATION IN PRESENCE OF COAGULOPATHY: A NATIONAL SURVEY AMONG CLINICIANS

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SUMMARY

Background. Coagulopathy in patients with an indication for a central venous catheter (CVC) is frequently corrected to prevent bleeding complications. However, retrospective studies support restrictive use of transfusion products. Platelet count thresholds vary significantly between guidelines, indicating uncertainty about the correct management in this clinical situation. We were interested in the current practice in the Netherlands.

Study design and methods. We conducted a mixed vignette and questionnaire web survey to investigate current practice and preferences for CVC placement. Clinical vignettes were used to quantify the tendency to administer platelet concentrate. A positive ß-coefficient is in favor of administering platelet concentrate.

Results. Ninety-seven physicians answered the survey questions. (36 critical care physicians, 14 hematologists, 20 radiologists and 27 anesthesiologist). Eighty-six physicians subsequently completed the clinical vignettes (response rate 71%). Preferences in favor of correcting thrombocytopenia prior CVC placement were platelet counts of 10 x 10^9 /l and 20 x 10^9 /l (ß = 3.9; ß = 3.2 respectively), the subclavian insertion site (ß = 0.8). An elevated INR (INR = 3; ß = 0.6) and an elevated aPTT (aPTT = 60 seconds; ß = 0.4) showed a positive trend towards platelet transfusion. Platelet transfusion was less likely in an emergency setting (ß = -0.4).

Reported transfusion thresholds for CVC placement varied from <10 x 10^9/L to 80 x 10^9/L for platelet count, from 1.0 to 10.0 for INR, and from 25 seconds to 150 seconds for aPTT. Implementation of ultrasound guidance as standard practice was limited.

Conclusion. Current transfusion practice prior to CVC placement is highly variable. Physicians adjust the decision to correct coagulopathy prior CVC placement based on clinical parameters, insertion site and technique applied.

INTRODUCTION

Central venous catheters (CVCs) provide vascular access, which enables blood sampling, hemodynamic monitoring, hemodialysis and the administration of medication, fluids and parenteral nutrition. Although pivotal for treatment, CVC-placement may cause serious complications, including pneumothorax, incorrect position, hemothorax and catheter related bloodstream infection.[1-4] Patient and procedural characteristics such as catheter insertion site, insertion technique and the presence of coagulopathy influence peri-procedural complications.

The use of real-time ultrasound during CVC insertion reduces the amount of complications compared to the traditional method based on anatomical landmarks.[5] Ultrasound guidance leads to fewer complications by reducing the number of unsuccessful placements, puncture attempts, hematomas, inadvertent arterial punctures and the time to placement.[5-9] Despite this evidence supporting ultrasonography for central venous cannulation, various authors showed limited implementation of real-time ultrasonography.[10-13] The current study presents the first survey amongst Dutch physicians.

Central veins commonly used for cannulization are the internal jugular vein, the subclavian vein and the femoral vein. Catheter site selection is based on the preference of the physician and patient related factors. A catheter located on the chest is more is more comfortable for ambulant patients as compared to the neck and groin region. The femoral vein is often presumed to have a higher infection rate, although literature does not support that the amount of catheter related bloodstream infections differs per site.[14] The internal jugular vein and femoral vein are relatively superficially located, allowing manual compression in case of a post-procedural bleeding. These factors may play a role in catheter site selection in patients with coagulopathy. We assumed that physicians adjust their preference for access site on the abovementioned clinical parameters. If patient related factors result in a strong preference for a specific insertion site, physicians may choose to accept possible disadvantages of that insertion site.

Patients in need for CVC frequently suffer from hemostatic disorders. In patients with coagulopathy, physicians are often hesitant to insert a CVC due to the perceived increased risk of bleeding. Based on guidelines, coagulopathy was defined as abnormal classical coagulation tests (i.e. aPTT >1.5 ULN and INR>1.5) or a reduced platelet count (platelet count $<50x10^9$/L) in the current study.[15-18]

Only limited evidence on the minimal preprocedural platelet count prior to central venous catheter placement is available. Classical coagulation tests, including PT, INR and platelet

count are poor predictors of bleeding complications after CVC placement.[19-21] With respect to platelet count, it remains a matter of debate what the minimal platelet count prior to central venous catheter placement should be.[19] Current guidelines are contradicting, and based on very-low-quality evidence. These international guidelines support correction of a platelet count varying from 20 to 50 x 10^9/L, prior to central venous catheter placement.[15-17,22] Recent evidence suggests that ultrasound guided CVC placement can safely be performed up to an uncorrected platelet count of 20 x 10^9/L.[23] Little is known about how this has influenced current practice. Data on physician's attitude towards the correction of thrombocytopenia prior to CVC placements and preferences on CVC placement in presence of coagulopathy is lacking.

The current study aims to designate factors that influence the clinical decision to transfuse platelets prior to central venous catheter insertion. This gives insight into the current transfusion practice and factors influencing the decision to transfuse platelets prophylactically. This can provide direction for expert-based guidelines and might reveal possible targets for the reduction of the use of transfusion products.

MATERIALS AND METHODS

Setting

We contacted the chiefs of the departments of critical care, hematology, (interventional) radiology and anesthesiology for participation in this study. Hospitals were included if an intensive care unit with five or more beds equipped for mechanical ventilation was present (N = 64). Chiefs were requested to indicate the physician in their department who was most experienced with central venous catheters and coagulopathy. An email invitation with a brief introduction on the aim of the study was sent to this physician. If either the chief, or the designated physician did not respond, at least two reminders were sent.

The survey

The questions in the survey were assigned in a fixed order. (The complete Survey Questionnaire and Clinical vignettes are provided in Supplement 2.) The survey began with characteristics of the physician, after which questions about current practice and preference on central venous catheter placement in the various centers were asked. Subsequently, local practice for transfusion triggers and policy were surveyed. Thereafter, the sixteen vignettes were posed. Finally, respondents were asked to indicate whether they support the National and International guidelines that advocates correction of a platelet count below 50 x 10^9/L prior to CVC placement. Space for a write-in was available.

To pre-test the survey, an independent representative of each specialty was asked to assess the survey questions for clarity and consistency. The content of the survey was not altered after this consultation.

The questions and vignettes were hosted on Survey Monkey® (http://www.surveymonkey.com). A bar representing progress in the survey was displayed. The survey could be paused and resumed at all times. The survey was opened between November 2015 and April 2016.

Vignettes

A vignette is a brief hypothetical scenario that provides information for participants to have an understanding of the scenario being depicted, asking how they would react in the given situation. Vignette-based surveys are particularly useful in the quantitative study of attitudes and preferences.[24] This method was validated to assess physician practice variation in clinical decision-making.[25] Physicians were asked to express their tendency to administer platelet concentrate with different combinations of factors. Vignettes were used to identify clinical factors that contribute to the decision to administer prophylactic platelets prior to CVC placement.

Elements that could influence the tendency to transfuse prophylactic platelets prior to central venous catheter placement were identified through previous studies and the clinical expertise of the authors. Risk factors for bleeding were presumed to influence the clinical decision to administer prophylactic platelet transfusion. Factors were divided in laboratory-, patient-, catheter- and procedure- related risk factors. Laboratory risk factors included classical coagulation parameters, e.g. platelet count, aPTT and PT.[20,26-28] Insertion site has been reported to influence bleeding complications. The femoral access site carries the largest risk of mechanical complications.[29] Subclavian catheterization is more likely to be complicated by hemothorax as compared to the internal jugular site.[29] We postulated that the absent possibility to apply manual compression on the subclavian site would improve the likelihood to transfuse prophylactic platelets, as compared to the femoral and jugular site.

To enhance feasibility, factors were reduced based on interdisciplinary consensus. (Supplement 1. Patient and catheter related factors that were presumed to be considered before administering platelet concentrate prior to CVC placement) Six factors remained after assessment for clinical relevance by a critical care physician, a hematologist and an interventional radiologist. The levels of the determinants were based on agreement. The factors and levels implemented in the vignettes are displayed in Table 1. Two factors with three levels, and four factors with two levels were embedded in the survey. A full factorial design would require 144 vignettes, which is not feasible. Therefore, the number

of representative clinical vignettes was reduced to 16 using an orthogonal main effects design (SPSS Version 22, SPSS, Inc., Chicago, IL)[24,30] Respondents were asked to rate the extent to which they were inclined to administer prophylactic platelet concentrate. A Likert scale was used ranging from 0 (totally disagree) to 7 (totally agree) to answer the question: "would you administer platelet concentrate prior to central venous catheter placement?" (Supplement 2. The Survey Questionnaire and Clinical vignettes)

TABLE 1. Factors and levels implemented in the vignette

Factor	Level
1. Severity of thrombocytopenia	Platelet count of $10x10^9$, $20x10^9$ or $50x10^9$
2. INR	1,5 or 3.
3. aPTT	30 seconds or 60 seconds.
4. Location	Jugular, femoral or subclavian vein
5. Setting	Emergency or elective.
6. Insertion technique	Ultrasound-guided or landmark.

Statistical analysis

Parametric data was expressed as medians with ranges, non-parametric data as means with standard deviations. Categorical variables were expressed as n (%). To test groups of continuous normally distributed variables, Student's t-test was used or, when more than two independent groups were involved, the ANOVA analysis. Likewise, if continuous data was not normally distributed, the Mann-Whitney U test was used or the Kruskal-Wallis test to compare three or more groups. Categorical variables were compared with the Chi-square test or Fisher's exact tests when appropriate. A p-value of less than 0.05 was considered to be significant. To correct for multiple testing, tests were followed by Bonferroni post-test. For the Kruskall-Wallis test, the incorporated Bonferroni post-test was using SPSS software. For the Chi-square test, with 12 tests, an adjusted p-value of 0.004 was considered to be significant.

The preferences for the vignettes were expressed as utilities. To test internal consistency, Chronbach's was determined for the clinical vignettes. Conjoint analysis was performed to calculate the relative weights for each level of the factor levels. This resulted in a utility score (common unit) for each factor level expressed as β with 95% confidence interval. The β value represents the direction of the preference, and its effect size. Higher values indicate greater preference. Negative β values indicate preferences against the positive direction of the statement, i.e. against the administration of prophylactic platelet concentrate. The

utility for a particular factor level is determined by multiplying the β with the defined factor category, i.e, one times β, two times β depending on the number of levels per factor. Analyses were carried out using SPSS software (Version 22, SPSS, Inc., Chicago, IL).

RESULTS

Chiefs of 122 departments gave consent for participation in the study. Of the 122 questionnaires sent, 97 physicians completed the questionnaire (response rate 80%). The responding physicians represented the fields of critical care (N = 36), anesthesiology (N = 27), radiology (N = 20) and hematology (N = 14). The physicians were employed in 54 hospitals. A total of 86 physicians completed the vignettes (response rate 71%). Characteristics of the responding physicians are listed in Table 2. No differences in age and years of experience per specialty were present. The responding hematologists worked more frequently in academic hospitals, compared to the respondents of other specialties.

TABLE 2. Characteristics of responding physicians

Medical Specialty	Critical care (n = 36)	Hematology (n = 14)	Radiology (n = 20)	Anesthesiology (n = 27)	Total (n = 97)
Age (SD)	44 ± 7	46 ± 14	47 ± 19	47 ± 7	46 ± 7
Years of experience %					
0-5	25	14	15	7	17
6-10	44	43	30	22	35
11-15	11	21	5	30	16
16-20	11	7	20	7	11
> 20	8	14	30	33	21
Hospital					
Academic	17	57*	20	15	28
Non-academic	83	43	80	85	72

These numbers represent the lowest platelet count, maximum aPTT and maximum INR that was prior to CVC placement, without preprocedural correction of hemostasis with blood products.
* Hematologists more frequently worked in an academic center as compared to other medical specialties, difference significant at the 0.05 level.

Questionnaire

Site of insertion

The first set of questions investigated current practice and preferences for central venous catheter placement. The respondents were asked to indicate their preferences for CVC insertion site. The jugular vein was most frequently preferred (54%), followed by the subclavian vein (37%), and the femoral vein (3%); 5% of the respondents did not have

a general preference for insertion site. The preferences for insertion site per discipline are displayed in Table 3. Hematologists differed significantly from radiologists and anesthesiologists, and prefered the subclavian and femoral site more frequently (Adjusted Residual of -2.7 for jugular site, $p < 0.05$). The majority of respondents (92%) believed an association between catheter location and catheter-related bloodstream infections exists. Most respondents considered the femoral vein to be at most risk for infection.

Physicians adjusted their preferences based on clinical parameters. If the catheter was expected to be in place for at least 5 days, the subclavian vein was preferred over the femoral and jugular site. In case of chemotherapy, the subclavian vein was preferred more frequently. No differences were found if the indication for CVC was hemodialysis (CVVH). In patients with increased bleeding tendency, the jugular and femoral vein were chosen above the subclavian vein.

TABLE 3. Preferences for catheter insertion site

	Subclavian vein	Internal Jugular vein	Femoral vein	No Preference
General preference (%)	37	54	3	5
Anesthesiology	26	70	0	4
Critical Care	50	47	0	3
Hematology	50	21[1]	14	14
Radiology	13	73	7	7
In increased bleeding tendency	3	66	29	2[2]
Expected indwelling time >5 days	61[3]	33[3]	2	3
Indication:				
Chemotherapy	58[4]	23[4]	1	18
CVVH	9	49	32	10

[1] Hematologist had a negative preference for the IJV compared to other disciplines. ($P < 0.05$)
[2] In increased bleeding tendency, physicians less frequently had no preference ($P < 0.01$)
[3] For an expected indwelling time of >5 days, the SCV was preferred over the IJV and FV ($P < 0.05$)
[4] For chemotherapy the SCV was preferred over the internal jugular vein ($P < 0.05$)

Landmark versus Ultrasound

The disciplines varied in the use of ultrasound for CVC placement. 86% of physicians performed central venous catheter placement in the previous year. All radiologists used ultrasound at all times, which differed from the other specialties (adjusted residual 5.2, $p < 0.01$). Only a minority of critical care physicians (39%), anesthesiologists (26%) and hematologists (14%) used ultrasound at all times. Almost a third of hematologists (29%), 17% of critical care physicians and 11% of anesthesiologists never used ultrasound for CVC placement. Disciplinary differences in the use of ultrasound, are displayed in Figure 1. The

physicians that always used ultrasound had a preference for the jugular site (Adjusted residual 4.0, $p < 0.01$). Contrastingly, physicians with a preference for the subclavian site made less use of ultrasound as compared to the other preferences (Adjusted residual -3.5, $p < 0.05$).

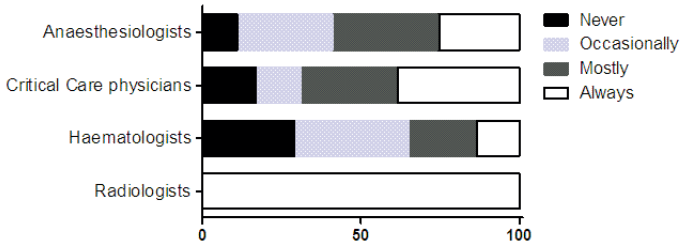


FIGURE 1: The use of ultrasound per medical specialty (%)

Correction of coagulopathy

The questionnaire revealed that interdisciplinary differences in triggers for correction of coagulopathy are present. The intensive care physicians had a more restrictive transfusion policy for platelet count and INR as compared to all other surveyed medical specialists (Kruskal Wallis test, $p = 0.047$ and $p = 0.045$ respectively). Furthermore, platelet transfusion practice varied amongst physicians. Of the respondents 46% indicated to correct a platelet count of 30-50 x 10^9 /L. This amount increased to 64% of physicians for a platelet count between 10-30 x 10^9/L. Hematologists corrected a low platelet count more frequently compared to the other surveyed disciplines. Only a minority of physicians stated to transfuse platelet concentrate if the patient uses a single platelet inhibitor (2%). This number increased to 17% if a patient uses double platelet aggregation. If thrombocytopenia is corrected prior to CVC placement, 63% of physicians stated to administer a fixed amount of one unit pooled donor platelet concentrate, while 38% indicated to determine the amount of transfused platelets based on platelet increment. Platelet increment was measured by 51% of physicians.

Bleeding complications

Of the surveyed physicians, 26% had seen at least one central venous catheter-related bleeding complication in the previous year, resulting in a total of 37 bleeding events (range 0-5). Two of these bleeding events occurred in patients with a platelet count below 50 x 10^9/L.

TABLE 4. Laboratory thresholds for correction of hemostasis per specialty

Medical Specialty	Critical care	Hematology	Radiology	Anesthesiology	Total
Platelet count	20 [<10-60] *	40 [<10-60]	50 [<10-60]	40 [10-80]	30 [10-80]
aPTT	60 [35-150]	40 [30-50]	45 [0-60]	50 [25-120]	50 [0-150]
PT (INR)	3 [1-10] *	1.5 [1-2.5]	2 [1.5-2.5]	2 [1.4-5]	2 [1-10]

These numbers represent the lowest platelet count, maximum aPTT and maximum INR that was prior to CVC placement, without preprocedural correction of hemostasis with blood products.
* Critical care physicians had a lower platelet count threshold and higher INR threshold as compared to other medical specialties, mean difference significant at the 0.05 level.

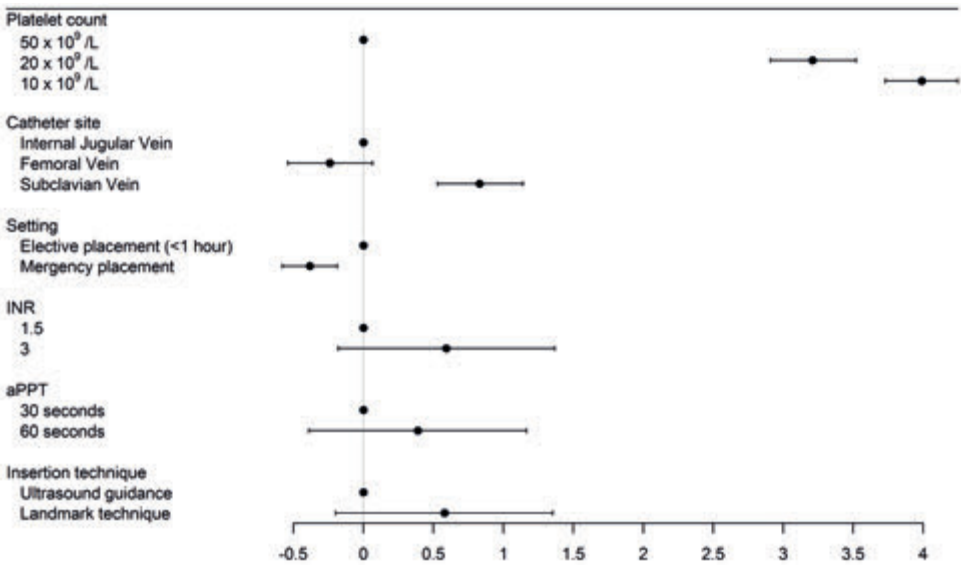


FIGURE 2. Relative preferences of all disciplines combined for determinants on the decision to administer prophylactic platelet concentrate

Vignette

The relative preferences of all disciplines in favor of administering prophylactic platelet transfusion prior to central venous catheterization are shown in Figure 2. The platelet count was the most important factor that contributed to the decision to administer platelet concentrate. A platelet count of 10 x 10^9 /L was a stronger positive preference for administering platelet concentrate (ß = 3.9 [3.7 – 4.3] vs. neutral) than a platelet count of 20 x 10^9 /L (ß = 3.2 [2.9 – 3.5] vs. neutral). The subclavian site had a positive preference for transfusion (ß =0.8 [0.5 – 1.1] vs neutral). In contrast, the femoral insertion site showed a negative trend for the administration of platelet concentrate (ß = -0.2 [-0.5 - 0.06] vs

neutral). A central venous line in an emergency setting was a negative determinant for platelet transfusion as compared to an elective setting (ß = -0.4 [-0.6 - - 0.2]) vs neutral). A patient with an INR of 3.0 showed a positive trend for platelet transfusion as compared to a patient with an INR of 1.5 (ß = 0.6 [-0.2 – 1.4] vs neutral). Likewise, a patient with an aPTT of 60 showed a stronger positive trend for platelet concentrate than an aPTT of 30 (ß = 0.4 [-0.4 – 1.2] vs neutral). The vignettes were reliable with a Cronbach's α = 0.901.

DISCUSSION

The main finding of this study are 1) current transfusion practice prior to CVC placement is highly variable 2) physicians adjust the decision to correct thrombocytopenia prior CVC placement based on clinical parameters, insertion site and technique applied 3) despite proven superiority, the use of ultrasound is still not well implemented among all disciplines involved in CVC placement.

The decision to administer platelet concentrate was influenced by patient and procedural factors, such as insertion site and technique used. Physicians were more inclined to administer prophylactic platelet concentrate in patients with an elevated INR or APTT. However, it is known that plasmatic coagulation disorders are not corrected by platelet concentrate. Our results suggest that physicians take multiple risk factors into account and construct a 'risk profile' for bleeding. However, current guidelines set the indication for prophylactic platelet transfusion solely based on absolute platelet count. This might explain why a considerable fraction of physicians did not adhere to current guidelines on correction of coagulopathy prior to CVC placement. This is also in line with the finding that absolute platelet count is a poor predictor for bleeding complications after central venous catheterization.[19,20].

The majority of physicians preferred the internal jugular vein as access site for CVC-placement in presence of coagulopathy. However, hematologist preferred the subclavian site. An explanation for this may be that the subclavian site might give more patient comfort compared to the other sites. On the other hand, the subclavian vein cannot be manually compressed in case of bleeding and is more difficult to visualize using ultrasound, in contrast to the other access sites. In line with this we showed that the catheter location influenced the decision to administer platelets. Consequently, physicians might adjust transfusion triggers based on the preferred catheter site and their ability to perform ultrasound guided CVC placement. Hence, physicians may correct coagulopathy in order to allow catheter placement in the subclavian site.

Ultrasound guidance for CVC placement was not routinely used by all physicians, despite the proven superiority of ultrasound guided CVC-placement over the landmark technique.[5-9] We did not look into motives for the omission of ultrasound during insertion. In a previous survey amongst emergency physicians in the United States, those who never used US considered insufficient training and lack of equipment as top barriers.[31] Other authors identified "no apparent need", and limited availability of ultrasound equipment as important reasons not to use ultrasound.[10,12,13] It was striking to observe that the implementation of ultrasound was limited among critical care physicians, while critical care physicians were most restrictive with the use of blood products to correct coagulopathy. The restrictive policy may be explained by the increasing evidence on transfusion related morbidity in this patient population.[32-34]

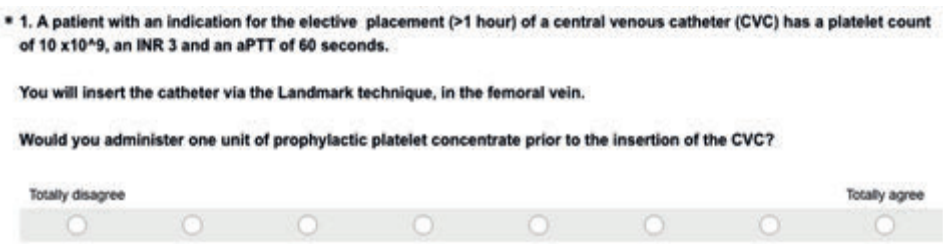
The vignettes showed a decreasing trend in the tendency to administer platelet concentrate if ultrasound was present during CVC placement. This is in line with retrospective studies that support a more restrictive transfusion policy, on the condition that CVC placement is performed by an experienced physician under ultrasound guidance.[23,35] The finding that the use of ultrasound for CVC placement is lacking is important to generalize the results of such trials on the correction of coagulopathy prior to CVC placement. Currently, a randomized trial is conducted on omitting correction of severe thrombocytopenia prior to ultrasound-guided CVC placement, by well-trained physicians (PACER Trial, registered at http://www.trialregister.nl, registration number NTR5653). If this study concludes that it is safe to omit correction of coagulopathy prior to CVC placement, implementation of the routine use of ultrasound in coagulopathic patients is warranted. Before lowering transfusion thresholds prior to CVC placement, training and education on application of ultrasound is essential among disciplines involved in CVC placement.

Our study had several limitations. First, the four medical specialties treat partially distinct patient populations, with various illnesses. Age and years of experience did not differ between respondents of different specialties. However, hematologists more frequently worked in an academic hospital. This might have affected our results. Second, the questionnaire and clinical vignette used a fixed clinical scenario which may not have been representative for all disciplines which may have influenced the outcome. Our overall response rate was good, but we were not able to reach all Dutch hospitals with at least 5 beds equipped for mechanical ventilation. However, we believe that the high rate of respondents of all disciplines from academic hospitals, large teaching hospitals and small regional hospitals ensures that our study results are representative practice of CVC placement in the presence of coagulopathy.

In conclusion, physicians adjust the decision to correct coagulopathy prior CVC placement based on clinical parameters, insertion site and technique applied. Ultrasonographic guidance during CVC placement is not implemented as standard care for all disciplines. Current practice for pre-procedural correction of hemostasis varied widely, with a trend towards a more restrictive transfusion policy. Well-designed studies are needed to determine the optimal transfusion trigger prior CVC placement in presence of coagulopathy.

SUPPLEMENT 1. Patient and catheter related factors that were presumed to be considered before administering platelet concentrate prior to CVC placement

1. Coagulation tests, e.g. platelet count, INR, aPTT
2. Patients with a history of failed catherization attempts
3. Previous surgery, scarring
4. BMI
5. Lever failure
6. Renal failure
7. Previous bleeding, other than CVC related
8. Indication for catheter placement (elective or emergency)
9. Experience of physician performing procedure
10. Catheter site
11. Large bore dialysis catheter vs standard CVC
12. Use of ultrasound



SUPPLEMENT 2. Example vignette

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4

PROPHYLACTIC PLATELET TRANSFUSION PRIOR TO CENTRAL VENOUS CATHETER PLACEMENT IN PATIENTS WITH THROMBOCYTOPENIA: STUDY PROTOCOL FOR A RANDOMISED CONTROLLED TRIAL

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TRIALS 2018 19:127

SUMMARY

Background. Severe thrombocytopenia should be corrected by prophylactic platelet transfusion prior to central venous catheter (CVC) insertion, according to national and international guidelines. Even though correction is thought to prevent bleeding complications, evidence supporting the routine administration of prophylactic platelets is absent. Furthermore, platelet transfusion bears inherent risk. Since the introduction of ultrasound-guided CVC placement, bleeding complication rates have decreased. The objective of the current trial is, therefore, to demonstrate that omitting prophylactic platelet transfusion prior to CVC placement in severely thrombocytopenic patients is non-inferior compared to prophylactic platelet transfusion.

Methods / Design. The PACER trial is an investigator-initiated, national, multicentre, single blinded, randomised controlled, non-inferior, two-arm trial in haematologic and / or intensive care patients with a platelet count of between 10-50 x 10^9 /L and an indication for central venous catheter placement. Consecutive patients are randomly assigned to either receive 1 unit of platelet concentrate, or receive no prophylactic platelet transfusion prior to CVC insertion. The primary endpoint is WHO grades 2-4 bleeding. Secondary endpoints are any bleeding complication, costs, length of intensive care and hospital stay and transfusion requirements.

Discussion. This is the first prospective, randomised controlled trial powered to test the hypothesis whether omitting forgoing platelet transfusion prior to central venous cannulation leads to an equal occurrence of clinical relevant bleeding complications in critically ill and haematologic patients with thrombocytopenia.

Trial registration. Nederlands Trial Registry, ID: NTR5653 (http://www.trialregister.nl/trialreg/index.asp), registered on 27 January 2016. Currently recruiting. Randomisation commenced on 23 February 2016.

INTRODUCTION

Central venous catheter (CVC) placement is a frequently applied medical intervention that enables both monitoring and treatment of patients. [1,2] The inserted cannula provides central venous access either in the neck region (subclavian vein or jugular vein) or groin region (femoral vein). CVCs form an essential element of treatment in various patient categories, mainly hematologic and intensive care patients. [1,2] The latter patient categories have an elevated risk for a low platelet count (thrombocytopenia), due to their treatment and the physiopathology of their illnesses. [3-5] Thrombocytopenia is associated with an increased bleeding risk for many invasive procedures. [6] However, abnormal traditional coagulation tests are known to be a poor predictor of peri-procedural bleeding. [7-9] Current national and international guidelines are conflicting, most recent Dutch and UK guidelines support prophylactic platelet transfusion below a platelet count of $50x10^9$/L, prior to CVC placement. [10,11] Others support the administration of prophylactic platelet transfusion below a platelet count of $20x10^9$/L, however the evidence supporting these recommendations is of low quality. [12,13] Current prophylactic platelet transfusion practice prior to CVC placement varies widely. [14]

In the last decades, more has become known about transfusion related morbidity and mortality; platelet transfusion bears a substantial risk for morbidity and mortality, including transfusion-related acute lung injury (TRALI), transfusion associated cardiac overload (TACO), allergic reactions, allo-immunisation and transfusion related infections. [15-21] In a prospective cohort study of consecutive ICU patients receiving blood transfusion products, 6% developed TACO. [22]. In the Netherlands, 0.32% of pooled platelet concentrates and 0.23% of apheresis platelet concentrate units are contaminated with bacteria. [23] Transfusion of platelets is an independent risk factor for the onset of nosocomial infections in ICU patients, with a hazard ratio of 1.40 (95% CI: 1.2-1.8). [24] Next to the burden of transfusion exposure, blood products are expensive and scarce. Critically ill and hematologic patients are at higher risk of transfusion related morbidity and mortality. [15,25] Altogether, these insights in transfusion related morbidity and mortality have made platelet transfusion without a very strict indication less desirable.

Recent retrospective studies suggest that the experience of the physician and the technique used (ultrasound vs. landmark) rather than the platelet count predicts bleeding complications. [26-28] The introduction of ultrasound (US) guidance for central venous catheter placement was a major improvement. [29] The use of US for CVC insertion results in a lower number of puncture attempts, arterial puncture, hematoma pneumothorax and hematothorax. [29-31] A meta-analyis showed a lower incidence of arterial puncture

with the use of US, namely 37/2009 (1.8%), compared to 196/2018 (9.7%) with anatomical landmarks (RR 0.25, 95% CI 0.15 to 0.42). Also, the occurrence of hematoma was reduced from 113/1512 (7.5%) to 24/1500 (1.6%), with a RR 0.30 (95% CI 0.19 to 0.46) [31]

Consequently, recent retrospective studies suggest that ultrasound guided central venous cannulation can safely be performed in patients with a platelet count above 20 x 10^9/L. [28,32,33] In a study with 604 CVC placements, in 193 patients with acute leukemia, manual compression to stop bleeding was required in 8 patients, no major bleeding was observed. [28] However, this study missed a control group and the administration of prophylactic platelet transfusion was not standardised, resulting in high risk of bias.

The current protocol describes the first randomised controlled trial to evaluate the effect of prophylactic platelet transfusion in hematologic and intensive care patients, in need of a central venous catheter.

METHODS/DESIGN

Study hypothesis

We hypothesize that the improved standards of CVC placement, e.g. the use of ultrasound, make correction of thrombocytopenia prior to CVC placement obsolete.

Study objective

The primary objective is to demonstrate that the omission of prophylactic platelet transfusion in severely thrombocytopenic patients does not increase the amount of bleeding complications related to CVC placement.

Primary endpoint

A procedure-related relevant bleeding, occurring within 24 hours after the procedure. A WHO grade 2-4 up to 24 hours of randomization is defined as relevant bleeding.

Secondary endpoints

- Platelet transfusion requirements within 24 hours of CVC placement
- Number of RBC transfusions within 24 hours of CVC placement
- WHO grade 1 bleeding within 24 hours of CVC placement
- Haematoma-size
- Haemoglobin level at 1 hour and 24 hours after CVC placement
- Platelet transfusion increment

- HEME-bleeding score (Additional file 1)
- Allergic transfusion reaction within 24 hours
- Onset of acute lung injury within 48 hours
- Length of hospital stay
- Costs

WHO Bleeding score.

Specified for Central Venous Catheter related bleeding*	
Grade 1	Haematoma <10 cm. Oozing.
Grade 2	Haematoma > 10 cm. Bleeding that requires more than 20 minutes of (manual) compression in order to cease. Bleeding not requiring red cell transfusion within 24 hours of onset and without hemodynamic instability.
Grade 3	Bleeding requiring red cell specifically for support of bleeding within 24 hours of onset and without hemodynamic instability.
Grade 4	Fatal bleeding. Bleeding associated with hemodynamic instability.

*All bleeding must be Central Venous Catheter related, within 24 hours after insertion.

Design

The PACER trial is a randomised controlled non-inferiority trial on prophylactic platelet transfusion prior to central venous catheter placement in patients with severe thrombocytopenia. It is an investigator-initiated, multicentre, parallel randomised controlled two-arm trial in hematologic and /or intensive care patients with thrombocytopenia and an indication for central venous catheter placement. The PACER trial will be conducted according to the principles of the Declaration of Helsinki as stated in the current version of Fortaleza, Brazil, 2013 [34] and in accordance with the Medical Research Involving Human Subjects Act (WMO). The Institutional Review Board of the Academic Medical Centre, Amsterdam, the Netherlands, approved the trial protocol under reference number 2015_27#B201662. The trial is registered at http://www.trialregister.nl (NTR5653). Intensive care patients will be provisionally included under a strategy of deferred consent, which is explained in detail in the *consent* section below. For patients at the department of haematology, written informed consent is obtained prior to randomisation.

CONSORT diagram

The CONSORT diagram of the PACER study is presented in Figure 1. Consecutive thrombocytopenic patients with an indication for central venous catheterisation are screened. Demographic data are registered regardless of meeting enrolment criteria using

a predefined screen log. If patients are excluded for participation, the reason(s) for exclusion are registered. For the screening of thrombocytopenia, routinely conducted laboratory values are used. The indication for a CVC is determined at physician's discretion.

Setting

The PACER trial is a multicentre study performed in six academic and five teaching hospitals in the Netherlands. We plan to include 392 patients in 36 months, with a potential limit of 462 patients to accommodate loss to follow up and possible drop out.

Study population

Thrombocytopenic patients with a platelet count of 10-50 x 10^9 are eligible for participation if a central line is indicated. Both patients who need elective and emergency line insertions are appropriate for randomisation. Both tunnelled and non-tunnelled CVC's are suitable for inclusion, as well as lines inserted for continuous venovenous haemodiafiltration. Notably, patients requiring replacement of central catheters are also eligible. Patients can participate in the study multiple times, however a patient can be randomized once per 24 hours. The central venous catheter should be expected to be in situ for at least 24 hours, to monitor 24-hour bleeding complications. Therefore, central lines for single plasmapheresis or the harvesting of stem cells will not be included. All other indications can be included, e.g. inotrope medication, lack of peripheral venous access, haemodialysis, haemodynamic monitoring, administration of irritating medication such as chemotherapy. According to guidelines, patients with an INR of >1.5 before line placement are excluded. [35] However, patients are eligible after correction of an elevated INR with fresh frozen plasma or prothrombin concentrate. Non-adult patients (age < 18 years) are excluded, as are patients with a history of congenital or acquired coagulation factor deficiency or bleeding diathesis. Also patients on therapeutic anticoagulant therapy are excluded. Of note, patients with a single platelet aggregation inhibitor and / or therapeutic unfractionated heparin that is discontinued at least one hour prior to insertion are considered to be eligible.

Consent

Informed consent will be obtained from all participants or a legal representative in case the former is impossible. As mentioned previously, this study involves two patient categories. In hematologic patients, lines are usually inserted electively for the administration of chemotherapy. Therefore, informed consent will be obtained before line placement. For the intensive care patient category, obtaining informed consent prior to CVC insertion is often not feasible. Therefore, for the intensive care setting patients may be included using the deferred consent procedure. We will randomise each patient at the intensive care unit (ICU) who meets the inclusion criteria directly before CVC placement. Informed consent

from the legal representative will be requested as soon as possible. If informed consent is denied by a legal representative, the patient is excluded and data will no longer be used. Thenceforth the patient is transfused according to the policy of the attending physician. If a patient dies before informed consent can be obtained, data is used. [36]

Randomisation and blinding

Randomisation will be performed using a dedicated, password protected, SSL–encrypted website with ALEA® software (TenALEA consortium, Amsterdam, the Netherlands). The researcher randomises the patient. Random block sizes are used. Block-randomisation will be stratified per centre and per patient population (ICU vs hematologic patients). Tunnelled lines are stratified per centre. Also, lines placed for dialysis / CVVH are stratified from central lines placed for other indications. This stratification takes place since dialysis / CVVH catheters are larger-bore catheters, made of stiffer materials and bear increased risk for bleeding. [37]

The proceduralist, the physician that inserts the catheter, is blinded for treatment allocation. This blinding takes place to prevent bias in ordering platelet transfusion in case of a peri-procedural bleeding complication. The proceduralist is not directly involved in patient care of the included patient. Blinding is not feasible for the patient and the treating medical staff. Obtained photographs of the insertion site will be analysed for haematoma size and bleeding post-hoc. This provides an additional blinded bleeding outcome.

Treatment arms

Included patients will be randomised into one of two groups. One group of patients will be allocated to not receiving platelet transfusion prior to placement of the CVC, this is the experimental group. The other group of patients will be transfused with one unit of platelet concentrate prior to placement of the catheter, this is the comparison group. All platelet products will be manufactured, screened and stored according to local Dutch standards, (Sanquin Blood Bank). Platelet concentrates are prepared from pooled buffy coats form 5 donors, and re-suspended in plasma, after which pooled platelet concentrates are leukoreduced by filtration. All platelet products are stored with gentle agitation at 20-24°C up to 7 days in the Netherlands. All other care will be according to standard practice as indicated by the treating physician.

Central venous catheter placement

All CVCs will be inserted percutaneously, under real time ultrasound guidance, using the Seldinger technique. In the transfusion-arm, catheters will be inserted as soon as possible after administration of one unit platelet concentrate. The type of ultrasound device,

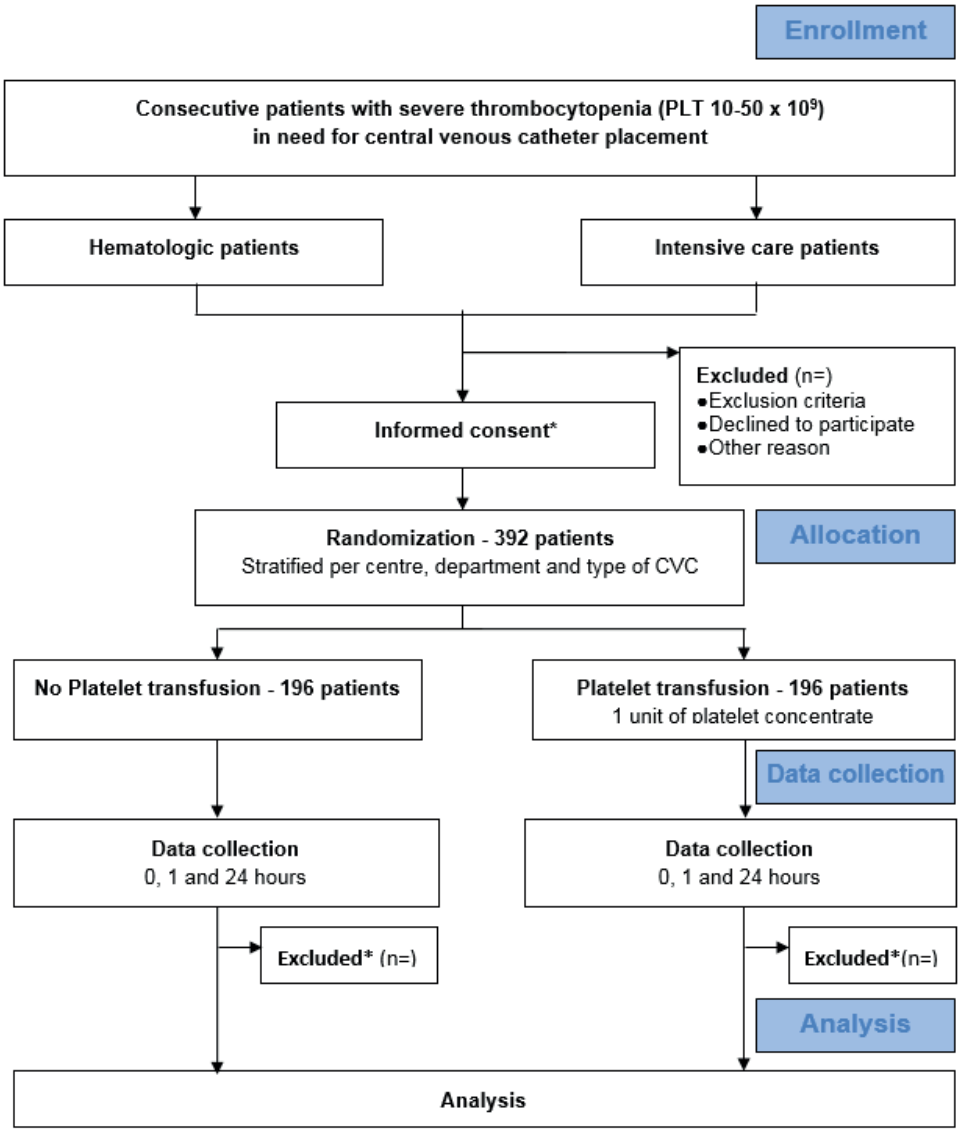

FIGURE 1. CONSORT diagram of PACER. * For hematologic patients, informed consent is required prior to randomization. For Intensive Care patients, randomization takes place via deferred consent. If consent is not obtained, data is excluded.

catheters and (local) anaesthesia is according to local hospital protocols. The puncture location will be determined by the puncturing physician. All procedures will be performed by experienced physicians, e.g. at least fifty previous line placements. [38] Proceduralists can be experienced residents or consultant physicians in Intensive Care, Anaesthesiology, Haematology, (Interventional) Radiology and Surgery. Procedural details, such as arterial puncture, vein lesion and number of puncture attempts are recorded.

Bleeding complications

Bleeding complications will be scored using WHO grades of bleeding specified for Central Venous Catheter placement, which are defined in the Appendix. The WHO bleeding score is adapted according to Zeidler (2011). Grade 1 consists of mild symptoms not requiring any intervention, for example local haematoma or oozing. Grade 2 bleedings are defined as mild symptoms requiring interventions, without haemodynamic instability or red blood cell transfusion. For the current study, this includes procedure-related bleeding that requires more than 20 minutes of manual compression to stop. Grade 3 bleedings are defined as procedure-related bleeding requiring red cell transfusion. Grade 4 bleedings are defined as bleedings associated with hemodynamic instability or death, defined as central venous catheter related bleeding associated with severe hemodynamic instability (hypotension; >50mm/Hg fall or >50% decrease in either systolic or diastolic blood pressure, with associated tachycardia (heart rate increase of > 20% for 20 minutes) and requiring RBC transfusion over routine transfusion needs or fatal bleeding. [39]

Furthermore, a distinction will be made between Minor and Major bleeding. Minor bleeding is defined as WHO bleeding scale 1 or 2. Major bleeding is defined as WHO grade 3 or 4 bleeding.. The proceduralist can administer rescue platelets at clinical indication. Rescue platelet concentrate can be given irrespective of treatment allocation, for which the proceduralist remains blinded.

In case of post procedural bleeding, physicians are encouraged to undertake the following steps:

1. Inspection of the insertion site.
2. Apply manual compression, for a maximum duration of 20 minutes.
3. Consider a skin suture at the insertion site.
4. Consider rescue platelet transfusion.
5. Consider the possibility of a radiological of surgical intervention.

Protocol violation

If platelet concentrate is administered to patients assigned to not receiving platelet concentrate, patients will not be excluded. Reasons for additional transfusion are noted. Patients will be analysed according to intention-to-treat and per protocol analysis. If line placement is unsuccessful due to technical reasons, data will also be analysed according to intention-to treat analysis. If blinding of the proceduralist is violated, this will be noted, however patient data will not be excluded. If the 24 hour time point is not completed, due to a CVC that is in situ for less than 24 hours, or in the case a patient deceases within this timeframe, obtained data will be analysed using missing data for the 24 hour time point.

Participant timeline and study flowchart

The participant timeline is presented in figure 2. The primary outcome of the study is procedure-related WHO bleeding 2-4, occurring within 24 hours after the procedure.

Secondary endpoints are subdivided into clinical outcome variables and health-economic outcome variables. WHO Grade 1 bleedings and subdivision on Minor and Major bleeding are scored as secondary outcome. Another clinical outcome variable is the validated HEME bleeding score [40]. Also, photographs of the insertion site at different time points are blindly scored. Other clinical variables include ICU– and hospital length of stay (LOS), ICU–, and hospital mortality, transfusion requirements and occurrence of transfusion reactions, such as TRALI and allergic reactions. Next to descriptive statistics, the effect of prophylactic platelet transfusion on the primary and secondary outcomes will be analysed using multiple logistic regression for patient-, procedural-, catheter- and transfusion- related characteristics. In patients receiving platelet transfusion, patient and transfusion related factors will be analysed to assess the relation to post transfusion platelet increment. To investigate the relationship between bleeding events and platelet count (post-transfusion platelet count if applicable) we divide platelet count into five groups: 10-20, 20-30, 30-40, 40-50 and above 50. *P* values of 0.05 will be accepted as statistically significant, values will be reported with and without correction for multiple testing.

Health-economic outcome are calculated in the cost-effectiveness analysis, where costs per procedure related bleeding event (primary clinical outcome) will be estimated. The economic evaluation will estimate costs (saved) per PC transfusion avoided. Results will be extrapolated to the national level to estimate the total impact on the health care budget per annum for the Netherlands in terms of cost reduction and increase in procedure related bleeding events.

Data collection

Inclusion is based on platelet count determined in the 24 hours prior to randomisation. Baseline parameters such as age, gender, height, weight and BMI are collected. Recorded clinical parameters include date of admission, diagnosis, cause of thrombocytopenia, indication for CVC, use of defined confounding medication, lever- and kidney failure, diffuse intravascular coagulopathy and fever. For intensive care patients, also APACHE score, admission diagnosis and information about setting during admission are obtained. For hematologic patients, information about the hematologic condition and the phase of therapy is collected. In all patients, the most recent PT (INR), aPTT and haemoglobin levels, prior to randomisation are recorded. Information about the CVC, such as type, indication and diameter is collected. Procedural details such as insertion site, number of punctures, inadvertent arterial puncture, manual compression and the administration of 'rescue platelets' are recorded. According to standard care, blood is collected at 1 and 24 hours after CVC placement. In this way the effect of the platelet concentrate, if applicable, can be measured. A photographic image of the insertion site is taken prior to CVC placement, as well as directly post-procedural and at 1 and 24 hours after CVC placement. Clinical bleeding will be assessed at these same time points. All administered transfusion of blood products will be registered, as well as their clinical indication. All radiologic and surgical interventions will be documented.

Follow up

The follow-up time for the primary outcome is 24 hours. Patient information about ICU- and hospital discharge or death, whichever comes first, are collected.

Data management

An eCRF for the PACER trial in Open Clinica is developed. All participating centres have 24-hour access to the eCRF. Data can be entered continuously for all participating patients. The principle investigator has access to the filled-out eCRFs. If data is entered incomplete or incorrect, the principle investigator can contact the participating centres for clarification.

Statistical considerations

We target to include 392 patients, with a potential limit of 462 patients to accommodate loss to follow up and a possible drop out. The expected rate of severe peri-procedural bleeding incidents is around 0%. [28,33,41-43] Major bleeding is defined as WHO grade 3: gross blood loss requiring transfusion, or grade 4: debilitating blood loss. (Table 1). Considering the low complication occurrence, sample sizes that are by far unrealistic within the proposed study are required to demonstrate non-inferiority with an acceptable non-inferiority limit of (<1%). We therefore also include WHO grade 2 bleedings in the definition

FIGURE 2. SPIRIT for the schedule of enrolment, interventions, and assessments.

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
TIMEPOINT	*-24*	-1	*CVC*	1 h	24 h	48 h	28 days	N = 392
Enrolment								
Eligibility screen	X							
Informed consent*	←	—	—	—	—	—	—	→
Allocation		X						
Interventions								
Platelet transfusion or Restrictive		X						
Insertion of CVC			X					
Assessments								
Laboratory values, platelet count and hemoglobin	X			X	X			
Assessment of catheter insertion site		X	X	X	X			
Transfusion reaction (TRALI, allergic reaction)		←	—	—	—	→		
Transfusions outside study protocol			←	—	→			
Alive at day 28							X	X

* Informed consent will be obtained from all participants or a legal representative in case the former is impossible. Intensive Care patients may be included using the deferred consent procedure. We will randomise each patient at the intensive care unit (ICU) who meets the inclusion criteria directly before CVC placement. Informed consent from the legal representative will be requested as soon as possible. If a patient deceases before informed consent can be obtained, data is used.

of the primary outcome. As the observed bleeding events will concern predominantly grade 2 bleedings, and probably no or only incidental bleedings with more severe grades 3 or 4, an upper limit reflecting an absolute risk increase of 2.5% more bleeding events as criterion to statistically demonstrate non-inferiority is considered both statistical and clinical acceptable. The sample size is increased by 2% to correct for lost to follow up and by 15% to correct for dropouts, e.g. refusal of consent. Using a non-inferiority design, a sample size of 196 patients (per arm) and a percentage bleeding events of 1% in the control group and an expected percentage bleeding events of 1% in the experimental group will result in a power of 80% to exclude that there is a significant bleeding rate in the experimental arm (>3.5% bleedings, absolute risk increase 2.5%, two group t-test with a 0.05 two sided significance level). As we do not anticipate any lost to follow-up – considering the short time-span of the study – we therefore intend to enrol 392 patients in total.

Statistical analysis

Statistical analysis will be based both on an intention-to-treat principle and a per-protocol approach. Baseline assessments and outcome parameters will be summarized using simple descriptive statistics. The main analysis focuses on a comparison between the trial treatment groups of the primary outcome, the occurrence of relevant bleedings, expressed in a relative risk estimate and absolute risk increase, with the associated 95% upper confidence limit. Non-inferiority is demonstrated if this interval does not exceed the non-inferiority limit of 2.5% absolute difference in favour of transfusion.

Withdrawal and replacement of individual patients

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. When deferred consent is not obtained after randomisation and provisional inclusion of a patient, the randomised subject will be replaced. These cases will be recorded in the randomisation log without patient-specific data. The randomisation subject will be replaced in order to retain properly distributed randomisation groups and stratification.

Study organization

The steering committee is composed of the principal investigators, the coordinating investigator and the local investigators in the participating ICUs and haematology departments. The coordinating investigator is responsible for administrative management and communication with the local investigators and provides assistance to the participating clinical sites in trial management, record keeping and data management. The coordinating investigator helps in setting up local training in the participating centres to ensure the

study is conducted according to the ICH–GCP guidelines, to guaranty integrity of data collection and to ensure timely completion of the case report forms. The local investigators provide structural and scientific leadership. They guarantee the integrity of data collection and ensure timely completion of the case report forms.

An independent monitor is installed to perform study monitoring. Remote monitoring by means of queries on the database will be done by a statistician and analysed by the monitor to signalize early aberrant patterns, trends, issues with consistency or credibility and other anomalies. On–site monitoring will comprise controlling presence and completeness of the research dossier and the informed consent forms, source data checks will be performed in the files of the first 3 patients of each participating centre, followed by at least 10% of files. Each centre will be visited at least once every year.

An independent Date Safety and Monitoring Board watches over the ethics of conducting the study in accordance with the declaration of Helsinki, monitors safety parameters and the overall conduct of the study. The DSMB is composed of 3 independent individuals (Dr. M.G.W. Dijkgraaf, Dr. M.C.A. Müller, Prof. dr. J.J. Zwaginga). The DSMB will meet by conference calls. The first took place before the first inclusion. Subsequent to this meeting the DSMB will meet approximately every six months. Also, a meeting will be scheduled after half of the total number of patients is enrolled.

As severe bleeding complications after central venous catheterisation are extremely rare, no bleeding related serious adverse event is expected. All unexpected adverse events will be reported to the DSMB. Any report and/or advice of the DSMB will be send to the sponsor of the study, the Academic Medical Centre, Amsterdam, The Netherlands. Should the sponsor decide not to fully implement advices of the DSMB, the sponsor will send the advice to the reviewing Institutional Review Board, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

DISCUSSION

This is the first randomised trial that investigates the safety of a lower platelet concentrate transfusion threshold prior to CVC placement, in a non-inferiority design. If this trial concludes that omission of prophylactic platelets in patients with a platelet count of 10×10^9/L and higher is safe, guidelines that advice routine administration of platelet concentrate might be adjusted. For the Netherlands alone, we calculated that a transfusion threshold of 10×10^9/L for prophylactic platelet transfusion prior CVC placement instead of 50×10^9/L could lead to a cost reduction of 9.2 million euros per year. [44]

Central venous catheterisation access is a frequently applied medical intervention; more than five million catheters are inserted in the United States each year. [45,46] A peripherally inserted central catheter (PICC-line) is often not an adequate alternative for percutaneous central vein cannulation, because it bears an increased risk for thrombosis, especially in hemato-oncologic and critically ill patients. [47]

Various factors associated with bleeding complications after CVC-placement have been identified. [29,38,48,49] The risk of bleeding after central venous catheterisation is multifactorial and is composed of procedural, patient and physician characteristics. Number of attempts, inadvertent arterial puncture, vein size, vein lesion, patient compliance, obesity and hyperinflation have also been associated with bleeding risk. [46,50] Therefore, it is difficult to assess the isolated effect of thrombocytopenia. Randomisation will divide patient related factors evenly over both treatment arms. All lines are placed by experienced proceduralists, under real-time ultrasound guidance.

A concern regarding safety could be the occurrence of major bleeding in the no platelet concentrate transfusion arm of the study. Based on previous observational studies we believe the risk for serious complications is limited. We monitor the patients intensively and rescue platelet concentrate is available. Prolonged manual compression, or a suture at the insertion site are suggested as safe and minimally invasive interventions to stop prolonged bleeding. [28,42,51] In contrast, patients randomised to not receiving platelet concentrate are not exposed to the inherent risk of platelet transfusion.

A potential shortcoming of the current trial is the heterogeneous patient population having different causes of thrombocytopenia. Our study includes the two patient populations in which thrombocytopenia and central venous cannulisation are most frequent. We believe this is also is a strength of our study, allowing results to be extrapolated to various patients categories.

In conclusion, this is the first prospective, randomised controlled non-inferiority trial powered to test the hypothesis whether not correcting thrombocytopenia prior to central venous cannulation does not lead to an increased occurrence of bleeding complications in critically ill and hematologic patients.

Trial status

Currently recruiting.

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5

THE ASSESSMENT OF BLEEDING SEVERITY IN THROMBOCYTOPENIC PATIENTS UNDERGOING MINIMALLY INVASIVE PROCEDURES IS HIGHLY VARIABLE

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MANUSCRIPT IN PREPARATION

SUMMARY

Background. Platelet transfusion guidelines rely on studies analyzing bleeding risk after invasive procedures. Reported bleeding incidences often depend more on definitions and methods of bleeding assessment than on actual bleeding occurence. The objective of our study was to systematically review bleeding definitions and assessment methods.

Study design and methods. We performed a systematic review identifying studies in thrombocytopenic patients (platelets <150x10^9/L) undergoing invasive procedures (central venous catheter placement, liver, renal and bone marrow biopsy or lumbar puncture), describing bleeding complications as outcome. Studies were retrieved from MEDLINE and transfusion guidelines. RCT's and cohort studies (prospective and retrospective) were eligible. Case reports/series were excluded. Relevant articles were reviewed to determine bleeding assessment methods.

Results. Thirty-two articles met the predefined inclusion criteria. All studies used a different approach to assess bleeding. Six studies (19%) did not provide a bleeding definition. Twenty-one studies (66%) used a bleeding definition of their own design. Four studies employed bleeding assessors, most studies were based on chart review. The bleeding incidence was highly variable, even between studies in comparable populations, undergoing equal interventions. The median (IQR) bleeding incidence was 12.2% (6.7%-28.6%) in prospective studies (n=9) and 0.8% (0.0%-4.3%) in retrospective studies (n=23).

Conclusion. We demonstrated high variability in both definition and assessment of bleeding, making studies difficult to interpret, reproduce and compare. This creates uncertainty about transfusion thresholds and hampers future studies in this area. To improve determination of proper thresholds for prophylactic platelet transfusion, consensus in bleeding definition and assessment is urgently needed.

INTRODUCTION

Patients with a low platelet count (thrombocytopenia) have an increased risk of both spontaneous and post-procedural bleeding.[1,2] Platelet transfusions are therefore recommended in various guidelines,[3-5] either when the platelet count drops below a certain threshold, or prior to invasive procedures. The clinical studies forming the basis of these guidelines are known to be of low quality,[3-6] essentially reducing the value of transfusion guidelines to the quality level of expert opinion.

Most studies designed to assess the optimal platelet transfusion trigger frequently include a clinical assessment of bleeding as outcome measure. A review of studies evaluating platelet transfusion triggers in patients with leukemia reported a spontaneous bleeding incidence that varied between 12 and 66%.7 The authors concluded that this wide variance was more likely a reflection of different methods of bleeding assessment than an actual difference in the occurrence of bleeding. A recent review on coagulopathy prior to central venous catheter (CVC) placement by our group, also found a large variance in the incidence of bleeding.[1]

Several bleeding scales have been developed to help clinicians and researchers assess bleeding. The most widely used of these is the World Health Organization (WHO) bleeding scale,[8] which was created to standardize toxicity reporting in cancer treatment. The Society of Interventional Radiology (SIR) has developed standards for reporting post-procedural complications that includes a bleeding scale.[9] These bleeding scales are ordinal in nature.

An ordinal scale assigns grades to bleeding of increasing severity, whereas a singular definition gives criteria of bleeding to which the answer is either yes or no. In principle, an ordinal bleeding scale renders more details on bleeding complications than a singular definition, provided it is clear enough to allow unambiguous usage. Especially the WHO bleeding scale suffers from subjectivity and while none of the frequently used bleeding scales have ever been formally tested for reproducibility,[10] a study on adjudication of the WHO scale revealed high inter-observer variability.[11]

Another problem with designing adequate bleeding scales is their clinical relevance. Historically, many studies have used WHO grade 2-4 as a surrogate outcome for bleeding complications, while grade 2 bleeding ("Mild blood loss") is widely regarded as clinically irrelevant.[12]

In this systematic review, we expect to find different bleeding incidences depending on the assessment methods and bleeding definitions used, but also depending on the study

design. Retrospective studies have been shown to be less accurate than prospective studies and heavily depend on chart-review. Minor bleeding in particular is not regularly recorded in clinical practice, and may therefore be underreported.[7,13]

The primary objective of our study was to systematically review the methods and definitions used to assess bleeding severity in clinical research on invasive procedures. The secondary objective was to investigate the role of the study design in the variability in bleeding incidence.

MATERIALS AND METHODS

Inclusion & exclusion criteria

We included clinical studies (randomized controlled trials and cohort studies), both prospective and retrospective, on the following invasive procedures: central venous catheter (CVC) placement, liver biopsy (LB), renal biopsy (RB), bone marrow biopsy (BMB) or lumbar puncture (LP). Included studies needed to have bleeding complications as their primary or secondary endpoint and had to include at least one thrombocytopenic ($<150x10^9$/L) patient. Animal studies and case reports/series were excluded. Additionally, we excluded studies that were unavailable in English or Dutch.

Search

We conducted a MEDLINE search in May 2019, for which we used the search strategy that was previously described by the AABB, for the development of platelet transfusion guidelines.1 Two authors independently reviewed citations for eligibility (EvdW, FvB), if any disagreement occurred a third author adjudicated (BB). We manually checked platelet transfusion guidelines to identify missing articles.3[-5] The complete search strategy is described in Appendix 1.

Assessment of risk of bias in included studies

For randomized controlled trials (RCT's), The Cochrane Collaboration tool for the assessment of the risk of bias was used.14 For observational studies, the Newcastle-Ottawa Scale was used.15 Overall study quality was assessed by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Method.16 The quality assessment is provided in Appendix 2.

Statistical analysis

Continuous data was described as mean (SD) if normally distributed or as median (IQR) if not normally distributed. Categorical data was described as number (%). Non-normally distributed data was analyzed with Mann-Whitney U-tests, confidence intervals of bleeding incidences were calculated with the Wilson method[17] and all statistical analyses were performed using R-Studio (version 1.1.453).

RESULTS

Study selection and characteristics

Our MEDLINE search yielded a total of 2692 articles (1190 BMB, 211 CVC insertion, 1247 LB & RB and 44 LP), and the manual search of transfusion guidelines yielded another 475 articles. After removal of duplicates 3018 articles were left, of which 32 met the predefined in- and exclusion criteria (Figure 1).

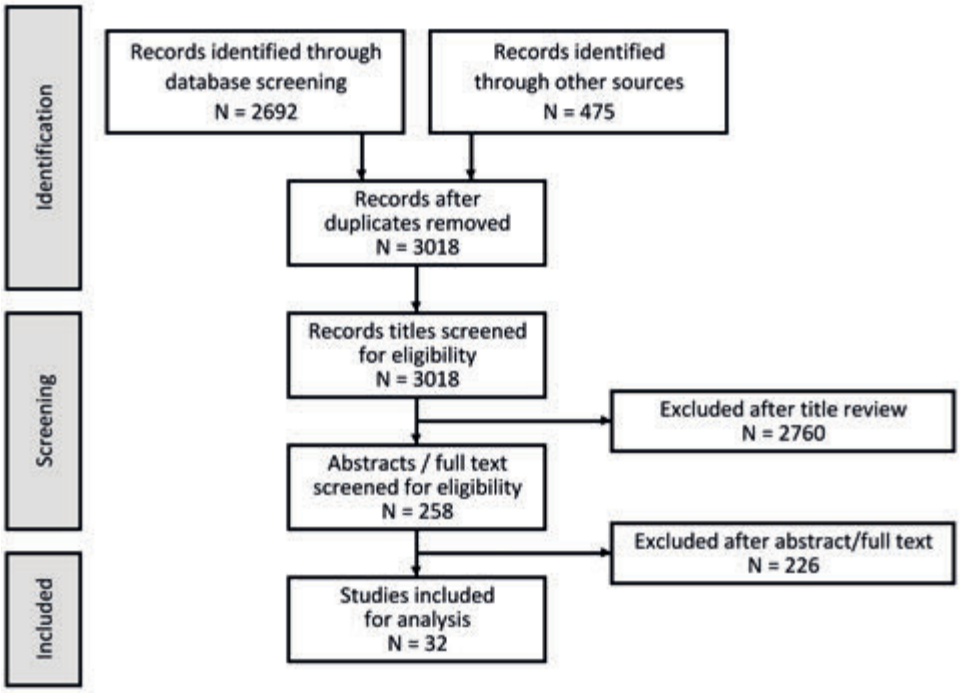

FIGURE 1. Study flow

All studies were cohort studies, 9 of which were prospective and 23 were retrospective. All but one of the studies had bleeding complications as their primary endpoint, the remaining one had bleeding as their secondary endpoint. There was reasonable variation in study types and populations studied (Table 1).

TABLE 1. Study characteristics

Number of participants, median (IQR)	296 (103 – 1150)
Publication year, median (IQR)	2012 (2000 – 2016)
Design	
RCT	0 (0%)
Prospective cohort	9 (28%)
Retrospective cohort	23 (72%)
Procedure type	
Central venous catheter	12 (38%)
Liver biopsy	8 (25%)
Renal biopsy	7 (22%)
Lumbar puncture	4 (13%)
Bone marrow biopsy	1 (3%)
Population	
General population	14 (44%)
Advanced liver disease patients*	5 (16%)
Hemato- / oncology*	4 (13%)
Coagulopathic patients	3 (9%)
TTP patients	2 (6%)
Other	4 (13%)

IQR = Interquartile Range, RCT = Randomized Controlled Trial, TTP = Thrombotic Thrombocytopenic Purpura. *Also includes studies in children.

Differences in bleeding definitions

Overall, 12 studies used an ordinal bleeding scale, 14 used a singular bleeding definition and 6 reported no bleeding definition at all. Of the 26 studies with a bleeding definition, 5 used an existing ordinal bleeding scale (2) or incorporated elements of an existing ordinal bleeding scale in their singular definition (3). 21 studies used a bleeding definition (ordinal scale or singular definition) of the researchers' own design (Table 2). When investigators designed their own ordinal scale, it was always a two-point scale (major and minor bleeding).

Table 2: Use of bleeding definitions in studies of minimally invasive procedures

		Categorical scale		Non-categorical definition		
Intervention	**N**	**Existing bleeding scale**	**Researchers' own design**	**Incorporating existing scale**	**Researchers' own design**	**No definition**
Total	**32**	**2 (6%)**	**10 (31%)**	**3 (9%)**	**11 (34%)**	**6 (19%)**
CVC placement	12	1 (8%)	5 (42%)	1 (8%)	3 (25%)	2 (17%)
Liver biopsy	8	1 (13%)	1 (13%)	0 (0%)	4 (50%)	2 (25%)
Renal biopsy	7	0 (0%)	4 (64%)	1 (14%)	2 (29%)	0 (0%)
Lumbar puncture	4	0 (0%)	0 (0%)	0 (0%)	2 (50%)	2 (50%)
BM biopsy	1	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)

BM = Bone Marrow; CVC = Central Venous Catheter.

The existing scales used in these studies included the Society of Interventional Radiology Technology Assessment Committee reporting standards (SIR)9 and the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE)18 (Table 3). A detailed overview of bleeding definitions for all included studies can be found in Table 4.

TABLE 3. Bleeding scales used in studies of minimally invasive procedures

Scale	Items
SIR	A: no therapy, no consequence; B: requiring nominal therapy, no consequence, including overnight admission for observation; C: requiring therapy, minor hospitalization <48h; D: requiring major therapy, unplanned increase in level of care, prolonged hospitalization >48h; E: permanent adverse sequelae; F: death
CTCAE*	1: mild symptoms not requiring invasive intervention; 2: mild symptoms requiring minimally invasive interventions or aspiration; 3: event indicating transfusion, radiological or surgical procedure; 4: life-threatening consequences necessitating major urgent intervention; 5: death

SIR = Society of Interventional; CTCAE = Common Terminology Criteria for Adverse Events. * Zeidler et al used an adapted form of CTCAE that included prolonged compression as grade 2 bleeding.

TABLE 4. Bleeding definitions and assessment methods for all included studies

			Bleeding definition*		
Study	**Year**	**Procedure**	**(Minor)**	**(Major)**	**Bleeding assessment / follow-up**
Ahmed et al	2016	LB (Transjugular)	Presence of an intraparenchymal liver hematoma, hemobilia, or subcapsular bleeding within 15 days following liver biopsy		Routine observation on nursing floor or interventional radiology recovery area for undetermined time. Review of records up to 15 days post-procedure.
Caturelli et al	1993	LB (US-guided percutaneous)	-		Frequent monitoring of vital signs, routine hematological studies, clinical and ultrasound examination of the abdomen within 6 hours.
Davis et al	1995	RB (US-guided percutaneous)	Drop in hematocrit >4 or ultrasound evidence of new perirenal hematoma within 6h of renal biopsy	Drop in hematocrit >6 within 6h of renal biopsy	Routine observation for 6 hours, with hematocrit check at 6 hours.
Doerfler et al	1996	CVC (Landmark)	-		Routine chest radiograph and nurses were instructed to report any evidence of bleeding or hematoma formation.
Duffy et al	2013	CVC (Mixed US-guided & landmark)	Requiring minimal or no intervention	Requiring surgical intervention or causing significant morbidity/ mortality	-
Estepp et al	2017	Lumbar puncture	Objective confirmation on diagnostic imaging of a spinal hematoma, or a clinical suspicion leading to diagnostic imaging in a symptomatic patient		-
Fisher et al	1999	CVC (Landmark)	Superficial oozing >24h without hemodynamic consequence, or superficial hematoma (visible or palpable)	Hemothorax or any other hemodynamically significant or life-threatening hemorrhage	Routine chest radiograph and daily inspection until catheter removal.
Foerster et al	2015	Lumbar puncture	-		Chart review at undetermined time.

TABLE 4. Continued

Study	Year	Procedure	Bleeding definition* (Minor)	Bleeding definition* (Major)	Bleeding assessment / follow-up
Foster et al	1992	CVC (Landmark)	Insertion site bleeding: hemorrhage requiring removal of catheter or surgical intervention, including placement of suture ligatures, not including bleeding arrested with manual pressure; Hemothorax: pleural opacity on X-thorax, confirmed by aspiration of blood on thoracocentesis; Mediastinal hematoma: collection of blood in mediastinum clinically evident from serial hematocrit concentrations, confirmed by appropriate density on X-thorax or CT; Subcutaneous hematoma: subcutaneous bleeding at insertion site requiring surgical intervention to arrest bleeding or evacuate clot		-
Haas et al	2010	CVC (US-guided)	SIR, excluding minor oozing not requiring any intervention other than brief manual compression		
Horlocker et al	1995	Lumbar puncture	-		Observation until discharge and hospital record review at 6 months.
Islam et al	2010	RB (US-guided)	Hematuria, blood transfusion after biopsy or ultrasound-detected hematoma formation		Routine ultrasound both post-procedure and at discharge.
Kamphuisen et al	2002	LB (Plugged percutaneous)	Acute bleeding event requiring blood transfusion		Close monitoring and twice daily hemoglobin check until discharge (average 4 days).
Kitchin et al	2018	LB (US-guided percutaneous)	CTCAE grade 1	≥ CTCAE grade 2	2-4 hours monitoring in nursing unit, next day telephone call and medical record review at 1 month by a single investigator.
Liu et al	2017	Bone marrow biopsy	≥ SIR grade C / biopsy site bleeding, postprocedural imaging showing hematoma, >2g/dL Hb drop and requirement of vasopressors and/or inotropes		1st hour vital signs monitoring every 15 minutes, then medical record review at 48 hours.
Manno et al	2008	RB (US-guided percutaneous)	Gross hematuria and/or subcapsular perinephric hematoma (<5cm diameter, defined as the product of longest and shortest diameter on two-dimensional US-images), spontaneously resolving without further intervention.	Requiring intervention for resolution (either transfusion of blood products or an invasive procedure) or leading to acute renal obstruction or failure, septicaemia or death.	Clinical and ultrasound evaluations within 24 hours, total observation at least 48 hours. Double entry of variables and checked by a third investigator.

TABLE 4. Continued

Study	Year	Procedure	Bleeding definition* (Minor)	Bleeding definition* (Major)	Bleeding assessment / follow-up
McVay	1990	LB (blind percutaneous)	Hb decrease <2,0g/dL, but RBC-transfusion for hypovolemia given	Hb decrease >2,0g/dL	Frequent monitoring of vital signs 1st 6 hours, routine hemoglobin check after 5 hours and often also the next day.
Monahan et al	2019	RB (US- & CT-guided percutaneous)	≥ CTCAE grade 3, within 3 months of biopsy		Routine imaging directly post-procedure and when clinically indicated. A telephone call after 1, 2 or 3 days. Review of medical record at day 1, 2 or 3 and after 3 months.
Mumtaz et al	2001	CVC (Landmark)	Bleeding arrested with digital manual pressure for approximately 20 minutes	Intervention necessary to stop hemmorhage, hematomas increasing in size, hemothorax, hemomediastinum	Routine chest radiograph. Medical record review at undetermined time.
Ning et al	2016	Lumbar puncture	Spinal, subdural, subarachnoid and epidural hematomas		Review of medical records at 1 week.
Olivieri et al	2016	CVC (Surgical)	Requiring minimal or no intervention	Requiring surgical intervention or causing significant morbidity/ mortality	Routine chest radiograph. Hemoglobin and platelet check within 24 hours. Medical record review at undetermined time.
Ong et al	2012	CVC (Mixed US-guided & landmark)	-		-
Pandey et al	2017	CVC (Landmark)	Requiring additional and non-expected hemostatic measures (compression bandage >15min; blood transfusions) and bleeding causing extension of hospital stay		Routine chest radiograph and observation for 6 hours. Blinded assessor.
Sandrasegaran et al	2016	LB (Mixed blind & US-guided percutaneous)	Acute hemoperitoneum; drop in hematocrit >2g/dL, requiring inotropic or blood transfusion support or need for embolization of hepatic artery branches		Review of medical records at 4 weeks
Sharma et al	1982	LB (blind percutaneous)	-		24 hours of bedrest. Frequent monitoring of vital functions for undetermined time.

TABLE 4. Continued

Study	Year	Procedure	Bleeding definition* (Minor)	Bleeding definition* (Major)	Bleeding assessment / follow-up
Soares et al	2008	RB (US-guided percutaneous)	All other procedure-related bleeding not meeting the criteria for major bleeding	Requiring 1 or more major interventions, such as blood transfusion, hospital admission, or interventional or surgical procedure.	Routine ultrasound post-procedure, observation at least 6 hours and review of clinical notes at 1 week.
Sun et al	2018	RB (US-guided percutaneous)	Not requiring intervention.	US or CT verified bleeding requiring blood transfusions, angiographic embolizations or surgical interventions.	Routine admission for 1 night with 6 hours of sandbag compression and imaging when signs of bleeding occurred.
Tokunaga et al	2013	LB (US-guided percutaneous)	Drop in Hb >2g/dl or need for blood transfusion		Review of clinical and laboratory data for undetermined period.
Vinson et al	2014	CVC (Mixed US-guided & landmark)	Oozing from a percutaneous puncture site or superficial hematoma <24hrs of CVC (includes use of manual pressure, no time-limit given); Minor with procedural intervention: requiring line removal, suture placement or administration of blood products	1) New postprocedural fluid collection or enlargement in the pleural cavity, mediastinum or neck <24hrs of CVC; 2) line-related bleeding causing hemodynamic compromise requiring blood or fluid replacement, vasopressors or surgery	Medical record review at 48 hours with complication assessment by 2 investigators and a third arbitrator from a pool of 4 trained abstractors. Additionally 5% randomly selected for independent review by a second investigator (97.8 – 100% interrater agreement).
Weigand et al	2009	CVC (US-guided)	Drop in Hb >1,5g/dl within 24 to 36 hours		Routine chest radiograph and a laboratory test at least once within 24 to 36 hours.
Xu et al	2017	RB (US-guided percutaneous)	Requiring intervention, including blood transfusion or invasive procedure (radiological or surgical) due to bleeding, within 1 week post-procedure		24 hours of bedrest, regular measurement of vital functions, imaging only on indication. Medical record review at undetermined time.
Zeidler et al	2011	CVC (Landmark)	CTCAE		Daily inspection by specialized nurses.

LB = Liver biopsy; RB = Renal biopsy; US = Ultrasound; CVC = Central venous catheter; CT = Computed Tomography; CTCAE = Common Terminology Criteria for Adverse Events. *If two colums are used for bleeding definition, a distinction was made between minor and major bleeding. If one column was used, nu such distinction was made.

Criteria

The criteria used to define bleeding could be categorized into 3 distinct categories: symptoms, interventions and laboratory results, which were all sometimes limited in time and/or size (Figure 2). General symptoms included oozing, subcutaneous hematoma and changes in hemodynamic function. Naturally, some symptoms differed between invasive procedures. Studies on CVC placement included hemothorax and mediastinal hematoma. Studies on LB included hemobilia, subcapsular bleeding and hemoperitoneum. Studies on RB included (subcapsular) perirenal hematoma and hematuria.

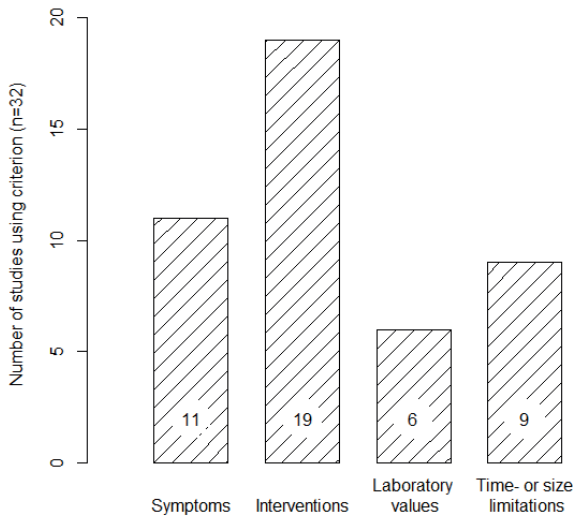

FIGURE 2. Criteria used in bleeding definitions

Studies on LP included spinal, subdural, subarachnoid and epidural hematoma. The study on BMB did not include specific symptoms.

Common interventional criteria included erythrocyte (RBC) transfusion, surgical and/or radiological intervention, which, together, often determined major bleeding, if such a distinction was made. Others included vasopressor or fluid therapy, extension of hospital stay, placement of suture ligaments, compression bandage or manual pressure, the latter of which was sometimes explicitly not included. Studies on CVC placement also included catheter removal, while one of the RB studies explicitly included angiographic embolization. Laboratory results used to define bleeding were a decrease in either hemoglobin (Hb) or hematocrit (Ht).

Some studies put size- or time-limitations on one or more of the prior criteria. Limitations in time were the most common, where the bleeding had to occur within a specified timeframe, varying between 24 hours and 3 months. In other studies symptoms and/or interventions needed a minimum duration, for instance manual compression for >15 – 20 minutes or oozing of >24 hours. Pertaining to size, one study limited minor bleeding to perinephric hematomas <5cm in diameter, another defined major bleeding as hematomas increasing in size.

Differences in bleeding assessment

In 5 studies there was no mention of routine clinical post-procedural care. Routine care included post-procedural imaging, laboratory and clinical examinations, (overnight) admission or observation. In 16/32 studies at least some details on bleeding assessment were described, in varying details, including chart review without further details on the procedure. Only one study used blinded bleeding assessors, although no details on the blinding procedure were given. Two studies used multiple bleeding assessors with an independent arbitrator, one of which also mentioned training their bleeding assessors. No other studies used trained bleeding assessors (Table 5).

TABLE 5. Use of bleeding assessors

	N (32)
Trained assessor	1 (3%)
Blinded assessor	1 (3%)
Multiple assessors & adjudication	2 (6%)

Variability in bleeding incidence

Although we restricted our study to five predefined invasive procedures, there was little overlap between studies, due to different subtypes of procedures and different study populations. We could identify 23 different combinations of patient populations and procedures, of which only 6 were represented by more than two studies. Bleeding incidences varied widely between groups, but even within groups we found non-overlapping 95% confidence intervals (Figure 3).

A significant difference in median bleeding incidence was observed between prospective studies (12.2% (6.7% - 28.6%)) and retrospective studies (0.8% (0.0% - 4.3), $p = 0.02$). We performed a *post-hoc* analysis on the ratio of major bleeding / minor bleeding, for 11 studies that reported separate major and minor bleeding incidences. The median ratio was

0.04 (0.03 – 0.11) in prospective studies (n = 3), meaning that for every major bleeding there were 25 minor bleeding episodes, and 0.4 (0.2 – 1.2) in retrospective studies (n = 8), meaning 5 minor bleeding episodes for every 2 major. This difference was not significant at $p = 0.4$.

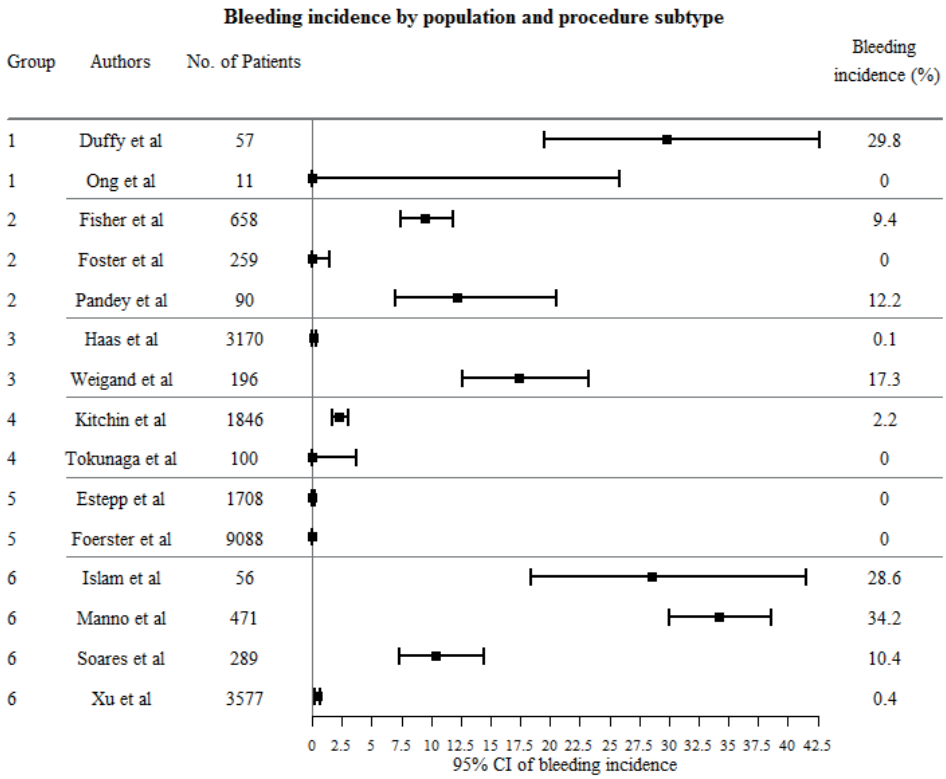

FIGURE 3. Bleeding incidence by population and procedure subtype. CI = Confidence Interval. 1 = CVC placement (ultrasound-guided and landmark) in thrombotic thrombocytopenic purpura patients, 2 = CVC placement (landmark) in advanced liver disease patients, 3 = CVC placement (ultrasound-guided) in general population, 4 = Liver biopsy (ultrasound-guided percutaneous) in general population, 5 = Lumbar puncture in paediatric cancer patients, 6 = Renal biopsy (ultrasound-guided percutaneous) in general population. Non-overlapping 95%-CI in groups 2, 3 and 6 signify difference in bleeding incidence within groups.

DISCUSSION

Invasive procedures in patients with thrombocytopenia form a common clinical problem and generate a great deal of uncertainty and discussion in daily clinical practice. This is reflected in the large variation in platelet thresholds used by different medical disciplines.[19] Despite the large number of studies performed, no clear answer could be given on which thresholds to use. In this study, we reviewed all studies on five frequently performed invasive procedures. We found a large variance in bleeding complications, even between studies assessing the same invasive procedure, mostly due to differences in the way clinical bleeding is assessed and defined, as suggested previously.[10,20]

The large proportion (21/32) of studies using a bleeding definition of investigators' own design forms a major problem, the impact of which is illustrated in the following example: a liver biopsy complicated by subcapsular bleeding requiring embolization and causing a 1g/dL drop in Hb they would be classified as major bleeding in one study (*Kitchin et al*[21]), but wouldn't even be classified as minor bleeding in another study (*McVay et al*[22]). This illustrates that the difference in bleeding definitions should be taken into account when interpreting these results. Moreover, in the 6 studies without bleeding definition it is impossible to interpret the results.

Five studies fully or partly used an existing bleeding scale, which seems to increase the validity of these studies. However, even these scales suffer from subjective criteria and have never been tested for inter-observer variability. One of these bleeding scales was used in the wrong context. The CTCAE scale was designed for toxicity reporting in cancer patients and therefore cannot be applied in patients undergoing an invasive procedure. Moreover, the CTCAE scale has no predefined cut-off between minor and major bleeding. Since researchers mostly report minor and major bleeding as separate entities, a clear distinction is needed.

Besides the two bleeding scales encountered in this review, many other bleeding scales have been published previously. *Koreth et al*[23] have already analyzed the majority of these scales, all of which are used in settings other than invasive procedures. Interestingly, the HEME bleeding assessment by *Arnold et al*,[24] which was specifically designed for an intensive care population, uses some objective criteria, like hemodynamic measures and specific bleeding sites, but retains subjectivity in defining major bleeding as bleeding requiring major therapeutic intervention. Another limitation of these interventional bleeding scales is the difference in the use of therapeutic interventions according to local clinical practice, as reported by *Koreth et al*.[23]

Methods of bleeding assessment varied also. Sixteen out of 32 reported their methods, which were mostly based on review of medical records, resulting in less accurate results than prospectively gathered data.[13] The amount of studies mentioning bleeding assessors was especially low (4/32), and none scored full marks with multiple trained, blinded bleeding assessors using independent adjudication. A systematic review on blinded versus non-blinded outcome assessors in RCTs showed that subjective binary endpoints suffer from bias when non-blinded assessors are used.[25] Furthermore, adjudication of the WHO-bleeding scale was shown to influence study results.[11]

The necessity of adjudicating results has not been demonstrated in all situations. For instance, multicenter research seems to have more benefit than single center research, and vague, subjective endpoints have more need of adjudication than well-defined, objective endpoints.[26-29] Not all measures allow for adjudication: a trial on thromboprophylaxis in intensive care patients showed that attribution of bleeding to anticoagulant use was too hard for an arbitrating committee, when so many different causes of bleeding co-existed.[30]

Chart review is the predominant assessment method in retrospective studies. Our results show a significantly lower reported bleeding incidence in retrospective studies compared to prospective studies. This difference could be explained by the fact that in retrospective studies subtle positive outcomes (i.e. minor bleedings) are missed easily, since the assessment and registration of minor bleeding is often not performed properly in general clinical practice.[7,13] The higher proportion of major bleeding that we found in retrospective studies further underlines this mechanism. However, due to the small number of prospective studies reporting minor and major bleeding, we were unable to demonstrate a statistically significant difference.

Our results support the hypothesis that reported bleeding incidence depends more on methods of assessment and bleeding definition than on actual bleeding tendency. This is in line with earlier results concerning both SAE reporting and clinical bleeding.[1,7,31] Also, we have shown that the way of reporting bleeding assessment is often insufficient. The lack of this essential information reduces the validity and hampers the reproducibility of these studies. A major concern is that these studies form the basis of both current clinical guidelines and sample size calculations for future studies. Clinicians and researchers should be aware of the importance of outcome assessment and bleeding definition.

Future research should focus on developing such a uniform, objective and practical bleeding definition. Through detailing current practices and common criteria in bleeding definitions, the results of this study could form the basis of such a uniform definition.

CONCLUSION

In studies of invasive procedures, in the context of low platelet count, bleeding is now a widely used primary endpoint. In our study, we demonstrate the high variability in definition and assessment of bleeding complications, making studies difficult to interpret, reproduce and compare. This has consequences for clinical practice (uncertainty about transfusion thresholds) and clinical research (imprecise sample-size calculations). There is a dire need of a consensus procedure-related bleeding definition in the field of transfusion medicine, in patients undergoing invasive procedures.

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APPENDICES

Appendix 1. MEDLINE search (Pubmed):

- ("Platelet Count"[Mesh] OR "Platelet Count"[tiab] OR "Platelet Counts"[tiab] OR "Platelet Number"[tiab] OR "Platelet Numbers"[tiab] OR "Blood Platelet Disorders"[Mesh] OR "Blood Platelet Disorders"[tiab] OR "Blood Platelet Disorder"[tiab] OR "Thrombocytopenia"[tiab] OR "Platelet Storage Pool Deficiency"[tiab]) AND (("Bone Marrow"[Mesh] AND "Biopsy"[Mesh]) OR "Bone Marrow Aspiration"[tiab] OR "Bone Marrow Biopsy"[tiab] OR "Bone Marrow Biopsies"[tiab])
- ("Platelet Count"[Mesh] OR "Platelet Count"[tiab] OR "Platelet Counts"[tiab] OR "Platelet Number"[tiab] OR "Platelet Numbers"[tiab] OR "Blood Platelet Disorders"[Mesh] OR "Blood Platelet Disorders"[tiab] OR "Blood Platelet Disorder"[tiab] OR "Thrombocytopenia"[tiab] OR "Platelet Storage Pool Deficiency"[tiab]) AND("Catheterization, Central Venous"[Mesh] OR "Central Catheterization"[tiab] OR "Central Catheterizations"[tiab] OR "Central Venous Catheterization"[tiab] OR "Central Venous Catheterizations"[tiab] OR "CVC"[tiab] OR "CVL"[tiab] OR "CVCs"[tiab] OR "Central Vein Catheterization"[tiab] OR "Central Vein Catheterizations"[tiab])
- ("Biopsy, Needle/adverse effects"[MAJR] OR "liver biopsy"[tiab] OR "renal biopsy"[tiab] OR "kidney biopsy" AND ("Platelet Count"[Mesh] OR "Platelet Count"[tiab] OR "Platelet Counts"[tiab] OR "Platelet Number"[tiab] OR "Platelet Numbers"[tiab] OR "Blood Platelet Disorders"[Mesh] OR "Blood Platelet Disorders"[tiab] OR "Blood Platelet Disorder"[tiab] OR Thrombocytopenia[tiab] OR "Platelet Storage Pool Deficiency"[tiab])
- ("Platelet Count"[Mesh] OR "Platelet Count"[tiab] OR "Platelet Counts"[tiab] OR "Platelet Number"[tiab] OR "Platelet Numbers"[tiab] OR "Blood Platelet Disorders"[Mesh] OR "Blood Platelet Disorders"[tiab] OR "Blood Platelet Disorder"[tiab] OR Thrombocytopenia[tiab] OR "Platelet Storage Pool Deficiency"[tiab]) AND ("Puncture, Lumbar"[Mesh] OR "lumbar punct*"[tiab] OR "Spinal puncture"[Mesh])

APPENDIX 2. GRADE assessment regarding bleeding incidence

Study	Year	Risk of Bias Serious -1; Very serious -2	Inconsistency Serious -1; Very serious -2	Indirectness Serious -1; Very serious -2	Imprecision Serious -1; Very serious -2	Publication bias Serious -1; Very serious -2	Large effect Large +1; Very large +2	Dose response Evidence of gradient +1	All plausible residual confounding would Reduce demonstrated effect +1; suggest spurious effect if no effect was observed +1	Total High: 2+; Moderate: 1+; Low: 0+; Very low: 1-
Ahmed et al	2016	-1	-1	-1	0	0	0	0	0	Very low
Caturelli et al	1993	-1	0	-1	-1	0	0	0	0	Very low
Davis et al	1995	-1	0	-1	0	0	0	0	0	Very low
Doerfler et al	1996	-1	0	0	-1	0	0	0	0	Very low
Duffy et al	2013	-1	0	0	0	0	0	0	0	Very low
Estepp et al	2017	-1	0	0	0	0	0	0	0	Very low
Fisher et al	1999	-1	0	0	0	0	0	0	0	Very low
Foerster et al	2015	-1	0	0	-1	0	0	0	0	Very low
Foster et al	1992	-1	0	0	-1	0	0	0	0	Very low
Haas et al	2010	-1	-1	0	0	0	0	0	0	Very low
Horlocker et al	1995	0	0	0	-1	0	0	0	0	Very low
Islam et al	2010	-1	0	0	0	0	0	0	0	Very low
Kamphuisen et al	2002	-1	0	0	-1	0	0	0	0	Very low
Kitchin et al	2018	-1	0	0	-1	0	0	1	0	Very low
Liu et al	2017	-1	0	0	0	0	0	0	0	Very low
Manno et al	2004	0	0	0	0	0	0	0	0	Low
McVay	1990	-1	0	-1	-1	0	0	0	0	Very low
Monahan et al	2019	0	0	0	0	0	0	0	0	Low
Mumtaz et al	2001	-1	-1	0	-1	0	0	0	0	Very low
Ning et al	2016	-1	0	0	0	0	0	1	0	Low
Olivieri et al	2016	-1	0	0	-1	0	0	0	0	Very low
Ong et al	2012	-2	0	0	-1	0	0	0	0	Very low
Pandey et al	2017	-1	0	0	0	0	0	0	0	Very low

APPENDIX 2. Continued

Study	Year	Risk of Bias Serious -1; Very serious -2	Inconsistency Serious -1; Very serious -2	Indirectness Serious -1; Very serious -2	Imprecision Serious -1; Very serious -2	Publication bias Serious -1; Very serious -2	Large effect Large +1; Very large +2	Dose response Evidence of gradient +1	All plausible residual confounding would Reduce demonstrated effect +1; suggest spurious effect if no effect was observed +1	Total High: 2+; Moderate: 1+; Low: 0+; Very low: 1-
Sandrasegaran et al	2016	0	0	0	0	0	0	0	0	Low
Sharma et al	1982	-1	0	0	-1	0	0	0	0	Very low
Soares et al	2008	-1	0	0	0	0	0	0	0	Very low
Sun et al	2018	-1	0	0	0	0	0	0	0	Very low
Tokunaga et al	2013	-1	0	-1	0	0	0	0	0	Very low
Vinson et al	2014	0	0	0	0	0	0	0	0	Low
Weigand et al	2009	-1	0	-1	0	0	0	0	0	Very low
Xu et al	2017	-1	0	0	-1	0	0	0	0	Very low
Zeidler et al	2011	-1	0	0	0	0	0	0	0	Very low

RCT = Randomised Controlled Trial; Observ = Observational Study

6

BIOTINYLATION OF PLATELETS FOR TRANSFUSION PURPOSES: A NOVEL METHOD TO LABEL PLATELETS IN A CLOSED SYSTEM

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SUMMARY

Background. Labeling of platelets is required to measure the recovery and survival of transfused platelets *in vivo*. Currently a radioactive method is used to label platelets. However, application of those radio-labeling methods are limited by both safety issues and the inability to isolate transfused platelets from the circulation. Biotinlabeled platelets (bioPLT) are an attractive non-radioactive option. However, no validated protocol to biotinylate platelets is currently available for human studies.

Study design and methods. Six platelet concentrates (PCs) derived from

pooled buffy coats were sub-aliquoted and biotinylated at day 1 and day 7 of storage. To distinguish the effect of the processing steps from the effects of biotin incubation, two control groups were used: 1) 'sham' samples were processed on day 1 and day 7 but without the biotinylation reagent and 2) control samples were assessed on day 1 and day 7 but without any processing other than the PC isolation. For the biotinylation procedure, 50 ml of PCs was washed twice and incubated with 5 mg/L biotin for 30 minutes in a closed system. Stability of the biotin label after irradiation and storage of the biotin-solution was quantified. As measure of platelet activation, phosphatidylserine exposure and CD62p expression were assessed.

Results. The biotin labeling density was reproducible. After biotinylation, 98.4% ± 0.9% of platelets were labeled. Platelet counts, pH and 'swirling' were within the range accepted by the Dutch blood bank for standard platelet products. Biotinylated platelets were not more activated compared to sham samples, but were more activated than the controls.

Conclusion. We developed a standardized and reproducible protocol according to Good Practice Guidelines (GPG) standards, for biotin-labeling of platelets for clinical purposes. This method can be applied as non-radioactive alternative assess survival and recovery of transfused platelets *in vivo*.

INTRODUCTION

Labeling of platelets is required to distinguish transfused platelets from the recipient's own circulating platelets. This enables the measurement of recovery and survival of transfused platelets *in vivo*.

Radiolabeling of platelets with the radioactive isotopes ^{111}Indium-oxin and/or ^{51}Chromium is currently the golden standard to test for survival and recovery of platelets.[1] This method is used to evaluate the effects of donor-, recipient- and platelet storage factors on platelet survival after transfusion in the recipient.[2] Also, radiolabeling is required by the FDA to analyze the effect of altered platelet storage protocols, such as new additive solutions and pathogen-reduction technologies.[3]However, radiolabeling exposes the recipient to potential harmful ionization. Therefore, this method cannot be used in vulnerable patients, in particular pediatric patients and neonates. Moreover, the use of radiolabeled platelets is strictly regulated, which limits it's applicability for research purposes. In Europe, radiolabeling is restricted to studies in healthy volunteers only. Therefore, a non-radioactive alternative to label platelets is desired. Biotin, a water-soluble vitamin (B8) can be used as a non-radioactive label for various cells.[4-9] The N-hydroxysuccinimide ester of the biotin reagent binds in a non-specific manner to cellsurface proteins of the platelet (Figure 1A, biotinylation of platelets). Unlike radiolabeling, biotin-labeling has the major advantage that it is possible to selectively isolate biotin-labeled platelets from the recipients' circulation. Also, a biotin label can even be used to trace multiple platelet populations concurrently by using different densities of the biotin label per platelet.

Recently, our group reported a protocol for the biotinylation of red cell concentrates, according to Good Practice Guidelines (GPG).[10] This product is currently available for clinical use and the first trials have started recruiting patients. (Registered at trialregister.nl: NTR 6596, NTR6492). However, labeling of platelets is difficult compared to erythrocytes, due to their propensity to become activated.

Biotin-labeling of platelets has been used in various animal models[7,11,12] and twenty years ago, biotin-labeled platelets (bioPLTs) were safely infused in humans for the first time.[13] In this pilot study, platelet recovery was measured after infusion of biotinylated platelets in ten healthy male subjects. However, this study was hampered by activation of the platelets during biotinylation. We developed a method to minimize platelet activation during biotinylation. Because platelets concentrates are particularly prone to bacterial contamination compared to other blood products, a method for biotinylating platelets in a closed system was developed.

In this manuscript, we describe the results of a method to produce a biotinylated platelet product in a closed system, in accordance to GPG, with minimal platelet activation, which can be used in clinical research in humans.

MATERIALS AND METHODS

Platelet concentrates

Platelet concentrates (PCs) were manufactured and stored by Sanquin Blood Bank, according to the Dutch Blood Bank standards. Whole blood (WB) collections (500 ml) were obtained from volunteer, non-remunerated donors. WB was centrifuged and separated after overnight hold into red cell concentrates, plasma and buffy coats. To obtain a PC, pooled buffy coats from five donors were re-suspended in 100% plasma or 65% PAS-E (Terumo BCT, Inc Lakewood CO USA) and 35% plasma and leukoreduced by filtration. Single donor apheresis PCs were obtained according to the manufacturer's instructions (Trima, Terumo BCT). PCs were stored under gentle agitation, at 20-24°C. Informed consent to use their blood for research purposes was obtained from all donors. The validation protocol was approved by the Sanquin department of Quality Assurance.

Preparation of the biotin solution

Sulfo-NHS-biotin was dissolved in phosphate-buffered saline (PBS,140.3 mmol/L NaCl, 10.9 mmol/L $Na_2HPO_4.2H_2O$, 1.8 mmol/L $NaH_2PO_4.2H_2O$, pH 7.4, Fresenius Kabi) to a concentration of 50 mg/L (EZ-link Sulfo-NHS--Biotin, 100mg; Thermo scientific). The sulfo-NHS-biotin-solution was sterilized by passing it through a 0.22 µM filter (Fresenius HemoCare, Fresenius Kabi) using a sterile connection device (Sterile Turbing Welder-Terumo BCT, TSCD II) and a 600-mL container (Compoflex, Fresenius Kabi). After filtration of the sulfo-NHS- biotin-solution, the biotinylation took place in a closed system, to prevent microbiological contamination. To obtain the final concentration of 5 mg/L, the sulfo-NHS-biotin solution was diluted 1:9 in Platelet Additive Solution (PAS-E: 0.030% $MgCl.12H_2O$, 0.037% KCl, 0.105% $NaH_2PO_4.2H_2O$, 0.318% $C_6H_5Na_3O_7.2H_2O$, 0.405% NaCl, 0.442 %, $C_2H_3NaO_2.3H_2O$, 0.769% $Na_2HPO_4.12H_2O$, pH: 7.1-7.5) (Terumo BCT), and divided in portions of 100 mL, and used within 30 minutes after diluting.

Standardized platelet biotinylation procedure

The biotinylation procedure is depicted in Figure 1B. Biotinylation of platelets was performed in a closed system. The full protocol for biotinylation of platelets is provided in Appendix 1. A 50 ml portion of PC was transferred to a small transfer bag. Transfusion of a biotinylated PC aliquot of 50 ml would theoretically achieve a bioPLT enrichment of 2.5-7%

in healthy subjects. Plasma proteins that could interfere with biotinylation were reduced by washing the platelet concentrate twice for PCs stored in plasma or once for PCs stored in 65% PAS-E. Prior to centrifugation, samples were acidified by ACD-A (Anticoagulant citrate dextrose Solution A, Terumo BCT) to prevent irreversible clumping during centrifuging (1700 x g for 10 minutes). For the first washing step, 100 ml of 8.5:1.5 PAS-E / ACD-A solution was added to the 50 mL of platelet concentrate. 150 ml of a 9:1 PAS-E/ACD-A solution was added or the second washing step. The washed platelets were incubated with the 100 mL biotin solution, for 30 minutes, under gentile agitation, at 22º Celsius. Twelve ml of ACD-A was added prior to centrifugation (1700 x g for 10 minutes), after which the biotinylated platelets were resuspended in PAS-E, to their original volume of 50 ml. To confirm biotinylation, samples of both biotinylated and unbiotinylated platelets were counterstained with Streptavidin 488 (1:200), Alexa Fluor 488 conjugate (Thermo Fisher Scientific, catalogue number: S32354), incubated for 30 minutes at room temperature, washed (1700 x g for 10 minutes) and measured by flow cytometry on a LSRII + HTS (BD Biosciences). Data were analyzed with FlowJo v(CFC).

Validation experiment

For the validation procedure, aliquots of six in plasma stored PCs were biotinylated at day 1 and day 7 of storage. At these time points a 'sham'-sample was also obtained from the same unit, in which all processing steps were identical to the biotinylated samples, except the incubation with sulfo-NHS-biotin, which was replaced by incubation with a 1:9 PBS-PAS-E solution. Platelets that were biotinylated after one day of storage were subsequently stored for two more days and tested for biotinylation and quality parameters (hence, at day three of storage after donation). To show applicability to various types of PCs, also three platelet concentrates that were obtained via apheresis and three PCs stored in 65% PAS-E were biotinylated at day 1 of storage.

Storage of bioPLT

Two methods of storage of bioPLT were tested: first 50 mL bioPLT and sham platelets were stored for three days after processing and then tested for quality parameters (Supplement Figure 1B). Since this led to inacceptable high platelet activation a second method was tested. We hypothesized that storage of a small sample in this storage bag caused the high platelet activation. Therefore, 50 ml of bioPLT was returned to the retained original PC, resulting in the original volume of approximately 330 ml consisting of labeled and unlabeled platelets (Supplement Figure 1C). Therefore triple staining was necessary to distinguish platelet activation of the unlabeled and labeled platelets, at day of biotinylation and day 4 to day 7 of storage.

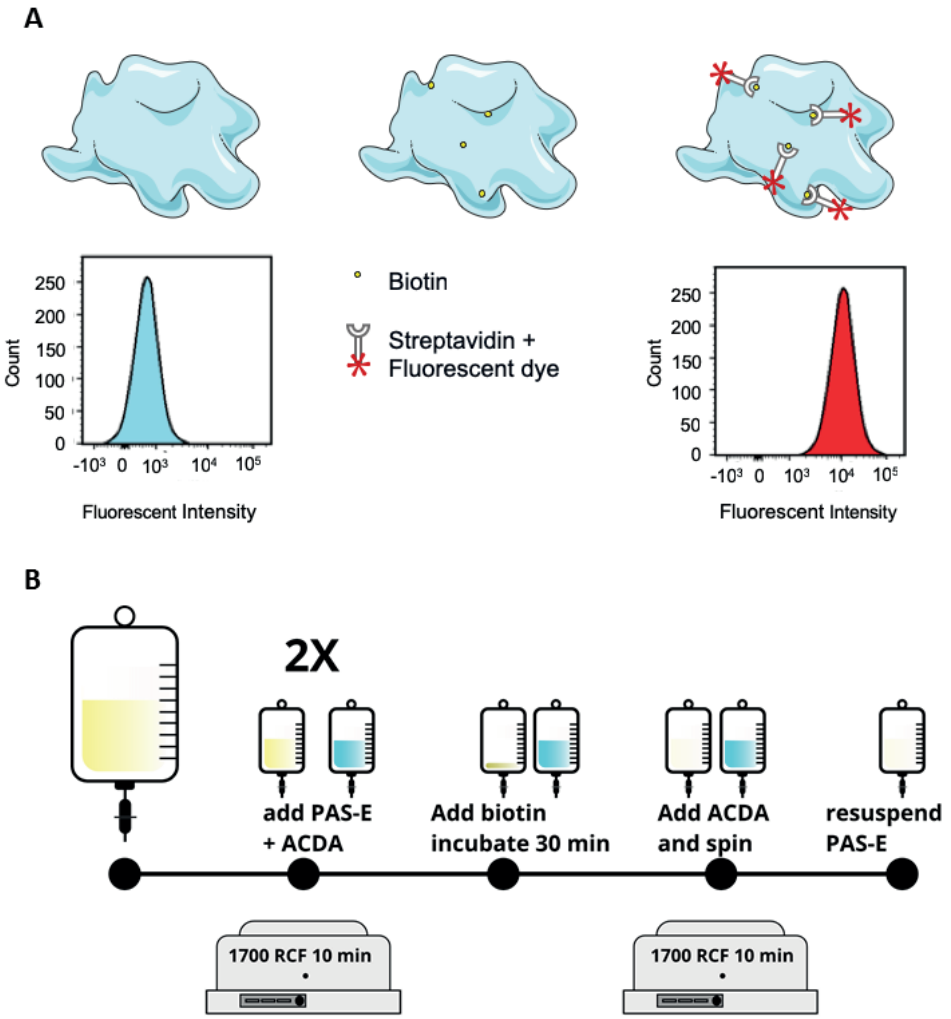

FIGURE 1. Biotinylation of platelets and biotinylation procedure. A. Platelets (top left), are incubated with sulfo-NHS-biotin (top middle), bioPLTs are counterstained with fluorescently labeled streptavidin (top right). Unlabeled platelets show a characteristic FACs histogram, the fluorescently labeled biotin-streptavidin causes the peak of the histogram to shift horizontally. **B.** A proportion of the PC was washed twice in PAS-E. Prior to each washing step, the PC was acidified to 10% ACD-A. Platelets were incubated with 5 mg/L biotin, diluted in PBS-PAS-E (1:9), for 30 minutes. BioPLTs were washed and resuspended in PAS-E.

Additional conditions

To evaluate the effect of storage of the sulfo-NHS-biotin solution on biotinylation, a part of the sulfo-NHS-biotin-PBS- solution was stored within 30 minutes after dilution at -30 (± 5) °C. After42 days the frozen sulfo-NHS- biotin-PBS- solution was thawed at 37°C in 10 minutes to approximately 20°C . After thawing, the sulfo-NHS-biotin-PBS- solution was diluted 10 times with PAS-E (at 20°C) to a final concentration of 5 mg/L, aliquoted to portions of 100 ml, and used within 30 minutes.

To analyze the effect of irradiation on the biotin label, biotin-labeled platelets were irradiated after labeling with a dose of 25 Gray (according to standard blood bank regulations). Samples were obtained prior and after irradiation to assess the effect of this treatment on the biotin label.

Platelet quality parameters

Ranges for quality parameters were pre-defined according to local blood bank guidelines and are expressed in Table 1. Blood gas analysis was performed to determine the pH of the platelet concentrates. (Rapidlab 1265, Siemens Medical Solution Diagnostics). Platelet counts were measured on an Advia 2120 (Siemens Medical Solutions Diagnostics).

The morphology of the platelets was assessed both non-invasive (swirl) and invasive (microscopically). To perform the platelet swirling test, the motion of the platelets was assessed visually by gently moving the bag in front of a light source. The swirl was recorded as positive (3), moderate (2), impaired (1) or absent (0). The test was performed by an independent, experienced, laboratory staff member, who was blinded for the intervention. Platelet morphology was also assessed by light microscopy, for which 50 µl of platelet concentrate was mixed with 250 µl 0.5% glutaraldehyde (Merck, Darmstadt Germany) in PBS. The fixed platelets were visualized with a 1000 times magnification (Axio, Zeis, Breda, the Netherlands).

Baseline platelet activation was assessed by CD62P expression.[14] The samples were incubated for 20 minutes at RT with PE-mouse anti-human CD62P (BD Pharmingen Biosciences) and FITC mouse antihuman CD61 (BD Pharmingen Biosciences. For the triple staining platelets were also incubated with Streptavidin 647 (1:200), Alexa Fluor 647 conjugate (Thermo Fisher Scientific, catalogue number: S32357). Immunoglobulin G1 Mouse PE conjugated (Immunotech SAS, Beckman Coulter, Marseille France) was used as isotype control for the CD62P activation. To assess platelets' ability to become activated, platelets were incubated simultaneously with CD61 and CD62P. Agonists used were 625 nM of thrombin receptor-activating peptide, (TRAP-6-amide/trifluoracetate salt, Bachem AG, Switzerland) or 125 µl/ L of adenosine di-phosphate (ADP, Chronolog, Havertown, USA).

The reaction was stopped after 20 minutes by adding formaldehyde 1% (Merck) in PBS.[15] Annexin V binding was assessed as a marker for phosphatidylserine (PS), as described.[16] Flow cytometry was performed using a LSRII + HTS (BD Biosciences). Data were analyzed with FlowJo v(CFC).

The nucleotide content of the platelets was assessed in neutralized perchloric acid extracts, which were stored at -80°C, until batch analysis with high-performance liquid chromatography using a cation exchange, column as described previously.[4,17]

Platelet concentrates were cultured by BacT/ALERT(Bio Merieux), both before and after the biotinylation procedure, to rule out bacterial contamination.

TABLE 1. Platelet quality parameters

Reference values	Biotinylation on day	platelet count (800-1600 x 109)	pH (6.3-7.5)	Swirl (>1)	Morphology >200	ATP*
Control (N=6)	day 1	1126 (1016-1202)	7.2 (7.2- 7.2)	3 (3-3)	270 (260-288)	42.6(5.3)
	day 7	1083 (1004-1152)	7.1 (7.1-7.2)	3 (3-3)	245 (233-250)	40.6(5.3)
Sham (N=6)	day 1	971 (890-1034)	7.1 (7.0-7.1)	3 (3-3)	245 (233-269)	34.2(3.6)
	day 7	997 (934-1113)	7.0 (7.0-7.1)	3 (3-3)	210 (205-215)	27.3(3.4)
Biotin (N=6)	day 1	1034 (965-1166)	7.0 (7.0-7.0)	3 (3-2.3)	258 (240-275)	33.2(4.4)
	day 7	949 (928-992)	7.01 (7.1-7.1)	3 (3-3)	213 (199-226)	29.6(4.9)

*No predefined range exists for ATP levels according to our local blood bank guidelines

Statistical analysis

Statistical analyses were performed in R, version 3.5.0. Variables were assessed for normality and corresponding statistical tests were performed (paired t-test for parametric data, Mann-Whitney U test for non-parametric data). Differences were considered to be statistically significant if the p-value was < 0.05.

RESULTS

Biotinylation of platelet concentrates

Six pooled buffy-coat derived PCs in plasma, three pooled buffy-coat derived PCs in 65% PAS-E/35% plasma and three apheresis PCs in plasma were biotinylated as described in the protocol (Appendix 1). After the biotinylation-procedure, 98.4% ± 0.9% and 99.0% % ± 0.9% of platelets were labeled with biotin at day 1 and day 7 of storage respectively (Figure 2). There was no difference in biotinylation of PCs obtained from pooled buffy coats and stored in plasma as compared to apheresis PCs and PCs stored in PAS-E. The unbiotinylated fraction and the biotinylated fraction of the PC could be visualized as two distinctive populations on flow cytometry. We confirmed that biotin labeling of platelets is still successful after 42 days of storage of the dissolved biotin solution at -30º (Figure 3). Irradiation of the biotin-labeled PC with a standard dose of 25 Gray did not affect the degree of biotinylation. (Figure 3). It was not possible to incubate the platelets with biotin and add ACD-A simultaneously. (Figure 3). This would have reduced one processing step. The reduction of biotinylation after additions of ACD-A is probably due to a lower pH.

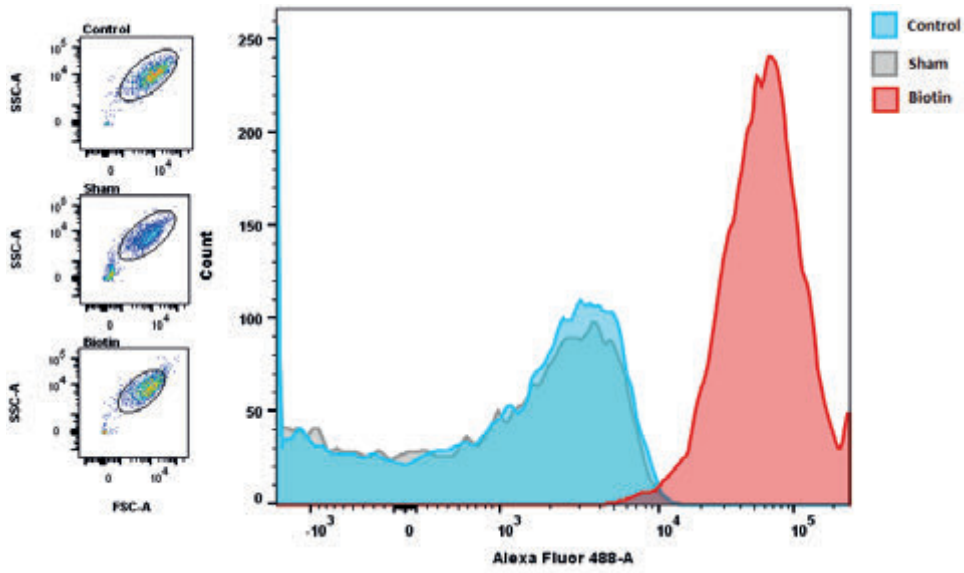

FIGURE 2. Flow-cytometric analysis of unlabeled (blue), sham (grey) and bioPLTs (red), after incubation with streptavidin-488. The bioPLT show a significant higher fluorescent intensity as compared to the sham and control platelets. 98.4% ± 0.9% of bioPLTs were biotinylated, and can be visualized as a distinct population. Scatters of all three populations are similar (left). Images are from a selected PC, but are representative for the other experiments (n=6).

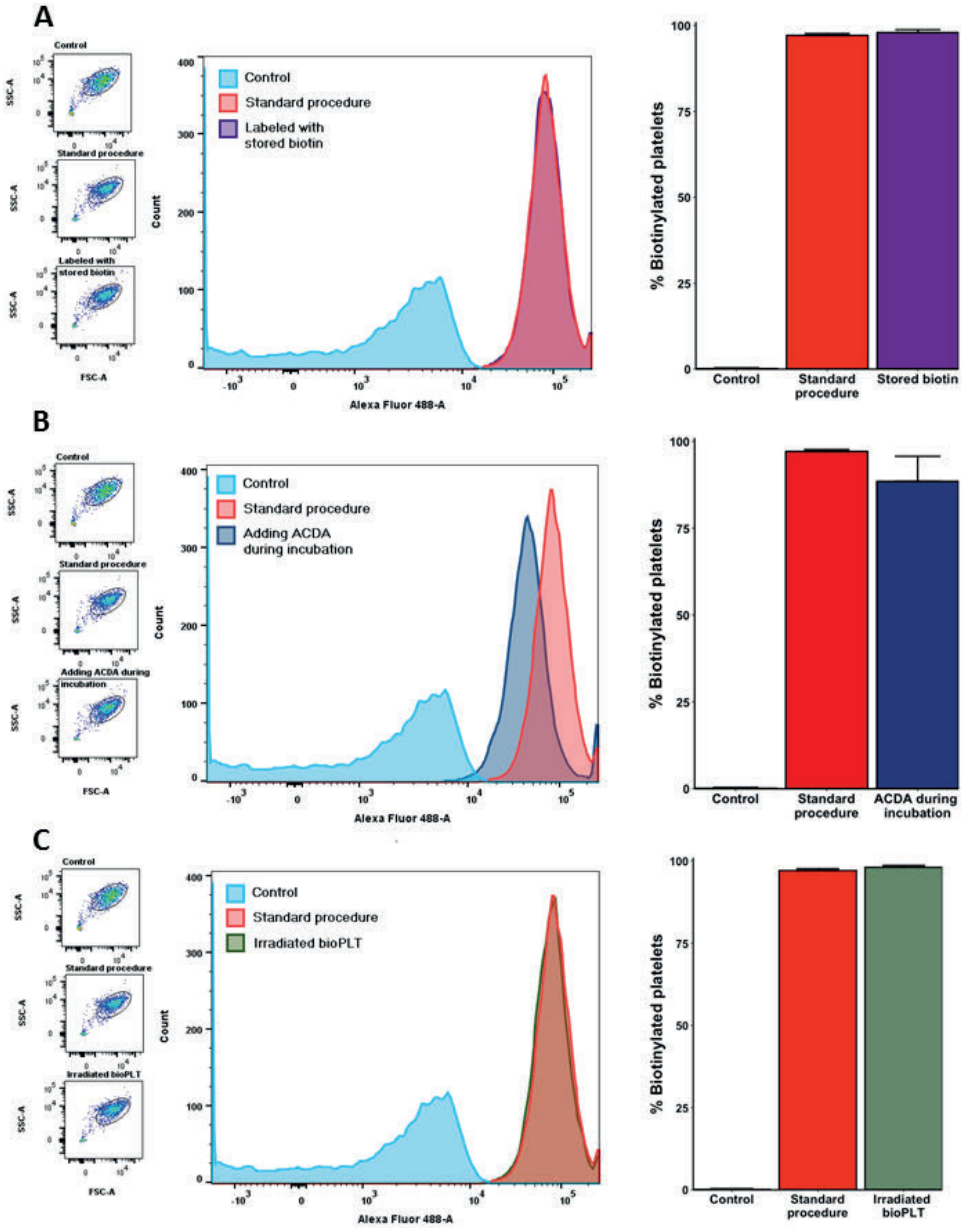

FIGURE 3. Biotinylation in various conditions. A. Biotinylation performed with biotin stored in PBS for 42 days at -30. There was no difference between freshly dissolved biotin and a stored biotin solution (mean of 97.9 and 97.1% respectively, n=3). **B.** Adding ACD-A simultaneoulsy with the biotin, led to a decrease of the amount of bioPLTs (mean of 88.5% versus 97.10%, n=3). **C.** Irradiation with 25 Grey did not influence the amount of bioPLTs (mean of 98.1%) .

Effects of the biotinylation procedure on platelet quality parameters

Platelet quality parameters were assessed to assure bioPLTs met the requirements of the Dutch blood bank. Ranges for quality parameters were pre-defined and are expressed in Table 1. Platelet counts, pH and 'swirling' score were within the range accepted by the Dutch blood bank for all products. Morphology scores were higher for control platelets, as compared to sham and bioPLTs. There was no significant difference between sham and bioPLT morphology scores, indicating that the difference to control was caused by the processing steps with repeated centrifugation steps and not by incubation with biotin. The procedure led to a marginal decrease in platelet count. Annexin V binding was not affected by the procedure (Figure 4). CD62P expression was increased to the same extent in both the sham and bioPLT (Figure 5). Hence, the processing steps, but not biotin itself led to activation of platelets. The percentage of CD62P activation met blood bank standard. All samples showed maximal response to thrombin receptor-activating peptide (Figure 5). Similar results were observed in apheresis derived PCs and in PAS-E stored PCs (supplement, table S1). All blood products were culture negative before and after biotinylation.

The effect of storage on platelet quality parameters

Platelet quality parameters were measured directly after biotinylation of a 50 mL aliquot of fresh (day 1) and stored (day 7) buffy-coat derived PCs. Both fresh and stored platelets fulfilled platelet quality standards after biotinylation (Table 1). Platelets that were biotinylated at day one of storage were subsequently stored for three more days and tested for stability of the biotin-label and platelet quality parameters. Storage of bioPLTs in the small aliquot volume of 50 ml led to substantial decrease of platelets quality (data not shown). This might be due to the sub-optimal storage and not to biotinylation itself. Therefore, for three PCs we transferred the biotinylated aliquot back to the retained fraction of the original PC and stored this PC for 7 days. BioPLT could be distinguished from the unbiotinylated platelets (Figure 6A). Both biotinylated and unbiotinylated showed minimal platelet activation, as expressed by CD62P expression (Figure 6B). We confirmed bioPLTs can be stored for 7 days using this method, all platelet quality markers met Dutch blood bank quality standard.

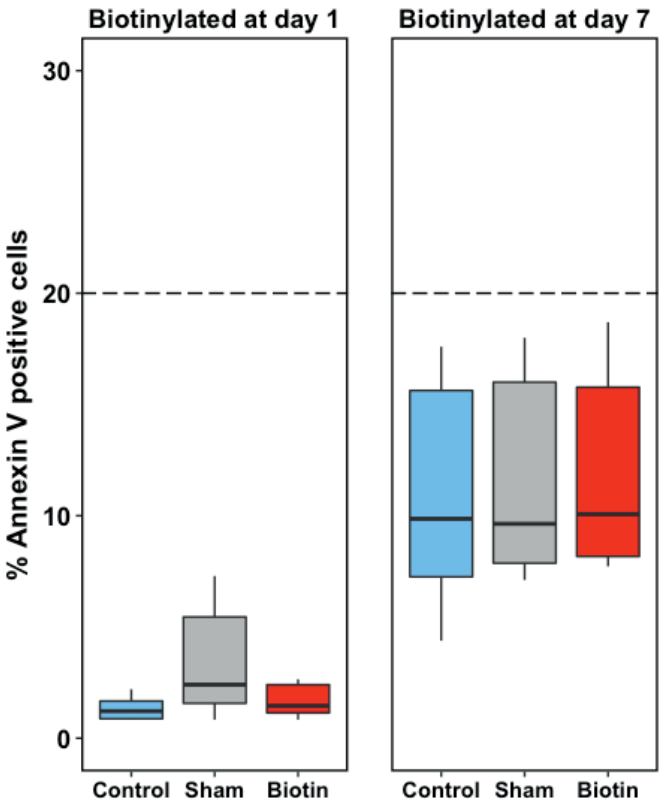

FIGURE 4. Annexin V expression of a fresh PC (day 1, left panel, n=6) and a stored PC (day 7, right panel, n=6). On day 1, the bioPLT did not show a statistically significant difference in Annexin V positive cells, as compared to control: 1.2% (0.9%-1.7%), p=0.56 and sham: 3.4% (1.6%-5.5%), p=0.16. At day 7, the bioPLT also showed no significant difference in annexin V positive cells: bioPLT: 10.1% (8.2%-15.8%) compared with the control: 9.9%(7.3%-15.6%), p=0.09 and the sham 9.6%(7.9%-16.0%), p=0.16.

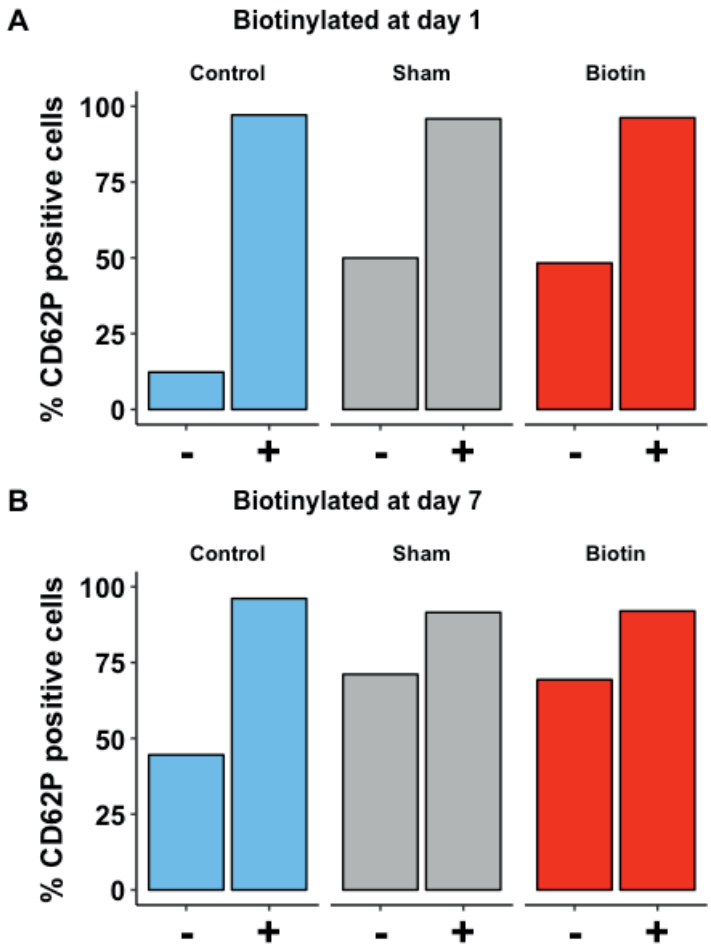

FIGURE 5. CD62P expression, of a fresh PC (day 1,panel A) and a stored PC (day 7, panel B). For each condition, left bars (-) represent the unstimulated state, right bars (+) represent CD62P expression in response to TRAP. After incubation with TRAP, all samples showed an increase in CD62P positive cells. At day 1, the number of CD62P positive cells increased in both the bioPLT 48.4%(41.7%-56.2%) and the sham 50.0%(41.7%-56.2%), as compared to control platelets 12,3%(9.5%-12.7%), p= 0.03. On day 7, more cells were CD62P positive in the bioPLT 69.60%(64.5%-70.3%) and sham 71.2%(66.4-75.7%) as compared to the control platelets: 44.6%(39.7%-50.3), p=0.03. No statistically significant difference was observed in comparing CD62P expression after incubation with TRAP in bioPLT with control (day 1: p=0.84, day 7 0.69) or sham platelets (day 1: p=0.11, day 7: 0.13).

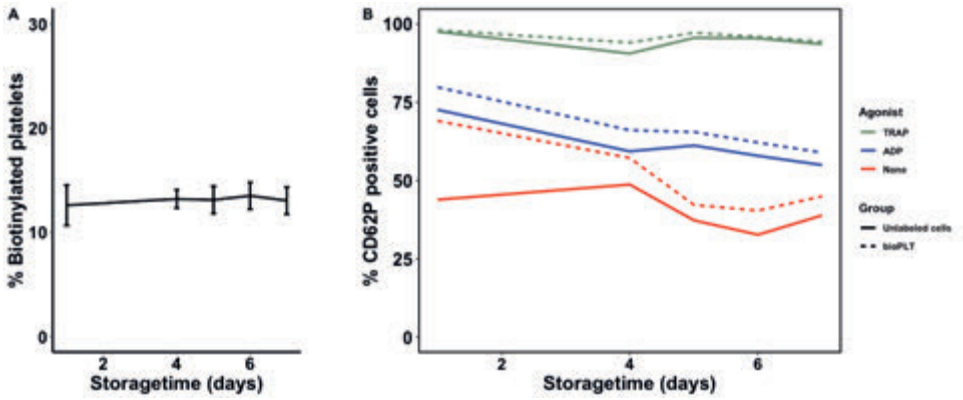

FIGURE 6. Stability of biotin label and CD62P expression of stored bioPLT. BioPLTs were returned to the retained fraction of the original PC, to enhance storage conditions. (n=3). **A** The percentage of biotinylated platelets remained stable througout 7 days of storage. (mean of 12.6% at day 1 and 13.0% at day 7). **B.** CD62P expression was assessed in unstimulated platelets (red), after stimulation with ADP (blue) and TRAP (green). CD62P expression was determined in both labelled (dotted lines) and unlabelled cells (continuous line). Measurements were performed at day 1,4,5,6 and 7. N=3

DISCUSSION

Here, we describe a reproducible protocol for biotin-labeling of PCs under GPG conditions in a closed system. Showing a low within and unit to unit variation. The findings will be of interest to blood banks and clinical researchers. BioPLTs can be used to evaluate the *in vivo* effect of new additive solutions, donor variability and the effect of transfusion in various patient categories.

The major advantage of biotin is that it enables *in vivo* tracing of transfused platelets without exposing the recipient to radiation. Moreover, bioPLTs enable tracing of multiple populations concurrently. Also, bioPLTs can be isolated from venous blood samples, thereby permitting direct population analysis for surface markers and metabolomics composition of the platelet subgroups. HLA discrepancy is another non-radioactive method to discriminate transfused platelets from the patients' circulating platelets.[18] However, this method inherently requires a HLA discrepancy, which excludes the possibility of tracing HLA-matched platelet transfusions or autologous transfusions in the recipient. Also, isolation and subanalyses of platelets are not possible with this method.

Biotinylation of platelets has previously been described under invalidated, experimental conditions[1,13] Our protocol fulfils GPG requirements. Since US Food and Drug Administration

(FDA) guidelines differ from European GPG requirements, the protocol needs to be validated according to the FDA standard before it can be implemented in the US. Our work can serve as a reference method.

We adapted the previous protocol on various crucial points (Table 2).[13] After sterile filtration of the sulfo-NHS-biotin-solution, the procedure took place in a closed-system, minimizing the risk of bacterial contamination. We added an extra washing step, to minimize nonspecific biotinylation of plasma proteins in the PC. Our group showed that PAS-E can be used to optimize platelet storage.[19] We tested whether PAS-E could be used to store the sulfo-NHS-biotin solution (for 42 days at (< -30°C). However, the quality of biotinylation decreased after storage of dissolved biotin in PAS-E. The biotin label remained stable when dissolved and stored at < -30ºC in PBS. Therefore, sulfo-NHS-biotin was dissolved and stored in PBS at a concentration of 50 mg/L. Shortly before biotinylation, the sulfo-NHS-biotin solution was diluted in PAS-E in a 1:9 ratio, to obtain a concentration of 5 mg/L. Since the reactive group of dissolved sulfo-NHS-biotin is instable, the sulfo-NHS-biotin-solution should either be used within 30 minutes, or after frozen storage at < -30º C, to be used within 42 days of storage. After thawing, the solution should be used within 30 minutes, to avoid hydrolysis. Since >97% of all platelets were biotinylated using our protocol, we found 30 minutes of incubation to be sufficient to adequately biotinylate platelets, instead of the previously described 45 minutes.[1]

TABLE 2. Adaptations on the previously described protocol

Protocol described by Van Der Meer[14]	Our protocol	Advantage
A platelet sample is centrifuged, the supernatant replaced by the biotin solution.	Centrifuged and supernatant removed twice before incubation.	Limits rest-biotinylation of proteins in PC.
Incubation in saline in which biotin is added at a final concentration of 25 mg/L.	The biotin solution was diluted 1:9 in PAS-E.	Less activation of the platelets.
Incubation for 45 minutes	Incubation for 30 minutes.	Time reducing.
Resuspended in saline/ACD.	Resuspended in PAS-E.	Superior storage conditions, mimics PC more realistically.
250 ml	50 ml	Minimal amount of traceable platelets.

BioPLTs and sham samples showed equal decreases in platelet quality parameters as compared to control platelets. Hence, platelets were affected by the processing steps, but not by biotin itself. The processing steps are similar to the steps in obtaining platelet volume reduced products, which have been in use for several years and showed, after correction for platelet loss during preparation, similar cell count increment as standard platelet concentrates.[20-22] Centrifugation of platelets has previously been suggested to activate platelets[23], which is not completely prevented by the addition of ACD-A. Radiolabeling of platelets also requires centrifugation steps.[24] Since the processing steps, and not the biotin led to platelet activation, similar results can be expected for radiolabeling. We found that exposure to saline or PBS is detrimental to platelet quality; incubating platelets in a PBS-sulfo-NHS-biotin or saline solution led to unacceptable high activation of platelets (data not shown). Therefore the PBS-biotin was diluted in PAS-E. To our knowledge, no data are available on the effect of radiolabeling on platelet quality parameters. However, in previous labeling studies, platelets were incubated in saline, both for radiolabeling and biotin-labeling,[13,24] which might be not optimal with respect to platelet quality. We recommend a comparative study to assess platelet activation in both bioPLTs and radiolabeled platelets.

An important limitation of our study is that biotin labeling has not yet been compared to radioactive labeling in humans. However, in dogs, survival of bioPLTs was comparable to platelets labeled with both ^{111}Indium-oxine or ^{51}Chromium.[7] BioPLTs survived normally after transfusion, and could be used for determining platelet life spans *in vivo*. The platelet lifespan curves of bioPLTs were in agreement with radiolabeled platelets.

Red blood cell labeling studies showed modification of antigens and the risk of antibody-formation against the biotin.[25,26] These antibodies did not affect red blood cell recovery and survival in the recipient. However, this limits the possibility of repeated transfusions with bioPLTs for clinical research. To minimize the risk of antibody formation we labeled at the lowest possible traceable biotin density. Future research should include the assessment of the formation of antibodies against bioPLTs. BioPLTs will be first administered in an autologous transfusion model in healthy volunteers (registered at trialregister.nl, NTR6493).

In conclusion, we developed a standardized, simple, reproducible, protocol according to GMP standards for biotin-labeling of platelets, as non-radioactive alternative to trace and isolate transfused platelets *in vivo*.

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APPENDICES

Appendix 1. Protocol to biotinylate platelets

1.0 Purpose

The aim of this protocol is to describe a technique for biotin-labeling of fresh or stored platelets with Sulfo-NHS-LC-Biotin, in a closed system. This protocol describes how to biotinylate 50 ml of platelet concentrate.

2.0 Equipment, supplies, and reagents

- 2.1 Sterile docking station, sealing device.
- 2.2 Calibrated, variable speed, swinging bucket centrifuge.
- 2.3 Device to remove supernatant of centrifuged platelets
- 2.4 Calibrated analytical electronic balance, accurate to ±0.00002 g.
- 2.5 Calibrated generic electronic scale, accurate to 0.5 g.
- 2.6 Kochers, clamps.
- 2.7 0.22 µ sterilizing filter unit.
- 2.8 PBS: Phosphate buffered saline.
- 2.9 ACD-A: anticoagulant citrate dextrose solution, Formula A.
- 2.10 PAS-E : Platelet Additive Solution.
- 2.11 Product bags for platelets, 150 ml.

3.0 Preparation of biotin solution

Note: Dissolved biotin needs to be used directly, or immediately after thawing of frozen product, as dissolved biotin is vulnerable to hydrolysis. Bound biotin is stable.

- 3.1. Biotin is dissolved in PBS at a concentration of 50 mg/L, and transferred in a 150 ml bag.
- 3.2. Filtrate biotin-solution by passing a 0.22 µ sterilizing filter.
- 3.3. Store within 15 minutes at -30º, or use within 30 minutes.

4.0 Preparation of required solutions

- 4.1 Prepare 150 ml of 15% ACD-A in 85% PAS-E in a sterile, labeled bag.
- 4.2 Prepare 100 ml of 10% ACD-A in 90% PAS-E in a sterile, labeled bag.
- 4.3 Prepare 11 ml of ACD-A in a sterile, labeled bag.
- 4.4 Maximal 30 minutes prior to the biotinylation procedure, thaw the stored 50 mg/L biotin-PBS solution, and mix 1 part biotin-PBS-solution to 9 parts PAS-E, to obtain a 5 mg/L biotin-PBS-PAS-E-solution. Fill out 100 ml in a sterile, labeled, bag.

5.0 Obtain the proportion of 50 ml platelet concentrate.

5.1 Transfer 52 grams (50 ml) of platelet concentrate to a sterile, labeled bag, using a sterile docking device.

6.0 Washing steps

Note: Before every centrifugation step, add a total of 10% ACD-A, to prevent thrombus formation due to centrifugation.

6.1 Dock the bag containing 50 ml of platelet concentrate to the 150 ml 15%ACD-A/85% PAS-E-solution bag. Add this 15%-ACD-A solution to the platelet concentrate.

6.2 Centrifuge the bags at 1700 x g for 10 minutes.

6.3 Remove the supernatant by using the empty bag, discard this bag.

6.4 Dock the bag containing the platelet pallet to the 100 ml 10% ACD-A/90% PAS-E bag. Resuspend the platelet pallet by gentle manipulation.

7.0 Incubation with biotin

7.1 Dock the 5 mg/L biotin-PBS-PAS-E-solution to the platelet pellet. Resuspend the platelet pellet by gentle manipulation.

7.2 Incubate for 30 minutes, at room temperature, under gently agitation.

8.0 Washing step and resuspending to original volume

8.1 Add the bag containing 12 ml of ACD-A to the biotinylated platelets.

8.2 Centrifuge at 1700 x g for 10 minutes.

8.3 Remove the supernatant by using the empty bag, discard this bag.

8.4 Dock PAS-E to the platelet pallet, bring to original volume (50 ml).

8.5 Resuspend the platelets pallet by gently manipulation.

8.9 Store the platelets under gentile agitation, at room temperature until use.

9.0 Test for biotinylation

9.1 Obtain sample, incubate for 30 min with streptavidin 488.

9.2 Flowcytometrically analyze the percentage of biotinylated platelets.

Appendix 2. Supplemental figures

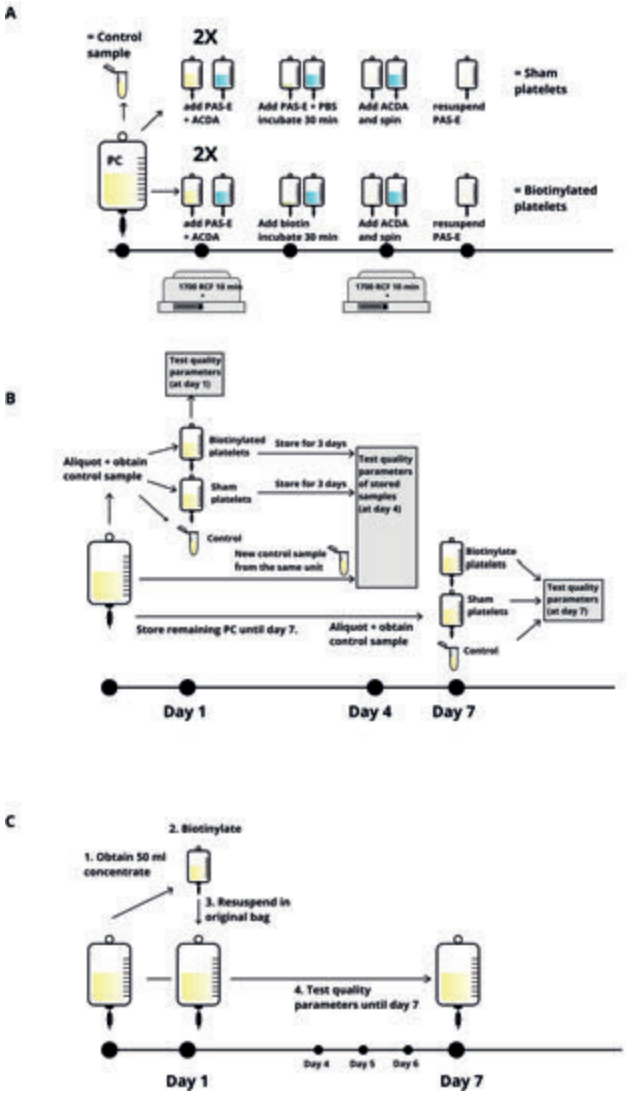

FIGURE S1. A) Procedural steps to obtain biotinylated platelet, sham platelets and the control sample. B) Validation experiment in which platelets were labeled at day 1 or day 7 of storage. Platelets labeled on day 1 of storage were also stored for 3 days separately to assess the effect of storage on the quality of the platelets. However this method of storage led to unacceptable high platelet activation. Therefor another method was designed: C) After labeling, the bioPLT were returned in its original bag. Here, the various parameters tested in biotinylated platelets fulfilled the quality criteria.

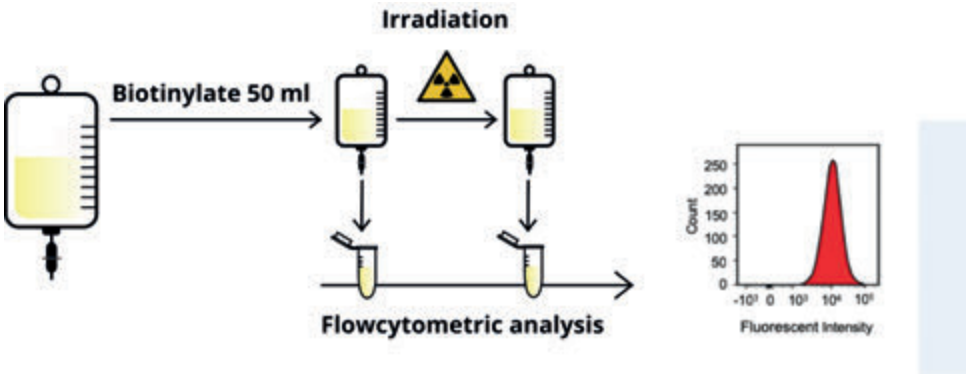

FIGURE S2. Samples from the biotinylated unit were obtained prior and after irradiation. From each unit both samples were tested for fluorescent intensity on the flocwcytometer to assess the effect of irradiation on the biotin label (n=3).

Appendix 3. Supplemental tables

TABLE S1. Platelet quality markers in apheresis PCs and PCs stored in 65% PAS-E

	Platelet count	pH	Annexin V positive cells (%)	Morphology score	CD62P expression (%)		
					Baseline	ADP stimulation	TRAP stimulation
Apheresis PCs (n=3)							
Control	1277 (134)	7.21 (0.054)	4 (1.5)	252 (12)	20.4 (5.8)	72.3 (2.7)	82.5 (27.8)
Sham	1129 (116)	7.062 (0.048)	3.7 (0.8)	252 (13)	57.1 (3)	63.4 (5.6)	75.9 (38.9)
Biotin	1095 (120)	7.089 (0.011)	4.2 (1.7)	227 (13)	54.5 (1.6)	59.2 (3)	98.5 (0.1)
PAS-E PCs (n=3)							
Control	867 (46)	7.162 (0.013)	4.1 (2.5)	267 (13)	19.1 (5)	48.4 (12.7)	97.9 (0.3)
Sham	917 (106)	7.058 (0.021)	4.8 (1.7)	265 (10)	42.8 (3.1)	48.9 (7)	97.6 (0.2)
Biotin	851 (80)	7.06 (0.019)	5.1 (1.6)	267 (13)	44.1 (6.8)	51.3 (3)	97.3 (0.3)

7

DONOR CHARACTERISTICS DO NOT INFLUENCE TRANSFUSION-RELATED ACUTE LUNG INJURY INCIDENCE IN A SECONDARY ANALYSIS OF TWO CASE-CONTROL STUDIES

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SUMMARY

Objective. To investigate the relation between donor characteristics and TRALI incidence.

Background. Transfusion-related acute lung injury (TRALI) is a potentially fatal complication of transfusion. In pre-clinical studies and several clinical studies, TRALI has been related to loss of product quality during red blood cell (RBC) storage, called the "storage lesion". Donor characteristics, as for example age, genetics and life style choices influence this "storage lesion". We hypothesized that donor sex, age and blood type is related to TRALI incidence.

Methods/Materials. We performed a secondary analysis of two cohort studies, designed to identify TRALI risk factors by matching TRALI patients to transfused controls. We obtained donor sex, age and blood type from the Dutch Blood Bank Sanquin and. investigated TRALI incidence in patients who were exposed to a certain donor characteristic. We used kruskal-wallis testing to compare the number of transfused products and chi-square testing to compare proportions of TRALI patients and transfused control.

Results. After implementation of the male-donor only plasma strategy, patients received more transfusion products from male donors. However, we did not detect a relation between TRALI incidence and donor sex. Both TRALI patients and transfused controls received mainly products from donors over 41 years old, but donor age did not influence TRALI risk. Donor blood type, the transfusion of blood type-compatible and blood type-matched products also had no influence on TRALI incidence.

Conclusion. We conclude that in two cohorts of TRALI patients, donor age, donor sex and donor blood type are unrelated to TRALI.

INTRODUCTION

Transfusion-related acute lung injury (TRALI) is a serious and potentially fatal complication of transfusion.[1] In the absence of biomarkers, TRALI is defined according to the TRALI conference and US National Heart, Lung and Blood Institute definition as onset of acute lung injury within 6 hours of blood transfusion without an additional risk factor for acute lung injury.[2,3] Up to 15.1% of transfused patients develops TRALI [1]. In approximately 80% of cases donor antibodies directed against the recipient's human leucocyte antigens (HLA) or human neutrophil alloantigen (HNA) induces pulmonary injury. However, not in all TRALI cases antibodies can be detected and cognate antibodies in the transfusion product do not always induce TRALI in the transfused patient.[4,5] The causative mechanism in these antibody-unrelated TRALI cases has not yet been identified, but increased storage time of cell containing blood products has been hypothesized to induce TRALI.[4] During storage the RBC product undergoes changes with a negative influence on product quality, which are known as the RBC "storage lesion".[6] However, recent randomized controlled trials did not show any protective effect of transfusion of fresh RBCs on lung injury compared to standard issued RBCs.[7-9] Several clinical studies in healthy volunteers also did not detect any relation between pulmonary function and age of the transfused product.[10-12] Ongoing research has revealed that donor characteristics, for example age,[13] sex,[14,15] genetic traits,[16] body weight[14,17] and smoking,[18] influence the *in vitro* "storage lesion". It has been hypothesized that these donor factors influence post-transfusion effects. However, limited evidence is available on the question whether - and to what extent - these factors also have an effect on lung injury. It has been demonstrated that products from males have lower *in vitro* quality.[14] Moreover, in a mouse model male sex has been related to reduced post-transfusion RBC recovery[13] but whether these factors are also related to lung injury is unknown. To our knowledge, no clinical studies have looked into the relation between TRALI and donor characteristics. We performed a secondary analysis of a retrospective and a prospective cohort of TRALI patients[19,20] to determine whether donor age, donor sex or donor blood type influenced the incidence of TRALI. We hypothesized that products from male donors, products from older donors and products from donors with a compatible blood type, but not a matching blood type would increase TRALI risk.

MATERIAL AND METHODS

We performed a secondary analysis on the transfusion data from a retrospective nested case control study and a prospective case control study, previously published by our study group.[19,20] All procedures in the studies were according to the declaration of Helsinki and were approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam.

Observational case control studies

The retrospective nested case control study was a study in which TRALI patients were matched to transfused controls without TRALI.[20] All patients admitted to our ICU from November 1, 2004, until October 1, 2007, were screened for onset of TRALI. Readmitted patients were excluded. Each TRALI case was matched with a control on sex, age (±10 years) and admission diagnosis.

The prospective case control study was performed in a cohort of adult cardiac surgery patients.[19] Consecutive patients scheduled for cardiac surgery were screened from November 2006 until February 2009. Patients with pulmonary thromboendarterectomy or emergency surgery were excluded. Patients that gave informed consent for the study, were observed for the onset of TRALI during surgery and afterwards up to 30 hours after admittance. TRALI cases were randomly matched with control patients in a 2:1 ratio. In this study, cases were not matched on potential risk factors. Potential risk factors for the onset of TRALI were collected.

In both cohorts, control cases were transfused patients that did not develop ALI and patients that did not receive transfusion and did not develop ALI. Suspected TRALI was defined using the consensus definition of ALI (new-onset hypoxemia or detoriation demonstrated by a PaO_2/FIO_2 <300 mm Hg within 6 hours after transfusion with bilateral pulmonatry changes in the absence of cardiogenic pulmonary oedema)[2,21,22].The studies were designed to investigate patient and transfusion product risk factors for development of TRALI in the included patient populations. A total of four TRALI patients were part of both cohorts as there was a small overlap in inclusion period for the retrospective and prospective study.

Statistical analysis

All statistical analyses are performed in R, version 3.3.1. Continuous data was inspected for normality with the use of density plots and histograms. As transfusion data was highly skewed we used non-parametric tests to test for statistical differences. Data is expressed in

median with interquartile range (IQR). The tests used are described below. A $p < 0.05$ was considered statistically significant. Due to the explorative, observational nature of our data we did not correct for multiple testing.

Sample size analysis

To get an indication whether our cohorts had enough power to investigate our hypotheses, we performed a power calculation on the relation between antibodies in donor products and TRALI. Human leukocyte antibodies class I and II are present in 14.3-26.7% of donors implicated in TRALI cases [5] compared to 7.1% in the male donor population.[23] In this series prevalence of HLA-antibodies in TRALI patients is at least doubled compared to the general donor population. To detect a similar difference in exposure to donor age, sex or blood type, we need at least 107 TRALI cases (alpha 5% and beta 80%).

Transfusion products

Each unit of RBC or plasma was counted as one transfusion product. Apheresis PLTs were counted as one product as well, but pooled PLT concentrates consist of five buffy coats from five separate donors, suspended in plasma of one of these donors. The characteristics from each of the five donors from the standard PLT products were counted as exposure to one donor. One pooled PLT concentrate thus added characteristics from five donors to our analysis. The products were produced according to Dutch Blood Bank standards. In 2007 the Dutch Blood Bank implemented male-only donor plasma for transfusion as preventive measure for TRALI.

Donor characteristics

Data on donor sex, donor AB0 blood type and donor age were obtained from the Dutch National Blood Bank Sanquin. We therefore performed various analyses to investigate the influence of a donor characteristic on TRALI incidence. We split up donor age in categories 18-30 years, 31-40 years, 41-50 years, 51-60 years and 61-70 years. Donor and patient blood type were categorized as "matched" if patients only received products from a donor with the same AB0 blood type. All patients who also received products from another blood type were categorized as "blood type compatible".

Step 1) First we investigated whether the total number of transfused products differed between categories of a donor characteristic, irrespective of whether patients received only one product type (RBC, PLT or plasma). For example, we counted the number of transfused RBCs from a female donor in TRALI patients, and compared this to the number in control patients with a chi-square test if the counts were more than 5 in each category. If the counts were less than 5 we used a Fisher's Exact Test (upper panel Figure 1).

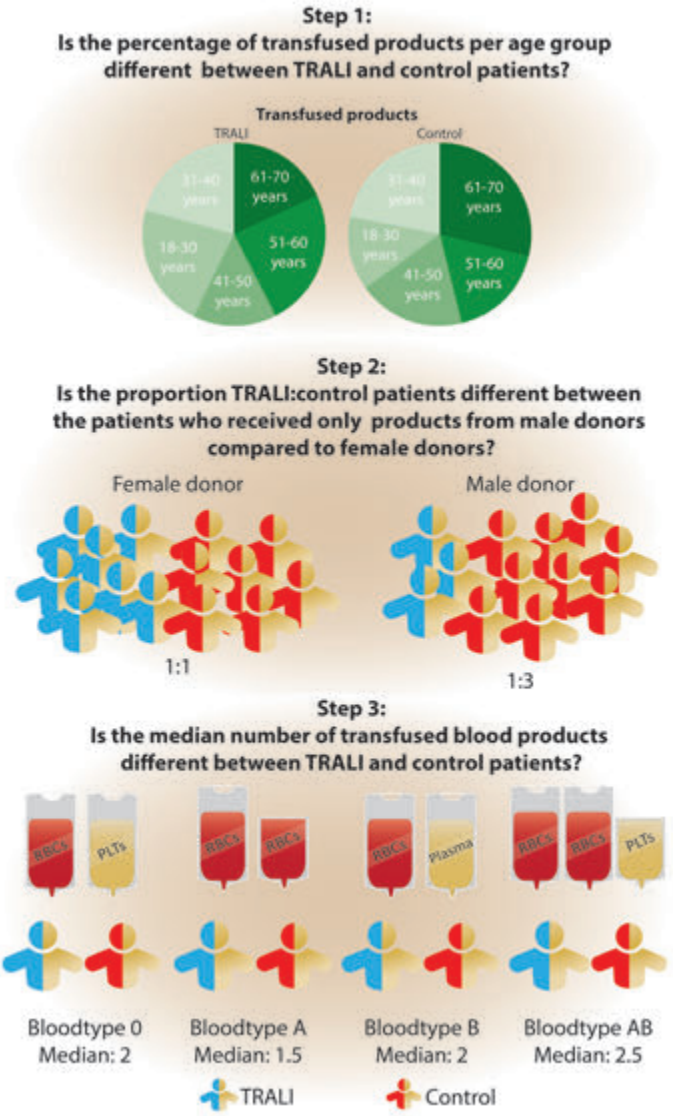

FIGURE 1. Analysis plan with hypothetical results. For each donor characteristic the same procedure was followed. Donor characteristics included donor age group (top panel), donor sex (middle panel) and donor blood type (lower panel). Step 1) First we investigated whether the total number of transfused products differed between categories of a donor characteristic, irrespective of whether patients received only one product type (RBC, PLT or plasma). Step 2) We then extracted all patients who only received products from one category and investigated whether TRALI was more prevalent in a certain category with chi-square test. 3) This was followed by analysis of the median number of transfused products per patient per category. Our analysis was set up as follows (Figure 1): Step 1)

Step 2) We then extracted all patients who only received products from one category and investigated whether TRALI was more prevalent in a certain category with a chi-square test. For example, we extracted the patients who only received products from donors aged 31-40 and compared the number of TRALI and control cases in this group (top panel Figure 1).

Step 3) This was followed by analysis of the median number of transfused products per patient per category. For example, we investigated whether TRALI patients on average received more products from donors with a higher age than control patients with a Kruskall-Wallis with post-hoc Nemenyi testing if $p<0.05$ (top panel Figure 1). We performed this analyses for all product types, irrespective of whether patients received only RBC, PLT or plasma or whether they received multiple product types. However, if we had enough cases of patients who only received either RBCs, PLTs or plasma, we split up this analyses per product type.

Supplemental information

We performed descriptive analyses on both the retrospective and prospective data. As the retrospective database represents a larger cohort than the prospective database, and as we performed a substantial number of analyses we only describe the data from the retrospective cohort in detail. The results of the analysis of the prospective cohort are presented in a summarized fashion in the supplement.

RESULTS

During the inclusion period of the retrospective nested case control study in total 5208 patients were admitted to the ICU. The incidence of TRALI in the screened cohort was 2.2 (14 of 5208), and 5.1% (114 of 235) per transfused patient. 109 patients were confirmed to the diagnostic criteria for TRALI and survived the first 48 hours of ICU admission. From the cohort of transfused patients who did not develop TRALI, 109 patients were randomly selected to serve as transfused control [20]. Baseline data are presented in Supplementary Table I.

Donor sex

Step 1) We split the data on product type and confirmed that TRALI patients received more products than control (chi-square test $p < 0.0001$; Figure 2). We investigated whether TRALI patients also received more products from females compared to control patients. However, the proportion of transfused products from males and females was comparable between TRALI and control (chi square test $p > 0.5$; Figure 2).

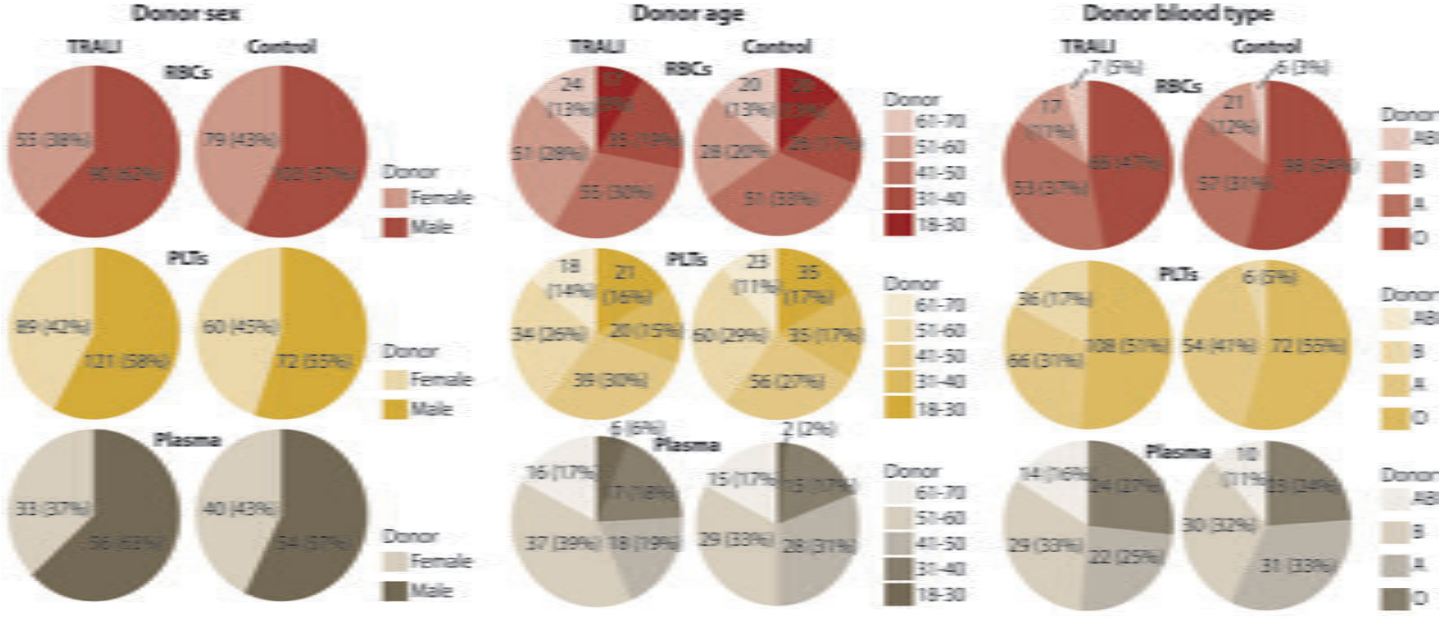

FIGURE 2. Retrospective study: number (%) of transfused products for each donor sex, donor age and donor blood type. TRALI patients received more transfusions than control patients ($p < 0.0001$). Both TRALI and control patients receive mainly plasma products from older donors ($p<0.05$) but age does not influence TRALI incidence. Both TRALI and control patients received mainly blood products from blood type 0 and A ($p<0.0001$) but the distribution of donor blood type is not different between controls and TRALI patients. Not all totals sum to 100 due to rounding of decimals.

Step 2) We extracted all patients who only received products from either males or females and investigated whether TRALI was more prevalent in either category (Table I). We also investigated whether a mismatch between donor and patient sex influenced TRALI incidence – e.g. is a male transfused with a product from a female (sex mismatch) more at risk to develop TRALI than a male transfused with a product from a male (sex match). However, the prevalence of TRALI was independent of donor sex and sex mismatch.

Step 3) We then inspected whether the median number of transfused products in the TRALI and transfused control group differed per product type with kruskall-wallis testing. We extracted all patients who only received RBCs or only received plasma but we did not have enough patients who only received PLTs from one sex to also inspect median transfusions in this subset. TRALI patients did not receive more products from either males or females compared to control (Supplementary Tables 3a and 3b).

TABLE I. Total patients who received RBCs, PLTs and plasma from either only males or only females. We used a chi-square test to investigate whether TRALI was related to donor sex. Donor sex did not influence the incidence of TRALI. The majority of these patients only received RBCs.

	Donor							
	Male				**Female**			
Recipient	**TRALI**	**Control**	**Total**	**p**	**TRALI**	**Control**	**Total**	**p**
Male	19	19	38*		12	10	22**	
Female	4	5	9***	1	6	14	20****	0.20

*25 patients received only RBCs
**21 patients received only RBCs
***5 patients received only RBCs
****18 patients received only RBCs

Donor age

Step 1) We investigated whether TRALI patients received more products from donors belonging to a certain age category compared to control. There was no difference in the number of transfused plasma, RBC or PLT products derived from one of the age categories between TRALI and control (chi square test $p > 0.5$; Figure 2).

Step 2) We extracted all patients who only received products from one of the age categories and investigated whether TRALI was more prevalent in a certain category. We did not have enough cases to also split up this set on product type, but the majority of patients only received RBCs (Table IIa). In this analysis the number of TRALI cases did not differ between the categories and donor age did not influence TRALI incidence.

Step 3) We analyzed the median number of transfused products per TRALI patient or transfused control per age category. We extracted all patients who only received RBCs (Table IIb). TRALI patients did not receive more products from either males or females compared to control (Table II). We did not have enough patients who only received plasma or PLTs from one age category to also inspect median transfusions in these subsets.

Donor blood type

The distribution of number of administered transfusion products amongst the different blood types is according to Dutch prevalence of blood types (Figure 2).[24] Type 0 blood products are used most frequently (46.1%), followed by A (33.2%), B (16.3%) and AB (4.3%). Step 1) We investigated whether TRALI patients received more products from one of the blood types compared to control. However, we did not detect any influence of blood type on TRALI incidence. The distribution of blood types was similar in TRALI patients and control (Figure 2).

TABLE IIA. Total patients who received RBCs, PLTs and plasma from a certain age category. We used a chi-square test to investigate whether TRALI was related to donor age. Donor age did not influence the incidence of TRALI. Three patients received both plasma and RBCs, six received only plasma, 61 received only RBCs.

Age (years)	Total	Control	TRALI	p
18-30	5	3	2	
31-40	10	4	6	
41-50	28	13	15	
51-60	18	9	9	
61-70	9	7	2	0.48

TABLE IIB. Number of transfused products from each age category. Three patients received both plasma and RBCs, six received only plasma, 61 received only RBCs. Kruskal-Wallis with post-hoc Nemenyi testing did not reveal any significant differences in the number of transfused products per age group.

	TRALI			Control		
Age (years)	No	Median	IQR	No	Median	IQR
18-30	2	1.00	1.00-1.00	3	1.00	1.00-1.50
31-40	6	1.00	1.00-1.00	4	1.00	1.00-1.00
41-50	15	1.00	1.00-2.00	13	1.00	1.00-1.00
51-60	9	1.00	1.00-1.00	9	1.00	1.00-2.00
61-70	2	1.00	1.00-1.00	7	1.00	1.00-1.00

Step 2) All patients who only received products from a donor with the same AB0 blood type were categorized as "matched". All patients who also received products from another blood type were categorized as "blood type compatible". We analyzed whether blood type compatible or blood type matched transfusion influenced the number of TRALI cases in each category. However, the number of cases per category was comparable between TRALI and transfused control cases (Table IIIa).

Step 3) We investigated whether the median number transfused products in TRALI patients was dependent of donor blood type or transfusion of blood type matched versus blood type-compatible products. The median number of transfused products were comparable in all donor blood type categories (Table IIIb). We did not detect any influence of blood type on the median number of transfusions in patients who only received RBCs (Supplementary Table IV). We did not have enough patients who only received PLTs or plasma to analyze these subsets.

TABLE IIIA. Total patients who received RBCs, PLTs and plasma from blood type matched donors only. All patients who also received compatible products from another blood type are categorized as "blood type compatible". We used a fisher-exact test to investigate whether TRALI was related to matched or compatible transfusion.

	Total	Control	TRALI	p
A matched	60	33	27	
AB matched	6	4	2	
B matched	22	9	13	
O matched	69	32	37	
Blood Type Compatible	45	17	28	0.41

TABLE IIIB. Number of transfused products from each donor blood type. Patients are categorized as "matched" if they only received products from a donor with the same AB0 blood type. All patients who also received compatible products from another blood type are categorized as "blood type compatible". Patients are not split on which type of blood product they received. Kruskall-wallis with nemenyi post-hoc testing did not reveal any statistical differences.

	TRALI			Control		
	No	Median	IQR	No	Median	IQR
A matched	27	2.00	1.00-6.00	33	2.00	1.00-6.00
AB matched	2	1.50	1.25-1.75	4	1.50	1.00-2.00
B matched	13	5.00	2.00-10.00	9	2.00	2.00-6.00
O matched	37	2.00	1.00-6.00	32	2.00	1.00-2.00
Blood Type Compatible	28	2.00	1.00-3.00	17	2.00	1.50-2.50

DISCUSSION

We investigated in a secondary analysis of a retrospective and a prospective cohort of TRALI patients[19,20] whether donor age, donor sex or donor blood type influenced the incidence of TRALI. We hypothesized that products from male donors, products from older donors and products from donors with a compatible blood type, but not a matching blood type would increase TRALI risk. However, our data did not support our hypotheses. We did not detect any relation between these donor characteristics and TRALI incidence.

The hypothesis that donor sex is related to complications and quality of RBC transfusions is a complex one. In several studies blood products from female donors have been related to increased mortality. In a previous study on single-transfused recipients, it was found that male recipients of female products had a higher hazard rate of mortality (hazard ratio 1.8, 95% confidence interval 1.2-2.7).[25] In a second study in a cohort of cardiac surgery patients, researchers found a hazard ratio of 2.28 (95% CI 1.67-3.12) for mortality after sex mismatch transfusion. However, this outcome was most likely confounded by disease severity as patients with more severe disease received more transfusion products and had a higher chance to have sex mismatch. The sex effect indeed disappeared after correction for these factors.[26] However, products from males have lower *in vitro* quality and have been related to decreased RBC recovery *in vivo*.[13] To complex matters even more, a clear association has been established between TRALI and plasma from female donors. HLA and HNA-antibodies develop when females are exposed during pregnancy to the antigens of their foetus which has inherited the alloantigen from the father. The prevalence of these antibodies increases rapidly with each pregnancy[27] and exclusion of female donors for plasma transfusion has resulted in a significant reduction in TRALI cases.[28] It is possible that we did not detect a donor sex effect due to heterogeneity of the transfusion products that have been administered in our study: the effect of male products may have been compensated by transfusion of female products. Moreover, the retrospective study was performed before and after implementation of the male-only plasma donor policy in 2007. This may have masked effects of male transfusions on TRALI incidence. However, no effect of sex was detected in the prospective cohort which only included patients after 2007. The size of the retrospective cohort also made it possible to isolate cases in which patients only received transfusions from one category, but this was not possible for all analyses. On the other hand it is also possible that donor sex does influence overall mortality, but has no influence on TRALI incidence.

We found no influence of blood type and donor age on the risk of TRALI. This result differs from *in vitro* studies.[29,30] *In vitro* research has revealed that there is large variation in the donor storage lesion. It is hypothesized that some of these donors are "bad storers" and

that genetic factors may account for these variations.[31,32] In a study that investigated the procoagulant effects of supernatant of stored RBCs, only a subset of these products induced coagulation. One could hypothesize that this effect is due to donor variation.[33] An explanation for disparate results is that *in vitro* studies can investigate these factors in a standardized fashion but do not take account for the fact that induction of complications and TRALI by transfusion products is mostly multi-factorial. Whether or not patients develop TRALI is influenced by both patient factors,[34] transfusion products factors[4,5] and possibly also donor factors. Heterogeneity in cohorts may mask any influence of donor characteristics, but also may reflect that additional predisposing factors are required to develop TRALI, even if the transfusion product is from a "bad storer".

The studies that have been published to this date have focused on the patient aspect. These studies used a cohort of patients to examine whether a donor characteristic had an effect on post-transfusion morbidity or mortality. It may be worthwhile to start on the side of the donor or transfusion product, in line with the lookback studies in antibody mediated TRALI:[35,36] it could be investigated whether products from these hypothetically "bad storers" are more often related to patient morbidity and mortality than products from "good storers".

Even though we used one of the largest cohorts of TRALI patients, our study still is limited by low numbers of inclusions. We performed a pre-study sample size calculation to estimate whether we would be able to detect any differences in exposure to a certain donor characteristic between TRALI and transfused controls. We based this on studies on prevalence on human leukocyte antibodies. These antibodies are present in 14.3-26.7% of TRALI cases[5] compared to 7.1% in the male donor population.[23] To detect a similar difference in exposure to donor age, sex or blood type, we needed at least 107 TRALI cases. However, more subtle influences from donor characteristics cannot be detected in our study.

Another limitation is that we made use of observational data. TRALI incidence is low which hampers design of a randomized controlled trial to investigate the influence of donor factors. Observational data from relatively large TRALI cohorts is the best evidence we can obtain from clinical trials at this moment. Still, this design has a high risk of bias. A third limitation is that the data from the donor screening was not always complete. This limits the conclusions which can be drawn on the effect of donor characteristics on TRALI incidence.

CONCLUSION

We performed a secondary analysis of a retrospective and a prospective cohort of TRALI patients whether donor age, donor sex or donor blood type influenced the incidence of TRALI. Our data did not support our hypotheses that these donor characteristics are related to TRALI incidence.

SUPPLEMENTARY RESULTS RETROSPECTIVE COHORT

TABLE 1. Baseline characteristics of the retrospective nested case-control study.

	TRALI (n = 109)	**No-TRALI (n = 109)**
Male sex, n (%)	70 (64)	66 (61)
Age in years, median (IQR)	62 (47-71)	57 (49-72)
APACHE II, mean (SD)	22 (8)	19 (8)
ICU admission category, n (%)		
Medicine	36 (32)	30 (28)
Respiratory	5 (5)	7 (6)
Cardiovascular	5 (5)	5 (5)
Neurology	3 (3)	3 (3)
Surgery	32 (29)	32 (29)
Cardiac surgery	27 (25)	31 (28)
Neurosurgery	1(1)	1 (1)
Massive transfusion, n (%)	37 (33)	16 (15)
RBCs transfused, median (IQR)	1 (0-2)	1 (0-2)
RBCs only, n (%)	45 (41)	51 (47)
PLTs transfused, median (IQR)	0 (0-6)	0 (0-0)
PLTs only, n (%)	15 (14)	2 (2)
plasma transfused, median (IQR)	0 (0-2)	0 (0-2)
plasma only, n (%)	12 (11)	9 (8%)

TABLE 2. Retrospective study: number of transfused products per donor characteristic, split on product type. The number between brackets is the percentage of total transfused products per product type.[20] *TRALI patients received more transfusions than control patients ($p < 0.0001$). †Both TRALI and control patients receive mainly plasma products from older donors ($p<0.05$) but age does not influence TRALI incidence. ‡Both TRALI and control patients received mainly blood products from blood type 0 and A ($p<0.0001$) but the distribution of donor blood type is not different between controls and TRALI patients. Not all totals sum to 100 due to rounding of decimals.

		TRALI*			Control		
		RBCs	PLTs	Plasma	RBCs	PLTs	Plasma
Donor Sex	Male	90 (62)	121 (58)	56 (63)	103 (57)	72 (55)	54 (57)
	Female	55 (38)	89 (42)	33 (47)	79 (33)	60 (45)	40 (43)
Donor Age (year)†	18-30	17 (9)	21 (16)	6 (6)	20 (13)	35 (17)	2 (2)
	31-40	35 (19)	20 (15)	17 (18)	26 (17)	35 (17)	15 (17)
	41-50	55 (30)	39 (30)	18 (19)	51 (33)	56 (27)	28 (31)
	51-60	51 (28)	34 (26)	37 (39)	28 (20)	60 (29)	29 (33)
	61-70	24 (13)	18 (14)	16 (17)	20 (13)	23 (11)	15 (17)
Donor BMI (kg/m^2)	<18	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	18-25	11 (69)	15 (65)	9 (69)	9 (50)	5 (29)	5 (50)
	26-30	2 (13)	7 (30)	4 (31)	6 (33)	8 (47)	5 (50)
	30-35	2 (13)	0 (0)	0 (0)	1 (6)	1 (6)	0 (0)
	>35	1 (6)	1 (4)	0 (0)	2 (11)	3 (18)	0 (0)
Donor Blood Type‡	0	68 (47)	108 (51)	24 (27)	98 (54)	72 (55)	23 (24)
	A	53 (37)	66 (31)	22 (25)	57 (31)	54 (41)	31 (33)
	B	17 (11)	36 (17)	29 (33)	21 (12)	6 (5)	30 (32)
	AB	7 (5)	0 (0)	14 (16)	6 (3)	0 (0)	10 (11)

TABLE 3A. Number of transfused products from male and female donors in patients who only received RBCs. Kruskal-Wallis with post-hoc Nemenyi testing did not reveal any significant differences in the number of transfused products per sex.

	Diagnosis		Donor			
Recipient	No	Diagnosis	Male Median	IQR	Female Median	IQR
Male	27	TRALI	1.00	1.00-2.00	1.00	1.00-1.00
Female	18	TRALI	1.00	1.00-2.00	1.00	1.00-1.00
Male	26	Control	1.00	1.00-2.00	1.00	1.00-2.00
Female	25	Control	1.00	1.00-1.00	1.00	1.00-1.00

TABLE 3B. Number of transfused products from male and female donors in patients who only received plasma. Kruskal-Wallis with post-hoc Nemenyi testing did not reveal any significant differences in the number of transfused products per sex.

	Diagnosis		Donor			
Recipient	**No**	**Diagnosis**	**Male Median**	**IQR**	**Female Median**	**IQR**
Male	30	TRALI	2.00	1.25-2.00	1.00	1.00-1.00
Female	15	TRALI	1.50	1.25-1.75	1.50	1.25-1.75
Male	30	Control	1.00	1.00-1.00	1.00	1.00-1.00
Female	11	Control	3.00	2.50-3.50	2.00	2.00-2.00

TABLE 4. Number of transfused products from each donor blood type for patients who only received RBCs. Patients are categorized as "matched" if they only received products from a donor with the same AB0 blood type. All patients who also received compatible products from another blood type are categorized as "blood type compatible". Kruskall-wallis did not reveal any statistical differences.

	TRALI			Control		
	No	**Median**	**IQR**	**No**	**Median**	**IQR**
A matched	13	1.00	1.00-1.00	16	1.00	1.00-1.25
AB matched	1	1.00	1.00-1.00	4	1.50	1.00-2.00
B matched	2	1.00	1.00-1.00	4	1.50	1.00-2.00
O matched	21	2.00	1.00-2.00	25	1.00	1.00-2.00
Blood Type Compatible	8	2.00	1.50-2.50	2	2.00	1.00-3.00

RESULTS PROSPECTIVE COHORT

In the prospective case control study of cardiac surgery patients, 16 patients developed TRALI. From the cohort of transfused patients cardiac surgery patients who did not develop TRALI, 32 patients were randomly selected to serve as transfused control [19]. The baseline characteristics of these patients are displayed in Supplementary Table 5. Supplementary Table 6 describes the number of transfused products per donor characteristic.

Donor sex

The results from the prospective cohort were comparable to the retrospective cohort with the exception that TRALI patients did not receive more transfusions than control patients.

Moreover, both TRALI and control received mainly products from males (chi square test $p < 0.0001$; Supplementary Table 5) but donor sex did not influence TRALI incidence (Supplementary Table 6 and 7).

Donor age

Both TRALI and control were mostly transfused with products from donors older than 41 years of age (chi square test $p < 0.05$; Supplementary Table 6). Donor age did not influence TRALI incidence (Supplementary Table 5; Supplementary Table 8).

Donor blood type

Approximately half of the patients in the prospective cohort received blood type-compatible transfusions. We did not detect any influence of the median blood type-compatible and blood-type matched transfusions on TRALI incidence. There also was no difference between TRALI and control patients (Supplementary Table 6; Supplementary Table 9).

TABLE 5. Baseline characteristics of the prospective case-control study in cardiac surgery patients.

	TRALI (n = 16)	**No-TRALI (n = 32)**
Male sex, n (%)	12 (75)	20 (63)
Age in years, median (IQR)	74 (71-79)	69 (62-75)
ASA-score* (SD)	3.3 (0.5)	3.1 (0.5)
ICU admission category, n (%)		
Cardiovascular	16 (100)	32 (100)
RBCs transfused, median (IQR)	3 (2-5)	2 (1-2)
RBCs only, n (%)	5 (31)	19 (59)
PLTs transfused, median (IQR)	1.5 (0-7.5)	0 (0-3)
PLTs only, n (%)	0 (0)	0 (0)
FFP transfused, median (IQR)	2 (0-4)	0 (0-2)
FFP only, n (%)	0 (0)	1 (3)

* American Society of Anesthesiologists Physical Status classification

TABLE 6. Number of transfused products per donor characteristic, split on product type. Both TRALI and control patients receive less products from females.[19] *Both TRALI and control patients receive more plasma products from older donors ($p<0.05$) but age does not influence TRALI incidence. †Both TRALI and control patients received more blood products from blood type 0 and A ($p<0.0001$) but the distribution of donor blood type is not different between controls and TRALI patients. Not all totals sum to 100 due to rounding of decimals.

		TRALI			Control		
		RBCs	**PLTs**	**Plasma**	**RBCs**	**PLTs**	**Plasma**
Donor Sex	Male	31 (57)	41 (62)	42 (93)	34 (59)	23 (48)	26 (93)
	Female	23 (43)	25 (38)	3 (7)	24 (41)	25 (52)	2 (7)
Donor Age (year)*	18-30	4 (7)	10 (15)	1 (2)	10 (17)	8 (17)	1 (4)
	31-40	16 (30)	7 (11)	5 (11)	7 (12)	11 (23)	11 (39)
	41-50	12 (22)	23 (35)	19 (42)	17 (29)	19 (40)	5 (18)
	51-60	17 (31)	19 (29)	12 (27)	19 (33)	6 (13)	8 (29)
	61-70	5 (9)	7 (11)	8 (18)	5 (9)	4 (8)	3 (11)
Donor Blood Type†	0	20 (43)	24 (50)	20 (44)	28 (52)	42 (65)	8 (29)
	A	18 (39)	24 (50)	8 (18)	18 (33)	24 (36)	15 (54)
	B	8 (17)	0 (0)	8 (19)	8 (15)	0 (0)	1 (4)
	AB	0 (0)	0 (0)	9 (20)	0 (0)	0 (0)	4 (14)

TABLE 7A. Total patients who received RBCs, PLTs and plasma from either only males or only females. We used a chi-square test to investigate whether TRALI was related to donor sex. Donor sex did not influence the incidence of TRALI. The majority of these patients only received RBCs. As these numbers are too small to perform meaningful statistical tests, we only provide descriptive data.

	Donor					
	Male			Female		
Recipient	**Total**	**Control**	**TRALI**	**Total**	**Control**	**TRALI**
Male	9*	6	3	1***	1	0
Female	5**	5	0	3***	1	2

*1 patient received both plasma, RBCs and PLTs, 1 patient received only plasma, 1 patient received both plasma and RBCs, the rest (6) received only RBCs

**all patients only received RBCs

***these patients only received RBCs

TABLE 7B. Number of transfused products from male and female donors irrespective of product type. Kruskal-wallis testing did not reveal any differences between groups.

	Diagnosis		Donor			
Recipient	**No**	**Diagnosis**	**Male Median**	**IQR**	**Female Median**	**IQR**
Male	12	TRALI	2.00	1.00-2.00	1.00	1.00-2.00
Female	4	TRALI	2.00	1.00-4.00	1.50	1.00-2.75
Male	12	Control	1.00	1.00-1.00	1.00	1.00-1.00
Female	19	Control	1.00	1.00-1.00	1.00	1.00-1.00

TABLE 7C. Number of transfused products from male and female donors in patients who only received RBCs. Kruskal-wallis testing did not reveal any differences between groups.

	Diagnosis		Donor			
Recipient	**No**	**Diagnosis**	**Male Median**	**IQR**	**Female Median**	**IQR**
Male	3	TRALI	2.00	2.00-2.00	2.00	1.50-3.00
Female	2	TRALI	1.00	1.00-1.00	1.00	1.00-1.00
Male	9	Control	1.00	1.00-1.25	1.00	1.00-1.00
Female	10	Control	1.00	1.00-1.00	1.00	1.00-1.00

TABLE 8A. Total patients who received RBCs, PLTs and plasma from a certain age category. We used a chi-square test to investigate whether TRALI was related to donor age. Donor age did not influence the incidence of TRALI.

Age (years)	**Total**	**Control**	**TRALI**	**p**
18-30	3	1	2	
31-40	7	5	2	
41-50	26	14	12	
51-60	22	10	12	
61-70	13	4	9	0.48

TABLE 8B. Number of transfused products from each age category. Kruskal-Wallis with post-hoc Nemenyi testing did not reveal any significant differences in the number of transfused products per age group.

	TRALI			Control		
Age (years)	**No**	**Median**	**IQR**	**No**	**Median**	**IQR**
18-30	1	1.00	1.00-1.00	2	1.00	1.00-1.00
31-40	5	1.00	1.00-1.00	2	1.50	1.25-1.75
41-50	14	1.00	1.00-2.00	12	1.00	1.00-1.00
51-60	10	1.00	1.00-1.00	12	1.00	1.00-2.00
61-70	4	1.00	1.00-1.00	9	1.00	1.00-1.00

TABLE 9A. Total patients who received RBCs, PLTs and plasma from blood type matched or blood type compatible donors. We used a fisher-exact test to investigate whether TRALI was related to matched or compatible transfusion but did not detect a relation between blood type and TRALI incidence.

Age (years)	**Control**	**TRALI**	**Total**
A matched	15	6	21
AB matched	2	0	2
B matched	2	2	4
O matched	4	10	14
Blood Type Compatible	4	2	6

TABLE 9B. Number of transfused products from each donor blood type. Patients are not split on which type of blood product they received. Kruskall-wallis with nemenyi post-hoc testing did not reveal any statistical differences.

	TRALI			Control		
Blood type	**No**	**Median**	**IQR**	**No**	**Median**	**IQR**
A matched	27	2.00	1.00-6.00	33	2.00	1.00-6.00
AB matched	2	1.50	1.25-1.75	4	1.50	1.00-2.00
B matched	13	5.00	2.00-10.00	9	2.00	2.00-6.00
O matched	37	2.00	1.00-6.00	32	2.00	
Blood Type Compatible	28	3.00	2.00-9.75	17	6.00	3.00-10.00

TABLE 9C. Number of transfused products from each donor blood type for patients who only received RBCs.

	TRALI			Control		
Age (years)	**No**	**Median**	**IQR**	**No**	**Median**	**IQR**
A matched	2	1.50	1.25-1.75	8	1.50	1.00-2.00
AB matched	0	-	-	2	2.00	2.00-2.00
B matched	1	2.00	2.00-2.00	2	2.00	2.00-2.00
O matched	1	4.00	4.00-4.00	7	1.00	1.00-2.00
Blood Type Compatible	1	2.00	2.00-2.00	0	-	-

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8

DIAGNOSTIC ACCURACY AND FEASIBILITY OF ULTRASOUND COMPARED TO CHEST X-RAY FOR CATHETER TIP CONFIRMATION AND DETECTION OF COMPLICATIONS AFTER CENTRAL VENOUS CATHETER PLACEMENT

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SUBMITTED

SUMMARY

Background. We aimed to systematically examine the utility of ultrasound for catheter tip confirmation and detection of complications after central venous catheter placement.

Methods. A systematic search of PubMed, Medline and the Cochrane Database, was performed by using predefined search terms. The protocol was registered in advance with number CRD42016041686. Studies comparing ultrasound to chest radiography after central venous catheter placement were eligible for this meta-analysis. Pre-specified data were extracted, to construct 2 x 2 contingency tables. Sensitivity, specificity, feasibility were calculated for correct catheter tip confirmation and pneumothorax. Time to test result was also extracted from the articles. Risk of bias was assessed by using the Quality Assessment of Diagnostic Accuracy Studies 2 tool.

Results. Thirteen studies with a total of 1205 central venous catheter placements met the inclusion criteria. Twelve studies were prospective. The meta-analysis showed a pooled sensitivity of 0.99 (95% confidence interval 0.98-0.99), and a pooled specificity of 0.82 (0.73-0.89) for the confirmation of correct catheter tip position after CVC placement. US showed a pooled sensitivity of 1.00 (0.69-1.00) and specificity of 1.00 (0.98-1.00) for the detection of post-procedural pneumothoraxes. Ultrasonic confirmation of the catheter tip was available 79 minutes before chest radiography result (range 10-288 min).

Conclusions. Ultrasonography is a reliable tool to confirm correct catheter tip position after CVC placement. US is highly accurate to detect iatrogenic post-procedural pneumothoraxes. Bedside ultrasonography reduces time to treatment. US is not feasible in a proportion of patients, in those patients US cannot replace chest radiography.

INTRODUCTION

Central venous catheter (CVC) placement is a frequently performed procedure for various indications. [1] The catheter provides vascular access that facilitates hemodynamic monitoring, blood sampling, hemodialysis and the administration of drugs, fluids and parenteral nutrition. [2,3] However, insertion of a CVC can induce mechanical complications including hemothorax, pneumothorax and malposition. [4,5] Correct position of the catheter tip is pivotal for functioning. Still, malpositioning occurs in approximately in 1-9% of the inserted catheters. [5] Malposition is associated with vessel perforation, cardiac tamponade, arterial cannulation and systemic embolism. [6]

Real-time ultrasonic guidance during CVC placement has been shown to improve success rates and decrease complications, compared to guidance based on traditional landmarks. [7] With ultrasound present, the operator can directly confirm catheter tip position and detect a potential pneumothorax. [8-10] Lung ultrasound has been suggested to be superior over chest radiography for the detection of pneumothorax in supine patients. [11] [12] Lung ultrasonography can easily be taught to physicians. [13] Since the vena cava-atrial junction is difficult to visualize by ultrasound, the physician can exclude the catheter tip is located in the internal jugular veins, the inferior cava vein and axillary veins. Catheter tip position can be performed by a "bubble test". The catheter tip can be detected via subcostal or epigastric view as a hyperechogenic line in the right atrium or in the vena cava. In the bubble test, saline is agitated with a mixture of air in a syringe to form microbubbles. These are administered in the CVC and allow indirect confirmation of the CVC by rapid entry of turbulent flow in the right atrium or vascular structures.

Various other methods have been suggested to confirm the correct position of the catheter tip and to rule out complications, such as electrocardiography, transoesophageal echocardiography or the manual palpation of a "fluid thrill". [14-17] However, chest radiography is considered the golden standard and is recommended after CVC placement. [18,19] Unfortunately, chest radiography has serious limitations. The accuracy in locating the catheter tip position is over-estimated and the patient is exposed to limited but harmful ionizing radiation. [20] Moreover the confirmation of catheter tip position by chest radiography is obtained after the insertion procedure, hence the manufacturing and interpretation of the chest radiography may cause delay in patient treatment. Detection of catheter misplacement on the chest radiography may lead to repeated manipulation of the catheter site, with an increased risk of catheter related bloodstream infection. [21]

We hypothesize US is a useful tool in confirming catheter tip position and detection of postprocedural complications. Therefore, we aim to systematically assess the diagnostic accuracy of US after CVC placement compared to chest radiography.

MATERIALS & METHODS

We adhered to the recommendations from the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement and the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) group. [22,23] A predetermined protocol was registered with PROSPERO, registration number CRD42016041686. We proposed the following PICO(S) question: **P**atients scheduled for central venous catheter placement in which the **I**ndex test of Chest radiography was **C**ompared to lung ultrasound for the **O**utcome of confirmation of correct catheter tip position and the exclusion of pneumothorax. Any **S**tudy design except case reports were eligible for inclusion.

Search Strategy

We identified all relevant trials that compared ultrasonography with chest radiography after central venous catheter placement. We searched MEDLINE, EMBASE, the Cochrane Central Register of Controlled trials and the Cochrane Database of Systematic Reviews, for relevant articles in any language up to September 2017. Search terms were "central venous catheter" and "Chest radiography". The search strategy is described in detail in Appendix 1. We reviewed bibliographies of review articles and the included studies identify additional articles. Clinicaltrials.gov was searched to identify ongoing and / or unpublished trials.

Study selection

Two authors (EW, AV) independently selected studies for inclusion. To be eligible for inclusion, both ultrasonography and chest radiography results had to be reported in the article. Articles had to report on an adult, human population. Peripherally Inserted Central Catheters were excluded. Catheters could be inserted under ultrasound guidance or via anatomical landmarks. Both conventional ultrasonography and contrast enhanced ultrasonography (CEUS) were eligible. Studies had to report on confirmation of correct catheter tip position or post-procedural complications. We required articles to supply sufficient data to construct 2 x 2 contingency tables. In case of duplicate data publication, we included only the most recent study. The systematic review process for study selection is shown in Figure 1.

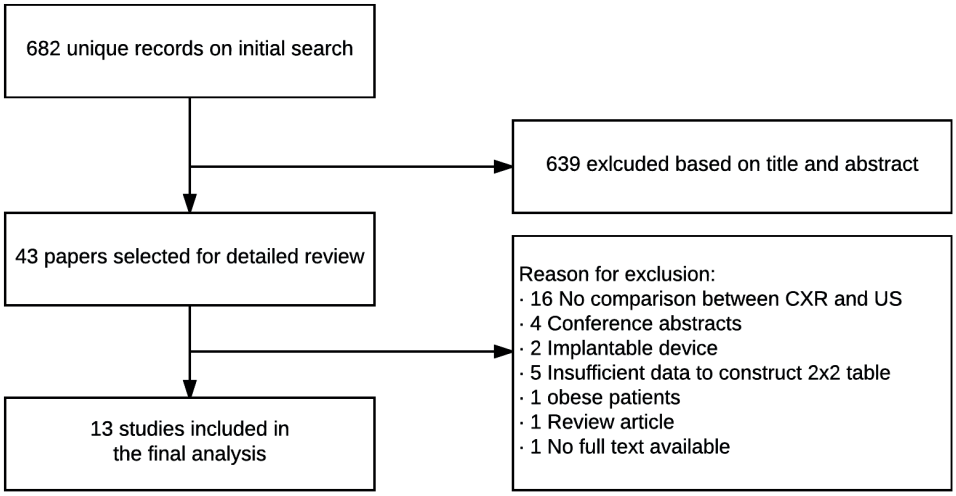

FIGURE 1. Meta-analysis flowchart.

Data extraction

Two authors independently abstracted data from the included studies by using a predefined form. We abstracted the number of patients and catheters, patient characteristics and information about the index test and the reference test. We noted the definitions for correct catheter tip position as mentioned in the articles. The number of malpositions detected by chest radiography and the number of malpositions detected by US were used for further analysis. Likewise, we counted the presence of pneumothorax as assessed by ultrasound compared to chest radiography.

Study Quality

Two authors assessed the studies for risk of bias and applicability using the Quality Assessment of Diagnostic Accuracy Studies 2, or QUADAS-2 tool. [24] A quality assessment was based on the following factors: patient selection, index test, reference standard and flow and timing. The risk of bias assessment consisted of two parts. First risk of bias was scored for patient selection, index test, reference test and flow and timing. Second concerns for applicability were assessed for patient selection, index test and reference test.

FIGURE 2. QUADRAS 2 results.

Data Synthesis and Analysis

Meta-analyses were performed using Review Manager 5.1 software for diagnostic accuracy studies. (Cochrane Collaboration, Oxford, UK). Chest radiography and ultrasonography test results for catheter misplacement and pneumothorax were extracted and entered in a 2 x 2 table. Ultrasound was the index test, chest radiography the reference test. True-positive (TP) results were defined as positive test results for both modalities. False-negative (FN) results were defined as positive reference test, while the index test was negative. True-negative (TN) results were defined as negative results on both modalities. False-positive (FP) results were defined as negative index test, while the reference test was positive. 2 x 2 tables were used to calculate sensitivities and specificities. We considered a diagnostic accuracy of 0.70 to be accurate. The Cochran Q test was used to calculate the I^2 statistics, a measure to quantify the amount of heterogeneity between studies. [25] Meta-analysis software was used to construct forest plots and to calculate I^2 statistics (Meta-DiSC, version 1.4; Unidad de Bioestadística Clínica del Hospital Ramón y Cajal de Madrid, Madrid, Spain). [26]

RESULTS

The electronic database searches identified 682 unique citations (Figure 1). Titles and abstracts were assessed for eligibility, after which 43 studies were reviewed in detail. Subsequently, 13 studies were included for the final analysis (Table 1). All studies were performed in industrialized countries. Twelve studies were prospective, one study was retrospective. In total, 1205 catheters insertions were analysed. Study characteristics are shown in Table 1. The studies were of moderate to high-quality, fulfilling 4 – 7 items of the 7 domain QUADAS checklist. (Figure 2.) We identified five registered trials at Clinicaltrials.gov, of which three are currently active. No unpublished studies with unknown status were registered.

Catheter tip malposition, diagnosed by chest radiography, ranged from 0% to 13.3% in the included studies. US had a pooled sensitivity for the detection of correct catheter tip position of 0.99 (95% confidence interval 0.98-0.99), with a pooled specificity of 0.82 (0.73-0.89). The 2 x 2 count data and the sensitivities and specificities are shown in Figure 3. The between-study heterogeneity was very high. Transthoracic echocardiography was not feasible in 0-14% of patients in the included studies. Abdominal surgery, severe obesity and chest tubes were reasons for inadequate views.

TABLE 1. Characteristics of included studies

Author	Inclusion time	Study design	CVC (n)	Index test	Setting
Baviskar [31]	2013 - 2015	Prospective	25	CEUS	Surgical ICU
Bedel [27]	2010	Prospective observational	101	US	ICU
Blans [32]	2015	Prospective observational	53	CEUS	General ward patients
Cortellaro [33]	NS	Prospective observational	71	CEUS	ED
Duran-Gehring, [40]	2012 - 2013	Prospective observational	54	CEUS	ED
Matsushima [29]	2009	Prospective observational	42	US	Surgical ICU
Maury [38]	1999	Prospective observational	84	US	ICU or ward
Meggiolaro [34]	2013	Prospective observational	105	CEUS	Preoperative CVC placement
Vezzani [39]	2008	Prospective observational	11	CEUS	Adult ICU
Weekes [28]	2013	Prospective convenience sample	152	NASF	ICU and ED
Wen [36]	2011 - 2012	Retrospective comparative	219	CEUS	Dialysis center
Wilson [35]	2012 2015	Prospective interventional	78	NASF	ICU and ED
Zanobetti [30]	2009 – 2011	Prospective observational	210	US	ED

Abbreviations: ICU = Intensive Care Unit, ED = Emergency Department; US = Ultrasound; CEUS: Contrast Enhanced Ultrasonography; NASF: Non-agitated Saline Flush.

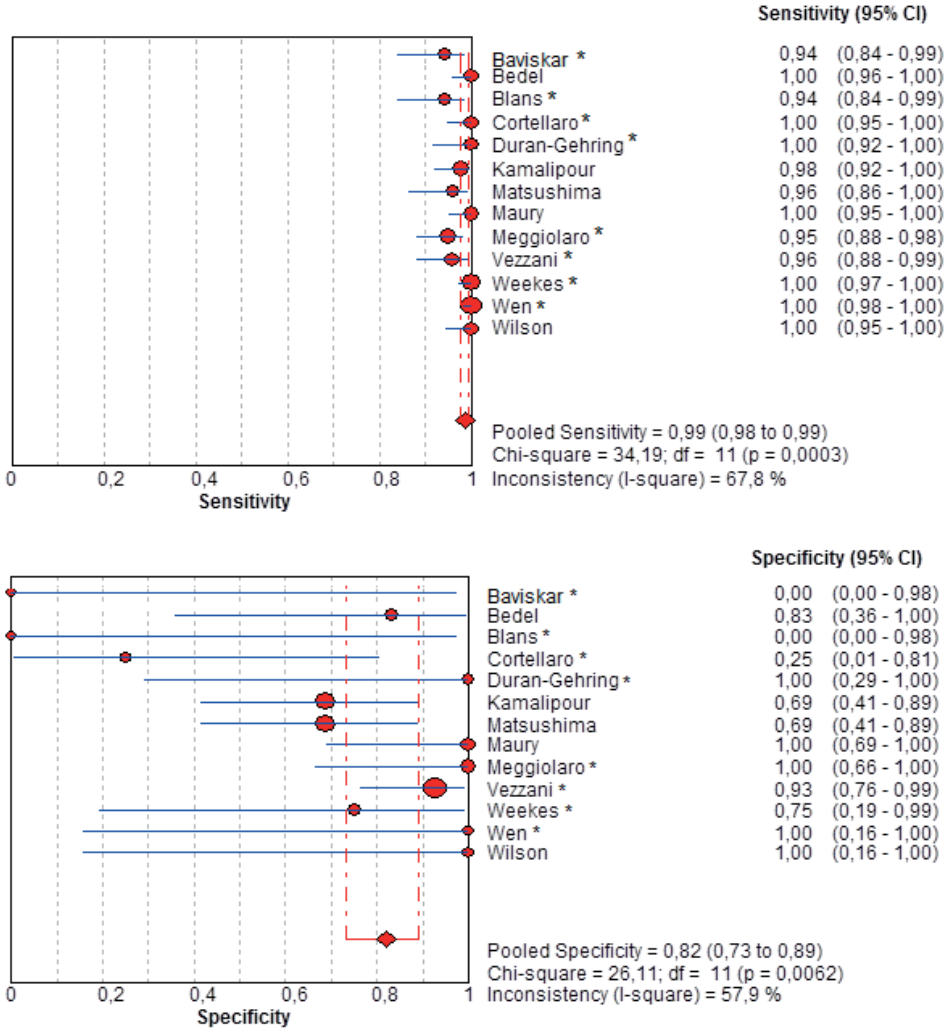

FIGURE 3. Forest plots with primary 2x2 data for sensitivity (top) and specificity (bottom) to confirm correct catheter tip position

Eight studies compared ultrasound with chest radiography for the detection of postprocedural pneumothorax. A total of eight iatrogenic pneumothoraxes (0.8%) detected by chest radiography, all were confirmed by US. US showed a pooled sensitivity of 1.00 (0.69-1.00) and specificity of 1.00 (0.98-1.00) for the detection of post-procedural

pneumothoraxes. The between-study heterogeneity was low for pneumothorax. We did not report sensitivity of pneumothorax detection in 4 studies as there were no events detected by the reference method (CXR).

Studies varied in the use of US for vein localisation and catheter guidance during insertion. Both US and landmark technique were used in four studies. [27-31] US was always used in four studies. [32-36] Three studies inserted catheters based on anatomical landmarks. [37] [38,39] The use of US for insertion was not specified in one article. [40]

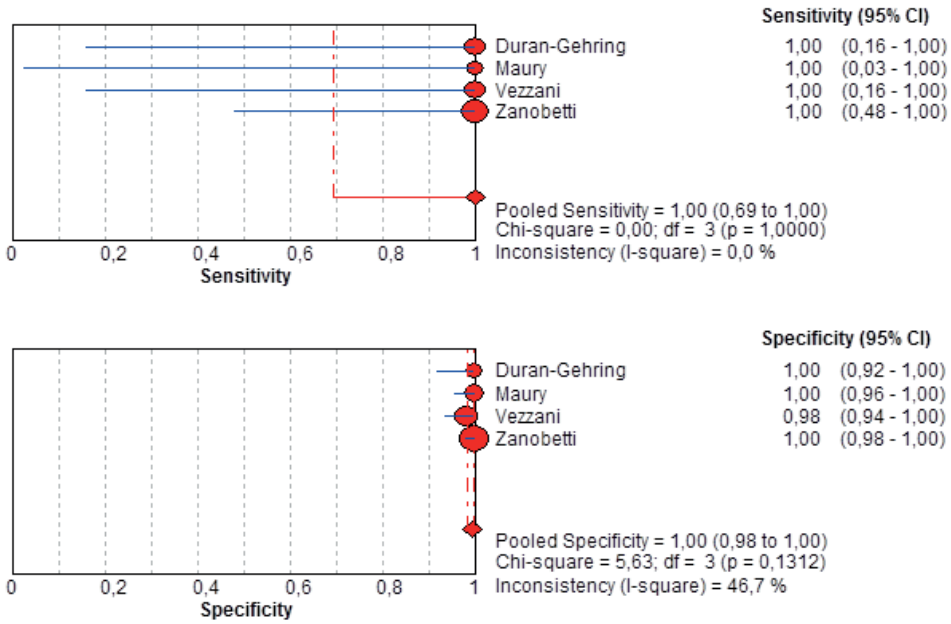

FIGURE 4. Sensitivity and specificity for CEUS to confirm correct catheter position.

DISCUSSION

The main finding of this systematic review and meta-analysis is that postprocedural ultrasonography has adequate sensitivity and specificity to detect correct catheter tip position and exclude a pneumothorax. Ultrasonography after CVC-placement is feasible in the majority of patients and saves time and minimizes radiation exposure.

Ultrasonography may also spare costs and resources compared to chest radiography, as portable chest radiography contribute up to 7.2% of total ICU costs. [41] Hirshberg calculated

that replacement of chest radiography with ultrasound after CVC placement could reduce over $50000 dollar on a yearly basis, based on approximately 2000 CVC placements in an ICU setting. [42] This may have direct consequences in high-resource settings in terms of cost-saving, but also a clear advantage for low-resource settings where systematic chest radiography is seldom available.

Two studies showed a low specificity for the detection of catheter malplacement. The absence of a catheter malposition led to a broad confidence interval and low specificity in one study. [43]

CEUS is an attractive method to diagnose correct position of the catheter tip. Five studies used a "bubble test" to confirm correct catheter tip position. Syringe size varied from 5 to 20 mL. The majority of studies used the method as described by Vezzani (Table 2) [39]. Only one study concluded CEUS could not substitute chest radiography. However, this negative study defined incorrect placement by CEUS as the absence or the appearance of only few bubbles or late (>2 seconds) echo-contrast entering the right atrium. This cut-off value of 2 seconds might be too high, since a cut off value of 500 ms has satisfactory sensitivity, specificity and inter-observer agreement for catheter misplacement. [34] Unfortunately, no standard protocol is available for catheter tip confirmation by US. The method of catheter tip confirmation by US varied amongst studies. A standard protocol that incorporates both direct subcostal view and a bubble test needs to be developed and validated, and learning curve defined. As suggested previously by Bolliger (2015) the "push-to-bubble" method can be used for identification of malposition of the CVC tip, whereas the ultrasound-guided localization of the CVC tip can be used to confirm correct catheter placement. [44]

Although this meta-analysis shows that ultrasonography has similar accuracy compared to chest radiography, ultrasonography has some well-defined practical limitations. Indeed, transthoracic echocardiography was not feasible in 0-14% of patients in the included studies. Therefore in a variable proportion of patients in which it is not possible to obtain adequate US images, chest radiography should be used as imaging method. Patients in which CXR should be used are patients with obesity, COPD, subcutaneous emphysema or (surgical) wounds. A hypovolemic or severely dehydrated status can impair real-time ultrasound visualisation of central veins. Additionally, the majority of the included studies were performed in non-ventilated patients outside the ICU. It is possible that ventilated patients yield less clear vascular US images.

Notwithstanding the potential advantages of a technique considered risk-free, it is important to acknowledge the greatest risk of ultrasound, which is the chance of false diagnosis. [45] People may underappreciate this as the risk of diagnostic error is conceptually separated

from the physical act of US examination. In addition, the vividness of visual images may activate the availability heuristic, leading to cognitive errors. [46] An important limitation of CEUS with air bubbles, is the occurrence of air embolism in case of an inadvertent arterial cannulation.

TABLE 2. Interpretation of Microbubble test according to Vezzani [39]

Characteristics	Interpretation
No bubbles	Negative test; an aberrant or extravascular, extracardiac placement should be considered
Few bubbles or appearance time > 2 seconds	Repeat test. If consistent: possible misplacement (in SV or IJV) or position too far from RA
Numerous indistinguishable bubbles; turbulent flow within the right atrium <2 seconds; direct visualization of catheter tip in right atrium	Negative test: intra-atrial misplacement
Numerous indistinguishable bubbles; linear flow from the SVC <2 seconds	Positive test; CVC correctly placed in the SVC

CVC = central venous catheter, IJV = internal jugular vein RA = right atrium; SVC = subclavian vein

In the current study, we defined chest radiography as reference method, since historically it has been considered the reference standard for confirming subclavian and internal jugular central venous catheter placement and evaluating for complications. [47] However, the accuracy of the reference method was limited: the sensitivity of chest radiography to detect a pneumothorax is inferior to US in the supine patient. [11,12,48] Similarly, US might be more accurate in the detection of catheter malposition compared to the thoracic radiograph. Vezzani reported two cases in which chest radiography considered the catheter tip to be positioned incorrect, which were missed by US. However, in at least one patient the chest radiography was of low quality, due to patient-related factors. These cases were considered to be false negative, while authors hypothesized this may have not been the case, since CEUS views were clear.

For the current study we used authors' definitions of correct catheter tip position. However, a recently published expert consensus statement concluded the preferred position for the catheter tip is the upper right atrium/caval-atrial junction. [49] The varying definitions of correct catheter tip position could have contributed to the wide range in occurrence of catheter malplacement between studies. An overview of definitions is provided in Appendix 3.

Chest radiography has been shown to be an inaccurate diagnostic tool to identify the junction between the SV. [20] Since the previously considered golden standard is imperfect, the results of the current study might underestimate the diagnostic accuracy of US.

From a practical point of view, it is a continuous debate if a post-procedural check should be omitted in uneventful procedures, where neither chest radiography, nor US may be necessary. Bailey et al. (2010) suggested that a post-procedural check could be omitted if the CVC placement is performed by an experience physician, with a maximum of two needle passes. [50] Patients in which catheter malposition or iatrogenic pneumothorax occur usually show signs and symptoms. On the contrary, others retain post-procedural imaging necessary as clinical factors alone could not reliably identify CVC tip misplacements. [51]

In our analysis, we excluded randomized controlled trials, because there was no comparison for ultrasound and chest radiography available in individual patients, which made it impossible to construct 2x2 tables. However, we identified one RCT, in which total of 60 patients were randomized to receive either a postprocedural chest radiography or ultrasound. [52] Indeed, ultrasound guided CVC placement and positioning reduced the use of bedside chest radiography and reduced the time to use of the CVC, with no pneumothoraces occurring during the study. Further prospective efforts are needed in alternative settings to compare the use of ultrasound versus radiography for the assessment of adverse events and catheter-tip position. Ideally, a randomized controlled trial would be conducted, to compare chest radiography, US and CT-images. However, such trial is ethically not feasible, due to the increase in radiation exposure.

This review has several limitations. First, the important heterogeneity observed for catheter tip position and time to catheter placement might be explained by the lack of methodological standardization and the high risk of bias for several studies. Statistical heterogeneity limits generalizations on diagnostic accuracy and did not seem to be explained by clinical factors such as operator experience and ultrasound techniques on a previous meta-analysis on the same topic. [53] For this reason no further subgroup analysis were performed in this paper in order to explain the statistical heterogeneity. Additionally, in ICU patients, the postprocedural CXR frequently performed is in antero-posterior view only, which makes differentiation of the vena cava from the right atrium particularly difficult. [54] Another possible explanation for the variation in malposition rate is the different insertion techniques applied during CVC insertion (e.g. anatomical landmarks versus ultrasound guidance). The level of training for both catheter placement and ultrasonography varied widely.

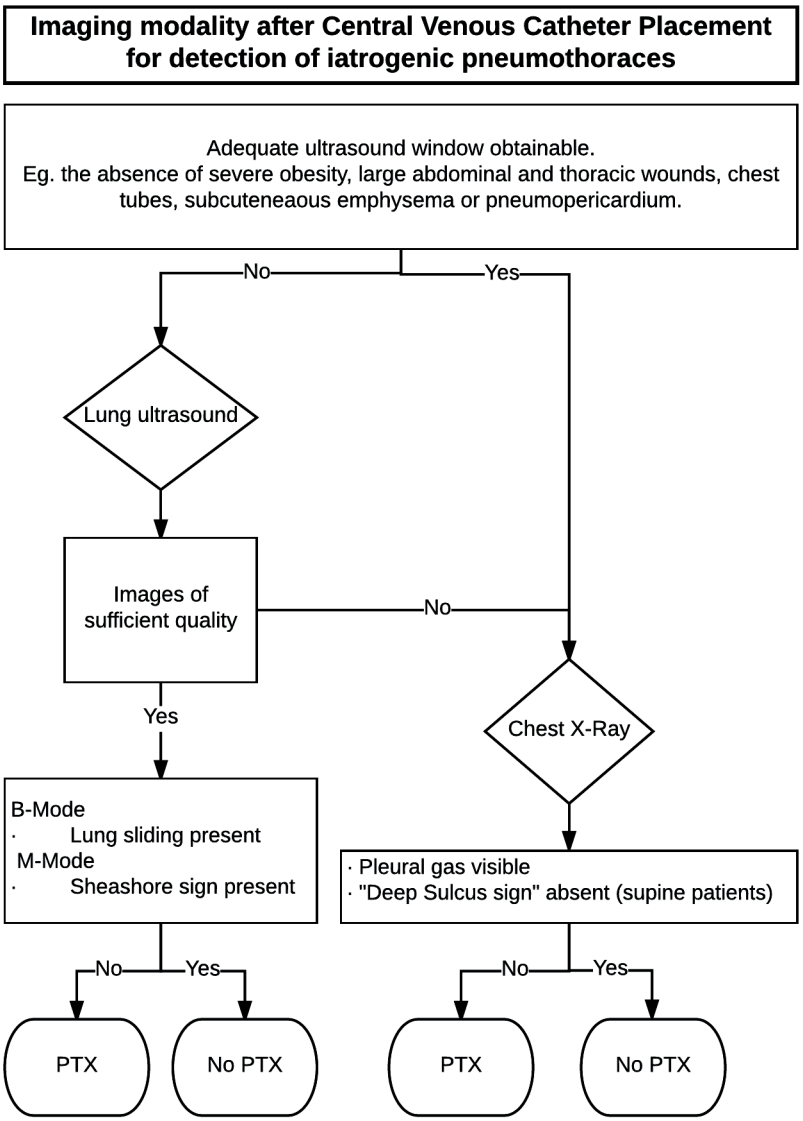

FIGURE 6. Imaging modality for the exclusion of pneumothoraxes

Ultrasound has consolidated utility for CVC placement procedure safety and success. [55] However, it's systematic post-procedural use for detecting correct position and exclude complications is still limited. This review shows implementation of systemic post-procedural ultrasonography for the confirmation of catheter tip position and the exclusion of pneumothorax is warranted.

In conclusion, a systematic review and meta-analysis of the published literature shows that US after CVC placement reduces time and costs, as compared to chest radiography. US has moderate diagnostic accuracy in confirmation of correct catheter tip position in the detection of iatrogenic pneumothorax. Chest radiography remains necessary in two specific patient groups: patients in which no clear ultrasonographic view can be obtained and patients with a high post-procedural risk of malposition. Further research should focus on developing and validating an uniform protocol for catheter tip detection.

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9

GENERAL SUMMARY

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SUMMARY OF THIS THESIS

This thesis focused on the risk benefit balance of platelet transfusion prior to invasive procedures in critically ill and hematologic patients. In the first part of this thesis, we focused on coagulopathy and minor invasive procedures. Hemostatic disorders are frequently corrected if a patient needs to undergo a minor invasive procedure, such as lumbar punctures, tracheostomy or CVC placement. The correction of hemostatic defects before CVC placement remains a matter of debate. Current national and international guidelines are conflicting and the recommended platelet count thresholds for inserting a central venous catheter vary from 20 to 50×10^9 per liter.

At the beginning point of this thesis, we wanted to review the incidence of bleeding after CVC placement in patients with disorders of hemostasis. We were also interested in clinical practice concerning prophylactic platelet transfusion and CVC placement.

Therefore, in **Chapter 2,** we conducted a nationwide mixed vignette and questionnaire survey to investigate current transfusion practice around CVC placement and to designate factors that influence the decision to transfuse platelets prophylactically. The most important finding of the study was that the current transfusion practice is highly variable. Maximal values of coagulation tests that were accepted without prophylactic correction varied between 1 and 10 for INR, and between 25 and 150 seconds for aPTT. The minimum platelet count that was accepted without preprocedural correction of thrombocytopenia varied from 10×10^9 to 80×10^9 per liter. Intensive care physicians accepted lower platelet counts as compared to the surveyed hematologists, radiologists and anesthesiologists. As expected, the chances of administering platelet transfusion increased with a lower platelet count. Physicians were less inclined to administer platelet concentrates to thrombocytopenic patients if the catheter needed to be placed in an emergency setting, as compared to elective catheter placement. On the other hand, physicians were more inclined to administer platelets if the catheter was placed in the subclavian vein, as compared to the internal jugular vein or femoral vein. A substantial proportion of physicians did not routinely use ultrasonography to guide central venous catheter placement. Whether ultrasound was used did not influence respondents' decision to administer platelets.

In **Chapter 3** the available literature on the insertion of central venous catheters in patients with severe coagulopathy was systematically reviewed. Severe coagulopathy was defined as any or a combination of the following laboratory results: a platelet count of 50×10^9 per liter or less, an INR above 1.5 or a partial thromboplastin time above 45 seconds. Serious or life-threatening bleeding after CVC placement appeared to be rare, even in coagulopathic patients. The reported total bleeding incidence varied from 0 to 32%. Definitions of minor

and major bleeding events varied widely across studies. Retrospective observational studies suggested no preprocedural correction of coagulopathy is required until a platelet count of $20x10^9$ per liter and an international normalized ratio of 3.0 as long as the intervention is performed by an experienced physician who applies ultrasound to guide this procedure. Our systematic review did not find evidence to support the correction of hemostatic defects prior to CVC insertion. However, quality of the studies was low. Most studies were observational studies performed in a selected patient population with a high risk of bias. Clearly there is a strong need for well-powered prospective randomized controlled trials to determine a save minimal platelet count in which CVC placement can be performed without prophylactic platelet transfusion.

In **Chapter 4,** the design of the PACER trial is described. This currently ongoing multicenter trial is the first randomized controlled trial investigating the necessity of prophylactic platelet transfusion prior to CVC placement in thrombocytopenic patients. We hypothesize that omitting platelet transfusion prior to central venous catheter placement is non-inferior to the prophylactic transfusion of one unit of platelet concentrate to prevent relevant bleeding complications. In this study, consecutive hematologic and critically ill patients, with a platelet count between $10\text{-}50x10^9$ per liter and an indication for a CVC, are eligible for participation. The physician performing the procedure should be experienced and compulsory to use ultrasound. Furthermore, the physician performing the procedure is blinded to the treatment arm the patient is randomized for. The primary endpoint of the study is WHO grade 2-4 bleeding, occurring within 24 hours after the procedure. Secondary endpoints include any bleeding complication, transfusion complications, transfusion requirements and costs. At the time this thesis was printed, the study is half way and is still recruiting patients according to schedule. The inclusion of participants is expected to be finished 2020.

In **Chapter 5,** we systematically reviewed the different scores and methods used to assess bleeding in clinical trials in thrombocytopenic patients undergoing a selected group of invasive procedures. We discussed the limitations and describe the important gaps in methodological reporting and identified confounders.

To decide on the optimal platelet count prior invasive procedures in thrombocytopenic patients, it is needed to understand the balance between improving hemostasis on one hand and the downside of platelet transfusion on the other hand. In Part II, we describe several studies that provide insight in this balance.

In **Chapter 6,** we developed and validated a novel method of *in vivo* labeling of transfused platelets by the use of biotin (vitamin 8), as an alternative to radioactive labeling. We

showed that biotin can be used to label platelets and validated the novel method. Biotin labeling did not result in platelet activation as compared to the "sham-sample", in which all procedural steps were identical, except for the incubation with biotin. The product met the predefined quality standards as set by the National Blood Bank Sanquin. We believe that this non-toxic assay can be very helpful in future research to investigate the clearance and half-life of transfused platelets in different clinical conditions.

In **Chapter 7,** we investigated whether donor characteristics influence the incidence of Transfusion Related Acute Lung Injury. In pre-clinical and several clinical studies, TRALI has been related to loss of product quality during red blood cell and platelet storage, called the "storage lesion". Donor characteristics, as for example age, genetics and sex, are known to influence this "storage lesion". Therefore, we hypothesized that donor sex, age and blood type could be related to TRALI incidence. To study this, a secondary analysis of two cohorts of TRALI patients was performed. The first cohort consisted of a retrospective nested case control study in ICU patients while the second cohort consisted of a prospective case control study in cardiac surgery patients. In both cohorts donor characteristics, including donor blood type, donor age, donor sex and the transfusion of blood type-compatible and blood type-matched products appeared not to be associated with an increased risk for TRALI.

In **Chapter 8,** we systematically reviewed the utility of post-procedural ultrasound instead of chest radiography to detect complications after central venous catheter placement. We found ultrasonography to be a reliable tool to confirm correct catheter tip position and rule out iatrogenic post-procedural pneumothoraxes after CVC placement. The implementation of this finding would strongly reduce the number of X-rays performed following CVC placement.

10

GENERAL DISCUSSION

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GENERAL DISCUSSION

This thesis focused on platelet transfusion thresholds and bleeding complications following (minor) invasive procedures. Many clinicians consider transfusion of blood products to be a harmless intervention. However, every transfusion product poses potential harm to the recipient, including transfusion-related acute lung injury (TRALI), transfusion associated cardiac overload (TACO), allergic reactions, allo-immunisation and transfusion related infections.

Recent evidence suggests that platelet transfusion increases the risk of adverse events and even death. Patients on antiplatelet therapy that suffered from a cerebral bleeding had a higher risk of death or worse neurological outcome after receiving even a single unit of platelet concentrate compared to placebo in a randomized multicenter trial.[1] This was an unexpected finding, and a sound explanation for the worse outcome after platelet transfusion was not found. Transfused platelets may contribute to pathologic thrombogenesis, since both thromboembolic and intracerebral bleeding complications occurred more frequently in the platelet transfusion group. Interestingly, thrombocytopenia itself is also strongly associated with mortality in critically ill patients,[2] suggesting that the rapid consumption of platelets in these patients, may trigger unidentified processes that lead to increased mortality. Therefore, correction of thrombocytopenia by transfusion might even worsen outcome. This is in line with a large observational trial in which increased mortality, and a higher occurrence of myocardial infarction was observed after platelet transfusion in patients with prothrombotic conditions, such as thrombocytopenic purpura and heparin-induced thrombocytopenia.[3] In preterm infants with severe thrombocytopenia, a lower threshold to transfuse platelets (l,e, more transfusions were given) compared to a higher threshold resulted in a higher rate of death and major bleeding.[4] Not only were platelets transfusions unable to prevent bleeding episodes, they were even harmful. Therefore, platelet transfusion should ideally only be administered to prevent bleeding and should be avoided if unnecessary.

In clinical practice, a pre-defined number of circulating platelets is traditionally used as a threshold to administer prophylactic platelet transfusions. Guidelines advise different cut-offs for various procedures and indications, institutional transfusion practice varies widely. This is because platelet transfusion threshold recommendations are based on very low-quality evidence, essentially reducing the value of transfusion guidelines to expert opinion. At the starting point of this thesis, more evidence was needed about both the beneficial and detrimental effects of platelet transfusion, to guide clinical decision-making.

Platelet transfusion prior to central venous catheter placement

In a systematic review of the literature, we found severe bleeding after central venous catheter placement to be rare, even in severely thrombocytopenic patients. However, most studies were retrospective and contained a high risk of bias. Reported bleeding incidence in comparable patient populations varied widely.

Multiple guidelines have implemented a platelet transfusion trigger of 20 x 10^9 per liter prior to CVC placement, based on only one observational study, of very low quality.[5] This was a retrospective observational study, in non-bleeding hematologic malignancy patients. In this study, all CVCs were placed under real-time ultrasonographic guidance, by experienced physicians. Conclusive evidence shows both the use of ultrasonography and the experience of the physician reduce the incidence of complications after CVC placement. Because of the higher risk of bleeding in thrombocytopenic patients, the use of ultrasound and the experience of the physician are even more important factors to take into account. Despite this evidence supporting ultrasonography for every central venous cannulation, various authors showed limited implementation of real-time ultrasonography.[6-9] In our questionnaire study, we found a substantial proportion of physicians do not routinely use ultrasonography to guide central venous catheter placement. Therefore, the conditions under which a platelet transfusion trigger of 20 x 10^9 per liter was found to be safe, are not met in the majority of centers in the Netherlands. Of note whether ultrasound was used did not influence respondents' decision to administer platelets.

In literature, all data on platelet transfusion triggers derive from retrospective studies in non-febrile patients, with isolated thrombocytopenia and no systematic comparative studies of the potency of platelet transfusion to prevent bleeding have been performed. Even though thrombocytopenia is irrefutably related to bleeding occurrence, the potency of prophylactic platelet transfusion to prevent this bleeding has never been proven. To overcome these knowledge gaps, we initiated the first randomized controlled clinical trial on the efficacy of prophylactic platelet transfusion prior to central venous catheter placement: the PACER trial. The PACER study is the first prospective randomized controlled trial to assess the efficacy of prophylactic platelet transfusion in a relevant clinical setting with both thrombocytopenia and an indication for a CVC: intensive care and hematologic malignancy patients. To date, no data on the minimal platelet transfusion threshold prior to CVC placement is available on thrombocytopenic critically ill patients or patients with a hematological malignancy is available.

In a questionnaire study we showed different disciplines accept different platelet transfusion thresholds for CVC placement. This could represent a cultural difference between specialties: for example, patients are monitored more closely at the intensive care unit, allowing a

rapid intervention if post-procedural bleeding occurs. However, it can also represent an actual difference in bleeding risk for various patient populations. The pathogenesis of thrombocytopenia in hematological malignancy patient categories differs from the pathophysiology of thrombocytopenia in critically ill patients. In hematological malignancy patients, thrombocytopenia is caused by bone marrow depression, both by hematologic malignancies and as result of chemotherapy. This results mainly in a reduced number of platelets which are functionally unaffected. In critically ill patients on the other hand, coagulopathy is more often multifactorial, leading to a combination of thrombocytopenia and thrombopathy which may be accompanied by coagulopathy. Also the response to platelet transfusion may differ between patient categories. In a paired prospective study, stored platelets resulted in a lower platelet recovery as compared to fresh platelets, this was only observed in critically ill patients and did not occur in hematologic malignancy patients.[10]

Critical illness is a well identified prognostic factor for complications of transfusion, including TRALI and TACO.[11-14] Critically ill patients might therefore respond differently to transfusion.

Future directives

The PACER trial will provide insight in the minimal safe platelet count prior to central venous catheter placement in a clinical relevant patient population. Following the results of the PACER study, future research should focus on differences between patient categories, to identify patients that benefit from transfusion. Also, well-powered clinical trials and threshold-recommendations for other interventions, such as tracheostomy, biopsies and lumbar punctures are highly warranted. Another area of interest is neurosurgery, as the PATCH trial showed harm for platelet transfusion in cerebral bleeding patients on anti-platelet drugs. The PACER trial may be the first step in several trials investigating the need for prophylactic platelet transfusion prior interventions. Future trials should explore the utility of point of care platelet function tests or a clinical algorithm to identify patients that would benefit from platelet transfusion. In clinical trials, absolute platelet counts have been researched, however, platelet function is not investigated. Future research should focus on more factors than only the absolute platelet count. Combining laboratory and clinical factors might predict bleeding more accurate than the platelet count. Besides their important role in hemostasis, it has become evident that platelets are contribute to inflammatory and immune responses, angiogenesis, atherosclerosis, and tumor groth.[15,16] The effect of platelet transfusion on these less known functions of platelets are to be researched.

Bleeding scores

To assess the effect of platelet transfusion, a valid outcome measure of the transfusion is warranted. A major problem in the field of platelet transfusion research is defining and scoring bleeding complications. Bleeding is used as a clinical relevant outcome measure. Clinical assessment of bleeding has some major limitations. The assessment of bleeding is prone to subjectivity, making the inter-observer variability an important issue. We showed definitions of bleeding and bleeding incidence in comparable study populations differ widely, suggesting methods to assess bleeding differ significantly among studies, hampering a good comparison of studies and difficulty defining guidelines with respect to prophylactic platelet infusion.

Future directives

Clinical research in the field of platelet transfusion would benefit from clear, reproducible and relevant tailored definitions for various interventions. The current WHO-bleeding scale is not adequate.

TRALI

In recent years, various preventative measures to decrease antibody-mediated TRALI have been implemented in the Netherlands. Initially female plasma was mitigated and subsequently fresh frozen plasma was substituted by pooled plasma, to dilute and bind the circulating antibodies responsible for TRALI.[17] However, these measures do not affect the risk of non-antibody mediated TRALI. Furthermore, other factors seem to play a role in the origin of severe transfusion side-effects. To gain inside in these factors, we performed a secondary analysis of two cohorts of TRALI patients. We showed that donor characteristics, such as blood type, age, sex and the transfusion of blood type-compatible and blood type-matched products were not associated with an increased risk for TRALI. However, we did not look into the difference between nullipara and women that had never given birth. In a recent large cohort trial, male red blood cell recipients of an ever-pregnant donor appeared to have an increased risk of mortality, as compared to male recipients receiving red blood cell transfusions from male donors or a never-pregnant female donors.[18] Interestingly, this effect was not observed in female recipients. The mechanism of this difference is to be scrutinized.

Future directives

Understanding donor and product bound factors that contribute to outcome and matching adequate donors per recipient are likely to improve transfusion outcomes. Platelets are known to deteriorate during storage, the effect and the possible relation to TRALI incidence would be an interesting future directive.

Biotin labeling

Currently, platelet count is the only available measure to assess the effect of platelet transfusion *in vivo*. However, platelet count does not discriminate the patient's own circulating platelets from the transfused platelets. In time after platelet transfusion, the ratio between a patients produced platelets and survival of transfused platelets is unknown. By labeling transfused platelets, it is possible to obtain insight in the survival, function and activation of transfused platelets. We demonstrated that biotin can be used to label transfused platelets for research purposes *in vivo*. The major advantage of biotin is that it enables *in vivo* tracing of transfused platelets, without exposing the recipient to radiation. Performing medical research with radiolabeled platelets is forbidden in Europe, because of the exposition to harmful radiation patients. Biotinlabelling of platelets, offers a method to analyze platelet recovery and survival, which can be used to evaluate the dynamics of platelet transfusions in different conditions. Also, by using magnetic beads, biotinylated platelets can be isolated from venous blood samples, thereby permitting direct population analysis for surface markers and metabolomics composition of the platelet subgroups.

It is important to realize that every transfusion product is modified and thereby different from autologous, functioning platelets. Because biotin binds to all proteins, all excess proteins need to be removed from the platelet concentrate prior to biotinylation. We did this by washing the platelets, via centrifugation. Corrected count increment (CCI) has already been shown to be lower for washed platelets, as compared to non-washed platelets.[19] In this study, the lower CCI was hypothesized to be the result of a reduction of absolute platelet number, and not by alteration in the platelet characteristics. Our research supports this finding, during every centrifugation, a marginal loss of platelets occurs.

In our study, platelets were activated by the procedure, as compared to the platelets in the 'control' sample. However, we showed that the procedure, and not the biotin itself induced platelet activation. The process to obtain platelet hyperconcentrate (platelets dissolved in small volume) is very similar to producing biotinylated platelets. Therefore, it is likely to assume that platelets in platelet hyperconcentrate are equally activated. Platelet hyperconcentrates have been administered to patients successfully for several years. Therefore, no problems are expected for administering biotinylated platelets.

Future directives

Biotinylation of platelets offers a novel, non-radioactive method to trace transfused platelets *in vivo,* with many possibilities for future research. Tracing transfused platelets in various patient categories and clinical conditions, can gain insight in the effectiveness of platelet transfusions.

Platelet labeling with biotin enables tracing of multiple populations concurrently. In this way, the influence of platelet product factors, such as storage time, additive solutions or donor factors on platelet performance in vivo can be analyzed. Hereby, platelet products can be improved. Biotinylated platelets will be first administered in an autologous transfusion model in healthy volunteers (registered at trialregister.nl, NTR6493).

Ultrasound instead of Chest X-ray after CVC placement

We found ultrasonography to be a reliable tool to confirm correct catheter tip position and rule out iatrogenic post-procedural pneumothoraxes after CVC placement. Traditionally, a chest X-ray is obtained after CVC placement, to confirm that the catheter tip is in the correct position and to rule out a pneumothorax. Even though radiography is considered golden standard, chest radiography has been shown to be an inaccurate diagnostic tool.[20] This makes it difficult to interpret the concordance between these two imaging modalities. The implementation of this finding would strongly reduce the number of X-rays performed following CVC placement.

Future directives

A well-powered trial that compares chest radiography to lung ultrasound is needed. Ideally, such a trial would not only compare diagnostic accuracy, both also at patient outcome, cost-effectiveness and clinical implementation.

Summary

We showed platelet transfusion thresholds in the Netherlands are highly variable between hospitals and specialties. The incidence of severe bleeding after central venous catheter placement in thrombocytopenic patients is rare. However, definitions of bleeding incidence vary between studies and are often incomplete, which makes it hard to interpret results or extrapolate results to guidelines. In addition, the available literature is mainly observational and has a high risk of bias. Therefore, we initiated the PACER-trial, in which the necessity of platelet transfusion prior to central venous catheter placement will be investigated. The results of this ongoing randomized controlled multicenter trial are expected in the near future. We designed and validated the use of biotin as a safe and non-radioactive method to label platelets *in vivo* which can give vital information on the half-life of transfused platelets and factors that are involved in the increment and clearance of transfused platelet concentrates. Lastly, we found ultrasonography to be a reliable tool to assess catheter tip position and rule out complications after central venous catheter placement.

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11

NEDERLANDSE SAMENVATTING VOOR NIET-MEDICI

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SAMENVATTING VOOR NIET-INGEWIJDEN

Achtergrond

Dit proefschrift gaat over bloedplaatjestransfusie alvorens het uitvoeren van kleine invasieve ingrepen patiënten met een laag bloedplaatjesgetal (trombocytopenie). Omdat bloedplaatjes een belangrijke rol spelen in de bloedstolling, hebben trombocytopene patiënten een verhoogd risico op bloedingen. Bij ongeveer de helft van de patiënten met hematologische maligniteiten treedt een bloeding op.[1,2] Trombocytopene patiënten hebben een verhoogd risico op een nabloeding bij het uitvoeren van een kleine invasieve ingreep, zoals het plaatsen van een centraal veneuze catheter of verrichten van een diagnostische of therapeutische punctie Ondanks dat een laag trombocytengetal geassocieerd is met verhoogd bloedingsrisico, is er geen bewijs dat het profylactisch corrigeren met trombocytentransfusie voor een ingreep dit risico op bloeding vermindert.[3,4] Trombocytentransfusie brengt echter wel risico's met zich mee. Daarom zal de arts bij elke patiënt de afweging moeten maken of het voordeel opweegt tegen de risico's.

Bloedplaatjes

Bloed bestaat voor 55% uit bloedplasma, waarin allerlei eiwitten zijn opgelost die onder andere van belang zijn voor de bloedstolling, voor 45% uit rode bloedcellen die het zuurstoftransport naar de weefsels verzorgen, en voor <1% uit witte bloedcellen, die verantwoordelijk zijn voor de afweer, en bloedplaatjes (trombocyten) die belangrijk zijn voor de bloedstolling. Desondanks zijn bloedplaatjes in absolute aantallen de meest voorkomende cellen in het bloed, met een normaal trombocytengetal van 150-400 x 10^9 per liter. Trombocyten zijn cellen zonder celkern, die ontstaan bij het uiteenvallen van in het beenmerg geproduceerde megakaryocyten. Een laag trombocytengetal kan ontstaan bij verminderde productie, verhoogd verbruik of ophoping in de milt.[5-7] Trombocytopenie komt in het bijzonder frequent voor bij patiënten met hematologische maligniteiten en ernstig zieke patiënten, die op de intensive care worden opgenomen. Bij 8-67% van de patiënten die op de intensive care worden opgenomen treedt trombocytopenie op, meestal door ernstige infectie en inflammatie, waardoor het stollingssysteem wordt geactiveerd en trombocyten worden verbruikt.[8,9] Het is bekend dat bij ernstig zieke patiënten, de ernst van de trombocytopenie samenhangt met de kans om te overlijden.[9]

Trombocytentransfusie kan worden gegeven om het trombocytengetal te corrigeren. In Nederland worden jaarlijks 250.000 trombocytentransfusies aan patiënten gegeven. (Sanquin Annual report 2017, www.sanquin.nl). Bloedplaatjes kunnen zowel profylactisch als therapeutisch gegeven worden. In patiënten zonder bloeding, die geen invasieve ingreep hoeven te ondergaan, wordt een afkapwaarde van 10x10^9 per liter gehanteerd.[1]

Dit is onderzocht in een gerandomiseerde klinische studie, waarbij de ene helft van de patiënten transfusie ontving als het bloedplaatjesgetal lager was dan $10x10^9$ per liter en de andere helft van de patiënten geen transfusie ontving. Er trad een bloeding op bij 43% van de patiënten die transfusie had ontvangen en bij 50% van de patiënten die geen transfusie had ontvangen. Ondanks dat transfusie het risico op bloedingen vermindert, is trombocytentransfusie dus vaak niet in staat op bloedingen te voorkómen. Over het optimale afkappunt voor het trombocytengetal alvorens verschillende invasieve procedures veilig uitgevoerd kunnen worden is weinig bekend. De studies over deze afkapwaardes hebben een hoog risico op bias en zijn meestal observationeel van aard. Hierdoor zijn aanbevelingen die worden gedaan in klinische richtlijnen gebaseerd op expert opinion, in plaats van medisch-wetenschappelijk bewijs.[10,11] Het optimale plaatjesgetal voor verschillende ingrepen, is daarom nog onduidelijk.

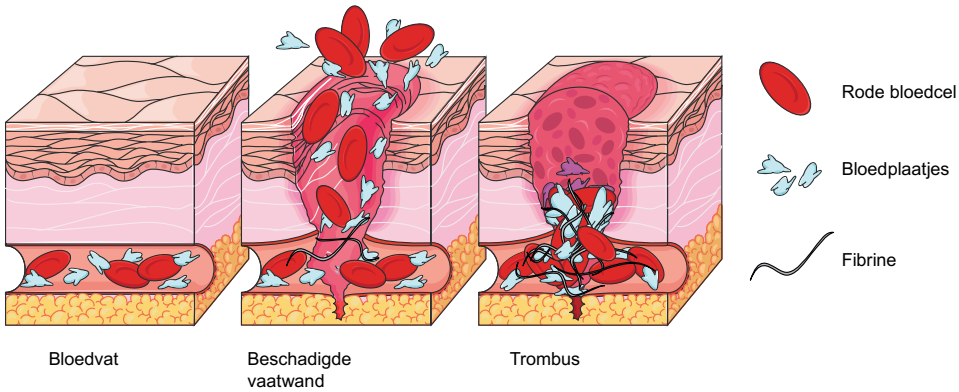

FIGURE 1. Bloedplaatjes en stolling In de normale situatie, zijn bloedplaatjes in overvloed aanwezig in de bloedsomloop (links), als het endotheel van een bloedvat wordt beschadigd (midden), raken bloedplaatjes geactiveerd en vormen ze een stabiele klont, samen met rode bloedcellen en fibrinedraden (rechts).

Nadelen van trombocytentransfusie

Transfusie van trombocyten brengt risico's voor de patiënt met zich mee (tabel 1). De reacties die op kunnen treden variëren van mild, zoals het ontstaan van galbulten en koorts na de transfusie, tot ernstige complicaties zoals acute longschade (transfusion related acute lunginjury: TRALI en transfusion related acute overload: TACO). TRALI is levensbedreigend, 91% van de TRALI patiënten moet beademd worden en 17-45% van de patiënten komen te overlijden. [12-14] Patiënten die op de afdelingen hematologie en intensive care zijn opgenomen, hebben een grotere kans op nadelige gevolgen van transfusie. [15,16]

TABEL 1. Risico's van trombocytentransfusie

Complicatie	Incidentie
Koorts reactie	7%
Allergische reactie	2%
TRALI	0.08-15.1%[13,15]
Bacteriële verontreiniging	0.23-0.32%[17,18]
Bacteriële sepsis	0.001%

Centraal veneuze lijnen

Een centraal veneuze lijn (CVL) is een soort infuus, dat ingebracht wordt in een grote ader, ondersleutelbeenader (vena subclavia) dan in de halsader (vena jugularis) of dijader (vena femoralis). Het voordeel hiervan is dat hierover medicatie gegeven kan worden die te beschadigend is voor kleine aders zoals chemotherapie, adrenaline en parenterale voeding. Het plaatsen van een CVL is een procedure die frequent wordt uitgevoerd, 8% van de patiënten in het ziekenhuis krijgt een CVL.[19] Na het plaatsen van een CVL kunnen complicaties optreden, zoals een klaplong (pneumothorax), verkeerde positive van de catheter, infectie of nabloeding.[20-23] Een nabloeding na het plaatsen van de lijn treedt op in 2-30% van de patiënten en is afhankelijk van de stollingsneiging van de patiënt, de ervaring van de arts die de lijn plaatst en of er wel of geen echo gebruikt wordt tijdens de lijnplaatsing.[24-27] [20-23] Door het gebruik van echo bij het plaatsen van een CVL is de procedure veel veiliger geworden.[28] De meeste van deze bloedingen zijn echter klein, zoals nadruppelen van de insteekopening op een blauwe plek bij de insteekopening.[29]

Het is bekend dat de klassieke laboratoriumtests om een inschatting te maken van de stollingsstatus van de patient, slecht in staat zijn te voorspellen bij welke patiënten daadwerkelijk een bloeding op zal treden na centraal veneuze lijnplaatsing.[3,4,30] Trombocytopene patiënten ontvangen vaak trombocytentransfusie alvorens een centrale lijn geplaats wordt. De huidige richtlijnen zijn tegenstrijdig en adviseren verschillende afkapwaarden.[10,31-33] Een retrospectieve studie in patiënten die opgenomen waren bij op de afdeling hematologie liet zien dat een trombocytengetal van $20x10^9$ per liter veilig is om een CVL te plaatsen.[26] Deze studie was echter retrospectief, observationeel en had een groot risico op bias. Daarom is het erg belangrijk deze vraag prospectief en gerandomiseerd te onderzoeken.

Samenvatting

Het eerste gedeelte van dit proefschrift is gericht op afwijkende bloedstolling voor kleine invasieve ingrepen. We gaven een overzicht van de bestaande medisch-wetenschappelijke

literatuur over het inbrengen van centraal veneuze lijnen bij ernstige stollingsstoornissen. Om een beeld krijgen van de huidige klinische praktijk en de factoren die artsen doen beslissen om een trombocytentransfusie te geven, voor een centraal veneuze lijnplaatsing hebben we een in **Hoofdstuk 2** een vragenlijststudie gedaan. Hiervoor hebben we alle Nederlandse ziekenhuizen die meer dan vijf beademingsbedden benaderd, een vragenlijst en klinische vignetten toegestuurd.

De belangrijkste uitkomst van de studie was dat er een grote variatie is in het trombocytengetal dat artsen accepteren voor het plaatsen van een CVL, dit getal bevond zich tussen 10-80 $\times$ 10^9 per liter. Dit geeft aan dat er veel onduidelijkheid en meningsverschillen zijn over de noodzaak van trombocytentransfusie voor deze indicatie. We vonden dat intensive care artsen een lager trombocytengetal accepteren in vergelijking met de andere ondervraagden. Artsen zijn minder geneigd om transfusie toe te dienen in een spoedsituatie. Artsen geven vaker trombocyten voor lijnplaatsing in de vena subclavia dan in de vena jugularis of vena femoralis. Ook vonden we dat veel artsen niet standaard echo gebruiken bij het plaatsen van een CVL, ondanks dat er uitgebreid bewijs is dat het gebruik ervan het risico op complicaties reduceert. [27,28,34-36]

Om te onderzoeken hoe vaak bloedingen optreden bij trombocytopene patiënten die een CVL krijgen, onderzochten we in **Hoofdstuk 3** systematisch naar de gepubliceerde medische literatuur over het centraal veneuze lijnen en ernstige stollingsstoornissen. We vonden dat het optreden van ernstige bloedingen zeldzaam is, zelfs bij patiënten met ernstige stollingsstoornissen.

In **Hoofdstuk 4** beschreven we de rationale en studieopzet van de PACER trial. Deze klinische studie zal antwoord geven op de vraag of het toedienen van trombocytenconcentraat voor centraal veneuze lijnplaatsing noodzakelijk is, bij patiënten met een ernstige trombocytopenie. Onze hypothese is dat het weglaten van trombocytentransfusie niet slechter is dan het wel geven van de transfusie. Hiervoor worden patiënten met hematologische maligniteiten en patiënten die opgenomen zijn op de intensive care, met een trombocytengetal 10-50x10^9/L die een centraal veneuze lijn moeten krijgen gerandomiseerd voor ofwel een zak trombocytenconcentraat, ofwel geen transfusie. De arts die de centraal veneuze lijn plaatst moet veel ervaring hebben met het plaatsen van centraal veneuze lijnen en hij of zij weet niet of de patiënt wel of geen trombocytentransfusie heeft gehad. Alle lijnen worden met behulp van een echo ingebracht. De belangrijkste uitkomstmaat is het optreden van bloedingen uit de insteekopening van de lijn, binnen 24 uur na lijnplaatsing. Daarnaast wordt gekeken naar alle complicaties van lijnplaatsing

en transfusie, de hoeveelheid transfusies die de patiënen nodig hebben en de kosten. De studie includeert momenteel nog patiënten, en zal naar verwachting worden afgerond in 2020.

In **Hoofdstuk 5** onderzochten we op welke wijze de mate van bloeding werd gedefinieerd en beoordeeld bij klinische studies naar trombocytopenie bij patiënten die een kleine invasieve ingreep moesten ondergaan. Het aantal bloedingen dat vóórkomt bij vergelijkbare patiëntengroepen in verschillende studies liep ver uiteen. Dit is waarschijnlijk het gevolg van verschillende definities en onderzoeksmethoden, en niet van en verschil in het daadwerkelijk aantal bloedingen. Dit maakt het erg moeilijk om onderzoeken te interpreteren en vertalen naar de klinische praktijk in richtlijnen. De meest gebruikte methode om bloedingen te scoren is de bloedingsscore van de World Health Organisation (WHO). Deze methode is echter niet ontwikkeld om bloedingen naar interventies te scoren, en daarmee te grofstoffelijk. Veel onderzoekers gebruiken daarom eigen definities, die helaas vaak lastig te reproduceren zijn.

In **Hoofstuk 6** ontwikkelden en valideerden we een nieuwe manier voor het labelen van bloedplaatjes in het lichaam. Om na transfusie van trombocyten de overleving in het lichaam (*in vivo)* te kunnen bepalen, is het nodig om de trombocyten te labelen, om de getransfundeerde trombocyten te kunnen onderscheiden van de in het lichaam aanwezige trombocyten. Momenteel is de gouden standaard hiervoor een radioactief label, maar omdat dit schadelijk is voor de ontvanger is het gebruik hiervan verboden in Europa. Daarnaast is het niet mogelijk om radioactief gelabelde getransfundeerde cellen uit de circulatie te isoleren na transfusie. Labeling met biotine (vitamine 8) is hiervoor een goed alternatief. We ontwikkelden een methode waarmee steriel en reproduceerbaar gelabeld kan worden. Om het effect van bewerkingsstappen te onderscheiden van het effect van het biotine label zelf, zijn ook "sham" samples verzameld. Door de methode werd activatie van de trombocyten veroorzaakt door de bewerkingsstappen, dit kwam echter niet door de biotine label zelf. Deze bewerkingsstappen komen overeen met het productie proces van bijvoorbeeld hyperconcentraten, een product dat al jarenlang veilig getransfundeerd wordt in patiënten.

In **Hoofdstuk 7** onderzochten we of eigenschappen van de donor invloed hadden op de kans op het ontwikkelen van TRALI in de ontvanger. Hiervoor gebruikten we de gegevens van twee eerder verzamelde cohorten van TRALI patiënten. Uit laboratoriumonderzoek blijkt dat de kwaliteit van het bloedproduct tijdens opslag structureel verschilt per donor. Van mannen, oudere donoren en donoren met metabool syndroom leiden is bekend dat de "opslaglaesie" ernstiger is dan van vrouwelijke donoren, jongeren donoren en donoren zonder metabool syndroom. Het is onbekend of deze factoren er ook voor zorgen dat de

bloedproducten tot meer complicaties leiden in de ontvanger. Wij wilden onderzoeken We onderzochten de hypothese dat geslacht, leeftijd en bloedgroep van invloed zou zijn op het optreden van TRALI. We vonden echter geen relatie tussen geslacht, leeftijd en bloedgroepcompatibiliteit en het risico op TRALI.

In **Hoofdstuk 8** onderzochten we of de wetenschappelijke inzichten in het ontstaan van TRALI en de geïmplementeerde strategieën om het risico erop te verminderen hebben geleid tot een veranderde herkenning van TRALI. Onze studie laat zien dat artsen geen gebruik maken van het "two-hit model". Bij milde ziekte en heftige reactie, kort na toediening van transfusie wordt TRALI herkend en gerapporteerd. In tegenstelling tot een studie van 10 jaar geleden, vonden artsen nu dat de duur van opslag van de bloedproducten niet van invloed was op het ontstaan van TRALI. Dit is in lijn met de wetenschappelijke kennis over dit ziektebeeld.

Tijdens het plaatsen van een centraal veneuze lijn kunnen verschillende complicaties ontstaan. Het uiteinde van de lijn kan zich op de verkeerde plaats bevinden, of er kan een klaplong (pneumothorax) ontstaan. Deze complicaties zijn niet altijd direct zichtbaar, daarom wordt er vaak een röntgenfoto gemaakt om te kijken of de lijn goed zit en uit te sluiten dat er een pneumothorax is opgetreden. Het maken van een röntgenfoto kost tijd en geld en is niet altijd direct mogelijk. een echoapparaat idealiter al aanwezig is tijdens het plaatsen van een lijn en door de arts direct gemaakt kan worden, wilden we in **Hoofdstuk 9** onderzoeken of echografie een longröntgenfoto kan vervangen bij het plaatsen van een centraal veneuze lijn. Hiervoor zochten we systematisch naar studies, en vonden we dat echo inderdaad bruikbaar is om complicaties na centraal veneuze lijnplaatsing uit te sluiten. Bovendien kunnen zo de kosten en stralingsbelasting van een röntgenfoto bespaard worden.

Conclusie

Het bloedplaatjesgetal waarop patiënten een transfusie krijgen loopt ver uiteen tussen verschillende ziekenhuizen en medische specialismen in Nederland. In de literatuur is het vóórkomen van ernstige bloedingen bij patiënten met een ernstige trombocytopenie zeldzaam. De definities van bloeding na centraal veneuze lijnplaatsing lopen ver uiteen, waardoor het moeilijk is de studies te interpreteren en te vertalen naar de klinische praktijk. De studies zijn laag van kwaliteit, observationeel van aard en hebben een hoog risico op bias. Daarom beschrijven we de opzet van de PACER trial, die de noodzaak van trombocytentransfusie alvorens het plaatsen van een centraal veneuze lijn onderzoekt. Deze gerandomiseerde klinische multicenter studie includeert momenteel patiënten, resultaten van deze studie zullen een antwoord geven op de vraag of het veilig is om trombocytopenie niet te corrigeren alvorens centraal veneuze lijnplaatsing. We

ontwikkelden en valideerden een veilige, en niet-radioactieve methode om trombocyten te labelen, waardoor getransfundeerde trombocyten *in vivo* vervolgd kunnen worden. Ten slotte vonden we dat echografie een goed alternatief is voor een long-röntgenfoto voor het uitsluiten van complicaties na het plaatsen van een centraal veneuze lijn.

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A

APPENDICES

PUBLICATION LIST

PHD PORTFOLIO

CURRICULUM VITAE

DANKWOORD

PUBLICATION LIST

- **van de Weerdt, E.K.,** de Bruin, S., Sijbrands, D., Vlaar, R., Gouwerok, E., Biemond, B.J., Vlaar, A.P.J., van Bruggen, R., de Korte, D. (2019). Biotinylation of platelets for transfusion purposes: a novel method to label platelets in a closed system. Transfusion. 00;1-10
- **van de Weerdt, E. K.**, Peters, A. L.Prinsze, F., de Korte, D., Juffermans, N. P., & Vlaar, A. P. J. (2019). Donor characteristics do not influence transfusion-related acute lung injury incidence in a secondary analysis of two case-control studies. *Transfusion Clinique et Biologique*, *26*(1), 10-17.
- **van de Weerdt, E. K.,** Biemond, B. J., Zeerleder, S. S., van Lienden, K. P., Binnekade, J. M., & Vlaar, A. P. (2018). Prophylactic platelet transfusion prior to central venous catheter placement in patients with thrombocytopenia: study protocol for a randomised controlled trial. *Trials*, *19*(1), 127.
- Peters, A. L., **van de Weerdt, E. K.,** Goudswaard, E. J., Binnekade, J. M., Zwaginga, J. J., Beckers, E. A., Zeerleder, S.S., van Kraaij, M.G.J., Juffermans N.P. & Vlaar, A. P. (2018). Reporting transfusion-related acute lung injury by clinical and preclinical disciplines. *Blood Transfusion*, *16*(3), 227.
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- **van de Weerdt, E. K.,** Biemond, B. J., Baake, B., Vermin, B., Binnekade, J. M., van Lienden, K. P., & Vlaar, A. P. (2017). Central venous catheter placement in coagulopathic patients: risk factors and incidence of bleeding complications. *Transfusion*, *57*(10), 2512-2525.
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- **van de Weerdt, E. K**., Determann, R. M., Biemond, B. J., van Lienden, K. P., & Vlaar, A. P. J. (2017). The inability to flush a central venous catheter. *Netherlands Journal of Critical Care*, 103.
- Meesters, M. I., Vonk, A., **van de Weerdt, E. K.,** Kamminga, S., & Boer, C. (2014). Level of agreement between laboratory and point-of-care prothrombin time before and after cardiopulmonary bypass in cardiac surgery. *Thrombosis research*, *133*(6), 1141-1144.

PHD PORTFOLIO

E.K. van de Weerdt

	Year	ECTS
Courses		
Basic Couse Legislation and Organisation for Clinical Researchers (BROK)	2015	0.7
Endnote	2015	0.1
Searching for a Systematic Review	2015	0.1
Practical biostatistics	2015	1.1
Clinical Epidemiology: Systematic Reviews	2016	3
Citation analysis and impact factors	2016	0.1
Project management	2016	0.6
The Next Big Thing	2017	6
Crash course: basic chemistry, biochemistry and molecular biology for MDs starting scientific research	2018	0.4
Scientific writing in English for publication	2018	1.5
Seminars, workshops and master classes		
Medical business masterclass	2016	1
Presentations		
Posterpresentation: "Donor characteristics do not influence transfusion-related acute lung injury incidence in a secondary analysis of two case-control studies"	2018	0.5
AABB annual conference 2018, Boston, USA Presentation: "Trombocytopenie", Congres update in transfusiegeneeskunde 2018, Zwolle	2018	0.5
Conferences		
AMSTOL symposium, AMC, Amsterdam	2016	0.25
Lage Landen Congres, Intensive Care Geneeskunde, Haarlem	2017	0.25
Attendence annual congress of the Dutch Society for Blood Transfusion, NVB, Ede, The Netherlands	2018	0.25
AABB annual conference 2018, Boston, USA	2018	0.75
Other		
Intensive Care Research Meeting (weekly)	2015-2018	12
Intensive Care Journal Club (monthly)	2015-2018	4
Laboratory of Experimental Intensive Care and Anesthesiology (LEICA) research meeting (weekly)	2015-2018	12
Supervising		
Bachelor Thesis	2018	1.0
Master Thesis	2018	1.0

CURRICULUM VITAE

Emma van de Weerdt was born on the 11th of September 1989 in Rhenen. After finishing her Gymnasium high school education in Veenendaal, she moved to Amsterdam were she obtained degrees in Beta-Gamma and Medicine. As a student she participated in various committees and boards (Opleidingscommissie VUmc, Studentenraad VUmc, Bestuur Medische Faculteitsvereniging aan het VUmc and Intreeweek CommissieUvA).

During her medical training she did internships in Mexico and Aruba. She participated in a research project at the department of anesthesiology, where her interest in blood platelets was evoked.

In 2015 she started a PhD project on platelets and invasive procedures, supervised by Prof. dr. Bart J. Biemond and dr. Alexander P.J. Vlaar, which resulted in this thesis.

During her PhD period, Emma ran four marathons: Amsterdam 2016, Berlin 2017 (PR: 3.57.50), Rotterdam 2018 and Loch Ness 2018. Recently, she started working as a doctor at the department of Oncology, in the Utrecht University Medical Centre.

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