The effect of prophylactic platelet transfusions on point-of-care coagulation parameters in conjunction to central venous catheter insertion—what effect do transfusions have and are they necessary?

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Centrala venkatetrar (CVK) är nålar som sätts in i hals- eller axelkärl och som används bland annat för att möjliggöra behandling av cancerpatienter med cellgifter (cytostatika). En del patienter har låga halter av blodplättar (trombocyter) i blodet. Ett tillskott av blodplättar, något som kallas blodplättstransfusion, ges ofta innan CVK-inläggning för att förhindra blödningar men det vetenskapliga underlaget för detta är dåligt. Vanliga blodprover som mäter blodets levringsförmåga (koagulationen) förutsäger inte blödning tillförlitligt. Nyare, patientnära metoder så som ROTEM och Multiplate kan bättre förutse patienters blödningsrisk vid operationer, men är inte välstuderade på patienter med blodcancer och låga halter av blodplättar. I denna studie studerades patienter med låga blodplättar som fick blodplättstransfusion inför CVK-inläggning. Målet med studien var att bättre förstå effekten av blodplättstransfusioner och analysera eventuella samband mellan vanliga blodprover, ROTEM/Multiplate och patientens egenskaper (t.ex. kön, ålder, vikt och diagnos).

I de preliminära resultaten såg man att blodplättstransfusionen hade god effekt på ROTEM. Effekten var större hos kvinnor och efter två påsar blodplättar och kvarstod fyra timmar. Multiplate indikerade mycket låg funktion hos blodplättarna både före och efter transfusion och funktionen var lägre hos kvinnor. De låga blodplättsfunktionsvärdena hos Multiplate bör valideras mot flödescytometri, som är den mest accepterade metoden för att mäta blodplättsfunktion, för att kunna bedöma hur användbar Multiplate är i handläggandet av patienter med låga antal blodplättar i blodet.

Abstract

Introduction: Platelet transfusions are often used when thrombocytopenic patients are facing a central venous catheter (CVC) insertion, but their use has been up to debate. Point-of-care devices such as thromboelastometry (ROTEM) and platelet aggregometry (Multiplate) better reflect in vivo haemostasis than routine coagulation tests. Aim of the study was to investigate the effects of platelet transfusions administered before CVC insertion, using ROTEM and Multiplate.

Materials and methods: Informed and signed consent was attained from all patients. Patients with a platelet count $< 50 \cdot 10^9$/L received platelet transfusion before CVC insertion (transfusion group, *n=14*). The patients' blood was analysed with routine coagulation and point-of-care tests (ROTEM, Multiplate) before as well as one and four hours after transfusion. A reference group with non-thrombocytopenic patients ($\geq 50 \cdot 10^9$/L) scheduled for CVC insertion was also analysed (*n=32*). Clinical data for patients as well as the age of the platelet units and the number of units used were recorded.

Results: The ROTEM clot firmness parameter, Extem MCF increased after platelet transfusion, with a sustained response after four hours. The increments in Extem MCF were significantly higher in women and when using two platelet units. The increments in Multiplate parameters after transfusion did not reach significance. The Multiplate adenosine diphosphate-agonised assay indicated severe platelet dysfunction before and after transfusion, and the values were significantly lower in women.

Conclusion: In patients with haematological malignancies and thrombocytopenia, ROTEM can be used to monitor the effect of a prophylactic platelet transfusion. Platelet transfusion strategies involving ROTEM and Multiplate should be tested in a larger study. The clinical significance of very low Multiplate values before and after platelet transfusions in severely thrombocytopenic patients needs to be verified with flow cytometry.

Introduction

A central venous catheter (CVC) is an ordinary part in the treatment of various malignancies with cytostatic drugs and stem cell transplants, but its use also extends to treatment of patients with malnutrition or difficult peripheral vein access as well as the monitoring of central venous pressure. Complications to CVC insertions include both infections and mechanical complications such as pneumothorax, haematoma, thrombosis, air embolism and arrhythmia. The risk of mechanical complications is dependent on patient-related factors, including the underlying disease, comorbidity, medications, and thrombocytopenia. Specifically, the risk of bleeding is thought to be increased in patients with thrombocytopenia, but the risk of severe bleeding is still low given that correct precautions are taken [1]. The clinical relevance of thrombocytopenia and the platelet transfusions that might accompany it in patients undergoing invasive procedures such as a CVC insertion is unclear.

In many cases, platelet transfusions have been used prophylactically before elective procedures when the platelet count is below $50 \cdot 10^9$/L, at the discretion of the physician [1]. This trigger is recommended for routine CVC insertion in current Swedish guidelines [2] and also for patients undergoing surgery [3]. However, a lower trigger is recommended in several studies [4,5] whereas others withhold CVC insertion as a safe procedure in these patients even without preprocedural platelet transfusions [6]. Moreover, the increment of platelet count might not reflect the outcome of platelet transfusions as its association with clinical bleeding is poor [7,8]. Assessments of platelet function, thromboelastometry (see below), transfusion intervals and clinical bleeding in relation to platelet transfusion offer additional insight in several studies, [9–11] but the effect of platelet transfusions remains incompletely investigated. With the cost and potential adverse effects of platelet transfusions—infectious and immunological [3]—it is important to further clarify their

clinical effect and relevance in the prevention of bleeding, both in general, as is being done in ongoing randomised controlled trials [12,13], but also prior to invasive procedures.

Point-of-care or "bedside" coagulation tests have been introduced into clinical practice as an addition to routine laboratory coagulation tests and their use has been the subject of plentiful research during the last decade. Advantages of these techniques include the fact that they use measurements of whole blood (as opposed to routine laboratory tests, which are run on plasma), which also provides information about platelet function (including its natural interactions with erythrocytes and leukocytes) as well as the possibility to assess clot structure. Furthermore, another advantage is the opportunity to perform the tests in the vicinity of the patient with small delays [14,15].

A prominent example, thromboelastography (TEG®) or thromboelastometry (ROTEM®) has become increasingly common and well documented in several clinical settings such as cardiac surgery [16,17], liver surgery [18,19], neurosurgery [20,21], obstetrics [22], and trauma [23–25]. Its underlying theory and technique have been reviewed elsewhere [14,26–28], but briefly, are based upon measuring the change of viscoelasticity as a clot forms, with the registration of several parameters. By adding different reagents, different aspects of the patient's coagulation state can be monitored [14]. Another point-of-care test, multiple electrode platelet aggregometry (e.g. Multiplate®) has been developed to specifically determine platelet function. Its use has extended from monitoring different anti-platelet therapies in cardiologic practice and cardiac surgery [29–31] to being used in immediate monitoring of trauma [32] and von Willebrand disease [33]. Its principle [28,34] relies on testing primary haemostasis in full blood (thrombin being inhibited by hirudin, thus preventing secondary haemostasis) by letting platelets aggregate on a pair of electrodes and measuring the increase in resistance, after addition of a substance agonising a

specific pathway in the platelets' cell signalling. In this way, platelet inhibition due to anti-platelet drugs or medical conditions can be monitored.

The aim of this study was to investigate the effect of platelet transfusions administered prior to CVC insertion by using thromboelastometry, platelet aggregometry and routine coagulation parameters as measures. Furthermore, the study attempted to investigate whether any effect was more pronounced at one or four hours after transfusion, and to identify baseline laboratory parameters and clinical factors that could affect the outcome. Also, the study includes non-thrombocytopenic patients undergoing CVC insertion (without prior transfusion) for gaining a better understanding of the relationship between routine coagulation parameters, point-of-care analyses, and clinical factors.

Material and Methods

Conduct was approved by the Regional ethics committee (Dnr 2011/626). Informed and signed consent was attained from all patients. The study is a prospective observational study, comprising two groups that were studied separately and independently. In one group, patients with a platelet count below $50 \cdot 10^9$/L scheduled for a CVC insertion with prior platelet transfusion were enrolled (transfusion group). Patients scheduled for CVC insertion with a platelet count above $50 \cdot 10^9$/L (not to be given transfusions) were enrolled into another group for a broader comparison of laboratory results and clinical data (reference group). Exclusion criteria for the transfusion group comprised an age < 18 years as well as administration of anticoagulant (warfarin, dabigatran, low molecular weight heparins) or anti-platelet drugs. For the reference group, the same exclusion criteria applied, with the exception of low molecular weight heparin (LMWH) treatment.

Transfusion group

Patients in the transfusion group received a platelet transfusion one hour ahead of CVC insertion, which was in accordance to established routine at the haematological and oncological units of the hospital. Blood sampling was performed before transfusion as well as one and four hours after transfusion. Ahead of transfusion (within six hours), blood was sampled for prothrombin time (PT-INR), activated partial thromboplastin time (aPTT), fibrinogen (2.7 mL vacutainer tubes containing 0.129M citrate, BD Vacutainer systems, Plymouth, UK) and platelet count (3 mL EDTA, BD Vacutainer systems) for analysis at the central laboratory. Blood was also sampled for ROTEM® and Multiplate® analysis at the intensive care unit. For ROTEM analysis, blood was sampled in a 2.7 mL vacutainer tube containing 0.129M citrate for ROTEM analysis (3.2% citrate, BD Vacutainer systems, Plymouth, UK) and preheated to 37 °C for 30 minutes. Analysis was performed with Extem and Fibtem assays as described by the manufacturer (Pentapharm, Switzerland). The

recorded ROTEM parameters included the clotting time (CT), clot formation time (CFT), alpha-angle (AA), and maximum clot firmness (MCF) for the Extem assay, and MCF for the Fibtem assay. For Multiplate analysis, blood was sampled in a 3.0 mL hirudin vacutainer tube (Verum Diagnostica GmbH, Germany) and conditioned to room temperature for 30 minutes before analysis was performed as described by the manufacturer (Verum Diagnostica GmbH, Germany). The platelet function was tested using agonisation by adenosine diphosphate, collagen, and thrombin receptor-activating peptide (ADP, COL, and TRAP assays respectively). For both ROTEM and Multiplate analyses, blood samples were mixed repeatedly immediately after blood withdrawal as well as before analyses. Blood samples were drawn either from venipuncture or from an established peripheral line or portacath (in which case flushing and excess blood withdrawal were performed in order to avoid heparinisation from the inner walls of the portacath).

The same batch of blood samples were collected again one hour after transfusion from the CVC immediately after its insertion. In addition, reference samples for analysis with ROTEM and Multiplate were also drawn from the steel needle before the guide-wire for the CVC was inserted, for comparison with blood sampled from the CVC. Four hours after transfusion, the batch of blood samples was again drawn from the CVC. At both times, a dummy sampling of 10 mL of blood was performed before sampling from the CVC.

Reference group

For the patients in the reference group, blood samples for PT-INR, aPTT, platelet count, fibrinogen, ROTEM, and Multiplate analyses were drawn once in conjunction to CVC insertion as described above. A reference sample from the steel needle was used also for this group.

Clinical data

For both groups, information regarding diagnosis, indication for CVC insertion, type and punctuation locale of the CVC, number of punctuation attempts, and occurrence of arterial puncture or air in the syringe after CVC insertion was attained from the CVC insertion record. Complications were categorised as arterial puncture, occurrence of air in the syringe, localised haematoma, haemothorax, pneumothorax, and others. 24 hours after CVC insertion, complications not registered in the CVC insertion record were checked for in post-insertion X-ray findings (pneumothorax, haemothorax) as well as in the patient records (occurrence of bleeding, haematoma, and others). Information regarding gender, age, height, weight, administration of antibiotic and antithrombotic medications, temperature prior to CVC insertion, and routine laboratory tests (haemoglobin, white blood cell count, PT-INR, aPTT, platelet count, fibrinogen) was also attained from patient records. For the patients in the transfusion group, the age, type (aphaeresis or buffy coat) and blood group of the platelet transfusion units as well as the number of units administered were noted. Any concurrent plasma transfusions were also noted. In the reference group, patients with a cancer diagnosis were stratified into a scoring system for assessing the risk of venous thromboembolism (VTE) in cancer patients [35].

Statistical analyses

Data were registered using OpenOffice Calc (version 3.3.0 for Mac OS, Oracle, USA). Statistical analyses and charts were made using SPSS (version 20 for Mac OS, IBM, USA). For calculating differences in the distributions of laboratory results before, one hour and four hours after transfusion, Friedman's related samples two-way analysis of variance (ANOVA) by ranks was used. For calculating differences between subgroups of patients within each group, the Mann-Whitney U test was used. The blood samples from the CVC were compared with the reference blood sample from the steel needle using the Wilcoxon signed-rank test. Correlation coefficients were calculated

using Spearman's rank correlation method. For all analyses, pairwise exclusion of missing samples was chosen. A p-value < 0.05 was considered significant.

Results

Transfusion group

A total of 14 patients (eight women and six men) were enrolled into this group. During the research period, four patients who met the inclusion criteria were not included. This was due to refusal in one case, understaffing in two cases, and ethical reasons in one case. Among the patients included, 10 patients had diagnoses considered highly malignant whereas four had diagnoses considered less malignant or benign (Table 1). Indications for CVC insertion included chemotherapy (nine patients), immunoglobulin treatment (one patient), allogenic stem cell transplantation (one patient), and blood sampling and drug administration (three patients). Ten patients received one unit of platelets; one patient also received plasma and was excluded from analyses except those solely based on baseline values. Four patients received two platelet units. Age of the platelet units varied from three to seven days (median five days). All units except one were of buffy coat type. All transfusions were ABO- and Rh-group compliant. During the time of CVC insertion, one patient was being treated with ciprofloxacin, four patients with vancomycin; all patients except one were treated with at least one antibiotic drug. More descriptive statistics and data from the CVC placement records are presented in Table 2. (Reference ranges for ROTEM and Multiplate are presented in the Appendix.) Complications occurred in one patient in form of a localised haematoma; one patient also experienced oozing bleeding during CVC insertion, which was not defined as a complication beforehand.

The differences in distribution of the different laboratory tests before and after transfusion are presented in Table 3 and Figure 1. (The patient also receiving plasma was excluded.) Adjusted significance was reached for the differences in platelet count (after one and four hours) and Extem MCF (after one hour only). Including patients receiving two platelet units, adjusted significance

was also reached for the difference in Extem CFT (significant difference only after four hours), Extem-Fibtem MCF, ADP, COL, and TRAP assays, and the difference in Extem MCF after four hours was also significant. The median Multiplate values were much lower than reference ranges both before and after transfusion, especially pronounced for the ADP parameter. There were no significant differences between any ROTEM or Multiplate values recorded at one and four hours after transfusion. The occurrence of missing data is presented in the Appendix. For seven patients, the steel needle reference sample was used instead of the CVC for analysis due to improper dummy sampling from the CVC.

Subgroup analysis showed significant differences between number of platelet units administered as well as gender, as shown in Table 4 and Figures 1 and 2. No significant differences were seen in BMI stratifications, diagnoses, or between patients younger or older than 60 years of age (not shown). Patients receiving two units of platelets had significantly higher Extem MCF increments after one hour as well as significant CFT decreases and platelet increments after four hours (excluding the patient who received plasma). Women had significantly lower baseline ADP, COL, TRAP values (all patients) and significantly higher one hour Extem MCF increments (within patients only receiving one unit of platelets and not plasma), as seen in Figures 2 and 3. Figure 1 indicates that only three patients had ADP values close to normal values at one hour after transfusion, and only two after four hours.

Correlation coefficients (patients receiving two units of platelets or plasma excluded) are presented in the Appendix. Of note, significant correlations were seen between Extem MCF one hour increment and gender, baseline Multiplate (ADP, COL, TRAP) parameters, and baseline Extem-Fibtem MCF (Figure 4) as well as between platelet increment and platelet age. For the Extem MCF increment at four hours, only significant correlation with ADP remained. The change of fibrinogen

level, which was significantly correlated with baseline Multiplate values at one hour after transfusion, was no longer significantly correlated with those values after four hours. No significant correlation was seen between baseline platelet count, baseline Multiplate parameters and Extem-Fibtem MCF, or between platelet, Multiplate and ROTEM increments (Figure 4). No correlation was seen between routine coagulation tests (including platelet count) and the various baseline values or increments measured by thromboelastometry or platelet aggregometry.

Reference group

32 patients were enrolled into this group (16 women and 16 men). 25 patients had a malignancy stated as diagnosis on the CVC placement chart (Table 1). 10 patients were treated with LMWH at the time of CVC insertion, of which two were considered high dose regimens. CVC placement statistics and indications for CVC insertion are summarised in Table 2. One complication occurred in form of an arterial puncture. CVC records were missing or incomplete in two cases and the number of attempts was not reported in an additional six cases. Descriptive statistics for patient characteristics and laboratory tests are summarised in Table 2. Correlation tables (using only patients without LMWH treatment as data) are presented in the Appendix. Of note, significant correlations were seen between Multiplate parameters and haemoglobin, white blood cell count and platelet count. Fibrinogen levels correlated significantly with Fibtem MCF, and Extem-Fibtem MCF correlated with Multiplate parameters and platelet count. PT-INR did not correlate with any other parameters, whereas aPTT correlated with several (see Appendix).

For the laboratory analyses, no significant differences were seen between male and female patients for the group as a whole. Selecting only patients without LMWH treatment revealed significantly higher ADP and COL values in women (Figure 3). Selecting only cancer patients among these revealed the same gender difference (not shown). Too few patients without either cancer diagnosis

and/or LMWH treatment were included to allow for comparison between patients with and without cancer, or between non-cancer patients with and without LMWH treatment. Using the VTE risk stratification score, the only significant difference was seen in platelet count, which is included as a criterion in the scoring system, although it can be noted that the only patient identified as a high risk patient displayed the highest ROTEM parameters in the entire group. No significant differences were seen between normal weight and over weight patients (BMI > 25) or between patients below and equal to/older than 60 years of age. For 19 patients, the reference blood sample from the steel needle prior to entering the CVC guidewire was drawn and analysed. A small but significant difference for Extem alpha-angle and Extem MCF values was seen when compared to the blood samples drawn from the CVC, with the more coagulating tendency seen in ROTEM parameters from the steel needle blood samples (Table 3).

Discussion

This is a preliminary report from an ongoing study aiming at 40 patients with thrombocytopenia and receiving prophylactic platelet transfusions before CVC. The number of observations is low and more observations are needed to make robust conclusions and prevent type I and II errors. The study saw increments in thromboelastometry (ROTEM) parameters after transfusion, of which the increment in Extem MCF reached statistical significance. The increment in Extem MCF was also significantly larger when using two platelet units. The findings support the notion that platelet transfusions have effect on thromboelastometry parameters, as seen in another study [11]. The increments in platelet function parameters, as measured by platelet aggregometry (Multiplate), did not reach statistical significance and the values were still very low after transfusion, suggesting that defects in platelet function sometimes remain even after transfusion. ROTEM and Multiplate values recorded at four hours after transfusion were not significantly different compared to values at one hour, indicating that the effects that the platelet transfusion has on these parameters might last four hours after transfusion. However, as seen in Figure 1 some patients' values (for both Extem MCF and ADP-agonised platelet function) improve after four hours, whereas the values decrease in others. This variability needs to be further studied.

This study saw significantly lower platelet function in women compared to men (as measured by the Multiplate device) in the transfusion group consisting mainly of haematological neoplasias, whereas platelet function was higher for women among the more varied cancer patients in the reference group. To the author's knowledge, no previous study has reported a gender difference in platelet function among patients with haematological malignancies. Previous studies of healthy volunteers as well as cardiovascular patients have noted either no gender difference or a higher platelet function in women [36–39]. No gender difference is established in Multiplate reference

values. Studies of platelet function in thrombocytopenic conditions are scarce, in part because of difficulties in differentiating platelet function and count, with the exception of flow cytometry. Still, it has been shown that there are multiple platelet defects in patients with acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) [40,41], that the degree of platelet function and activation predicts haemorrhage [42] and that patients with AML and MDS have lower platelet function than patients with immune thrombocytopenic purpura [43]. These studies did not elaborate on gender differences.

The effects of a prophylactic platelet transfusion, other than on the platelet count, are not completely understood; there is no clear association between platelet count and clinical bleeding in thrombocytopenic patients [7,8]. Several studies have attempted to assess the effectiveness of platelet transfusions by studying platelet function by various means [9,10]. One study found that rotational thromboelastometry detected improvements in coagulation after transfusion of platelets in thrombocytopenic patients, whereas the INR and APTT parameters remained unchanged [11]. Another study noted a negative correlation between thromboelastometry clot firmness increments and clinical bleeding status after transfusion, while no correlation with platelet increments was seen [44].

This study showed significantly higher increments in clot firmness (Extem MCF) one hour after transfusion in the female patients, as measured by thromboelastometry. It is unclear whether it is the gender or the low baseline platelet function that corresponds to this; the number of patients was too small to allow for further statistical analysis within each sex. The smaller plasma volume in women might create this difference, although a similarly pronounced difference in the platelet increment, also affected by the plasma volume, was not seen. Moreover, unlike gender, the baseline ADP parameter remained significantly correlated with clot firmness increment after four hours in this

dataset. Interestingly, ADP was also found to be the most sensitive parameter of the three Multiplate parameters when identifying patients with high platelet transfusion requirements in cardiac surgery [31]. The low platelet function and ADP levels might reflect a pathophysiological characteristic in these patients that suppress platelets and/or coagulation and is more frequently present in women. If so, this could account for part of the gender difference in Extem MCF increment. Nevertheless, the increase in thromboelastometry parameters and in particular the clot firmness was not correlated with the measured improvement in platelet function or count, implying that interactions between transfused platelets, native platelets, the coagulation cascade, and the patient's disease, cytokine levels, and white blood cells [45] are complex. Further research is needed to verify if baseline ADP or other markers of platelet function could play a role as a similar preprocedural test. The lower platelet function (measured by Multiplate) in women seen among these patients as well as the relationships between platelet function and transfusion outcome in this study should be investigated further by using flow cytometry in order to validate its clinical significance—is there a platelet defect that needs to be treated with more units of platelet concentrates or is the Multiplate device inadequate at low platelet counts? The platelet function also needs to be linked to bleeding complications which are infrequent in CVC insertions (see below). Additionally, renal function and platelet volume should be taken into account, as these parameters clinically affect coagulation [46,47].

The next question, which this study is not able to respond, is the clinical perspective: what role does thrombocytopenia play in CVC insertion, and are platelet transfusions necessary in order to prevent bleeding in the clinical perspective, and if so when? A few studies have attempted to investigate CVC insertions in patients with disorders of haemostasis. One retrospective study found that a platelet count below $50 \cdot 10^9$/L was associated with an increased risk of bleeding; however all the bleedings were easily corrected with a cutaneous suture and the authors did not recommend use of

preprocedural platelet or plasma transfusions [48]. Two retrospective studies have indicated that CVC insertion is safe without prophylactic transfusion in patients with platelet counts as low as 20-25 · 10^9/L [4,5]. In one of these studies, there was no significant difference in bleeding incidence between patients with and without abnormal results on coagulation tests, or between those with and without risk factors for bleeding; only a platelet count lower than 25 · 10^9/L was found to be an independent risk factor for bleeding (when compared with a count ≥ 100 · 10^9/L) [4]. The other study saw only three bleeding complications in 3,170 CVC insertions; none occurred in thrombocytopenic patients [5]. Doerfler et al. noted the platelet count as a risk factor for minor bleeding, but concluded CVC insertion is safe for patients with uncorrected disorders of haemostasis, if performed by experienced physicians [6]. Several authors believe that the experience of the physician is important [1,6,49]. For comparison, studies investigating the influence of elevated PT-INR found no association with increased bleeding complications [50,51]. A meta-analysis saw no evidence that routine coagulation tests predict bleeding in patients undergoing invasive procedures [52].

Altogether, CVC insertion seems to be a safe procedure in thrombocytopenic patients, there is no clear cut association between the level of thrombocytopenia and the risk of bleeding, and the role of platelet transfusions is unclear. Since transfusions carry infectious and immunological complications that put some patients in danger [3], clarification in this area of interest is required. It needs to be decided how coagulation should be measured, when and in which patients (if at all) platelet transfusions are to be administered, what the goal of platelet transfusions should be, and the number of platelet units required to achieve such a goal. The lack of change in routine coagulation parameters after transfusion seen in this study, as well as their inability to predict clinical bleeding noted in other studies indicate that current practice does not answer adequately to these issues and there is reason to review the use and dependence of these tests before invasive procedures. For

example, in this study, the Extem MCF parameter, which has been argued to be useful for assessing platelet transfusion requirements [53] and which increment has been linked to decreased clinical bleeding [44], was within reference range after transfusion in only four patients in this study, of which two already had normal values before transfusion. Moreover, the measured platelet function in this study was still very low after transfusion. More research is needed to show if thromboelastometry or platelet function parameters can predict bleeding and platelet transfusion effect in thrombocytopenic patients, even though these methods, especially thromboelastometry, have been validated in other situations of haemostasis management, sometimes favourably to conventional tests [17,28]. Even if platelet transfusions before CVC insertions would be discouraged in the future, the previously mentioned findings in this study might prove useful to the issue of whether using platelet transfusions in the setting of other invasive procedures.

The Multiplate device, like other methods of measuring platelet function (apart from flow cytometry), has been reported to show results reflecting the platelet count when the platelet count falls [54,55] as well as the concentration of red and white blood cells [54,56], although one study admits there are clear variations, with thrombocytopenic patients showing normal results and vice versa [57]. While there might well be an association between Multiplate parameters and platelet count in general (as seen in the reference group), there was no correlation between platelet count and Multiplate parameters *within* the group of thrombocytopenic patients in this study (see Figure 4), nor was there a significant correlation between increments in platelet count and Multiplate parameters after transfusion, suggesting that Multiplate parameters do not merely follow the platelet count in the setting of thrombocytopenia. In all, the Multiplate device might prove useful for patients with thrombocytopenia. As stated above, the device should be validated against flow cytometry when analysing these patients.

Both the characteristics of the platelet unit and patient characteristics influence the outcome of transfusion. There have been differing indications of how the storage time affects outcome, with most authors arguing that storage should be limited to five days [58]. The type of platelet product has also been under consideration, with recent studies noting no clear difference between aphaeresis versus pooled platelet concentrates in regard to transfusion intervals or clinical bleeding [59,60]. This study confirms the association between platelet age and platelet increment; however, no significant effect on ROTEM or Multiplate parameters could be demonstrated, even though significance was almost reached for increments in Multiplate parameters at four hours after transfusion.

Conclusion

The results from this study are preliminary and must be interpreted carefully as the number of patients is small. However, the results indicate that in patients with haematological malignancies and thrombocytopenia, prophylactic platelet transfusions have effect on thromboelastometry parameters as measured by ROTEM, of which the increase in clot firmness (Extem MCF) reached significance and was significantly larger when two platelet units were used. Increments in platelet function parameters, as measured with platelet aggregometry by the Multiplate device, were seen but did not reach statistical significance. The increments seen one hour after transfusion were not significantly different after four hours, which could indicate that the effects of platelet transfusion last until that time. It is also indicated that platelet age does not correlate with thromboelastometry or platelet aggregometry increments after transfusion. Among these patients, women had a higher increase in Extem MCF after platelet transfusion compared to men, which was not due to a higher increment in platelet count or function after platelet transfusion. Also, the measured platelet function before transfusion was lower in women. These findings could indicate gender differences in pathophysiology and response to platelet transfusion in this population. Further research is needed to clarify if ROTEM or Multiplate parameters could predict the effect of platelet transfusions and play a role in coagulation screening and transfusion strategies prior to invasive procedures, as current guidelines for prophylactic platelet transfusions in conjunction to CVC insertion should be reviewed. The Multiplate device might prove useful in the setting of thrombocytopenia, but should be validated against flow cytometry.

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Tables.

Table 1. Cross tabulation chart displaying the distribution of gender and diagnoses for both groups.

			Gender		Total
			Male	Female	
Transfusion group	Diagnosis	Highly malignant	4 (3 AML, 1 MCL)	6 (2 ALL, 2 LCBCL, 1 AML, 1 APL)	10
		Less malignant/Benign	2 (MDS, ITP)	2 (CLL, MDS)	4
Total			6	8	14
Reference group	Diagnosis	Cancer	12 (3 LCBCL, 2 AML, 2 testicular, undefined lymphoma, myeloma, plasmacytoma, pharynx, oesophagus)	13 (3 AML, 3 gynaecological, 2 neuroendocrine, HL, breast, pancreatic, sarcoma, duodenal)	25
		Not cancer	4 (WG, infection, respiratory insufficiency, undiagnosed)	3 (2 infections, WG)	7
Total			16	16	32

Legend: Exact significance in Fisher's exact test for the transfusion group is 1 (0.59 1-sided); the exact significances for the reference group is 1 (0.5). AML, acute myeloid leukemia; MCL, mantle-cell lymphoma; ALL, acute lyphatic leukemia; LCBCL, large-cellular B-cell lymphoma; APL, acute promyeolocytic leukemia; MDS, myelodysplastic syndrome; ITP, immune thrombocytopenic purpura; CLL, chronic lymphatic leukemia; HL, Hodgkin's lymphoma; WG, Wegener's granulomatosis (granulomatosis with polyangitis).

Table 2. Descriptive statistics and baseline data from CVC placement records for both groups.

		Transfusion group (*n=14*)	**Reference group (*n=32*)**
	Age	55.5 (25-80)	65 (20-87)
	BMI	25.3 (18.7-29.2)	25.6 (19.6-36)
	Temperature	36.8 (36-40)	36.8 (36.0-37.8)
Routine	Haemoglobin (g/L)	96 (81-121)	115.5 (89-156)
	WBC (· 10^9/L)	4.6 (0.1-187)	7.4 (2.3-53.7)
	PT-INR	1.15 (1.0-1.5)	1.1 (0.9-1.5)
	APTT (s)	32 (26-41)	35 (26-59)
	Platelets (· 10^9/L)	24 (8-43)	230 (36-687)
	Fibrinogen (g/L)	2.9 (1.5-7.1)	4.2 (1.9-7.4)
ROTEM	Extem CT (s)	63 (47-216)	43 (31-88)
	Extem CFT (s)	321 (47-4429)	68.5 (16-166)
	Extem AA (°)	69 (13-82)	78.5 (59-87)
	Extem MCF (mm)	38 (12-56)	66 (53-84)
	Fibtem MCF (mm)	12.5 (3-38)	22 (12-61)
	Extem-Fibtem MCF (mm)	17.5 (9-28)	43 (23-51)
Multiplate	ADP (AUC)	3 (0-27)	82.5 (19-202)
	COL (AUC)	7.5 (0-50)	102 (23-168)
	TRAP (AUC)	4 (0-45)	126 (34-213)
CVC indication	Chemotherapy	9 (64.3%)	19 (59.4%)
	SCT	1 (7.1%)	1 (3.1%)
	Other	4 (28.6%)	12 (37.5%)
CVC locale	Internal jugular vein	7 (50%)	20 (66.7%)
	Subclavian vein	7 (50%)	9 (30.0%)
	Other	0	1 (3.3%)
No. of attempts	1	10 (71.5%)	17 (70.8%)
	2	1 (7.1%)	3 (12.5%)
	>2	3 (21.4%)	4 (16.7%)
Complications	No	13 (92.9%)	29 (96.7%)
	Yes	1 (7.1%)	1 (3.3%)

Legend: Laboratory data are presented as medians and range in parentheses. CVC, central venous catheter; WBC, white cell blood count; PT-INR, prothrombin international normalised ratio; aPTT, activated partial thromboplastin time; Extem, tissue factor-activated assay; Fibtem; tissue factor-activated assay with platelet inhibition; CT, clotting time; CFT, clot formation time; AA, alpha-angle; MCF, maximum clot firmness; ADP, adenosine diphosphate-agonised assay; COL, collagen-agonised assay; TRAP; thrombin receptor-activating peptide-agonised assay; SCT, stem cell transplantation. For the reference group, data from CVC charts were missing in two cases and incomplete in six cases.

Table 3. Medians and ranges for coagulation parameters before, one hour after and four hours after transfusion in the transfusion group (only patients receiving one platelet unit and no plasma). Comparison between CVC and steel needle samples for the reference group.

	Transfusion group			**Reference group**	
	Before transfusion	**One hour**	**Four hours**	**CVC**	**Steel needle**
Platelets (· 10^9/L)	24 (8-41)	40 (19-55)*	35 (16-52)*	230 (36-687)	NA
PT-INR	1.1 (0.9-1.4)	1.1 (0.9-1.4)	1.15 (1.0-1.4)	1.1 (0.9-1.5)	NA
aPTT (s)	31.5 (26-40)	30 (25-42)	33.5 (28-41)	35 (26-59)	NA
Fibrinogen (g/L)	3.8 (1.8-7.1)	3.9 (1.8-6.9)	3.9 (1.8-6.6)	4.2 (1.9-7.4)	NA
Extem CT (s)	53.5 (47-83)	48.5 (45-85)	56 (43-84)	43 (31-88)	44 (36-76)
Extem CFT (s)	170 (67-859)	126.5 (67-383)	150 (51-390)	68.5 (16-166)	65 (30-159)
Extem AA (°)	80 (29-82)	79.5 (39-81)	80 (42-82)	78.5 (59-87)	79 (60-84)*
Extem MCF (mm)	42.5 (26-56)	47.5 (36-57)*	47 (35-61)	66 (53-84)	68 (52-83)*
Fibtem MCF (mm)	22.5 (9-38)	23.5 (10-36)	24.5 (7-35)	22 (12-61)	22 (9-58)
Extem – Fibtem MCF (mm)	17.5 (11-26)	22.5 (20-28)	25 (17-29)	43 (23-51)	NA
ADP (AUC)	5 (0-27)	7 (0-45)	5 (0-99)	82.5 (19-202)	100 (17-140)
COL (AUC)	12 (0-50)	16 (0-91)	13 (0-124)	102 (23-168)	109 (33-155)
TRAP (AUC)	4 (0-45)	21 (0-173)	13 (0-163)	126 (34-213)	137 (38-199)

Legend: Cells containing an asterisk denote statistically significant differences in distribution ($p < 0.05$) (compared to before transfusion in the transfusion group; compared to CVC samples in the reference group), after adjusting for the number of times of measurement (for the transfusion group). For the reference group, data from all patients are presented, but data from only 19 of those patients were used for statistical comparison with steel needle samples. CVC, central venous catheter; WBC, white cell blood count; PT-INR, prothrombin international normalised ratio; aPTT, activated partial thromboplastin time; Extem, tissue factor-activated assay; Fibtem; tissue factor-activated assay with platelet inhibition; CT, clotting time; CFT, clot formation time; AA, alpha-angle; MCF, maximum clot firmness; ADP, adenosine diphosphate-agonised assay; COL, collagen-agonised assay; TRAP; thrombin receptor-activating peptide-agonised assay.

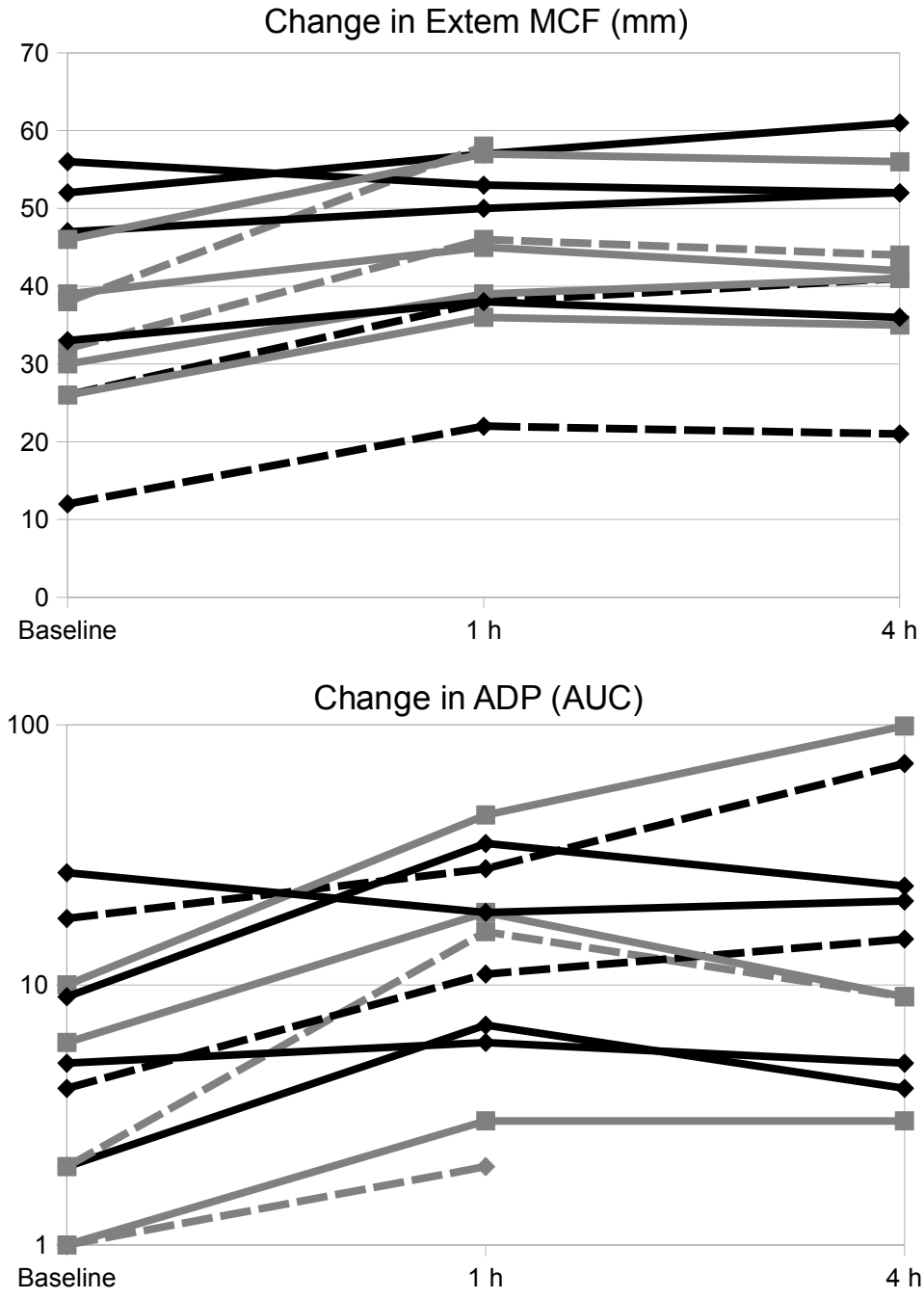
Table 4. Comparisons between gender and the number of platelets units administered in the transfusion group.

	Gender (1 platelet unit)		**No. of platelet units**	
	Men	Women	1 bag	2 bags
Δ PT-INR	0 (-0.1–0.1)	0 (-0.2 – 0)	0 (-0.2 – 0.1)	0 (0 – 0.2)
Δ APTT (s)	0 (0 – 2)	-1 (-14 – 2)	0 (-14 – 2)	-4 (-10 – -1)
Δ Platelets (· 10^9/L)	13.5 (7 – 24)	18 (6 – 22)	16 (6 – 24)	21 (19 – 43)
Δ Fibrinogen (g/L)	-0.2 (-0.2 – -0.1)	-0.05 (-0.3 – 0)	-0.1 (-0.3 – 0)	-0.55 (-0.8 – -0.3)
Δ Extem CT (s)	-3 (-5 – -2)	-2 (-14 – 2)	-3 (-14 – 2)	-13.5 (-43 – 10)
Δ Extem CFT (s)	-11.5 (-194 – 22)	-139.5 (-497 – 9)	-43.5 (-497 – 22)	-462.5 (-2927 – 20)
Δ Extem AA (°)	-0.5 (-3 – 0)	-0.5 (-2 – 10)	-0.5 (-3 – 10)	12.5 (-3 – 21)
Δ Extem MCF (mm)	4 (-3 – 8)*	9.5 (6 – 11)*	5.5 (-3 – 11)*	13 (10 – 20)*
Δ Fibtem MCF (mm)	-0.5 (-8 – 3)	1.5 (-1 – 6)	1 (-8 – 6)	0 (-3 – 2)
Δ Extem-Fibtem MCF (mm)	2 (-1 – 13)	7.5 (3 – 10)	5.5 (-1 – 13)	12 (8 – 17)
Δ ADP (AUC)	3 (-8 – 26)	2 (0 – 35)	2 (-8 – 35)	8.5 (1 – 14)
Δ COL (AUC)	12.5 (-3 – 41)	2 (0 – 46)	6 (-3 – 46)	28 (4 – 45)
Δ TRAP (AUC)	9.5 (-9 – 100)	1 (-1 – 128)	6 (-9 – 128)	29.5 (2 – 56)

Legend. Laboratory data are presented as medians and range in parentheses. Cells containing an asterisk denote statistically significant differences in distribution between the two groups in each analysis (men vs. women; 1 bag vs. 2 bags; $p < 0.05$). PT-INR, prothrombin international normalised ratio; aPTT, activated partial thromboplastin time; Extem, tissue factor-activated assay; Fibtem; tissue factor-activated assay with platelet inhibition; CT, clotting time; CFT, clot formation time; AA, alpha-angle; MCF, maximum clot firmness; ADP, adenosine diphosphate-agonised assay; COL, collagen-agonised assay; TRAP; thrombin receptor-activating peptide-agonised assay. Δ denotes increments.

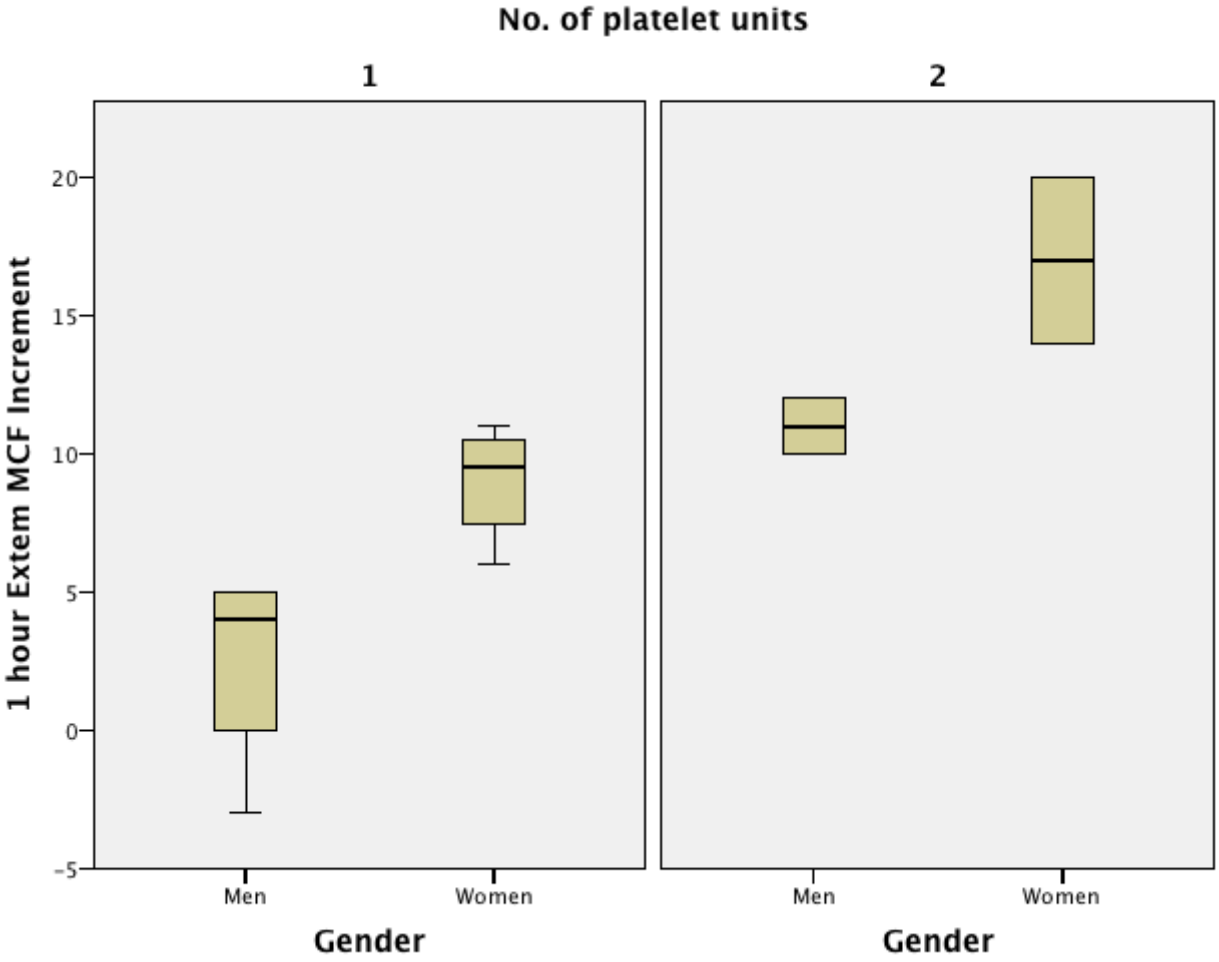
Figures

Figure 1. Change in clot firmness and ADP-agonised platelet function as seen in individual patients before and after platelet transfusion.



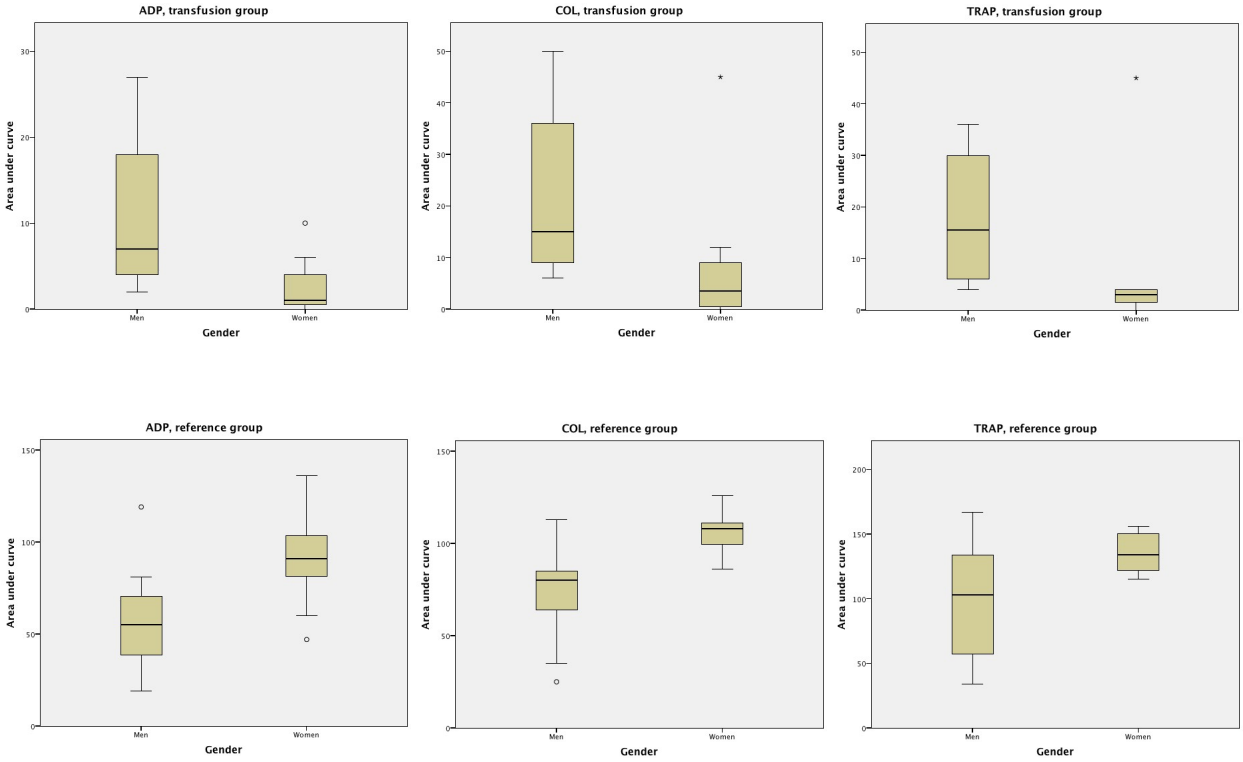
Grey lines indicate female patients; jagged lines indicate patients receiving two platelet units. Patients receiving plasma or having missing values are omitted. The lower chart has a logarithmic scale, and two patients with zero values are omitted. Extem; tissue factor-activated assay; MCF, maximum clot firmness (in mm); ADP, adenosine diphosphate-agonised assay; AUC, area under curve.

Figure 2. The difference in maximum clot firmness increments one hour after transfusion divided by gender and number of transfused platelet units.



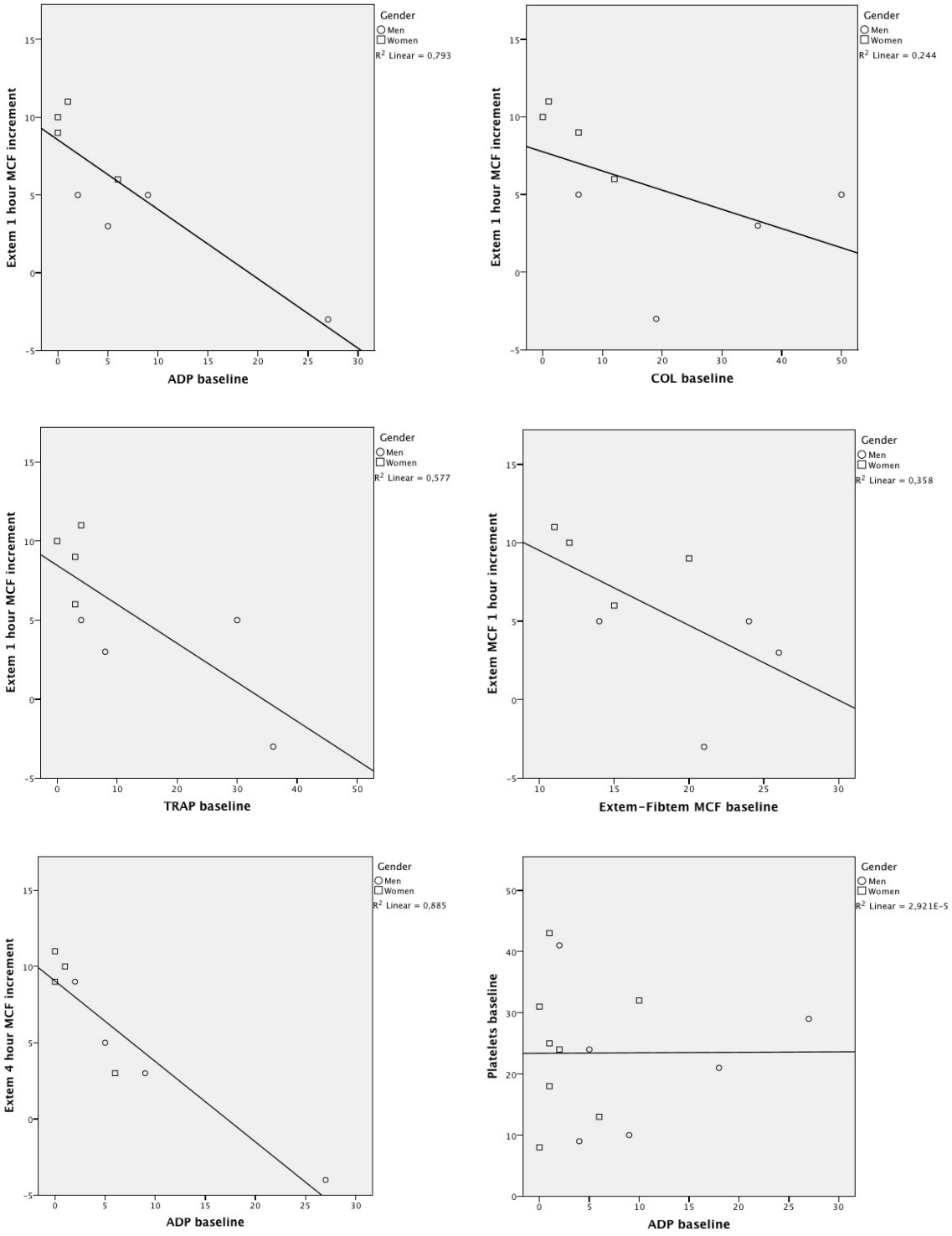
Legend: The patient receiving plasma transfusion was excluded from these calculations. Extem; tissue factor-activated assay; MCF, maximum clot firmness (in mm).

Figure 3. Gender differences in baseline Multiplate values for the transfusion and reference groups.



Legend: Differences in distribution reached significance ($p < 0.05$) for ADP, COL and TRAP assays in the transfusion group (top row) and for ADP and COL in the reference group (excluding patients under LMWH treatment). ADP, adenosine diphosphate-agonised assay; COL, collagen-agonised assay; TRAP; thrombin receptor-activating peptide-agonised assay.

Figure 4. Scatter dot diagrams for selected significant correlations in the transfusion group.



Legend: unit for ADP, COL, TRAP scales is area under curve. Units for Extem-Fibtem MCF scale is millimetres. Unit for platelet count is $\cdot 10^9$/L. ADP, adenosine diphosphate-agonised assay; COL, collagen-agonised assay; TRAP; thrombin receptor-activating peptide-agonised assay. Extem, tissue factor-activated assay; Fibtem; tissue factor-activated assay with platelet inhibition; MCF, maximum clot firmness.

Appendix.

Correlation tables with selected variables.

Transfusion group

		Female	Platelets	Fibrinogen	Ex-Fib MCF	ADP	COL	TRAP	Platelet age	API 1h	Δ MCF 1h	Δ ADP 1h	Δ COL 1h	Δ TRAP 1h	Δ Fibr. 1 h	API 4h	Δ MCF 4h	Δ ADP 4h	Δ COL 4h	Δ TRAP 4h	Δ Fibr. 4h
Female	*r*	1	-,173	,000	-,655	-,435	-,478	-,480	,046	,173	,878**	,130	,000	,000	,437	,058	,552	,221	-,043	,173	,144
Platelets	*r*	-,173	1	,071	,119	,167	,134	,395	-,158	-,183	-,359	-,017	,017	-,033	-,342	,171	,241	,009	-,042	-,133	-,036
Fibrinogen	*r*	,000	,071	1	-,314	,523	-,214	,393	,189	-,036	-,086	-,054	-,234	-,179	-,270	-,148	-,429	-,148	-,179	-,071	-,321
Ex-Fib MCF	*r*	-,655	,119	-,314	1	,587	,910**	,615	,230	-,286	-,790*	-,096	,299	,262	-,638	,037	-,530	-,123	,335	,048	-,143
ADP	*r*	-,435	,167	,523	,587	1	,803**	,840**	,106	-,109	-,771*	,315	,395	,393	-,891**	,074	-,927**	,282	,370	,343	-,414
COL	*r*	-,478	,134	-,214	,910**	,803**	1	,793*	-,066	,042	-,789*	,437	,651	,628	-,865*	,390	-,697	,423	,651	,519	-,036
TRAP	*r*	-,480	,395	,393	,615	,840**	,793*	1	-,199	,202	-,758*	,278	,384	,370	-,955**	,454	-,598	,266	,430	,345	-,286
Platelet age	*r*	,046	-,158	,189	,230	,106	-,066	-,199	1	-,764*	-,328	-,450	-,291	-,264	,219	-,764*	-,381	-,525	-,278	-,395	-,510
API 1h	*r*	,173	-,183	-,036	-,286	-,109	,042	,202	-,764*	1	,479	,368	,192	,200	-,270	,854**	,374	,426	,251	,367	,143
Δ MCF 1h	*r*	,878**	-,359	-,086	-,790*	-,771*	-,789*	-,758*	-,328	,479	1	,157	-,096	-,084	,638	,334	,727*	,253	-,102	,168	,486
Δ ADP 1h	*r*	,130	-,017	-,054	-,096	,315	,437	,278	-,450	,368	,157	1	,920**	,929**	-,555	,614	-,152	,983**	,866**	,937**	,252
Δ COL 1h	*r*	,000	,017	-,234	,299	,395	,651	,384	-,291	,192	-,096	,920**	1	,996**	-,636	,565	-,248	,898**	,975**	,946**	,342
Δ TRAP 1h	*r*	,000	-,033	-,179	,262	,393	,628	,370	-,264	,200	-,084	,929**	,996**	1	-,631	,561	-,277	,894**	,979**	,950**	,286
Δ Fibr. 1h	*r*	,437	-,342	-,270	-,638	-,891**	-,865*	-,955**	,219	-,270	,638	-,555	-,636	-,631	1	-,524	,841*	-,524	-,631	-,595	,234
API 4h	*r*	,058	,171	-,148	,037	,074	,390	,454	-,764*	,854**	,334	,614	,565	,561	-,524	1	,374	,625	,634	,610	,296
Δ MCF 4h	*r*	,552	,241	-,429	-,530	-,927**	-,697	-,598	-,381	,374	,727*	-,152	-,248	-,277	,841*	,374	1	-,056	-,224	-,169	,600
Δ ADP 4h	*r*	,221	,009	-,148	-,123	,282	,423	,266	-,525	,426	,253	,983**	,898**	,894**	-,524	,625	-,056	1	,829**	,936**	,296
Δ COL 4h	*r*	-,043	-,042	-,179	,335	,370	,651	,430	-,278	,251	-,102	,866**	,975**	,979**	-,631	,634	-,224	,829**	1	,937**	,286
Δ TRAP 4h	*r*	,173	-,133	-,071	,048	,343	,519	,345	-,395	,367	,168	,937**	,946**	,950**	-,595	,610	-,169	,936**	,937**	1	,321
Δ Fibr. 4h	*r*	,144	-,036	-,321	-,143	-,414	-,036	-,286	-,510	,143	,486	,252	,342	,286	,234	,296	,600	,296	,286	,321	1

Legend. One asterisk (*) denotes significance at the 0.05 level (2-tailed), two asterisks (**) denote significance at the 0.01 level (2-tailed). Only patients receiving one unit of platelets and not plasma were analysed. Hb, haemoglobin; WBC, white cell blood count; fibr., fibrinogen; PT-INR, prothrombin international normalised ratio; aPTT, activated partial thromboplastin time; API, absolute platelet increment; Extem, tissue factor-activated assay; Fibtem; tissue factor-activated assay with platelet inhibition; CT, clotting time; CFT, clot formation time; AA, alpha-angle; MCF, maximum clot firmness; ADP, adenosine diphosphate-agonised assay; COL, collagen-agonised assay; TRAP; thrombin receptor-activating peptide-agonised assay. Δ denotes increments.

Reference group

		Hb	WBC	PT-INR	aPTT	Platelets	Fibrinogen	Extem CT	Extem CFT	Extem AA	Extem MCF	Fibtem MCF	Extem - Fibtem MCF	ADP	COL	TRAP	Female	Cancer
Haemoglobin	*r*	1	,201	-,193	-,095	,431*	,104	-,396	,117	-,310	,158	-,304	,457*	,359	,387	,573**	-,043	,230
WBC	*r*	,201	1	-,163	,082	-,016	,144	-,001	,336	-,223	-,022	-,157	,042	,400	,529*	,582**	,316	,063
PT-INR	*r*	-,193	-,163	1	,167	-,261	,041	-,128	-,049	,046	-,058	,198	-,295	-,288	-,230	-,398	-,283	,065
aPTT	*r*	-,095	,082	,167	1	-,423*	,760**	,421	-,071	,335	,060	,600**	-,572**	-,322	-,388	-,306	-,144	-,136
Platelets	*r*	,431*	-,016	-,261	-,423*	1	-,436	-,334	-,479*	,143	,670**	-,130	,811**	,718**	,624**	,409	,322	,532*
Fibrinogen	*r*	,104	,144	,041	,760**	-,436	1	,228	,018	,244	-,051	,713**	-,637*	-,339	-,278	-,320	-,170	-,591*
Extem CT	*r*	-,396	-,001	-,128	,421	-,334	,228	1	-,310	,410	-,043	,397	-,454*	-,367	-,447*	-,312	-,253	-,442*
Extem CFT	*r*	,117	,336	-,049	-,071	-,479*	,018	-,310	1	-,880**	-,830**	-,594**	-,244	-,174	-,002	,341	,007	-,115
Extem AA	*r*	-,310	-,223	,046	,335	,143	,244	,410	-,880**	1	,713**	,792**	-,071	-,025	-,190	-,446*	-,072	-,010
Extem MCF	*r*	,158	-,022	-,058	,060	,670**	-,051	-,043	-,830**	,713**	1	,489*	,434*	,533*	,376	-,010	,187	,410
Fibtem MCF	*r*	-,304	-,157	,198	,600**	-,130	,713**	,397	-,594**	,792**	,489*	1	-,507*	-,186	-,299	-,494*	-,043	-,220
Extem - Fibtem MCF	*r*	,457*	,042	-,295	-,572**	,811**	-,637*	-,454*	-,244	-,071	,434*	-,507*	1	,656**	,639**	,443*	,194	,493*
ADP	*r*	,359	,400	-,288	-,322	,718**	-,339	-,367	-,174	-,025	,533*	-,186	,656**	1	,886**	,687**	,580**	,470*
COL	*r*	,387	,529*	-,230	-,388	,624**	-,278	-,447*	-,002	-,190	,376	-,299	,639**	,886**	1	,723**	,653**	,314
TRAP	*r*	,573**	,582**	-,398	-,306	,409	-,320	-,312	,341	-,446*	-,010	-,494*	,443*	,687**	,723**	1	,423*	,198
Female	*r*	-,043	,316	-,283	-,144	,322	-,170	-,253	,007	-,072	,187	-,043	,194	,580**	,653**	,423*	1	,132
Cancer	*r*	,230	,063	,065	-,136	,532*	-,591*	-,442*	-,115	-,010	,410	-,220	,493*	,470*	,314	,198	,132	1

Legend. One asterisk (*) denotes significance at the 0.05 level (2-tailed), two asterisks (**) denote significance at the 0.01 level (2-tailed). Only patients without low molecular heparin treatment were analysed. Hb, haemoglobin; WBC, white cell blood count; fibr., fibrinogen; PT-INR, prothrombin international normalised ratio; aPTT, activated partial thromboplastin time; API, absolute platelet increment; Extem, tissue factor-activated assay; Fibtem; tissue factor-activated assay with platelet inhibition; CT, clotting time; CFT, clot formation time; AA, alpha-angle; MCF, maximum clot firmness; ADP, adenosine diphosphate-agonised assay; COL, collagen-agonised assay; TRAP; thrombin receptor-activating peptide-agonised assay. Δ denotes increments.

Missing data for laboratory analyses.

	PT-INR, aPTT, platelets	Fibrinogen	ROTEM
Transfusion group	Missing 4 hour samples for 2 patients. Missing baseline aPTT for 1 patient.	Missing samples for 2 patients. Missing baseline sample for 1 patient.	No analysis for 1 patient due to technical error.
Reference group		Missing samples for 9 patients. >7.5 g/L in 1 patient.	Steel needle sampling cancelled after 19 patients.

Reference ranges for ROTEM and Multiplate assays.

Extem CT	Extem CFT	Extem AA	Extem MCF	Fibtem MCF	Extem-Fibtem MCF	ADP	COL	TRAP
38 – 79 s	34 – 159 s	63 – 83°	50 – 72 mm	9 – 25 mm	41 – 48 mm	57 – 113 AUC	72 – 125 AUC	84 – 128 AUC

The reference ranges are as given by the manufacturers (Pentapharm, Switzerland for ROTEM and Verum Diagnostica GmbH, Germany for Multiplate).