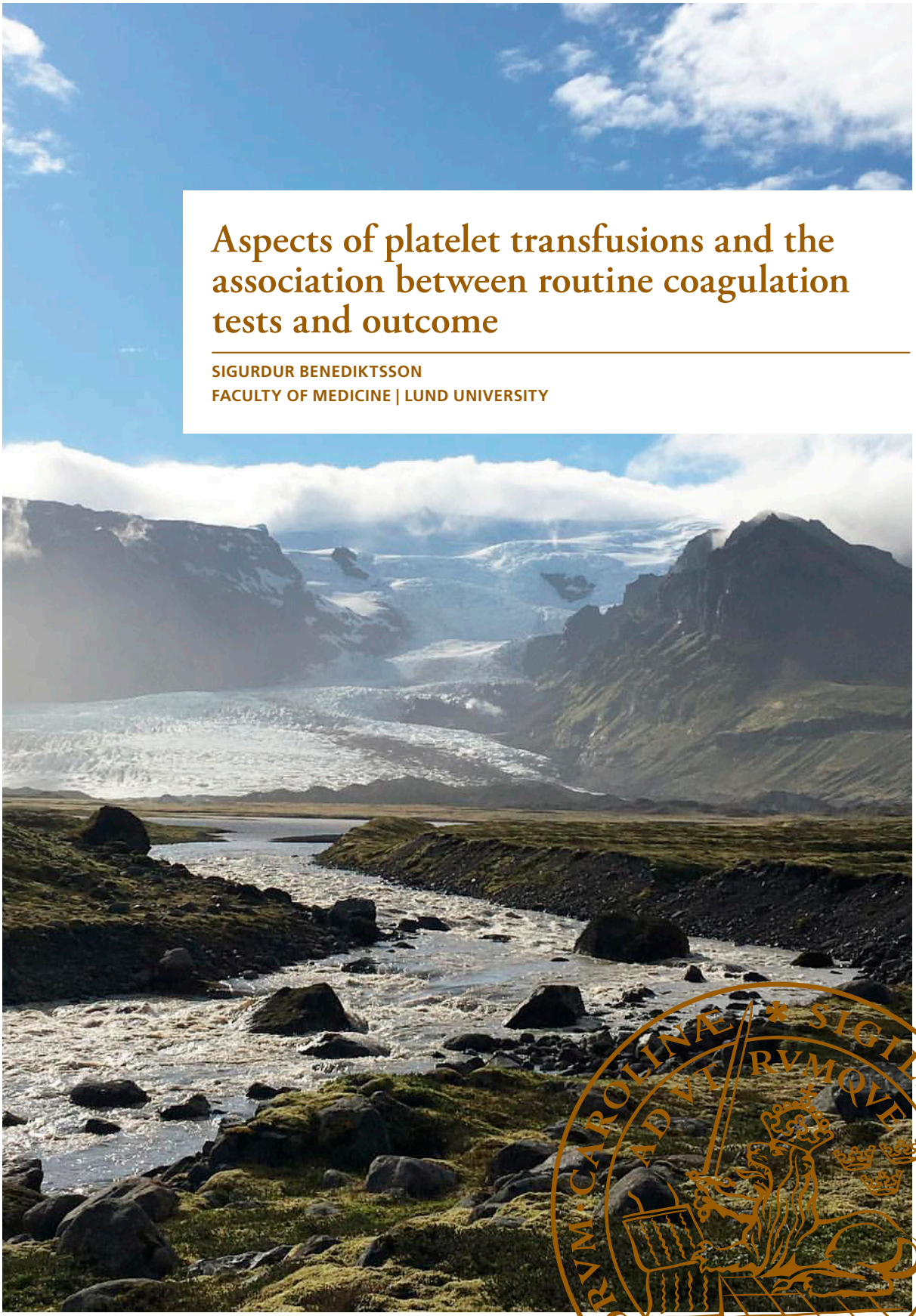
Aspects of platelet transfusions and the association between routine coagulation tests and outcome

Benediktsson, Sigurdur

2019

Aspects of platelet transfusions and the association between routine coagulation tests and outcome

SIGURDUR BENEDIKTSSON
FACULTY OF MEDICINE | LUND UNIVERSITY



Printed by Media-Tryck, Lund 2019 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2019:119
ISBN 978-91-7619-848-3
ISSN 1652-8220

Aspects of platelet transfusions and the association between routine coagulation tests and outcome

Aspects of platelet transfusions and the association between routine coagulation tests and outcome

Sigurdur Benediktsson

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Belfrage lecture hall at Skåne University Hospital in Lund.
December 6, 2019, 9:00 AM.

Faculty opponent
Christian Rylander, Sahlgrenska University Hospital

Organization LUND UNIVERSITY	**Document name** DOCTORAL DISSERTATION
	Date of issue December 6th 2019
Author Sigurdur Benediktsson	Sponsoring organization

Title Aspects of platelet transfusions and the association between routine coagulation tests and outcome

Abstract

Patients with chemotherapy-induced bone marrow aplasia often develop severe thrombocytopenia which requires platelet transfusion. However, up to 25% of the patients show a less than adequate response to the transfusion. This phenomenon is called platelet refractoriness and is associated with increased morbidity and mortality as well as longer hospital stays and increased costs.

It is well documented in the critical care setting that both a low platelet count (PLC) and a decline in PLC is associated with increased mortality. However, it is unknown whether the results of routine coagulation tests on admission to ICU may predict mortality and morbidity.

Study I, performed on hematological patients receiving platelet transfusions, showed that platelet increment, measured as corrected count increment (CCI), declined in a linear manner from hours 1 to 24 post-transfusion. In patients with bone marrow aplasia due to chemotherapy for either acute leukemia or stem cell transplantation, the CCI declined 2.0% ± 0.6% (mean ± 95% confidence interval) per hour. In patients who were transfused prior to an intervention, the CCI declined 2.8% ± 1.2% per hour.

Study II, a sub-study of Study I, showed that platelet increment did not correlate with endothelial damage measured with the endothelial cell markers syndecan-1, soluble thrombomodulin and vascular endothelial growth factor.

Study III, a retrospective cohort study, showed that prolonged APTT and increased PT-INR on admission to the ICU correlated with increased mortality in patients with severe sepsis or septic shock.

Study IV, a retrospective study on an unselected ICU cohort, showed that prolonged APTT on admission to the ICU correlated with increased mortality and the need for vasopressors and renal replacement therapy. Increased PT-INR was, on the other hand, not associated with mortality, but was nevertheless associated with the need for vasopressors and invasive ventilation.

The findings in Studies III and IV were adjusted for severity of illness by adding the simplified acute physiology score 3 (SAPS 3) to the regression models. This means that APTT and PT-INR on admission to the ICU have a prognostic value which is not accounted for in the SAPS 3 model.

Key words

Classification system and/or index terms (if any)

Supplementary bibliographical information		**Language** English
ISSN and key title 1652-8220		**ISBN** 978-91-7619-848-3
Recipient's notes	**Number of pages** 59	Price
	Security classification	

Signature  Date 2019-10-31

Aspects of platelet transfusions and the association between routine coagulation tests and outcome

Sigurdur Benediktsson
MD

LUND
UNIVERSITY

Cover: Kvíárjökull glacier, photo by Kristbjörg Heidur Olsen

Lund University
Faculty of Medicine
Department of Clinical Sciences
Anaesthesiology and Intensive Care

ISSN 1652-8220
ISBN 978-91-7619-848-3
Lund University, Faculty of Medicine Doctoral Dissertation Series 2019:119

Printed in Sweden by Media-Tryck, Lund University
Lund 2019

To my family

Table of contents

Original studies

This thesis is based on the following studies, which will be referred to in the text by their Roman numerals.

I Benediktsson S, Lazarevic V, Nilsson L, Kjeldsen-Kragh J, Schött U, Kander T. Linear decline of corrected platelet count increment within 24 hours after platelet transfusion in haematological patients: a prospective observational study. Eur J Haematol. 2017;99(6):559-68.

II Benediktsson S, Kander T, Ostrowski SR, Johansson PI, Thomas OD, Schött U. Platelet increment is not associated with endothelial damage in haematological patients: a prospective observational study. Scand J Clin Lab Invest. 2019;79(6):395-403.

III Benediktsson S, Frigyesi A, Kander T. Routine coagulation tests on ICU admission are associated with mortality in sepsis: an observational study. Acta Anaesthesiol Scand. 2017;61(7):790-6.

IV Benediktsson S, Hansen C, Frigyesi A, Kander T. Coagulation tests on admission correlate with mortality and morbidity in general ICU patients: an observational study. *Resubmitted after revision.* Acta Anaesthesiol Scand.

Abbreviations

AKIN-crea	Acute kidney injury network–creatinine score
AML	Acute myeloblastic leukemia
APACHE	Acute physiology and chronic health evaluation
APTT	Activated partial thromboplastin time
BC	Buffy coat
CCI	Corrected count increment
CRRT	Continuous renal replacement therapy
CVC	Central venous catheter
DAF	Days alive and free
DIC	Disseminated intravascular coagulation
PLC	Platelet count
PRP	Platelet rich plasma
PT-INR	Prothrombin time–international normalized ratio
SAPS	Simplified acute physiology score
sTM	Soluble thrombomodulin
TA-GVHD	Transfusion-associated graft-versus-host disease
VEGF	Vascular endothelial growth factor

Background

Platelet transfusions

History of hemostasis

The word hemostasis has its origins in ancient Greek and means motionlessness, or stopping, of the blood. The first written attempt to try to explain the physiology of coagulation is found in the Hippocratic collection (5th century BCE). Hippocrates (c. 460–370 BCE) hypothesized that a normal volume of blood was the basis for a healthy life and that excess blood volume would make a patient sick. He also described what he called "the solid blood" and introduced the term "thrombus." The Greek philosopher Plato (c. 428–347 BCE) believed that blood contained "fibers" which facilitated the formation of the "thrombus," upon which Aristotle (384–322 BCE) later elaborated, stating that blood would not clot if the "solid fibers" were removed [1].

The "solid fibers" hypothesis turned out to be quite accurate when in 1905 Morawitz proposed that four substances are involved in the formation of the clot [2]. His formula stated that fibrinogen (factor I) is converted to fibrin, which is facilitated by thrombin, which is produced from prothrombin (factor II) in the presence of "thrombokinase" (factor III) and calcium (factor IV). Currently, the defined coagulation factors and cofactors are more than twenty, and knowledge on the coagulation system is constantly evolving [3, 4].

History of platelets

In the late 19th century, scientists began to describe "particles" in blood other than erythrocytes and leukocytes. Max Schultze was the first to describe platelets as uncolored spherules or granules which he called "granular masses." The first scientist to describe platelets as the third morphological component in the blood alongside red and white cells was Giulio Bizzozero in 1869. Later on, he also demonstrated the function of the platelets by inserting a fine needle into the arterial walls of anesthetized guinea pigs and rabbits. He observed that the platelets gathered at the damaged spot, quickly aggregated from a few to hundreds, attracted white blood cells, and at last built a clot obstructing the blood flow in the vessel. Bizzozero wrote:

> whereas under normal conditions the platelets float isolated in the plasma, when subject to an influence that leads to thrombosis, they adhere to one another to form an accumulation. The blood platelets, free in the blood stream and being hurried along are held up by other platelets that they come into contact with as they become stickier than they are under normal condition. [5]

Bizzozero's study was the first physiological study on platelets in history and was to become the fundament for future research in the field [6].

History of platelet transfusions

Even if the existence of platelets had been known since the 1870s, the first successful platelet transfusion did not take place until 1910. W.W. Duke wrote a case report describing a 20-year-old man who was suffering from extreme mucocutaneous bleeding due to thrombocytopenia with a platelet count (PLC) of only 6×10^9/L. On vital indication, he was given a "large" amount of whole blood which dramatically ceased the bleeding and increased his PLC to 123×10^9/L [7].

Due to many obstacles along the way, platelet concentrates did not become readily available for the treatment of thrombocytopenia until 1959. This revolutionizing treatment option lowered the percentage of leukemia patients dying from hemorrhage from 67% to 37% [8]. Another milestone was achieved when Scott Murphy and Frank Gardner showed in 1970 that platelets could be stored at 22 ± 2°C for up to 72 hours without losing their hemostatic function [9]. Further improvements have since been made, and nowadays, platelet concentrates can be stored for up to seven days before being administered [7].

Preparation of platelet concentrates

The most straightforward way to transfuse platelets to a thrombocytopenic patient is to infuse whole blood, as described above. However, the amount of blood needed to obtain a considerable number of platelets in the transfusion can easily cause transfusion-related circulatory overload (TACO). Additionally, due to the storage of whole blood in refrigerators, the platelets lose their function and the labile coagulation factors (factors V and VIII) deteriorate [10, 11]. For these reasons and many more, platelet concentrates are now manufactured either from whole blood, which requires four to six donors, or by apheresis from a single donor.

In the apheresis method, the blood from the donor is processed through a cell separator which separates the platelets with a small amount of plasma from other parts of the blood and collects them directly into a concentration bag. The procedure is based on an in-line centrifuge and delivers a leucocyte-reduced product. Normally, two units of platelets are obtained from a single donor.

Platelet concentrates from whole blood can either be produced with the platelet-rich-plasma (PRP) method or with the buffy-coat (BC) method, which is the most commonly used technique in Europe. Whole blood is first collected into a bag containing an anticoagulant–preservative solution to prevent it from clotting. In Sweden, a solution consisting of citrate, phosphate, and dextrose is used. The citrate binds to calcium and thereby inhibits both the coagulation system and platelets from activating. The phosphate is needed for ATP synthesis, keeping the Na^+/K^+-pump in the cell membrane functioning properly, and dextrose is an energy source for the cells [12-14].

Both whole blood methods take advantage of the different specific gravities of erythrocytes (1.08–1.09), plasma (1.03–1.04), and platelets (1.023) to accomplish the separation. In the PRP method, whole blood is subjected to a soft spin to produce PRP, and then PRP is spun down further at a higher speed to obtain the platelet concentrate. The platelets are then stored either straight in a small amount of plasma or diluted in a synthetic medium [13, 15].

In the BC method, whole blood is centrifuged at a high speed and then the lighter layer containing plasma and the heavy layer containing erythrocytes can be separated from the buffy coat consisting of leukocytes and platelets in a small amount of plasma. After overnight storage of the BCs, four to six BCs are pooled, platelet additive solution (PAS) is added and the pool is centrifuged. Finally, an automated extractor lets the platelet-rich supernatant pass through a leukocyte filter before it reaches the final platelet storage container. Four units of whole blood are needed to obtain at least 200×10^9 platelets which is considered as the minimum platelet content for one transfusion unit [13, 15].

Unlike erythrocytes, platelets are very dependent on aerobic metabolism. Studies have shown that under anaerobic conditions, the platelets lose their function and shape [16]. Since the optimal storage temperature is 22 ± 2°C, the metabolic rate is quite high, and thus there is a need for storage bag permeability in order for both oxygen to flow in and for carbon dioxide to flow out. The bags are therefore produced in a large but thin format and from a material with good gaseous permeability [12, 14].

Prevention of complications

Bacterial infection transmitted via transfusion is a rare but very severe complication which accounts for > 10% of transfusion-associated fatalities. Studies have shown that the risk for transfusion-associated bacteremia is higher after platelet transfusion than erythrocyte transfusion. Even small numbers of bacteria introduced to a platelet concentrate during preparation can grow excessively while the bag is stored for up to a week at room temperature [17, 18].

Several methods have therefore been developed to detect bacterial growth in platelet concentrates. This is primarily done with an automated bacterial culture which is drawn from the bag the day after blood or platelet collection. Other passive methods include analysis of the oxygen tension and glucose levels or detection by dielectrophoresis and cytometry [14].

Another method to prevent bacterial contamination is pathogen reduction, for example by adding amotosalen to the concentrate which binds to the helical regions of DNA and RNA. When exposed to ultraviolet light, the amotosalen cross-links with nucleic acid, thereby preventing replication [19].

Transfusion-associated graft-versus-host disease (TA-GVHD) is another severe complication of platelet transfusion. When this occurs, T-lymphocytes from the donor initiate an immune response against the recipient's lymphoid tissue with catastrophic consequences. The risk of TA-GVHD depends on the degree of HLA disparity between the donor and the recipient and on the number and viability of the foreign lymphocytes. Patients with immunodeficiency are at the highest risk of developing this syndrome, and the mortality rate is > 90% [20].

Leucocyte filtration is used to reduce the number of leucocytes in platelet concentrates and is now standard practice in Sweden. However, this method is not sufficient to prevent TA-GVHD in vulnerable patients. To prevent the remaining lymphocytes from proliferating, the concentrates are irradiated prior to transfusion to patients with immunosuppression [21].

Platelet refractoriness

The PLC of an average adult patient who is transfused with one unit of platelets will increase by approximately 25×10^9/L measured within 10 to 60 min of transfusion [22]. This rise normally returns to baseline within two or three days. However, up to 25% of hematological patients show a less than adequate response to the transfusion [23]. This phenomenon is called platelet refractoriness and is associated with increased morbidity and mortality as well as longer hospital stays and increased costs [24, 25]. Brubaker et al. transfused patients with indium-111-labeled platelets and showed that 1 hour after transfusion, 35% of the platelets could be found in the spleen and 25% in the liver [26].

The simplest way to define platelet refractoriness is by measuring the post-transfusion platelet increment (PPI) where an increment of $> 10 \times 10^9$/L, 10 to 60 min after transfusion, is considered an adequate response. This method is convenient in the clinical setting but does not take into account the number of platelets given and the size of the patient in which they are distributed. However, the corrected count increment (CCI) formula factors in both transfused platelets and calculated body surface area, as shown below.

$$CCI = \frac{(Posttransfusion\ PLC - Pretransfusion\ PLC)\ (10^9/L)\ x\ Body\ surface\ area\ (m^2)\ x\ 10^5}{platelets\ transfused\ (10^9)}$$

The American Society of Clinical Oncology defines platelet refractoriness as 1-h CCI value below 5,000, although some researchers use 7,500 or 10,000 as a cut-off [27].

Platelet refractoriness can be derived from both immune and non-immune causes. The immune causes include alloimmunization to human leukocyte antigen or human platelet antigen due to prior exposure through transfusion, transplantation, or pregnancy. The non-immune causes include fever, sepsis, splenomegaly, TA-GVHD, disseminated intravascular coagulation (DIC), and medications [28].

The development of CCI within the first 24 hours after transfusion has been investigated in several studies. Bishop et al. measured CCI 1 hour and 20 hours after transfusion and found that the two values correlated quite well, where the 20-hour CCI was on average 67% of the 1-hour CCI [29]. Brubaker et al. investigated CCI with short intervals in the first 2 hours after transfusion and subsequently 24 hours after transfusion and found that the transfused platelets had not reached equilibrium with other organs until 60 min after transfusion [26].

Endothelium

The endothelium is a monolayer of endothelial cells which forms the inner lining of arteries, veins, and capillaries, as well as the lymphatic system. The human body contains more than one trillion endothelial cells, covering more than 1000 m^2 but only weighing approximately 100 g. Due to their unique location, where they come into contact with the circulating blood, endothelial cells have the opportunity to participate in many processes in the body [30]. The vasomotor tone is mediated by the endothelium as well as the maintenance of blood fluidity. The endothelium also plays a central role in regulating inflammation and the balance between coagulation and anticoagulation. Some of its tasks are performed throughout the whole vascular bed, for instance, the regulation of hemostasis, while others are only performed in specific organs [31].

Glycocalyx

The endothelial glycocalyx is a thin layer lining the vascular endothelium. It consists mainly of carbohydrates which are anchored to the endothelium by proteoglycans and glycoproteins. This forms a mesh in which soluble molecules, either from plasma or the endothelium, are incorporated. The equilibrium between this layer of soluble substances and the flowing blood is dynamic, which is apparent by the glycocalyx

continuously changing thickness and composition. Additionally, the glycocalyx is sensitive to both shear-induced shedding as well as enzymatic degradation, which limits its function [32].

Endothelial damage

The function of the protective glycocalyx and signs of its impairment have attracted increased attention from researchers in recent years. Signs of endothelial damage have been associated with several different conditions including ischemia and hypoxia, inflammation and sepsis, atherosclerosis, diabetes, and renal disease [33]. For example, the capillary leakage seen in sepsis is considered to be caused by endothelial damage leading to the movement of fluid and larger molecules from the circulation into the interstitium [34]. Many authors have proposed that refractoriness to platelet transfusion might also be related to endothelial damage, but no studies have yet been published on the subject [22, 24, 29].

The shedding of glycoproteins from the glycocalyx can both stimulate and inhibit the coagulation system. Shedded heparans work as anticoagulants, similarly to heparin, and the unprotected endothelium can ignite the formation of a clot [33, 35].

Damage to the glycocalyx can be investigated by analyzing the concentrations of the shedded proteins; the most studied ones are heparan sulphate, syndecan-1, hyaluronic acid, and chondroitin sulphate [33]. The endothelial cell markers studied in this thesis are further described below.

Syndecan-1

Syndecan-1 is a heparan sulphate proteoglycan expressed on the cell surface that binds to many mediators involved in different diseases. Through its molecular interactions, syndecan-1 is involved in leukocyte recruitment, cancer proliferation and invasion, matrix remodeling, angiogenesis, and microbial attachment and entry. Syndecan-1 levels correlate with levels of inflammatory cytokines, such as TNF-α and IL-1, and are also associated with increased coagulopathy and mortality in trauma patients [34].

Thrombomodulin

Thrombomodulin is a glycosylated transmembrane protein expressed on the surface of endothelial cells where it functions as a cofactor for thrombin. When combined with thrombin, the complex stimulates protein C activation and thereby works as an anticoagulant. Recent studies have revealed that thrombomodulin has additional functions, some of which are independent of the thrombin-protein C pathway [36, 37]. These include fibrinolysis, inflammation, and embryogenesis. Activated leukocytes can

cleave thrombomodulin from its connection to the endothelium, releasing it into the circulation, which is referred to as soluble thrombomodulin (sTM) [38].

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a signal protein which plays a central role in both physiological and pathological angiogenesis. The biological effect of VEGF on endothelial cells is mediated through two receptor tyrosine kinases inducing proliferation, migration, and increased permeability [39]. Platelets store VEGF in their α-granules, which they release at the site of injury, stimulating the endothelial cells to repair the damage [40, 41].

Epidemiology in intensive care

Prediction of outcome and severity scoring

Prediction of patient outcomes is one of the cornerstones of clinical medicine at all levels. Outcome prediction aims at finding and measuring markers of illness and correlating them with outcomes of interest. Due to the relatively high risk of death among critically ill patients, mortality is often the focus of outcome prediction in the intensive care setting [42].

Several severity scoring systems have been developed to predict outcome in the intensive care setting. Almost all of these systems predict mortality within a defined time frame, including the acute physiology and chronic health evaluation (APACHE) series of scores, the mortality prediction model (MPM), and the simplified acute physiology score (SAPS) series of scores. Other severity scores have been designed to predict outcome in certain subgroups of patients such as the injury severity score (ISS) for trauma patients and survival after venoarterial-extracorporeal membrane oxygenator (ECMO) (SAVE) for patients receiving ECMO treatment [42].

Severity scores predicting mortality are useful tools in several aspects of intensive care. Firstly, they enable comparison of the performance of different ICUs by adjusting for the severity of illness measured by the severity score. Making a crude comparison of mortality between two units treating dissimilar groups of patients is understandably meaningless. Secondly, severity scoring systems can be used to predict length of stay and resource requirements after elective surgery, and they are therefore helpful to level out the demand for postoperative beds. Thirdly, the scoring systems can be used to compare and adjust for the severity of illness in clinical studies [42, 43].

Simplified acute physiology score

The first version of SAPS was designed in France in 1985 and was meant to be simpler and less time-consuming than the older acute physiology score (APS). SAPS was even simpler than the dominant APACHE I and showed equivalent performance in predicting mortality risk [44]. Variables included in the severity score were chosen by a group of experts, and defined ranges of values gave certain points which were summed up to a total score which in turn was converted into a predicted mortality risk.

In 1993, the SAPS score was expanded to SAPS 2, including 17 variables which were chosen and graded using logistic regression. The cohort was much larger than the previous one, with more than 13,000 patients from 10 countries included. The predictive power of the system increased when compared to its predecessor, but with preserved simplicity [45].

The third version of the SAPS model was developed in 2005 based on a database of 19,577 admissions from 307 ICUs spread over all continents. The score is divided into three categories or boxes:

- Box I: Patient characteristics before ICU admission: age, comorbidities, location in the hospital before ICU admission, etc.
- Box II: Reason for ICU admission, surgical status, infection, etc.
- Box III: Physiological derangement at ICU admission.

Interestingly, one half of the predictive power in the model is derived from Box I (i.e., patient characteristics), unrelated to the physiological derangement and the reason for ICU admission.

Scores from the boxes are added up and converted to an estimated mortality rate at hospital discharge. The equation used for the conversion is customized for the actual geographical area and adjusted over time. SAPS 3 is registered into a national database for all patients admitted to ICUs in Sweden [42, 46, 47].

Morbidity measures

Mortality is without a doubt the main outcome measure in intensive care, but organ dysfunction is also important for several reasons. Due to a low mortality rate in some ICU trials, researchers need very large treatment groups to reach sufficient statistical power. For example, almost all randomized controlled trials on vasopressors in septic shock with mortality as the primary endpoint have been negative, but when organ dysfunction is investigated, significant differences are found between treatment groups [48]. Another important reason for paying more attention to organ dysfunction is the fact that even if the patients survive the first four weeks after admission, the degree of organ dysfunction seems to correlate with mortality later on, as was shown by Linder et al. [49].

Measurements of organ dysfunction are not as straightforward as they are for mortality and are continuously debated. A simple and objective way to tackle the problem is to count the days of received organ support, i.e., vasopressors for circulatory failure, invasive ventilation for respiratory failure, and renal replacement therapy (RRT) for kidney failure. However, since mortality must be weighted in the measurement in some form, many authors report days alive and free (DAF) from certain organ support, which is most often based on the first 28 days after admission to the ICU.

Aims of the studies

Study I

To investigate the longitudinal development of platelet increment during the first 24 hours after platelet transfusion in patients with chemotherapy-induced bone marrow aplasia. Another aim is to study whether the increment differs between the four predefined groups of patients.

Study II

To investigate whether the patients in Study I show signs of endothelial damage in conjunction with platelet transfusion and if that potential damage correlates with the platelet increment.

Study III

To investigate whether activated partial thromboplastin time (APTT) and prothrombin time (PT-INR), on admission to ICU, correlate with mortality in patients with severe sepsis or septic shock.

Study IV

To investigate whether activated partial thromboplastin time (APTT) and prothrombin time (PT-INR), on admission to ICU, correlate with mortality and organ failure in unselected patients with critical illness.

Materials and methods

Ethics

All studies were approved by the Regional Ethical Review Board, Lund, Sweden. Participating patients in Studies I and II signed a written consent form prior to their inclusion. Patients in Studies III and IV were offered the chance to opt out of the cohort.

Coagulation tests

Platelet count

Platelet count (PLC) measures the number of platelets in a blood sample but does not assess their function in any way. Platelet counts in Studies I and II were measured at the accredited hospital laboratory using the impedance method (Sysmex XN-10, Sysmex Corp., Kobe, Japan). Ethylenediamine tetraacetic acid (EDTA) blood is diluted with Cellpack DCL/DST and injected into the cell detector where it is focused with sheath fluid. This thin jet of fluid flows through the aperture containing two electrodes which detect the increase in impedance that occurs when each cell passes by (Figure 1).

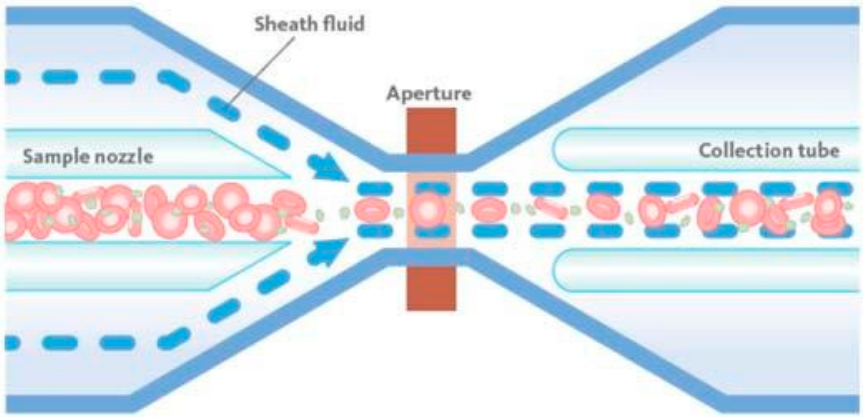


Figure 1. Impedance method to measure platelet count. (©Sysmex Europe GmbH)

In cases of suspected interference in the measurement, flow cytometry was used instead. Manual counting was used if both automated methods failed.

The above described methods were also used to determine the PLC in the platelet concentrates.

Activated partial thromboplastin time

Activated partial thromboplastin time (APTT) is a test used to detect defects in the intrinsic part and the common pathway of the coagulation system [50]. The test is sensitive to defects or shortages of factors II, VIII, IX, XI, XII, X, and fibrinogen. APTT is also widely used to monitor the effect of heparin [51].

APTT was analyzed in citrate plasma at the accredited hospital laboratory using a Sysmex 5100 instrument (Sysmex Corp., Kobe, Japan). The citrate tubes were centrifuged for 20 min at 2000 g and then a reagent was added to the plasma which enabled the activation of factors XI and XII, prekallikrein, and high-molecular-weight kininogen. The mix was incubated for 5 min at 37°C and then recalcificated with $CaCl_2$. The coagulation time was measured until a clot was formed, i.e., until a certain threshold of light absorbance was reached. The method is reagent-dependent, and during our study period, the reagent at our laboratory was changed from Dade to actin-FSL. All test results prior to September 2012 were converted to new values using a formula provided by the laboratory, $y = 0.45x + 12.2$.

Prothrombin time–international normalized ratio

Prothrombin time–international normalized ratio (PT-INR) is a test used to measure the activity in the extrinsic pathway of the coagulation system and detects the lack of or defects in factors II, VII, and X [51]. The method is widely used to monitor the effects of warfarin and other vitamin K antagonists. The INR concept was introduced by the World Health Organization to prevent problems related to different sensitivities of thromboplastin reagents produced by various manufacturers.

PT-INR was analyzed in citrate plasma at the accredited hospital laboratory using a Sysmex 5100 instrument (Sysmex Corp., Kobe, Japan). The citrate tubes were centrifuged for 20 min at 2000 g and then the plasma was mixed with Owren reagent, which contains thromboplastin, calcium, and bovine plasma lacking factors II, VII, and X. The time it takes for the clot to form is proportional to the function of factors II, VII, and X from the patient, as all the other factors are available in excessive amounts. The clot formation time is determined in the same way as for APTT, by measuring light absorbance.

Corrected count increment

To adjust for the patients' blood volumes and for the varying number of platelets in the platelet concentrates, corrected count increment (CCI) was calculated using the formula described in the Background section.

Study I

In this prospective observational study, platelet increments were studied in patients with chemotherapy-induced bone marrow aplasia. PLC was measured before, immediately after, and then 1, 4, 8, 16, and 24 hours after the platelet transfusion. The patients were divided into four study groups and one control group (Table 1). A central venous access was used to obtain the blood samples, except for the pre-transfusion and first post-transfusion samples in Group 4. The blood was sampled through a vacutainer system into 3 ml Vacutainer® spray-coated K2EDTA tubes (BD, Plymouth, UK).

Table 1. Patient groups.

GROUP	DESCRIPTION
1	Acute leukemia[a]
2	Autologous stem cell transplantation
3	Allogeneic stem cell transplantation
4	Prophylactic transfusion before CVC insertion
5	Control group

[a] Acute myeloblastic leukemia (AML), Acute lymphoblastic leukemia (ALL), Myelodysplastic syndrome (MDS), Mixed phenotype acute leukemia (MPAL). CVC: Central venous catheter.

The patients in Groups 1 to 3 had a morning PLC of < 10×10^9/L and received a platelet transfusion at the discretion of the treating physician. Patients in Group 4 were transfused prior to the insertion of a central venous catheter. Group 5 consisted of patients with a morning PLC of 10–20 $\times$ 10^9/L who did not receive a platelet transfusion during the observation period but underwent the same series of blood tests previously described. This was done to confirm that the observed platelet increments in the other groups were related to the transfusions and nothing else.

The platelet concentrates were prepared from either four pooled interim platelet units or by apheresis from a single donor. The concentrates were suspended in 200 ml of platelet additive solution PAS-E (T-PAS+, Terumo BCT). The PLC in each unit was measured approximately 1 hour prior to transfusion.

Study II

This study was a predefined sub-study to Study I. Blood samples were collected into K2EDTA tubes from all the blood sample series in Study I, centrifuged at the accredited hospital laboratory, and the plasma frozen to -86°C within 2 hours of sample time.

The endothelial cell markers syndecan-1, sTM, and VEGF were analyzed at the Section for Transfusion Medicine, Capital Region Blood Bank, Copenhagen University Hospital, Denmark. The plasma vials were defrosted and the endothelial cell markers analyzed with immunoassays according to the manufacturers' recommendations.

Study III

In this retrospective cohort study, we investigated patients with severe sepsis or septic shock who were admitted to the general ICU at Skåne University Hospital in Lund, Sweden, between 2 November 2007 and 9 December 2014. The admitting physician diagnosed the patients according to the "Sepsis 2" criteria defined at the International Sepsis Definition Conference 2001 [52].

Blood samples for analyses of APTT and PT-INR were drawn from an arterial line within 90 min of admission using a vacutainer system into 2.7 ml Vacutainer® citrate tubes (BD, Plymouth, UK) and analyzed at the accredited hospital laboratory.

Mortality data was acquired through the National Population Registry and the SAPS 3 score was extracted from the Swedish Intensive Care Registry.

Study IV

In this retrospective cohort study, we investigated patients regardless of diagnosis who were admitted to the general ICU at Skåne University Hospital in Lund, Sweden, between 1 November 2007 and 31 October 2017. Blood sampling, analysis and extraction of mortality data, and SAPS 3 scoring was done in the same manner as in Study III.

Circulatory and respiratory failure during the first 28 days after admission was measured as received organ support in the form of vasopressors (norepinephrine and vasopressin) and invasive ventilation, respectively. Both variables were reported as days alive and free (DAF) by subtracting days with support from 28 or days alive if shorter than 28 days. Continuous renal replacement therapy (CRRT) was reported as the occurrence of CRRT during the first 28 days after admission. To enable investigation of patients with milder renal failure (i.e., not requiring CRRT), we classified them according to their worst acute kidney injury network (AKIN)–creatinine score (AKIN-crea) the first 28 days after admission [53].

Statistics

Study I

Linear regression with estimation of the best fit line was used to evaluate the decline in CCI over time. To compare differences in CCI in the first 24 hours between groups, area under the curve (AUC) was used. AUC was calculated according to the trapezoidal approximation, presented as mean CCI per hour.

To compare the patients' responses to platelet transfusion between Groups 1–4, the one-way ANOVA or Kruskal–Wallis test was used for quantitative variables, and the Chi-squared test was used for qualitative variables.

Multiple linear regression was used to investigate whether CCI in the transfused patients was affected by background data.

Study II

Concentrations of endothelial cell markers measured before transfusion were set as the baseline, and the later measurements were presented as the differences from baseline, referred to as delta (Δ). The Friedman test was used to analyze differences in concentration over time. Concentrations were compared to baseline using Dunn's multiple comparisons test. The Kruskal–Wallis test was used to compare baseline and Δ concentrations between groups. Correlation analyses were calculated using Spearman's correlation tests.

Study III

Cox regression was used to analyze the correlation between the three independent variables—APTT, PT-INR, and SAPS 3—and mortality. The proportional hazard assumption was proven valid prior to the analysis.

Study IV

Cox regression was used to analyze the correlation between the independent variables—APTT, PT-INR, and SAPS 3—and mortality. The proportional hazard assumption was proven valid prior to the analysis.

The distribution of DAF vasopressor and DAF ventilator was U-formed, with the majority of patients having either low or high scores. Since the distribution pattern is not applicable in any commonly used regression model, these variables were dichotomized using more than one day of treatment as the cut-off (i.e., DAF < 27). The distribution of AKIN-crea was also uneven, with almost 80% of the patients in AKIN class 0. Therefore, this variable was also dichotomized into AKIN 0 vs AKIN 1–3. All binominal variables were analyzed using logistic regression. The goodness of fit was proven valid with the Pearson test.

Software

The statistical analyses for all the studies were done using GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA), R Core Team software (2012; R Foundation for Statistical Computing, Vienna, Austria), and SPSS Statistics version 24 (SPSS Inc., Chicago, Illinois, USA).

Databases containing data from all studies were created in Microsoft Excel (2010 and later), which was also used for the descriptive statistics.

Results

Study I

Fifty-four transfusion occasions in 43 patients were studied (Table 2).

Table 2. Transfusions by group.

GROUP	DESCRIPTION	OBSERVED TRANSFUSIONS
1	Acute leukemia[a]	19
2	Autologous stem cell transplantation	7
3	Allogeneic stem cell transplantation	16
4	Prophylactic transfusion before CVC insertion	12

[a] Acute myeloblastic leukemia (AML), Acute lymphoblastic leukemia (ALL), Myelodysplastic syndrome (MDS), Mixed phenotype acute leukemia (MPAL).
CVC: Central venous catheter.

The patients in Groups 1 to 3 had a median pre-transfusion PLC of 7×10^9/L (5–8 interquartile range) which increased to 19 (14–25) 1-hour post-transfusion. The same values for Group 4 were 17 (13–26) pre-transfusion and 36 (24–30) post-transfusion. Platelet refractoriness, defined as CCI < 5000 1-hour post-transfusion, was found in three cases in Groups 1 to 3 but none were found in Group 4.

The decline in CCI from immediately post-transfusion to 1-hour post-transfusion was 2100 ± 950 (mean ± standard deviation) in Groups 1 to 3, while Group 4 showed a slight increase: 570 ± 300 (Figure 2). From hour 1 to hour 24, the decline in CCI can be described as a linear function with a certain decline in CCI per hour (Figure 3). The decline for Groups 1 to 3 was 2.0% ± 0.6% (95% CI) per hour and 2.8% ± 1.2% per hour in Group 4.

There were no significant differences between Groups 1 to 4, neither in terms of the decline in CCI nor in terms of the mean CCI during the first 24 hours after transfusion.

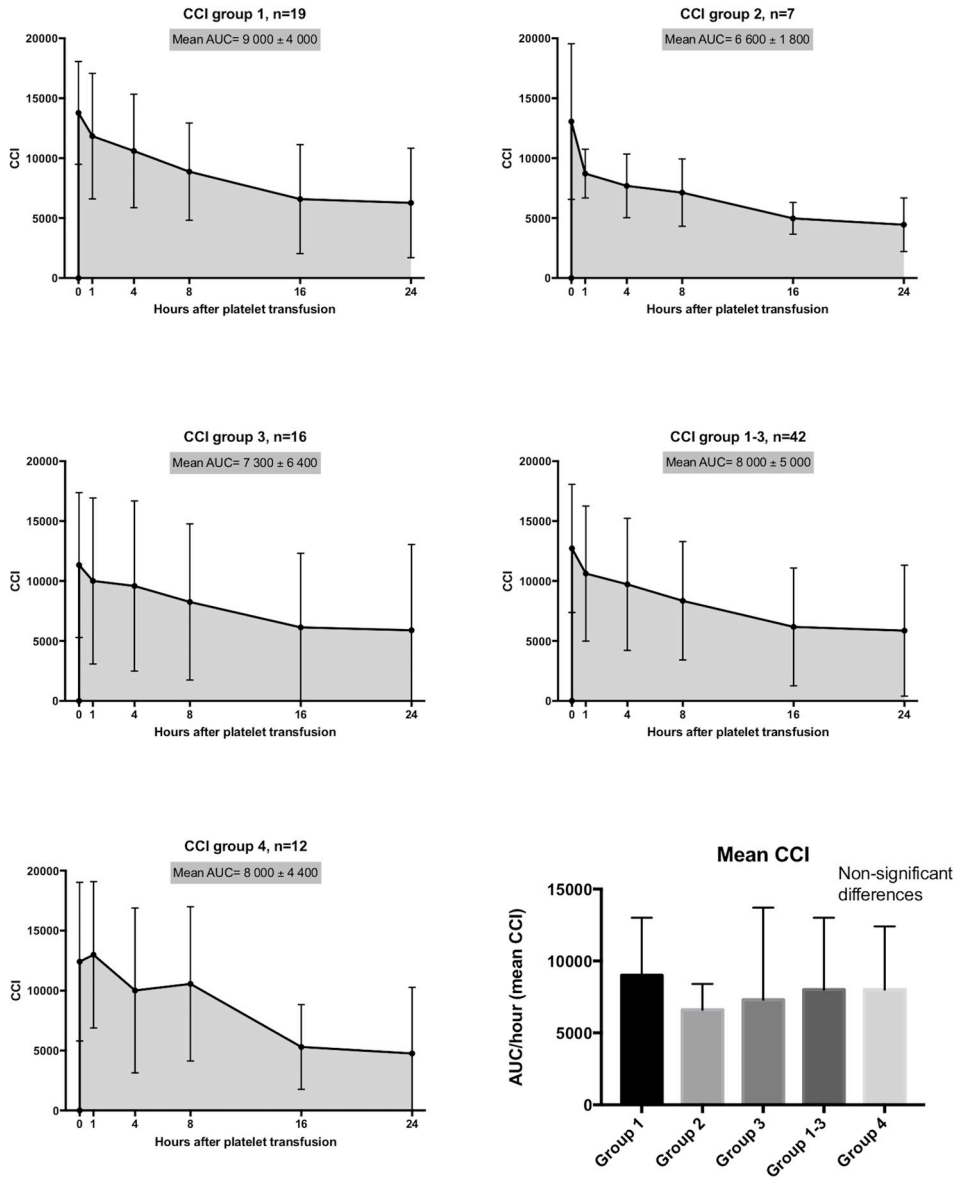


Figure 2. CCI ± standard deviation. Area under the curve (AUC) is filled in with gray color. The time 0 h after platelet transfusion represents the blood sample taken directly after the completion of the platelet transfusion. The mean CCI diagram is presented with mean ± standard deviation, and significance is tested with one-way ANOVA. Group 1: Patients receiving chemotherapy with diagnoses of acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, or mixed phenotype acute leukemia. Group 2: Patients undergoing autologous stem cell transplantation during current admission. Group 3: Patients undergoing allogeneic stem cell transplantation during current admission. Group 4: Patients receiving platelet transfusion before interventions.

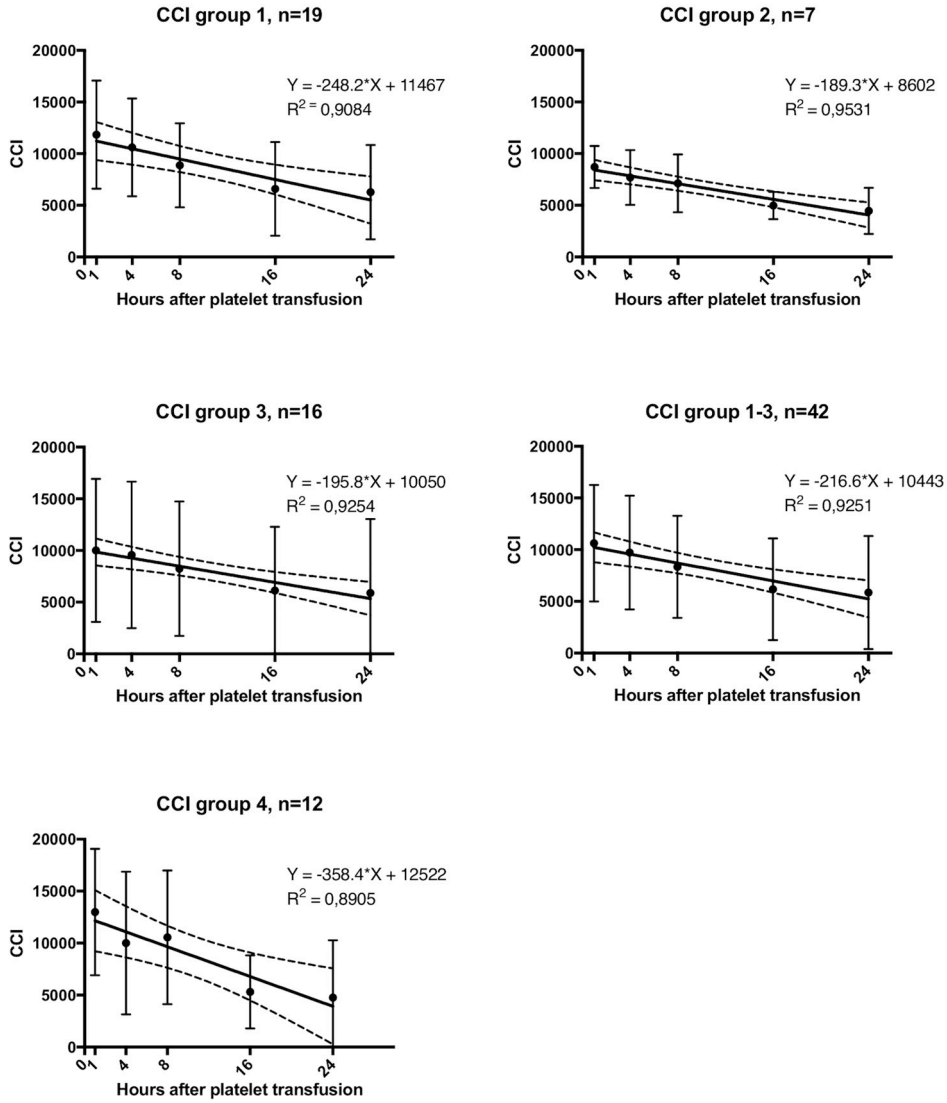


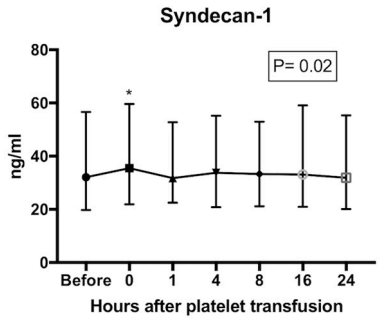
Figure 3. Linear regression with best fit line of corrected count increment (CCI) from 1 to 24 h post-transfusion, presented with mean and 95% confidence interval (dashed lines). The equation for the best fit line and the coefficient of determination (R^2) for each group is presented in the designated diagrams. Group 1: Patients receiving chemotherapy with diagnoses of acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, or mixed phenotype acute leukemia. Group 2: Patients undergoing autologous stem cell transplantation during current admission. Group 3: Patients undergoing allogeneic stem cell transplantation during current admission. Group 4: Patients receiving platelet transfusion before interventions.

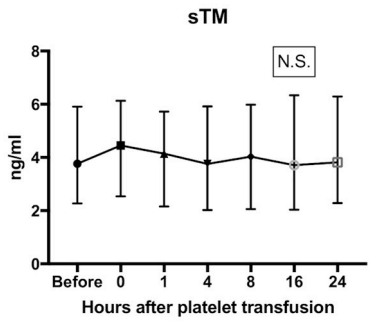
Study II

No significant changes of the endothelial cell markers were detected compared to baseline, except for a transient rise in syndecan-1 immediately after transfusion ($p = .02$) (Figure 4).

Baseline concentrations of the endothelial cell markers were compared between the predefined groups (Figure 5). No significant differences were found regarding syndecan-1 and sTM; however, the VEGF concentrations were higher in Group 4 when compared to the other groups ($p = .02$). Additionally, Δ concentrations before and after transfusion were compared between the groups and no significant differences were found.

Correlations between CCI and Δ concentrations before and after transfusion were also analyzed for all three cell markers without signs of significant association.





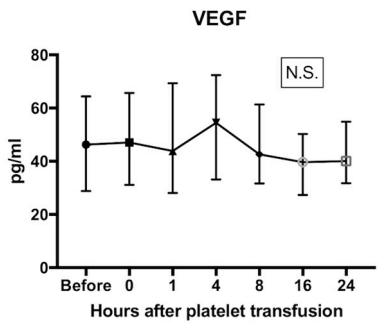
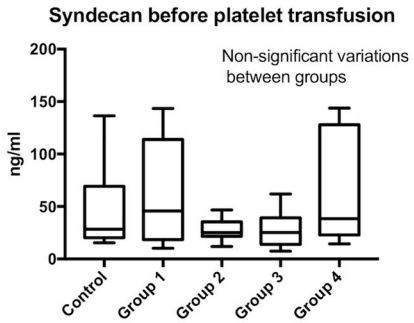
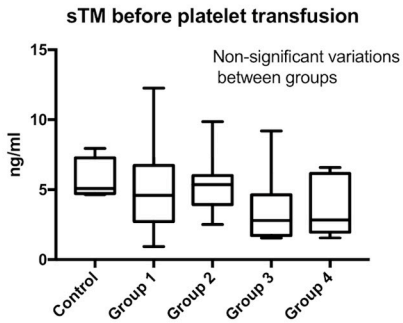


Figure 4. Plasma concentrations of endothelial cell markers. Groups 1–4 aggregated. Friedman test revealed a p value of .02 for differences between all time points for syndecan-1. Concentrations of the endothelial cell markers at the baseline (before platelet transfusion) were compared with concentrations from all other time points using Dunn's multiple comparisons test, which showed a p value of .02 for syndecan-1 directly after transfusion (0 h). The numbers in the squares represent significance for the Friedman tests. N.S.: non-significant; sTM: soluble thrombomodulin; VEGF: vascular endothelial growth factor.





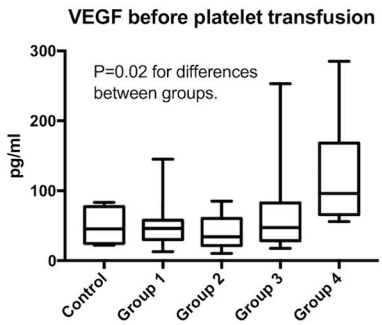


Figure 5. Comparison of plasma concentrations of the endothelial cell markers at baseline. A Kruskal–Wallis test was used to compare the groups. Group 1: Patients with AML, ALL, MPAL, or MDS. Group 2: Patients receiving autologous stem cell transplantation (SCT). Group 3: Patients receiving allogeneic SCT. Group 4: Patients scheduled for prophylactic platelet transfusion prior to CVC insertion. Control group: Patients not scheduled for platelet transfusion. N.S.: non-significant. sTM: soluble thrombomodulin; VEGF: vascular endothelial growth factor.

Study III

Six hundred and forty-seven patients with severe sepsis or septic shock were included in the study. The Cox regression showed that all the independent variables—SAPS 3, APTT, and PT-INR—were significantly associated with mortality (Table 3 and Figure 6).

Table 3. Correlations from the Cox regression between the independent variables and mortality.

Independent variables	Hazard ratio	95% CI of hazard ratio	p value
SAPS 3	1.036	1.028–1.044	< 0.001
APTT	1.014	1.006–1.023	< 0.01
PT-INR	1.422	1.117–1.811	< 0.01

CI: Confidence interval.

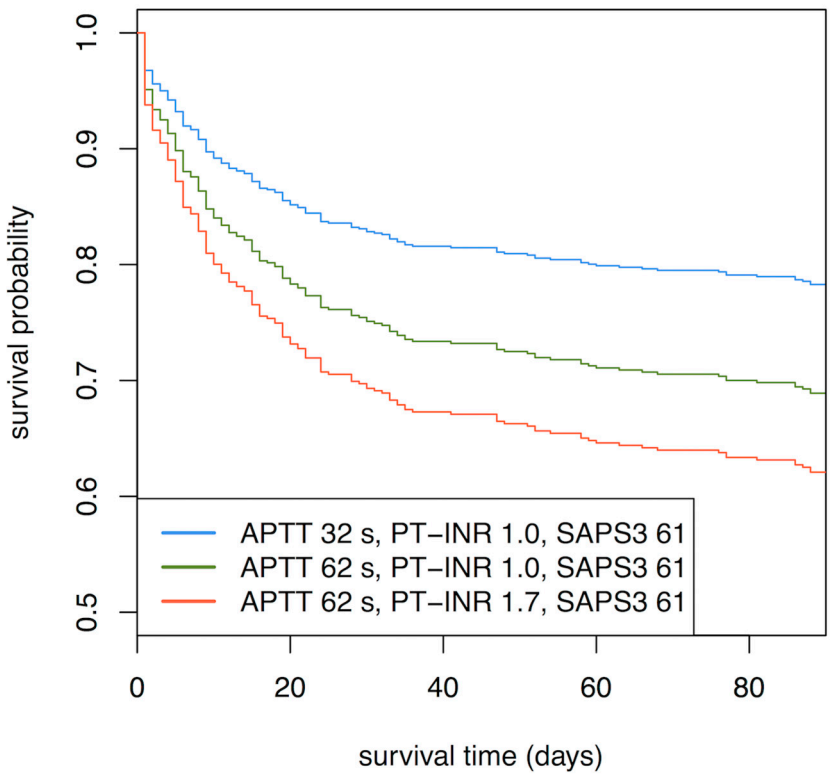


Figure 6. Estimated survival probability. Survival curves for different assumed values of activated partial thromboplastin time (APTT) and prothrombin time–international normalized ratio (PT-INR) given a fixed simplified acute physiologic score 3 (SAPS 3) of 61 (25th percentile in our material), which corresponds to an estimated mortality risk (EMR) of 0.21. APTT 32 s is within the normal range (26–33 s) and is the 25th percentile in our material. APTT 62 s is abnormal and is the 95th percentile in our material. PT-INR 1.0 is normal and 1.7 is the 75th percentile in our material.

Study IV

Three thousand five hundred and eighty-five critically ill patients were included in the study.

Mortality analyses

The SAPS 3 score correlated strongly with mortality in the Cox regression. APTT also correlated significantly, whereas PT-INR did not (Table 4 and Figure 7).

Table 4. Correlations from the Cox regression between the independent variables and mortality.

Independent variables	Hazard ratio	95% CI of hazard ratio	p value
SAPS 3	1.043	1.035–1.050	< 0.001
APTT	1.008	1.001–1.016	0.03
PT-INR	1.090	0.876–1.357	0.44[a]

[a] Not significant, $p \geq 0.05$.

Morbidity analyses

The logistic regression showed that SAPS 3 correlated strongly with all morbidity outcomes, and APTT was significantly associated with the need for vasopressor and CRRT, as well as the occurrence of renal failure. PT-INR correlated with the need for vasopressor and invasive ventilation but not the renal outcomes (Table 5 and Figure 7).

Table 5. Correlations between independent variables and outcome. 95% CI of OR.

Independent variables	DAF vasopressor	DAF ventilator	CRRT	AKIN-crea
SAPS 3	1.075–1.089	1.063–1.075	1.040–1.058	1.037–1.049
APTT	1.009–1.034	0.998–1.017[a]	1.016–1.037	1.009–1.028
PT-INR	1.112–2.014	1.135–1.847	0.863–1.635[a]	0.626–1.034[a]

All outcomes were analyzed in separate logistic regression models as described in the materials and methods section.
CI: Confidence interval; DAF: Days alive and free; OR: Odds ratio; CRRT: Continuous renal replacement therapy; AKIN-crea: Acute kidney injury network (AKIN)-creatinine.
[a] Not significant, $p \geq 0.05$.

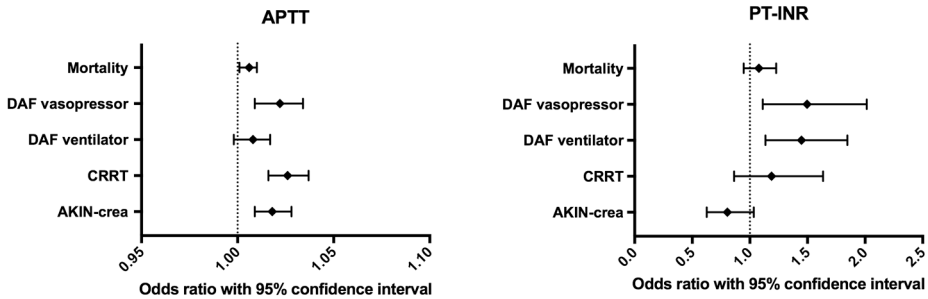


Figure 7. Forest plot of the correlation between the coagulation tests, APTT and PT-INR, and outcome.
All outcomes were analyzed in separate multivariable regression models as described in the materials and methods section. DAF: Days alive and free; CRRT: Continuous renal replacement therapy; AKIN-crea: Acute kidney injury network (AKIN)-creatinine class 1 or worse.

Discussion

Platelet increment

In Study I, we showed that platelet increment after transfusion, measured as CCI, declined in a linear manner from hours 1 to 24 post-transfusion. Patients with bone marrow aplasia due to chemotherapy for either acute leukemia or stem cell transplantation (Groups 1–3) declined 2.0% ± 0.6% (mean ± 95% CI) per hour in their CCI. Patients who were transfused prior to an intervention (Group 4) declined 2.8% ± 1.2% per hour. We did not detect any significant differences between the groups, neither in terms of the rate of CCI decline nor in terms of the mean CCI in the first 24 hours post-transfusion.

Only a few studies have previously described the development of CCI during the first 24 hours after platelet transfusion. Bishop et al. measured CCI 1 and 20 hours post-transfusion and found that the two values correlated strongly; moreover, the 20-hour value was on average 64% of the 1-hour value (i.e., the 1-hour value had declined on average 36% over a 19-hour period) [29]. The patients in Groups 1 to 3 in our study declined on average 2.0% per hour in CCI (i.e., 38% in 19 hours), so the two studies show very similar results. Bishop et al.'s study had no CCI measurements between hours 1 and 20, whereas our study has three measurements (4, 8, and 16 hours), which enables us to show the linearity of the decline.

Brubaker et al. investigated CCI with short intervals during the first 2 hours after transfusion and then 24 hours after transfusion, and they found that the transfused platelets did not reach intravascular equilibrium until at least 60 min after transfusion, from which they concluded that a 10-min count should not be used to detect platelet refractoriness [26]. The CCI values were nevertheless stable during the first 2 hours in all groups, except for the group that had platelet-associated antibodies, where the CCI declined more rapidly. In our study, we chose to divide the patients into groups based on their clinical status rather than their immunological status in hopes of finding differences that could be helpful for the treating physicians. We did not find any differences between the groups; however, a type-II error is possible considering the size of the groups (i.e., the study is probably underpowered to show these potential differences). The power calculations for the study were based on detecting platelet increments after transfusion, which was the primary outcome of the study.

The number of platelets in each transfusion concentrate in the study varied from 153 $\times 10^9$ to 302 $\times 10^9$, and 10 out of a total of 54 concentrates contained less than 200 $\times 10^9$ platelets, which is the minimum according to quality standards. This finding underlines the importance of measuring the PLC of the delivered product both to ensure that standards are met and to enable the treating physician to interpret the increment in the patient in the correct context.

Patients with thrombocytopenia are often in need of interventions with associated risks of bleeding, such as the insertion of central venous catheters (CVCs). Guidelines for the threshold of a prophylactic transfusion vary significantly between countries. For example, they are 10×10^9/L in Germany and 50×10^9/L in the UK [54, 55]. Evidence in this field is scarce, and a recent Cochrane systematic review showed that due to the lack of randomized trials, no certain threshold can be recommended [56]. The tradition at our institution is to transfuse a patient who is about to receive a CVC if their PLC is lower than 50×10^9/L. Our team has previously shown that there is no significant difference between the PLC increments 1 and 4 hours after transfusion [57]. In Study 1, we demonstrated further that the increment in Group 4 declined by 2.8% per hour from hour 1 to hour 24 which means that 8 hours post-transfusion, we still have approximately 80% (100-7×2.8) of the 1-hour increment in the patient. This finding is important in the clinical setting when timing interventions in relation to the prophylactic transfusion.

One limitation of the study is that the study patients were in different phases of their bone marrow aplasia when included. Some were in regeneration phase meaning that their own platelet production contributed considerably to the PLC while others were in a degenerative phase with minimal platelet production. This confounding factor is one of the reasons why the study was limited to the first 24 hours after transfusion.

Platelet increments and endothelial damage

Study II showed that the investigated endothelial cell markers are not associated with platelet increments in hematology patients.

Plasma concentrations of all the endothelial cell markers were within normal ranges before perfusion. A transient rise in syndecan-1 was observed directly after transfusion when compared to baseline values, but no other significant differences were detected over time—neither for syndecan-1 nor the other cell markers. The transient rise in syndecan-1 could potentially be related to high concentrations of syndecan-1 in the transfusion concentrates, as has been described for VEGF [58]. However, unlike VEGF, syndecan-1 is not shed from platelets, and the concentration found in donor plasma is diluted by the platelet additive solution which makes this explanation unlikely. Furthermore, Larsen et al. investigated syndecan-1 levels in plasma after

platelet transfusion in patients with acute myeloblastic leukemia (AML) and found no significant changes up to 24 h [59]. Another possible explanation for the rise in syndecan-1 directly after transfusion lies in the infused volume load. Chappel et al. showed how a fluid challenge of 20 ml/kg during elective surgery resulted in an 80% rise in syndecan-1 [60]. Although the infused volume (median 303 ml) in Study I is much less than 20 ml/kg, this is a reasonable possibility.

Baseline concentrations of VEGF were significantly higher in Group 4 when compared to the other groups. It is well documented that VEGF is expressed by solid tumors and facilitates angiogenesis, which is necessary for growth and invasion. Recent evidence suggests that hematological malignancies also behave in a similar manner. Bone marrow in AML patients shows signs of increased vascularization and tumor cells in hematological malignancies express and secrete VEGF [61-63]. The patients in Group 4 had higher median leucocyte counts when compared to Groups 1 to 3: 0.9 versus 0.1, respectively ($p < .001$). It is possible that the different VEGF levels are simply representing the different leucocyte counts between the groups. Furthermore, the PLC values at baseline were considerably higher in Group 4 than in the other groups. Platelets release VEGF when activated, and several studies have described the correlation between PLC and VEGF in patients with solid tumors [64-66]. The second possible explanation for the higher VEGF levels in Group 4 is therefore simply the different platelet levels between the groups. A third possible explanation could be related to the manner in which the blood samples were collected. The baseline samples in Group 4 were retrieved via peripheral cannulation and not a CVC. This has been associated with the release of p-selectin, the value of which in turn correlates with VEGF [67, 68].

It is worth mentioning that all comparison between the groups in Study II should be interpreted with caution. The groups were small, meaning that only large differences between the groups were detectable.

In conclusion, no evidence of endothelial damage was found either before or after platelet transfusion. A transient increase in syndecan-1 was observed directly after platelet transfusion, but with no correlation to platelet increment measured with CCI. Patients who were scheduled for a CVC insertion had higher baseline concentrations of VEGF, which could be related to their higher PLC or to the peripheral venous sampling.

Routine coagulation tests and outcome

Study III showed how prolonged APTT and increased PT-INR on admission to the ICU correlate with increased mortality in patients with severe sepsis or septic shock. This association is adjusted for the severity of illness by adding the SAPS 3 score as an

independent variable in the Cox regression. This adjustment is necessary to correct for the covariance of the coagulation tests with SAPS 3, as we are only interested in what these tests can tell us beyond what we already know about the patients' prognosis via the SAPS 3 score. The SAPS 3 score is registered for all patients admitted to ICUs in Sweden, and is at that time point a well-validated prognostication tool.

It is well documented that both a low PLC and a decline in PLC is associated with increased mortality in critically ill patients, both with and without sepsis [69-71]. However, the evidence for the association between coagulation tests and mortality is limited. Thromboelastograms (TEG) from septic patients have been investigated in several studies that reveal that hypocoagulation correlates with both mortality and bleeding risk [72-74]. Massion et al. studied 39 septic patients and found that both APTT and PT-INR were prolonged on admission to the ICU but only APTT was associated with mortality [75]. This finding was not adjusted for severity of illness, but when the APACHE II score was added to the regression model, only APTT on day 3 correlated with mortality. The strength of our findings in Study III lies in the size of the cohort and the adjustment made by adding the SAPS 3 score to the regression model.

The results of Study III describe an association between coagulation tests and survival, although it is unclear whether causality exists. However, one reasonable explanation for this correlation is the over-activation of the coagulation system that is often seen in septic patients which, in its most severe form, leads to disseminated intravascular coagulation (DIC) [76]. This activation results in the consumption of platelets, intravascular fibrin formation, and finally, the thrombotic occlusion of small and midsize vessels [77, 78]. Both APTT and PT-INR can be elevated in DIC [79]. It is well known that patients with septic shock who develop DIC during their ICU stay have a higher mortality risk and suffer more severe organ failure [80, 81]. However, it is debated to which extent organ failure is related to the DIC itself or whether signs of DIC are just surrogates for severity of illness. Nonetheless, data from animal studies show that fibrin depositions in the lungs, kidneys, liver, and brain can be prevented by treating the underlying coagulopathy [82, 83].

Another possible explanation for the correlation between the coagulation tests and survival is that either sepsis itself or the treatment for sepsis (i.e., intravenous fluids and vasoactive drugs) trigger shedding of the endothelial glycocalyx, which may in turn affect APTT and PT-INR [84, 85].

In Study IV, we investigated a large unselected cohort of ICU patients and showed that APTT prolongation on admission was associated with mortality, kidney failure, and the need for vasopressors and CRRT. PT-INR was, on the other hand, not associated with mortality, but it was nevertheless associated with the need for vasopressors and invasive ventilation. Again, SAPS 3 was included as an independent variable in all

regression models, meaning that the findings are independent of severity of illness as predicted by SAPS 3.

Only a few studies have described this relationship in unselected ICU cohorts. Walsh et al. investigated nearly 2,000 ICU admissions in the UK and found that PT-INR > 1.5 on admission was independently associated with ICU mortality after adjusting for severity of illness with the APACHE II score [86]. These results differ from our findings, which could be related to the different methodologies used. Whereas they used logistic regression with a relatively low cut-off (PT-INR > 1.5), we used Cox regression, which uses the whole range of the continuous independent variable. They used the APACHE score in their regression model, which possibly adjusts otherwise for the severity of illness and therefore influences the results. Fei et al. studied a similar cohort in China and also found that PT-INR on admission correlates with ICU mortality, but the regression was not adjusted for severity of illness, implying that the results should be interpreted with caution [87]. They also analyzed APTT and found no correlation with mortality. No studies were found describing the correlation between coagulation tests and the need for organ support in unselected ICU cohorts.

We found that APTT correlated with both mortality and all morbidity measures, except for the need for invasive ventilation, while PT-INR was only associated with the need for vasopressor and invasive ventilation. One can speculate that the reason for this lies in the nature of the coagulation tests, as APTT measures a larger share of the coagulation system and thus may possibly be more sensitive to derangements in the system.

Both Studies III and IV are single-center studies, which limits the generalization of our findings. They are retrospective in nature, although most of the data is registered prospectively. It should also be mentioned that our findings describe correlations in large cohorts of patients and should not be used for prognostication on an individual basis.

In summary, APTT and PT-INR on admission to ICU correlate with both mortality and organ failure in critically ill patients. These findings were adjusted for severity of illness, meaning that these blood tests have a prognostic value which is not accounted for in the SAPS 3 model. If this relationship can be confirmed in a larger setting, adding these variables to the prognostication models could possibly enhance their predictive value.

Main conclusions

- There is a linear decline in platelet increment 1 to 24 hours after platelet transfusion in patients with chemotherapy-induced bone marrow aplasia. *Study I*
- No differences in platelet increment were detected between the patient groups with acute leukemia, autologous SCT, allogeneic SCT, and those who were transfused before a CVC insertion. *Study I*
- Nineteen percent of the investigated platelet concentrates contained less than 200×10^9 platelets, which is the minimum according to quality standards. This fact underlines the need for measuring platelet count in each unit during concentrate production. *Study I*
- No signs of endothelial damage were detected before or after platelet transfusion in patients with chemotherapy-induced bone marrow aplasia. *Study II*
- Endothelial cell markers did not correlate with platelet increment in patients with chemotherapy-induced bone marrow aplasia. *Study II*
- APTT prolongation and PT-INR increase on ICU admission correlate independently with increased mortality in patients with severe sepsis and septic shock. *Study III*
- APTT prolongation on ICU admission correlates independently with increased mortality and kidney failure and the need for vasopressors and CRRT in an unselected ICU cohort. *Study IV*
- PT-INR increase on ICU admission correlates independently with the need for vasopressors and invasive ventilation in an unselected ICU cohort. *Study IV*

Sammanfattning på svenska

Trombocyter är små celler som produceras i benmärgen och spelar en central roll vid blodlevring när en skada uppstår i kärlväggen. Patienter med olika blodsjukdomar behandlas ofta med cellgifter som gör att benmärgen sviktar och trombocytantalet i blodet sjunker. Lågt trombocytantal kan leda till både spontana blödningar, t.ex. i hjärnan, och blödningar i samband med ingrepp, såsom inläggning av en central venkateter. I förebyggande syfte behandlas dessa patienter med trombocyttransfusioner men vissa av patienterna är ”refraktära” till behandlingen, vilket betyder att trombocytantalet i patienten stiger otillräckligt efter given transfusion.

I delarbete I studerade vi hur cellgiftsbehandlade patienter som erhöll trombocyttransfusioner steg i trombocytantal och hur trombocytantalet härefter successivt sjönk de första 24 timmarna efter given transfusion. Endast ett fåtal studier är gjorda inom ämnet men de beskriver framför allt hur trombocytantalet utvecklas de första fyra timmarna efter avslutad transfusion. Därför genomfördes delarbete I där trombocytantalet mättes innan transfusion, direkt efter och sedan 1, 4, 8, 16, och 24 timmar efter avslutad transfusion. Femtio fyra transfusionstillfällen observerades och delades upp i grupper beroende på sjukdomstillstånd. Vi kunde visa att trombocytantalet föll linjärt med 2.0% ± 0,6% (medel ± 95% konfidensintervall) per timme från timme 1 till 24 hos patienter med akut leukemi och dem som behandlades inför stamcellstransplantation. För patienter som erhöll trombocyttransfusion inför inläggning av en central venkateter föll trombocytantalet med 2,8% ± 1,2% per timme.

Inom ramen för delarbete I mättes antalet trombocyter i varje transfusionspåse för att kunna jämföra patienternas stegring i trombocytantal på ett standardiserat sätt. Minsta antalet trombocyter i en transfusionspåse var 153×10^9, det högsta antalet var 304×10^9 och i 19% av transfusionspåsarna var antalet trombocyter mindre än den önskvärda mängden, 200×10^9. Fyndet har lett till att Transfusionsmedicin i Lund planerar att införa mätning av antalet trombocyter i varje transfusionspåse.

I delarbete II undersökte vi om patienterna i delarbete I visade tecken på endotelskada före eller efter trombocyttransfusion och om den eventuella skadan står i samband med hur väl patienterna tillgodogör sig de transfunderade trombocyterna. Det finns många riskfaktorer för att inte kunna tillgodogöra sig trombocyttransfusioner men fortfarande är mycket oklart kring patofysiologin. En hypotes som har föreslagits är att ytan av kärlväggens insida, det så kallade endotelet, kan ha betydelse för hur väl transfunderade

trombocyter stannar kvar i blodbanan. Vi mätte tre olika endotelskademarkörer från alla provtillfällen i delstudie I men hittade inga tecken på endotelskada.

Kritiskt sjuka patienter med lågt trombocytantal vid ankomst till en intensivvårdsavdelning (IVA) har sämre överlevnad än de som har ett normalt trombocytantal. Det är däremot oklart om resultat av rutin koagulationstester såsom partiell tromboplastin-tid (APT-tid) och protrombinkomplex (PK-INR) vid ankomst till IVA korrelerar med dödlighet och sjuklighet.

Delarbete III var en registerstudie omfattande alla patienter med allvarlig sepsis eller septisk chock som blev inlagda på IVA, Skånes universitetssjukhus i Lund, 2007-2014. I analyserna korrigerades för allvarlighetsgraden av patienternas sjuklighet i övrigt, mätt med Simplified Acute Physiology Score 3 (SAPS 3). Delarbete III visade att både ökad APT-tid och ökat PK-INR vid ankomst till IVA hos patienter med allvarlig sepsis eller septisk chock korrelerar med ökad dödlighet.

Delarbete IV var också en registerstudie som dock omfattade alla patienter inlagda på IVA 2007-2017. Syftet med studien var att undersöka om det finns ett samband mellan rutin koagulationstester vid ankomst till IVA och graden av organsvikt som patienterna utvecklar. Justering för allvarlighetsgraden av sjuklighet i övrigt gjordes på samma sätt som i delarbete III. Det visade sig att förlängning av APT-tid korrelerade med ökad dödlighet och svikt i cirkulation och njurfunktion. Stegring i PK-INR korrelerade med svikt i både cirkulation och respiration.

Sammantaget indikerar våra fynd i delarbeten III och IV att både APT-tid och PK-INR vid ankomst till IVA representerar sjuklighet som inte är inräknad i den etablerade SAPS 3 modellen.

Acknowledgements and grants

Many people have helped me during my work with this thesis. Many thanks to all of you!

In particular I would like to thank:

Thomas Kander, my supervisor, scientific and clinical mentor and friend, for dragging me into the world of science and showing me how research and clinical work complement each other. Thank you for all the patience and enthusiasm along the way. You are one of a kind.

Ulf Schött, my co-supervisor, former head supervisor, scientific mentor and friend, for your endless support and positivity. Thanks for your wise advice both on work but not least on the game of life.

Attila Frigyesi, my co-supervisor, statistical right-hand, colleague and friend. It has been an invaluable asset to have you in the team and I hope that our play with regression models has just begun.

Claudia Hansen, Pär I. Johansson, Jens Kjeldsen-Kragh, Vladimir Lazarevic, Lars Nilsson, Sisse Ostrowski and Owain Thomas, my other co-authors, for great cooperation and invaluable contributions to the presented papers.

The nurses at the Hematology Clinic, Skåne University Hospital, for your enormous effort when collecting the blood samples for Studies I and II.

The physicians at the Hematology Clinic, Skåne University Hospital, for your help with inclusion of patients to Studies I and II.

The staff at the Department of Transfusion Medicine, Skåne University Hospital, for all your help when preparing the transfusion concentrates for Studies I and II.

Maria Jönsson and her colleagues at the Department of Laboratory Medicine, Skåne University Hospital, for all your help when analyzing and preparing the blood samples from Studies I and II.

Ann Svensson Gustafsson ICU nurse and Jan Karlsson, computer technician, for invaluable help with data extraction from the data management system for Studies III and IV.

Mikael Bodelsson, professor and colleague, for giving me the opportunity to teach at the university and providing me with research time.

Bengt Roth, Marie Martinsson, Carolina Samuelsson and Anders Rehn, former and present heads of the Department of Anesthesiology and Intensive Care, for your support in my clinical and scientific work.

Peter Bentzer, my mentor, for your good advice both on and off the shooting range.

Colleagues and friends at the Department of Anesthesia and Intensive Care, for making the work enjoyable every day.

Ólöf Kristjana Bjarnadóttir, my friend and neighbor, for all your good advice and support regarding thesis writing and baking.

Ingólfur and Þórhildur, my friends and extra-grandparents to my daughters. I thank you from the bottom of my heart for your unconditional love for my daughters and for all the help during the years.

Agnes and Benedikt, my parents, for bringing me up and encouraging me to study when my focus was elsewhere.

Sigurlaug and Agnes, my sisters, for teaching me martial arts from early childhood and always being there for me and my little family.

Hildur, Edda, Steinunn and Sólveig, my beloved daughters, for constantly putting things in the right perspective.

Kristbjörg, my love and best friend, for being an outstanding wife and mother. Thank you for your understanding and patience for my drawbacks. Your love and excellent sense of humor light up my life.

Grants

This work was supported by the Skåne county council´s research and development foundation, the Faculty of Medicine, Lund University, and the Department of Anesthesia and Intensive Care, Skåne University Hospital, Sweden. This is gratefully acknowledged.

References

1. Tsoucalas G, Karamanou M, Papaioannou TG, Sgantzos M. Theories about blood coagulation in the writings of ancient greek medico-philosophers. Curr Pharm Des. 2017;23(9):1275-8.
2. Morawitz P. Die Chemie der Blutgerinnung. Ergebnisse der Physiologie. 1905;4(1):307-422.
3. Marder VJ. Hemostasis and thrombosis : basic principles and clinical practice. Philadelphia: LWW; 2012. p.51-52.
4. Palta S, Saroa R, Palta A. Overview of the coagulation system. Indian J Anaesth. 2014;58(5):515-23.
5. Bizzozero G. Sul midollo delle ossa. Napoli: Tipografia Italiana. 1869.
6. Ribatti D, Crivellato E. Giulio Bizzozero and the discovery of platelets. Leuk Res. 2007;31(10):1339-41.
7. Blajchman MA. Platelet transfusions: an historical perspective. ASH Education Program Book. Washington: American Society of Hematology; 2008. p. 197.
8. Bayer WL, Bodensteiner DC, Tilzer LL, Adams ME. Use of platelets and other transfusion products in patients with malignancy. Semin Thromb Hemost. 1992;18(4):380-91.
9. Murphy S, Sayar SN, Gardner FH. Storage of platelet concentrates at 22°C. Blood. 1970;35(4):549-57.
10. Cardigan R, Lawrie AS, Mackie IJ, Williamson LM. The quality of fresh-frozen plasma produced from whole blood stored at 4 degrees C overnight. Transfusion (Paris). 2005;45(8):1342-8.
11. Smith JF, Ness PM, Moroff G, Luban NL. Retention of coagulation factors in plasma frozen after extended holding at 1-6 degrees C. Vox Sang. 2000;78(1):28-30.
12. Nordberg J. Kompendium i transfusionsmedicin. Sweden: Klinisk immunologi och transfusionsmedicin, Skåne University Hospital; 2017.
13. Strauss D. Component preparation and manufacturing. In: Shaz BH, Hillyer CD, Reyes Gil M, editors. Transfusion medicine and hemostasis. New York: Elsevier; 2019. p. 53-8.
14. Tynngård N. Preparation, storage and quality control of platelet concentrates. Transfus Apher Sci. 2009;41(2):97-104.
15. Gulliksson H. Defining the optimal storage conditions for the long-term storage of platelets. Transfus Med Rev. 2003;17(3):209-15.
16. van der Meer PF, de Korte D. Platelet preservation: agitation and containers. Transfus Apher Sci. 2011;44(3):297-304.

17. Sazama K. Reports of 355 transfusion-associated deaths: 1976 through 1985. Transfusion (Paris). 1990;30(7):583-90.
18. Kuehnert MJ, Roth VR, Haley NR, Gregory KR, Elder KV, Schreiber GB, et al. Transfusion-transmitted bacterial infectionin the United States, 1998 through 2000. Transfusion (Paris). 2001;41(12):1493-9.
19. Lin L, Cook DN, Wiesehahn GP, Alfonso R, Behrman B, Cimino GD, et al. Photochemical inactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. Transfusion (Paris). 1997;37(4):423-35.
20. Brubaker DB. Human posttransfusion graft-versus-host disease. Vox Sang. 1983;45(6):401-20.
21. Chapman J, Finney RD, Forman K, Kelsey P, Knowles SM, Napier JAF, et al. Guidelines on gamma irradiation of blood components for the prevention of transfusion-associated graft-versus-host disease. Transfus Med. 1996;6(3):261-71.
22. Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, Kao K-J, et al. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood. 2005;105(10):4106-14.
23. Legler TJ, Fischer I, Dittmann J, Simson G, Lynen R, Humpe A, et al. Frequency and causes of refractoriness in multiply transfused patients. Ann Hematol. 1997;74(4):185-9.
24. Kerkhoffs JL, Eikenboom JC, van de Watering LM, van Wordragen-Vlaswinkel RJ, Wijermans PW, Brand A. The clinical impact of platelet refractoriness: correlation with bleeding and survival. Transfusion (Paris). 2008;48(9):1959-65.
25. Meehan KR, Matias CO, Rathore SS, Sandler SG, Kallich J, LaBrecque J, et al. Platelet transfusions: utilization and associated costs in a tertiary care hospital. Am J Hematol. 2000;64(4):251-6.
26. Brubaker DB, Marcus C, Holmes E. Intravascular and total body platelet equilibrium in healthy volunteers and in thrombocytopenic patients transfused with single donor platelets. Am J Hematol. 1998;58(3):165-76.
27. Schiffer CA, Anderson KC, Bennett CL, Bernstein S, Elting LS, Goldsmith M, et al. Platelet transfusion for patients with cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol. 2001;19(5):1519-38.
28. Hod E, Schwartz J. Platelet transfusion refractoriness. Br J Haematol. 2008;142(3):348-60.
29. Bishop JF, Matthews JP, McGrath K, Yuen K, Wolf MM, Szer J. Factors influencing 20-hour increments after platelet transfusion. Transfusion (Paris). 1991;31(5):392-6.
30. Jaffe EA. Cell biology of endothelial cells. Hum Pathol. 1987;18(3):234-9.
31. Félétou M. The Endothelium: part 1: multiple functions of the endothelial cells - focus on endothelium-derived vasoactive mediators: San Rafael (CA): Morgan & Claypool Life Sciences; 2011. p. 1-3.
32. Reitsma S, Slaaf D, Vink H, van Zandvoort M, oude Egbrink M. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Archiv: Eur J Phys. 2007;454(3):345-59.

33. Becker BF, Leipert S, Jacob M, Salmon AHJ, Chappell D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. Br J Clin Pharmacol. 2015;80(3):389-402.
34. Chelazzi C, Villa G, Mancinelli P, De Gaudio AR, Adembri C. Glycocalyx and sepsis-induced alterations in vascular permeability. Crit Care. 2015;19:26.
35. Schött U, Solomon C, Fries D, Bentzer P. The endothelial glycocalyx and its disruption, protection and regeneration: a narrative review. Scand J Trauma Resusc Emerg Med. 2016;24:48.
36. Conway EM, Van de Wouwer M, Pollefeyt S, Jurk K, Van Aken H, De Vriese A, et al. The lectin-like domain of thrombomodulin confers protection from neutrophil-mediated tissue damage by suppressing adhesion molecule expression via nuclear factor kappaB and mitogen-activated protein kinase pathways. J Exp Med. 2002;196(5):565-77.
37. Ikeguchi H, Maruyama S, Morita Y, Fujita Y, Kato T, Natori Y, et al. Effects of human soluble thrombomodulin on experimental glomerulonephritis. Kidney Int. 2002;61(2):490-501.
38. Weiler H, Isermann BH. Thrombomodulin. J Thromb Haemost. 2003;1(7):1515-24.
39. Ferrara N, Gerber H-P, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003;9(6):669-76.
40. Maloney JP, Silliman CC, Ambruso DR, Wang J, Tuder RM, Voelkel NF. In vitro release of vascular endothelial growth factor during platelet aggregation. Am J Physiol Heart Circ Physiol. 1998;275(3):H1054-H61.
41. Tsai W-C, Li Y-H, Huang Y-Y, Lin C-C, Chao T-H, Chen J-H. Plasma vascular endothelial growth factor as a marker for early vascular damage in hypertension. Clin Sci. 2005;109(1):39-43.
42. Barlow CJ, Pilcher D. Severity scoring and outcome prediction. In: Bersten A, Handy J, editors. Oh's Intensive Care Manual. USA: Elsevier; 2019. p. 19-33.
43. Becker RB, Zimmerman JE, Knaus WA, Wagner DP, Seneff MG, Draper EA, et al. The use of APACHE III to evaluate ICU length of stay, resource use, and mortality after coronary artery by-pass surgery. The Journal of Cardiovascular Surgery. 1995;36(1):1-11.
44. Le Gall JR, Loirat P, Alperovitch A, Glaser P, Granthil C, Mathieu D, et al. A simplified acute physiology score for ICU patients. Crit Care Med. 1984;12(11):975-7.
45. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA. 1993;270(24):2957-63.
46. Moreno RP, Metnitz PGH, Almeida E, Jordan B, Bauer P, Campos RA, et al. SAPS 3--From evaluation of the patient to evaluation of the intensive care unit. Part 2: Development of a prognostic model for hospital mortality at ICU admission. Intensive Care Med. 2005;31(10):1345-55.
47. Metnitz PGH, Moreno RP, Almeida E, Jordan B, Bauer P, Campos RA, et al. SAPS 3--From evaluation of the patient to evaluation of the intensive care unit.

Part 1: Objectives, methods and cohort description. Intensive Care Med. 2005;31(10):1336-44.
48. Russell JA, Lee T, Singer J, De Backer D, Annane D. Days alive and free as an alternative to a mortality outcome in pivotal vasopressor and septic shock trials. J Crit Care. 2018;47:333-7.
49. Linder A, Lee T, Fisher J, Singer J, Boyd J, Walley KR, et al. Short-term organ dysfunction Is associated with long-term (10-Yr) mortality of septic shock. Crit Care Med. 2016;44(8):e728-e36.
50. White II GC. The partial thromboplastin time: defining an era in coagulation. J Thromb Haemost. 2003;1(11):2267-70.
51. Levy JH, Szlam F, Wolberg AS, Winkler A. Clinical use of the activated partial thromboplastin time and prothrombin time for screening: a review of the literature and current guidelines for testing. Clin Lab Med. 2014;34(3):453-77.
52. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Intensive Care Med. 2003;29(4):530-8.
53. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. Acute kidney injury network: report of an initiative to improve outcomes in acute kidney injury. Crit Care. 2007;11(2):R31.
54. Sibrowski W. Platelet Concentrates. Transfusion medicine and hemotherapy : offizielles Organ der Deutschen Gesellschaft fur Transfusionsmedizin und Immunhamatologie. 2009;36(6):372-82.
55. British Committee for Standards in H, Blood Transfusion Task Force. Guidelines for the use of platelet transfusions. Br J Haematol. 2003;122(1):10-23.
56. Estcourt LJ, Desborough M, Hopewell S, Doree C, Stanworth SJ. Comparison of different platelet transfusion thresholds prior to insertion of central lines in patients with thrombocytopenia. Cochrane Database Syst Rev. 2015;12(12):CD011771.
57. Kander T, Tanaka KA, Norström E, Persson J, Schött U. The effect and duration of prophylactic platelet transfusions before insertion of a central venous catheter in patients with bone marrow failure evaluated with point-of-care methods and flow cytometry. Anesth Analg. 2014;119(4):882-90.
58. Wen Y-H, Lin C-J, Sun Y-C, Chang P-Y, Wang H-Y, Lu J-J, et al. Sustained or or higher levels of growth factors in platelet-rich plasma during 7-day storage. Clin Chim Acta. 2018;483:89-93.
59. Larsen AM, Leinøe EB, Johansson PI, Larsen R, Wantzin P, Birgens H, et al. Haemostatic function and biomarkers of endothelial damage before and after platelet transfusion in patients with acute myeloid leukaemia. Transfusion Medicine (Oxford, England). 2015;25(3):174-83.
60. Chappell D, Bruegger D, Potzel J, Jacob M, Brettner F, Conzen P, et al. Hypervolemia increases release of atrial natriuretic peptide and shedding of the endothelial glycocalyx. Crit Care. 2014;18(5):1-8.
61. Kumar S, Witzig TE, Timm M, Haug J, Wellik L, Fonseca R, et al. Expression of VEGF and its receptors by myeloma cells. Leukemia. 2003;17(10):2025-31.

62. Hussong JW, Rodgers GM, Shami PJ. Evidence of increased angiogenesis in patients with acute myeloid leukemia. Blood. 2000;95(1):309-13.
63. Chand R, Chandra H, Chandra S, Verma SK. Role of microvessel density and vascular endothelial growth factor in angiogenesis of hematological malignancies. Bone Marrow Res. 2016;2016:1-4.
64. Salgado R, Vermeulen PB, Benoy I, Weytjens R, Huget P, Van Marck E, et al. Platelet number and interleukin-6 correlate with VEGF but not with bFGF serum levels of advanced cancer patients. Br J Cancer. 1999;80(5-6):892-7.
65. George ML, Eccles SA, Tutton MG, Abulafi AM, Swift RI. Correlation of plasma and serum vascular endothelial growth factor levels with platelet count in colorectal cancer: clinical evidence of platelet scavenging? Clin Cancer Res. 2000;6(8):3147-52.
66. Verheul HM, Hoekman K, Luykx-de Bakker S, Eekman CA, Folman CC, Broxterman HJ, et al. Platelet: transporter of vascular endothelial growth factor. Clin Cancer Res. 1997;3(12 Pt 1):2187-90.
67. Qi C-L, Wei B, Ye J, Yang Y, Li B, Zhang Q-Q, et al. P-selectin-mediated platelet adhesion promotes the metastasis of murine melanoma cells. PLoS One. 2014;9(3):e91320.
68. Milburn JA, Ford I, Cassar K, Fluck N, Brittenden J. Platelet activation, coagulation activation and C-reactive protein in simultaneous samples from the vascular access and peripheral veins of haemodialysis patients. Int J Lab Hematol. 2012;34(1):52-8.
69. Kompoti M, Michalia M, Peppa P, Nanou F, Charitidi M, Sartzi M, et al. Thrombocytopenia: Incidence and clinical impact on ICU mortality. Crit Care. 2011;15:S155-S6.
70. Faviere W, Boechat T. Sepsis: Thrombocytopenia is bad, not recovering thrombocytopenia is too bad. Crit Care. 2011;15:S155.
71. Vanderschueren S, De Weerdt A, Malbrain M, Vankersschaever D, Frans E, Wilmer A, et al. Thrombocytopenia and prognosis in intensive care. Crit Care Med. 2000;28(6):1871-6.
72. Schmittinger CA, Dünser MW, Torgersen C, Hasibeder WR, Luckner G, Mayr V, et al. Rotational thrombelastometry to assess the coagulation system in severe sepsis and the systemic inflammatory response syndrome: A prospective, controlled trial. Intensive Care Med. 2009;35:S162.
73. Ostrowski SR, Windelov NA, Ibsen M, Haase N, Perner A, Johansson PI. Consecutive thrombelastography clot strength profiles in patients with severe sepsis and their association with 28-day mortality: a prospective study. J Crit Care. 2013;28(3):317 e1-11.
74. Haase N, Ostrowski SR, Wetterslev J, Lange T, Moller MH, Tousi H, et al. Thromboelastography in patients with severe sepsis: a prospective cohort study. Intensive Care Med. 2014;41(1):77-85.
75. Massion PB, Peters P, Ledoux D, Zimermann V, Canivet JL, Massion PP, et al. Persistent hypocoagulability in patients with septic shock predicts greater hospital mortality: Impact of impaired thrombin generation. Intensive Care Med. 2012;38(8):1326-35.

76. Scully M, Levi M. How we manage haemostasis during sepsis. Br J Haematol. 2019;185(2):209-18.
77. Levi M. Disseminated intravascular coagulation. Crit Care Med. 2007;35(9):2191-5.
78. Levi M, ten Cate H. Disseminated Intravascular Coagulation. N Engl J Med. 1999;341(8):586-92.
79. Chandrashekar V. DIC score: Statistical relationship with PT, APTT, and simplified scoring systems with combinations of PT and APTT. ISRN Hematol. 2012;2012:1-4.
80. Gando S, Shiraishi A, Yamakawa K, Ogura H, Saitoh D, Fujishima S, et al. Role of disseminated intravascular coagulation in severe sepsis. Thromb Res. 2019;178:182-8.
81. Fourrier F, Chopin C, Goudemand J, Hendrycx S, Caron C, Rime A, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation: compared patterns of antithrombin III, protein C, and protein S deficiencies. Chest. 1992;101(3):816-23.
82. Levi M, van der Poll T. Coagulation and sepsis. Thromb Res. 2017;149:38-44.
83. van der Poll T, van de Veerdonk FL, Scicluna BP, Netea MG. The immunopathology of sepsis and potential therapeutic targets. Nat Rev Immunol. 2017;17(7):407-20.
84. Rahbar E, Cardenas JC, Baimukanova G, Usadi B, Bruhn R, Pati S, et al. Endothelial glycocalyx shedding and vascular permeability in severely injured trauma patients. J Transl Med. 2015;13:117.
85. Schott U, Solomon C, Fries D, Bentzer P. The endothelial glycocalyx and its disruption, protection and regeneration: a narrative review. Scand J Trauma Resusc Emerg Med. 2016;24:48.
86. Walsh TS, Stanworth SJ, Prescott RJ, Lee RJ, Watson DM, Wyncoll D. Prevalence, management, and outcomes of critically ill patients with prothrombin time prolongation in United Kingdom intensive care units. Crit Care Med. 2010;38(10):1939-46.
87. Fei A, Lin Q, Liu J, Wang F, Wang H, Pan S. The relationship between coagulation abnormality and mortality in ICU patients: a prospective, observational study. Sci Rep. 2015;5:9391.

Appendix

I Benediktsson S, Lazarevic V, Nilsson L, Kjeldsen-Kragh J, Schött U, Kander T. Linear decline of corrected platelet count increment within 24 hours after platelet transfusion in haematological patients: a prospective observational study. Eur J Haematol. 2017;99(6):559-68.

II Benediktsson S, Kander T, Ostrowski SR, Johansson PI, Thomas OD, Schött U. Platelet increment is not associated with endothelial damage in haematological patients: a prospective observational study. Scand J Clin Lab Invest. 2019;79(6):395-403.

III Benediktsson S, Frigyesi A, Kander T. Routine coagulation tests on ICU admission are associated with mortality in sepsis: an observational study. Acta Anaesthesiol Scand. 2017;61(7):790-6.

IV Benediktsson S, Hansen C, Frigyesi A, Kander T. Coagulation tests on admission correlate with mortality and morbidity in general ICU patients: an observational study. *Resubmitted after revision.* Acta Anaesthesiol Scand.