COLLECTION, PRODUCTION AND STORAGE OF BLOOD COMPONENTS

Original article

Cryopreserved platelets and amotosalen-treated plasma in an experimental clot formation set-up

Per Sandgren[1,2], Kristina Ehn[1,2], Linda Larsson[1,2], Michael Uhlin[1,2], Agneta Wikman[1,2]

[1]*Department of Clinical Immunology and Transfusion Medicine (KITM), Karolinska University Hospital, Stockholm, Sweden;*
[2]*Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institute Stockholm, Sweden*

Background - Amotosalen treatment of plasma and cryopreservation of platelets affect the quality and potentially the interplay between platelets and coagulation factors. We set up an experimental clot formation study to test the hypothesis that amotosalen treatment of plasma affects the interaction with different platelet preparations.

Materials and methods - Pooled plasma units (n=16) were subjected to coagulation tests before and after pathogen inactivation with amotosalen treatment (PI) and aliquots were frozen at −80°C. Fresh and cryopreserved platelets were analyzed for phenotypic and activity markers. Finally, coagulation properties of different combinations of platelets and plasma, before and after PI, were analyzed by viscoelastography (ROTEM).

Results – PI of plasma reduced the concentration of several coagulation factors ($p<0.01$). Cryopreservation altered phenotypic expression and reduced the platelets' ability to respond to agonists ($p<0.0001$). The interplay between all plasma derivatives and cryopreserved platelets resulted in shortened coagulation time ($p<0.0001$) but prolonged clot formation time and reduced clot strength ($p<0.0001$) as compared to the interaction between fresh platelets with different plasma variants. PI of the plasma does not seem to have a major impact on coagulation time, clot formation time or clot strength.

Discussion - Our data show that the reduced concentration of coagulation factors after PI treatment of plasma are negligible measured by viscoelastography, with fresh and cryopreserved platelets in this experimental clot formation setup, and that platelets play a more pronounced role. Cryopreserved platelets are more activated and result in reduced clot stability.

Keywords: *platelets cryopreservation, amotosalen-treated plasma.*

INTRODUCTION

Transfusion of platelet and plasma products provides an important contribution to hemostasis to stop bleeding, and current clinical recommendations are to transfuse combinations of platelets, plasma and red cells to individuals with massive bleeding[1,2]. This has been shown to improve hemostasis[3], regulated by the interplay between platelets and coagulation factors[4]. The physiological process of hemostasis involves complex interactions between endothelial cells, platelets, von Willebrand factor and coagulation

Arrived: 25 October 2021
Revision accepted: 7 February 2022
Correspondence: Per Sandgren
e-mail: per.sandgren@sll.se

factors. The coagulation factors interact with platelets by binding to platelet receptors directly, indirectly or by cleaving platelet receptors through clot formation[5-9]. Accordingly, preserved platelet function and coagulation are crucial factors for a purposeful interplay between platelets and the coagulation system. Considered from a transfusion perspective, it is reasonable to assume that the interplay between platelets and plasma-based coagulation factors may be affected by different techniques used in the preparation of blood components.

It is well known that collection, preparation, and storage of platelet concentrates can result in platelet storage lesions including changes in metabolism and surface receptors[10-12]. This might affect their ability to promote hemostasis. Addition of different pathogen inactivation (PI) technologies[13-15] as well as cryopreservation procedures[16-18] are no exception to this notion. Plasma products are also affected by freezing and PI treatment, mainly through effects on various coagulation factors[19-21]. Over the years, these blood components have been well studied separately, while the potential effects on the interplay between platelets and plasma derivatives has not been previously described. As the availability of and requests for PI-treated products and platelets increase, it is important to evaluate how these new techniques affect both the products and their combinations. Hence, we set up an experimental study comparing combinations of platelet and plasma derivatives. The main objective of the experiments was to test the hypothesis that the interplay between platelets and plasma may be influenced by the pre-transfusion *in vitro* preparation procedures. This may be of value in determining the clinical effectiveness of transfusing such components in combination.

MATERIALS AND METHODS

General overview of the experiments

Samples (n=16) of whole blood plasma units (blood group AB) in pools of five were subjected to conventional coagulation tests, measurement of the concentrations of coagulation factors and viscoelastography before and immediately after amotosalen-treatment (PI). Thereafter, aliquots from one randomly selected pool were frozen at (–80°C) before and after PI. Fresh and cryopreserved platelet products (n=16) were initially analyzed for phenotypic and activity markers, and further tested diluted in the aliquoted plasma for coagulation properties as schematically illustrated in **Figure 1**. The experimental combinations for investigation of the interplay between the different platelet and plasma derivatives were subjected to viscoelastography and are listed in **Table I**.

Blood component preparation and *in vitro* testing

Preparation of pooled plasma for characterization before and after pathogen inactivation and further aliquoted and tested in combinations with platelets

AB plasma (n=40) was prepared from 450 mL±10% whole blood units. In short, the whole blood was centrifuged (3,130 *g*, 11 min, Macospin, Macopharma [Tourcoing, France]) and separated into red blood cells, plasma and buffy coat within 8 hours of donation. The plasma was leukoreduced through filtration. Afterwards, the plasma (270.0±10.9 mL) was combined in pools of 5 units (n=8 pools), then split into two sub-pools of identical content using DONOpack (2×1,500 mL pooling bags, Lmb Technologie GmbH, Schwaig, Germany). One of the sub-pools was kept for aliquoting before PI, whereas the other was amotosalen-treated with the INTERCEPT blood system and the associated INT31 processing set for plasma (Cerus Europe B.V., Amersfoort, the Netherlands). Aliquots of the pre- and post-PI sub-pools were prepared for testing with the different platelet derivatives. Each sub-pool of plasma tested in the study unit met the processing requirements to undergo INTERCEPT blood system treatment.

Preparation of platelet derivatives for characterization and experimental combinations with plasma derivatives

Double-dose platelet concentrates (n=16) were prepared from pools of eight buffy coats in additive solution (SSP+)

Table I - *Experimental combinations for Rotem analysis (n=16)*

Platelet product	Plasma product	Assembly
BC platelets (day 1)	Base plasma (day 1)	A
BC platelets (day 1)	PI plasma	B
Cryopreserved BC platelets	Base plasma (day 1)	C
Cryopreserved BC platelets	PI plasma	D

*Coagulation properties of conventional and cryopreserved platelets in combinations with conventional and amotosalen treated plasma products were assessed by Rotem analysis.
BC: buffy coat, PI: pathogen inactivation with amotosalen.

Blood Transfus 2023; 21: 137-145; doi: 10.2450/2022.0279-21

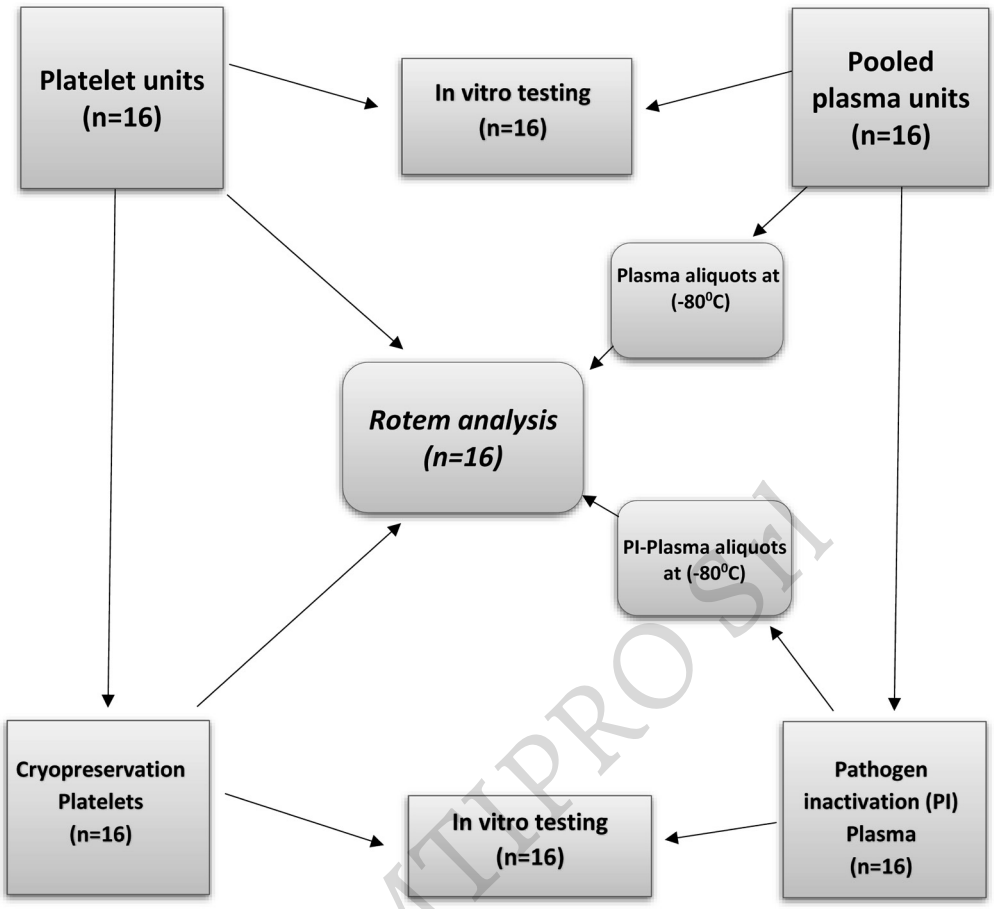

Figure 1 - General overview of the experiments
Pooled plasma units (n=16) were tested for conventional coagulation parameters, concentrations of coagulation factors and viscoelastography before and directly after pathogen inactivation treatment with amotosalen (PI). A randomly collected pooled plasma unit was aliquoted and frozen at −80°C before and after PI. Fresh (day 1) and cryopreserved platelet products (n=16) were analyzed for phenotypic and activity markers, and further tested diluted in the aliquoted plasma for coagulation properties. BC: buffy coat.

as recently described[22,23] and platelet samples (n=16), used to determine the baseline *in vitro* quality (on day 1 after collection and preparation) and subsequent interplay with the plasma derivates, were collected. For cryopreservation, a total of 16 untreated platelet units from buffy coats were transferred to a freezing bag (Macopharma) using a sterile connection device (Terumo BCT, Lakewood, CO, USA). A mixture of 25% DMSO/NaCl (50 mL) was then sterile docked, and the solution added to the platelet concentrates. After centrifugation at 1,200 *g* for 10 min, as much as possible of the supernatant was removed, leaving on average 0.5 mL freezing medium in approximately 10 mL of platelet suspension. The freezing bags, containing approximately 10 mL of platelet suspension (5% DMSO), were immediately frozen with a fast-uncontrolled freezing rate protocol and then stored, and subsequently thawed, and resuspended as recently described[24]. All thawed and reconstituted units were kept at room temperature for 1 hour prior to analysis. The *in vitro* properties of the cryopreserved platelets were analyzed after freezing and in combination with the plasma derivatives as outlined in **Table I**.

In vitro analysis of plasma

Samples (n=16) of pooled plasma were frozen, before and after PI and stored at –30°C until coagulation analyses were done, using routine measurements, at the Department of Clinical Chemistry, Karolinska University Hospital (Sweden). Total protein was analyzed on a Cobas 8000 instrument (Roche Diagnostics, Basel, Switzerland), activated partial thromboplastin time, prothrombin time and the international normalized ratio were documented, fibrinogen was assayed by Clauss coagulation photometry, antithrombin III was measured using a Sysmex CS 5100 instrument (Sysmex, Kobe, Japan), coagulation factors V, VIII, XIII, and VII were assayed by coagulation turbidimetry, von Willebrand factor by photometry, protein S-free by immunological turbidimetry and protein S by a chromogenic test of enzymatic activity on a hemostasis analyser BCS-XP, (Siemens, Munich, Germany). Viscoelastography of the plasma samples, before and after PI, was done on a TEG6S (Haemonetics Corp, Boston, MA, USA) using a cartridge loaded with citrated kaolin (CK), tissue factor/kaolin (RapidTEG [Haemonetics]), heparinase/kaolin (HK) and a platelet inhibitor (citrated functional fibrinogen [CFF]). The parameters CK R-time (coagulation time) and CFF maximum amplitude (MA) (fibrinogen level) were evaluated.

In vitro analysis on the different platelet derivates

In vitro parameters including recovery, before and after freezing, of platelet counts and mean platelet volume, were measured using the CA 620 Cellguard (Boule Medical, Stockholm, Sweden). pH (at 37°C) and the extracellular metabolic environment (glucose, lactate and bicarbonate) were studied using routine blood gas equipment (ABL 800, Radiometer Medical ApS, Copenhagen, Denmark). Flow cytometric analyses for the expression of a conformational epitope on the GPIIb/IIIa complex of activated platelets was assessed using the FITC-conjugated monoclonal antibody PAC-1 (IgM, Becton Dickinson, Franklin Lakes, NJ, USA); for P-selectin, PE-conjugated CD62P (Beckman Coulter, Brea, CA, USA); for GPIb, FITC-conjugated CD42b (Beckman Coulter); for signaling endothelial cell adhesion molecule[31], PECAM-1 (Sigma-Aldrich, St. Louis, MI, USA); for GPIIb, PE-conjugated CD61 (Beckman Coulter, Indianapolis, IN, USA); for collagen receptor detection, FITC-conjugated GPVI (Pharmingen BD Biosciences, San Jose, CA, USA) and for changes in the platelet mitochondria transmembrane potential (Δψ), the mitochondrial permeability transition detection kit MitoPT JC-1 (Immuno-Chemistry Technologies, LCC, Bloomington, MN, USA). All were demonstrated using a CytoFLEX Flow Cytometer (Beckman Coulter Life Sciences) as previously described[17,22,24].

Thromboelastometry

To assess the interplay between different platelet and plasma coagulation factors (**Table I**) in the coagulation cascade, thromboelastometry using a ROTEM delta 3000 (TEM International, GmbH, Munich, Germany) was performed. The different platelet samples were diluted approximately 1: 8, in either base AB plasma or plasma exposed to PI technology (Intercept, Cerus), obtained from the same pooled unit, to a platelet concentration comparable to human levels (200×10^9/L). The mixed plasma-platelet combinations were then analyzed in ROTEM, by using EXTEM as the read out according to the manufacturer's instructions. Clotting time (CT) which reflects the time until the clot starts to form; influenced by pro- and anti-coagulation factors, clot formation time (CFT), the time that a clot takes to increase from 2 to 20 mm above baseline and maximum clot firmness (MCF) which reflects the maximum tensile strength of the thrombus were recorded.

Statistical analyses

The mean values and standard deviations are given unless otherwise indicated. A paired *t*-test was assessed to determine the statistical significance of the differences between the pooled plasma before and after PI. Two-way analysis of variance, including Tukey's multiple comparison test, was performed to distinguish differences between the platelet groups and their interaction with the plasma derivatives. The analyses were carried out using GraphPad Prism version 9.0.0 (121) (GraphPad, San Diego, CA, USA).

RESULTS

Amotosalen treatment reduced several plasma coagulations factors

This experiment compared the concentration of coagulation factors and plasma function before and after treatment with amotosalen. PI significantly reduced several coagulation factors, including fibrinogen but did not affect the TEG variables CK R and CFF MA, expressing coagulation factors and functional fibrinogen properties,

respectively (**Table II**) while the levels of factor XIII, von Willebrand factor, and protein S remained unaffected after PI treatment (**Table II**).

Cryopreservation affects platelet *in vitro* variables in multiple ways

In general, platelet concentration, pH, the extracellular glucose and lactate concentrations and bicarbonate calculation were altered after reconstitution of the cryopreserved platelet units, as outlined in **Table III**. The percentages of platelets expressing GPIIb, GPVI and GPIb were decreased after cryopreservation (**Table IV**). In contrast, we observed increased activation after cryopreservation, reflected by increased cell surface expression of P-selectin (**Table IV**) as well as spontaneous PAC-1 binding, obvious in the cryopreserved units (**Table IV**) Consequently, cryopreservation reduced the platelets' ability to respond to ADP and collagen as assessed by measurement of the active conformation of GPIIb/IIIa by using PAC-1 as the readout (**Table IV**). Accordingly, $\Delta\psi$ (JC-1-positive cells) was also found to be reduced by cryopreservation (**Table IV**).

Table II - *In vitro properties of plasma units before and after amotosalen treatment*

Variable	Before PI	After PI
Total protein (g/L)	63.88±1.64	62.25±2.46*
Fibrinogen (g/L)	2.23±0.12	1.70±0.10**
APTT (sec)	34.0±1.02	38.0±1.30**
PT (INR)	1.02±0.03	1.14±0.05**
AT3 (kIE/L)	0.94±0.03	0.90±0.04**
TEG CK R	12.44±3.14	12.44±3.85
TEG CFF MA	23.69±1.74	24.19±2.32
FV	0.91±0.08	0.77±0.05**
FVIII	0.91±0.19	0.61±0.12**
FXIII	1.19±0.13	1.13±0.11
FVII	0.90±0.05	0.80±0.05*
vWF	1.01±0.30	0.95±0.25
Protein S	0.93±0.09	0.87±0.09
Protein C	1.03±0.06	0.90±0.06**

Data are presented as mean ± standard deviation. Results are for before pathogen inactivation (PI) treatment (i.e. on pooled units before PI) and after PI (n=16), directly after the procedure. *p<0.05 compared to pre-PI. **p<0.01 compared to pre-PI.
APTT: activated partial thromboplastin time; PT: prothrombin time; INR: international normalized ratio; AT3: antithrombin III; TEG CK R: clotting time on thromboelastography; TEG CFF MA: fibrinogen-nased clot firmness on thromboelastography; FV: factor V; FVIII: factor VIII; FXIII: factor XIII; FVII: factor VII; vWF: von Willebrand factor.

Table III - *Basic variables of the platelet units before and after cryopreservation*

Variable	Before cryopreservation	After cryopreservation
N. of platelets ($\times10^9$/L)	1,390±182	982±203*
Mean platelet volume (fL)	9.5±0.3	9.9±0.6
pH (37°C)	6.964±0.028	7.097±0.056*
Glucose (mmol/L)	8.1±0.6	17.1±1.2*
Lactate (mmol/L)	6.9±0.6	3.3±0.5*
Bicarbonate (calculated)	9.4±0.5	18.0±1.1*

Data are presented as mean ± standard deviation (n=16). Results are presented for pre- cryopreservation (i.e. on day 1 after collection day prior to freezing) and post-cryopreservation, 2 hours after thawing. *p<0.05 compared to pre-cryopreservation.

Table IV - *Effects of cryopreservation on platelet variables*

Variable	Type of platelet concentrate	Expression (%)
GPIIb (%)	Conventional	98.5±1.3
	Cryopreserved	95.9±3.9**
GPVI (%)	Conventional	98.2±0.9
	Cryopreserved	86.4±6.3**
GPIb (%)	Conventional	97.6±2.8
	Cryopreserved	90.2±5.7**
P-selectin (%)	Conventional	8.4±4.2
	Cryopreserved	76.9±8.1**
PAC-1 (%)	**Type of platelet concentrate**	**Expression (%)**
Spontaneous	Conventional	3.4±3.8
	Cryopreserved	27.3±10.1**
ADP	Conventional	72.1±12.5
	Cryopreserved	30.6±7.8**
Collagen	Conventional	72.4±15.0
	Cryopreserved	51.6±12.8**
Δψ (% JC-1$^+$ platelets)	Conventional	90.0±8.4
	Cryopreserved	47.3±12.5**

Expression of platelet surface markers GPIIb, GPVI, GPIb, P-selectin and the active conformation of GPIIb/IIIa determined with PAC-1 and Δψ is presented as percentages in all cases. Results are presented for platelet concentrates pre-cryopreservation (Conventional) (i.e. on day 1 of storage before freezing) and post-cryopreservation (2- and 24 hours after thawing) as mean ± standard deviation (n=16). *p<0.05 and **p<0.01 *vs* pre-cryopreservation for cryopreserved platelet concentrates.

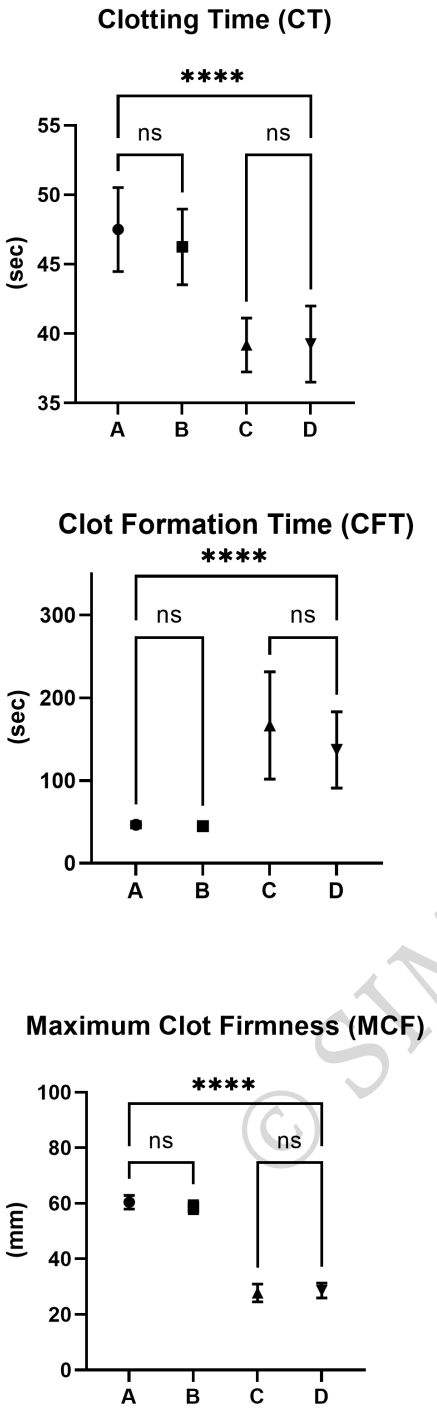

Figure 2 - Interplay between the platelet and plasma derivatives

EXTEM clotting time, clot formation time and maximum clot firmness assessed on the following combinations: (A) buffy coat platelets (day 1)/base plasma (day 1); (B) buffy coat platelets (day 1)/pathogen inactivated plasma; (C) cryopreserved buffy coat platelets/ base plasma (day 1); (D) cryopreserved buffy coat platelets/pathogen-inactivated plasma. Results are presented as mean + standard deviation (n=16). ns: not statistically significantly different; *p<0.05 and ****p<0.0001 multiple comparison between groups.

Viscoelastic properties were affected by cryopreservation rather than by amosotalen treatment

Initially, we hypothesized that PI of plasma could affect the interaction of the plasma with platelets. To test this hypothesis, Amosotalen-treated plasma, fresh platelets and cryopreserved platelets were tested in combinations. All cryopreservation combinations resulted in shortened EXTEM - CT but prolonged CFT and reduced MCF, significantly as compared with the fresh platelet combinations (**Figure 2**). Overall, the PI-treatment of the plasma seems not to have any impact on CT, CFT or MCF, clot-activated by tissue factor (**Figure 2**). Consequently, CFT as well as the MCF activated by tissue factor thromboplastin was similarly unaffected by the PI-plasma (**Figure 2**).

DISCUSSION

It is well known that blood component manufacturing affects different platelet and plasma derivatives in multiple ways and much work has been carried out to develop new platelet and plasma products that, through their contribution to hemostasis, can prevent or stop bleeding[13,17,19-21,25]. Overall, these studies show that additional manufacturing steps to increase safety and availability, such as PI techniques and cryopreservation of platelets, further affect the *in vitro* function of the products. Initially, our data indicated multiple *in vitro* effects of cryopreservation of the platelets and pathogen reduction of the plasma. The data presented are in line with those of recent studies on such platelet derivatives, showing phenotypic and functional deficiencies as compared with fresh platelets[17, 24], and previous data on photochemical treatment of plasma, showing a moderate reduction of labile coagulation factors[19-21]. The effects of PI treatment on differently prepared platelet and plasma products have been investigated in previous studies. Agey *et al.*[26] showed reduced clot strength in ROTEM but unaltered platelet metabolism in PI-treated cold-stored platelets after 14 days. In our study cryopreserved platelets were reconstituted in fresh plasma and showed an activated profile and reduced ROTEM clot stability. In a study by Kamyszek *et al.*[27] cryoprecipitate prepared from PI-treated and untreated plasma was analyzed with proteomics. It was found that PI treatment did not affect the coagulation factor content but reduced platelet-derived proteins. This is in line with the findings

Blood Transfus 2023; 21: 137-145; doi: 10.2450/2022.0279-21

of Erickson *et al.*[28] who demonstrated stable levels of pro- and anticoagulant factors in PI-treated plasma 5 days after thawing, except for the labile factors V, VIII and protein S. The different results from our study and other studies showing reductions of coagulation factors in PI-treated plasma may be explained by methodology, treatment of samples and time to analysis. However, the main objective in our study, differently from that of other studies, was to evaluate the interaction between manipulated plasma and platelet components.

Importantly, our ROTEM results confirm that all tested combinations supported clot formation in this setup, regardless of the platelets and plasma used, but with differences in the variables. The CT intervals for all tests were within the normal range, suggesting that the coagulation factors are functioning normally, regardless of PI treatment of the plasma or not[29-31]. However, all cryopreservation combinations shortened the CT and prolonged the CFT as compared with the other groups, suggesting that there is some impact on clot propagation in such combinations. This finding is, however, not especially specific and could be attributed to either the concentration of platelets, platelet dysfunction or low fibrinogen[32]. It can be ruled out that differences in platelet concentration or differences in the plasma content might have contributed to the differences observed since all platelet samples were diluted in equivalent AB plasma (before and after PI treatment) to a concentration comparable to human levels (200×10^9/L) before all ROTEM runs. Hypothetically, the shortened CT in the cryopreserved combinations might, therefore, have been caused by release of substances promoting hemostasis from the procoagulant platelets as was found in previous studies[33,34]. Functional GPIIb/IIIa, as well as maintained fibrinogen concentrations in the plasma, are necessary for clot propagation. The prolonged CFT found in the cryopreserved combinations could, therefore, have been due to reduced capacity to transform the GPIIb/IIIa receptors into the active conformation, which is supported by the findings from our flow cytometry measurements. The MCF for EXTEM was also uncharacteristically low in all cryopreserved combinations[31,32], suggesting that clot stability was somehow affected as compared to that with the fresh platelet combinations. A low MCF could also be attributed to low fibrinogen or hyperfibrinolysis[29-32]. but since our results do not confirm a deterioration in MCF in the PI-combinations, it can be ruled out that the reduction in MCF is linked to a reduction in fibrinogen levels caused by the amotosalen treatment. Given that none of the amplitudes narrowed with time, hyperfibrinolysis is clearly not the problem. Therefore, our data indicate that platelets play a more pronounced role in this blood component interaction setup and that the contribution from the PI-treated plasma to adverse interaction effects is negligible.

Hemostasis is, however, a complex interactive process involving adhesion of platelets to damaged endothelium, formation of a platelet plug (aggregation), formation of a fibrin network to stabilize the plug, clot retraction and finally fibrinolysis[35]. Platelet plug formation and coagulation (primary and secondary hemostasis) are generally initiated simultaneously upon diverse activation pathways, which means that these two processes are communicating with each other jointly during the coagulation process. Initially, coagulation factors share (or compete for) membrane receptors (or binding sites) on resting platelets[4,36]. Once platelets are activated, they provide additional binding sites with greater affinity for the activated coagulation factors than for the unactivated coagulation factors[4,36]. Our data indicate that these multifactorial events lead to a loss in mechanical force caused by the blood component manufacturing processes. Balanced platelet function and coagulation are crucial for stable blood circulation. If one of the factors involved is not functioning correctly, this may lead to impaired hemostasis, which can be clinically expressed as bleeding complications. Abnormalities in GPIb and αIIbβ3 have been associated with moderate to severe bleeding symptoms. Impaired prothrombin consumption can be adjusted by the addition of human factor VIII or factor VIII-von Willebrand factor, which might indicate the essential role of the factor VIII/von Willebrand factor-GPIb interaction in the activation of coagulation[37]. Uncharacteristic αIIbβ3 expression results in malfunctioning clot retraction due to decreased binding of fibrinogen to platelets[37]. Immobilized protein C also affects platelet binding and activation signaling through GPIbα under shear conditions[38]. The ability of platelets to bind protein C might, therefore, stimulate platelet activation and limit thrombosis situations by localizing the anticoagulant role of the

protein C system. GPIbα expressed on activated platelets has also been shown to contribute to the localization of factor VII to the surface of activated platelets[39] and induction of platelet GPVI shedding results in down-regulation of GPVI under procoagulant conditions[40], all strengthening the importance of preserved platelet and plasma function. Our data show that the interplay between platelets and plasma is influenced by the pretransfusion *in vitro* preparation procedures and that manufacturing effects on the platelets play a more pronounced role. In a future perspective, the extent to which interactions may compromise hemostatic function could be helpful in identifying the clinical effectiveness of transfusing different blood components in combination. However, the interpretation of our data is limited by the fact that the interactions between blood components were evaluated in an *in vitro* system which cannot completely reflect the *in vivo* effects. This discrepancy warrants additional studies to shed more light onto the link between the present *in vitro* data and the real *in vivo* interplay between platelets and plasma derivatives after transfusion.

CONCLUSIONS

Our ROTEM results show that all tested combinations of cryopreserved or fresh platelets with amosotalen-treated or –untreated plasma supported clot formation, but there was a tendency for a diminished interaction related to the platelet manufacturing rather than to PI treatment of the plasma. Thus, our study confirmed that PI of plasma reduces coagulation factors to some extent and that cryopreservation of platelets causes several phenotypic alterations and functional deficiencies compared with fresh platelets. These effects may influence the interplay between platelets and plasma after transfusion, warranting an increased attention to studies focusing on blood component interplay *in vivo*.

ETHICAL APPROVAL

The Swedish Regional Ethics Review Boards advisory opinion was that the Ethics Review Authority had no ethical objections regarding this research project (Dnr. 2021-01321).

ACKNOWLEDGMENTS

The Authors thank Mrs Gunilla Gryfelt at Karolinska University Hospital (KITM), Huddinge for her skillful technical assistance.

AUTHORSHIP CONTRIBUTIONS

PS designed the study together with AW. PS was responsible for selected methods, performed the statistics and wrote the first draft including figures and tables. KE performed the laboratory experiments, summarized the data, and contributed intellectually to the results section; LL contributed to the plasma part of the study in terms of design, expert knowledge, laboratory experiments and as an active co-author in the Materials and methods section of the manuscript. MU gave valuable intellectual input regarding the overall study design as well as the final version of the manuscript. AW (senior author) designed the study and wrote the later versions of the manuscript together with PS. All Authors actively contributed to the review and approved its submission

The Authors declare no conflicts of interest.

REFERENCES

1. Agren A, Edgren G, Kardell M, et al. In vitro combinations of red blood cell, plasma and platelet components evaluated by thromboelastography. Blood Transfus 2014; **12**: 491-6.
2. Schmidt DE, Halmin M, Wikman A, et al. Relative effects of plasma, fibrinogen concentrate, and factor XIII on ROTEM coagulation profiles in an in vitro model of massive transfusion in trauma. Scand J Clin Lab Invest 2017; **77**: 397-405.
3. Holcomb JB, Tilley BC, Baraniuk S, et al. Transfusion of plasma, platelets, and red blood cells in a 1: 1: 1 vs a 1: 1: 2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. JAMA 2015; **313**: 471-82.
4. Sang Y, Roest M, de Laat B, et al. Interplay between platelets and coagulation. Blood Rev 2020; **46**: 100733.
5. Dubois C, Panicot-Dubois L, Merrill-Skoloff G, et al. Glycoprotein VI-dependent and -independent pathways of thrombus formation in vivo. Blood 2006; **107**: 3902-6.
6. Heemskerk JW, Mattheij NJ, Cosemans JM. Platelet-based coagulation: different populations, different functions. J Thromb Haemost 2013; **11**: 2-16.
7. Ninivaggi M, Feijge MA, Baaten CC, et al. Additive roles of platelets and fibrinogen in whole-blood fibrin clot formation upon dilution as assessed by thromboelastometry. Thromb Haemost 2014; **111**: 447-57.
8. Swieringa F, Spronk HMH, Heemskerk JWM, et al. Integrating platelet and coagulation activation in fibrin clot formation. Res Pract Thromb Haemost 2018; **2**: 450-60.
9. Versteeg HH, Heemskerk JW, Levi M, Reitsma PH. New fundamentals in hemostasis. Physiol Rev 2013; **93**: 327-58.
10. Cauwenberghs S, van Pampus E, Curvers J, et al. Hemostatic and signaling functions of transfused platelets. Transfus Med Rev 2007; **21**: 287-94.
11. Seghatchian J. Platelet storage lesion: an update on the impact of various leukoreduction processes on the biological response modifiers. Transfus Apher Sci 2006; **34**: 125-30.
12. Seghatchian J, Krailadsiri P. The platelet storage lesion. Transfus Med Rev 1997; **11**: 130-44.
13. Knutson F, Alfonso R, Dupuis K, et al. Photochemical inactivation of bacteria and HIV in buffy-coat-derived platelet concentrates under conditions that preserve in vitro platelet function. Vox Sang 2000; **78**: 209-16.

14. Sandgren P, Berlin G, Tynngard N. Treatment of platelet concentrates with ultraviolet C light for pathogen reduction increases cytokine accumulation. Transfusion 2016; **56**: 1377-83.
15. Zeddies S, De Cuyper IM, van der Meer PF, et al. Pathogen reduction treatment using riboflavin and ultraviolet light impairs platelet reactivity toward specific agonists in vitro. Transfusion 2014; **54**: 2292-300.
16. Jimenez-Marco T, Ballester-Servera C, Quetglas-Oliver M, et al. Cryopreservation of platelets treated with riboflavin and UV light and stored at -80 degrees C for 1 year. Transfusion 2021; **61**: 1235-46.
17. Meinke S, Wikman A, Gryfelt G, et al. Cryopreservation of buffy coat-derived platelet concentrates photochemically treated with amotosalen and UVA light. Transfusion 2018; **58**: 2657-68.
18. Valeri CR, Ragno G, Khuri S. Freezing human platelets with 6 percent dimethyl sulfoxide with removal of the supernatant solution before freezing and storage at -80 degrees C without postthaw processing. Transfusion 2005; **45**: 1890-8.
19. Irsch J, Lin L. Pathogen inactivation of platelet and plasma blood components for transfusion using the INTERCEPT Blood System. Transfus Med Hemother 2011; **38**: 19-31.
20. Prowse C. Properties of pathogen-inactivated plasma components. Transfus Med Rev 2009; **23**: 124-33.
21. Singh Y, Sawyer LS, Pinkoski LS, et al. Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function. Transfusion 2006; **46**: 1168-77.
22. Ohlsson S, Diedrich B, Uhlin M, et al. Optimized processing for pathogen inactivation of double-dose buffy-coat platelet concentrates: maintained in vitro quality over 7-day storage. Vox Sang 2018; **113**: 611-21.
23. Sandgren P, Diedrich B. Pathogen inactivation of double-dose buffy-coat platelet concentrates photochemically treated with amotosalen and UVA light: preservation of in vitro function. Vox Sang 2015; **108**: 340-9.
24. Tynngard N, Bell A, Gryfelt G et al. Cryopreservation of buffy coat derived platelets: Paired in vitro characterization using uncontrolled versus controlled freezing rate protocols. Transfusion 2021; **61**: 546-56.
25. Johnson LN, Winter KM, Reid S, et al. Cryopreservation of buffy-coat-derived platelet concentrates in dimethyl sulfoxide and platelet additive solution. Cryobiology 2011; **62**: 100-6.
26. Agey A, Reddoch-Cardenas K, McIntosh C, et al. Effects of Intercept pathogen reduction treatment on extended cold storage of apheresis platelets. Transfusion 2021; **61**: 167-77.
27. Kamyszek RW, Foster MW, Evans BA, et al. The effect of pathogen inactivation on cryoprecipitate: a functional and quantitative evaluation. Blood Transfus 2020; **18**: 454-64.
28. Erickson A, Waldhaus K, David T, et al. Plasma treated with amotosalen and ultraviolet A light retains activity for hemostasis after 5 days post-thaw storage at 1 to 6°C. Transfusion 2017; **57**: 997-1006.
29. Nielsen VG. A comparison of the thrombelastograph and the ROTEM. Blood Coagul Fibrinolysis 2007; **18**: 247-52.
30. Nielsen VG, Kirklin JK, Hoogendoorn H, et al. Thrombelastographic method to quantify the contribution of factor XIII to coagulation kinetics. Blood Coagul Fibrinolysis 2007; **18**: 145-50.
31. Sankarankutty A, Nascimento B, Teodoro da Luz L, et al. TEG(R) and ROTEM(R) in trauma: similar test but different results? World J Emerg Surg 2012; **7** (Suppl 1): S3
32. Theusinger OM, Schroder CM, Eismon J, et al. The influence of laboratory coagulation tests and clotting factor levels on Rotation Thromboelastometry (ROTEM(R)) during major surgery with hemorrhage. Anesth Analg 2013; **117**: 314-21.
33. Tegegn TZ, De Paoli SH, Orecna M, et al. Characterization of procoagulant extracellular vesicles and platelet membrane disintegration in DMSO-cryopreserved platelets. J Extracell Vesicles 2016; **5**: 30422.
34. Tynngard N, Wikman A, Uhlin M, et al. Haemostatic responsiveness and release of biological response modifiers following cryopreservation of platelets treated with amotosalen and ultraviolet A light. Blood Transfus 2020; **18**: 191-9.
35. Tynngard N, Lindahl TL, Ramstrom S. Assays of different aspects of haemostasis - what do they measure? Thromb J 2015; **13**: 8.
36. Gale AJ. Continuing education course #2: current understanding of hemostasis. Toxicol Pathol 2011; **39**: 273-80.
37. Bellucci S, Girma JP, Lozano M, et al. Impaired prothrombin consumption in Bernard-Soulier syndrome is corrected in vitro by human factor VIII. Thromb Haemost 1997; **77**: 383-6.
38. White TC, Berny MA, Tucker EI, et al. Protein C supports platelet binding and activation under flow: role of glycoprotein Ib and apolipoprotein E receptor 2. J Thromb Haemost 2008; **6**: 995-1002.
39. Weeterings C, de Groot PG, Adelmeijer J, et al. The glycoprotein Ib-IX-V complex contributes to tissue factor-independent thrombin generation by recombinant factor VIIa on the activated platelet surface. Blood 2008; **112**: 3227-33.
40. Al-Tamimi M, Grigoriadis G, Tran H, et al. Coagulation-induced shedding of platelet glycoprotein VI mediated by factor Xa. Blood 2011; **117**: 3912-20.