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Predictive haemostatic biomarkers and transfusion efficacy, insights from fresh frozen plasma use in surgical patients, preliminary results

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Abstract

The aim of the study was to examine various haemostasis values to identify the most relevant biological indicators for detecting significant haemorrhage, to determine the effectiveness of fresh frozen plasma (FFP) transfusion. Our findings suggest that a low prothrombin time, elevated Von Willebrand Antigen, increased plasma fibrinogen, and reduced Ca2 + levels are associated with challenges in achieving proper haemostasis. However, measurements of factors II, V, VII, VIII, IX, X, XI, XIII, protein C, and protein S do not appear to be linked to difficulties in achieving adequate haemostasis. Additionally, the administration of FFP appears to impact factors V, VII, X, and II.

Trial registration EudraCT number: 2019-002898-64.

Keywords Transfusion yield, Transfusion threshold, Fresh frozen plasma, Haemostasis

Introduction

Massive haemorrhage occurring in the surgical setting is a rare but highly critical event. While mechanical methods can be employed in certain cases to achieve haemostasis, medicinal interventions become necessary in others such as administration of haemostatic products [1].

The understanding of the mechanisms underlying the activation of the haemostasis cascade has improved, revealing two primary categories: Disseminated Intravascular Coagulation (DIC), observed in patients with inflammation and commonly found in intensive care settings [2], and Trauma Induced Coagulopathy (TIC) in patients with multiple injuries [3].

To establish an optimal strategy in these challenging situations, transfusion algorithms have been developed. A potential solution lies in the identification of measurable parameters that can predict future bleeding incidents [4]. This assumption postulates that excessive consumption of clotting proteins contributes to uncontrollable haemorrhage.

Ethical approval for this study (Ethical Committee N°P2019-422) was obtained from the ethical committee of Erasme hospital, Anderlecht, Belgium (chairperson Prof J-M BOEYNAEMS) on October 19th, 2019. An EudraCT file was created, receiving the reference 2019-002898-64. Written informed consent was obtained from all participating patients at least 48 h prior to surgery. The study adhered to CARE® & EQUATOR® guidelines.

The objective of this study was to evaluate various haemostasis and hematologic biomarkers to identify the

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most relevant ones for detecting major haemorrhage and determining the yield of fresh frozen plasma transfusion. Working hypothesis suggests that monitoring the evolution of specific haemostasis parameters could enable prediction of the patient's haemostatic condition.

Material and methods

We conducted an observational cohort study at the Charleroi academic hospital (Centre Hospitalier Universitaire de Charleroi, Hôpital Marie Curie) in Belgium, following a prospective and monocentric design. The inclusion period for participants was from October 2019 to March 2020.

Population

The study population consisted of patients scheduled for elective surgery who were identified as having high risk of requiring blood transfusion based on the assessment of guidelines established by the British committee for standards in Hematology. These guidelines were routinely used in the department to evaluate preoperative bleeding risk. Demographic information, including age, weight, height, type of surgery, and interruption date for anticoagulant or antiplatelet treatment, was collected.

Anaesthesiology protocol

The anaesthetic maintenance protocol was following local guidelines. The blood pressure target was a minimum of 65 mmHg mean arterial pressure for all patients. The methods to achieve this target was at the discretion of the anaesthetist (crystalloid or colloid expansion, norepinephrine) but had to be recorded in the investigator's notebook.

The transfusion decision was made by the anaesthesiologist physician in charge of the patient following European society of anesthesiology guidelines [1]. When possible, bleeding volumes were collected using a CellSaver® (Haemonetics Corporation, Boston, United States of America). The CellSaver® was fully assembled before the start of surgery and made available to the surgeons throughout. In all cases where it was used, it was used throughout the surgery. The entire circuit was anticoagulated with 5 000 IU of unfractionated heparin in a 500 mL bag of 0.9% NaCl. For all patients, the volume recovered either by the CellSaver® or by conventional aspiration was measured at the end of the procedure. If the CellSaver® was able to return a volume to the patient, it was administered as soon as the minimum volume was reached.

Failure of haemostasis was determined by the surgical team in charge of the patient when surgical control of the haemorrhage could not be achieved.

Endpoints

The primary endpoint of the study was to evaluate changes in blood sample parameters during haemostatic degradation, corrective transfusion, and postoperative period to assess the performance of fresh frozen plasma (FFP) transfusion. During surgery, a comprehensive coagulation sample test was performed every hour, measuring various parameters such as Von Willebrand antigen (vWF:Ag), Von Willebrand Ristocetin cofactor (vWF:RCof), Prothrombin Time Index (PTI), activated partial thromboplastin time (APTT), fibrinogen plasma level, thrombin time (TT), factor II, factor V, factor VII, factor VIII, factor IX, factor X, factor XI, factor XIII, protein C, and protein S. Additionally, blood gas analysis, including pH, PaO2, PaCO2, HCO3-, lactate, and ionized calcium, was conducted every hour. The sampling process began after induction and continued until the completion of surgery. In case of transfusion, two additional samples were collected, one before the transfusion and one immediately after. Six hours following the conclusion of surgery, a new series of samples was taken to evaluate the postoperative haemostasis.

All samples were sent directly to the hospital's central laboratory. Routine tests (PT, APTT, Fibrinogen) were carried out immediately, so that clinicians could analyse them if necessary. The rest of the factors were analysed at a much later date during special procedures carried out by the laboratory once a sufficient number of patients had been reached. Tests were carried out every 14 days in the hospital for targeted screening of certain deficiencies in other patients. The maximum storage time for samples was therefore 14 days. The samples were stored after centrifugation at a temperature of – 80 °C.

Statistical analysis

Due to the small number of patients and the very preliminary context, no statistical analysis was carried out. Only the raw results were presented.

Results

Seven patients were included in the study, with two receiving FFP (patients #5 and #6) and five not receiving FFP (patients #1, #2, #3, #4, and #7) (Table 1). Among the patients, three had undergone trauma in the seven days preceding the operation and required damage control surgery on the same day (patients #1, #3, and #6). Two other patients (patients #2 and #7) were enrolled due to planned surgery associated with haemorrhage. Patient #4 underwent liver resection, and the cardiac surgery patient (#5) underwent valvuloplasty due to valvular endocarditis.

Table 1 Epidemiologic, demographic and transfusion data

	Surgery type	Duration (hh:mm)	Blood loss (mL)	Crystalloids (mL)	Colloids (mL)	Cell Saver (mL)	Calcium gluconate (gram)	Fresh Frozen Plasma (unit)	Red blood cell concentrates (unit)	Tranexanic acid (mg)
Patient #1	Orthopaedic	8:40	1470	4000	0	750	1g	0	0	1000
Patient #2	Orthopaedic	9:46	2330	4000	1000	705	1g	0	0	1000
Patient #3	Orthopaedic	5:15	3100	3000	500	600	1g	0	0	1000
Patient #4	Liver	6:48	875	2000	0	600	1g	0	0	1000
Patient #5	Cardiac	**6:11**	**1000**	**2000**	**0**	**0**	**1g**	**4**	**4**	**2236**
Patient #6	Orthopaedic	**2:52**	**1500**	**3000**	**0**	**0**	**2g**	**2**	**2**	**1000**
Patient #7	Orthopaedic	5:18	2200	3000	1000	0	1g	0	0	1000

Patient #5 and #6 (in yellow) received fresh frozen plasma.

Patients #5 and #6 received transfusions of FFP because the surgical team was unable to achieve satisfactory haemostasis and bleeding control. None of the patients received FFP solely for haemodynamic reasons.

Regarding the patients classified as inflammatory, who had previously undergone surgery within the last seven days (patients #1, #3, #5, and #6), their preoperative haemostasis was generally normal, except for patient #6 who exhibited disrupted coagulation throughout their hospital stay. Notably, none of the patients were taking anticoagulants or platelet aggregation inhibitors before the surgery. Coagulation factor assays and haematological tests were also conducted in patient #6, who exhibited a slight decrease in factor VII (68%, normal = 70%) and thrombocytosis (platelet count = 700,000 platelets per microliter). This patient was discharged from the hospital with persistent abnormal haemostasis test results.

A red blood cell transfusion was performed at the end of the operation for patient #5 and at the beginning for patient #6 due to anaemia with a haemoglobin level below 7 g/dL. FFP was administered at 210 min and 270 min for patient #5 and at 120 min for patient #6 (Table 2).

Patients #5 and #6 were not able to benefit from blood return via the CellSaver® due to sepsis present at the time of surgery. Patient #7 was unable to benefit from cell saver restitution due to incorrect assembly of the kit and therefore the inability of the kit to supply a sufficient volume of blood.

The changes in biological parameters over time are depicted in the figure. Data was missing for some

Table 2 Fluid and other therapeutics management

	Blood loss (mL)	Crystalloids (mL)	Colloids (mL)	Cell Saver (mL)	Calcium gluconate (gram)	Fresh Frozen Plasma (unit)	Red blood cell concentrates (unit)	Tranexanic acid (mg)
Patient #1	1470	4000	0	750	1g	0	0	1000
Patient #2	2330	4000	1000	705	1g	0	0	1000
Patient #3	3100	3000	500	600	1g	0	0	1000
Patient #4	875	2000	0	600	1g	0	0	1000
Patient #5	**1000**	**2000**	**0**	**0**	**1g**	**4**	**4**	**2236**
Patient #6	**1500**	**3000**	**0**	**0**	**2g**	**2**	**2**	**1000**
Patient #7	2200	3000	1000	0	1g	0	0	1000

Patient #5 and #6 (in yellow) received fresh frozen plasma

Fig. 1 Biologic values evolution during the surgery. *Lactates* blood lactate (mmol/L), *Ca2+* blood ionized calcium (mmol/L), *Hb* Haemoglobin (g/dL), *vWF:Ag* von Willebrand factor level, antigen (%), *vWF:RCof* von Willebrand factor activity, Ristocetin cofactor (%), *PT* Prothrombin Time (%), *APTT* activated partial thromboplastin time (sec), *INR* International Normalised Ratio, *Fibrinogen* Fibrinogen plasma level (g/L), *TT* Thrombin time (sec). Factor II: blood Factor II (%), Factor V: blood Factor V (%), Factor VII: blood Factor VII (%), Factor VIII: blood Factor VIII (%), Factor IX: blood Factor IX (%), Factor X: blood Factor X (%), Factor XI: blood Factor XI (%), Factor XIII: blood Factor XIII (%), Protein C: blood protein C (%), Protein S: blood protein S (%)

samples, volumes of samples taken were not sufficient to carry out all the analyses (Fig. 1).

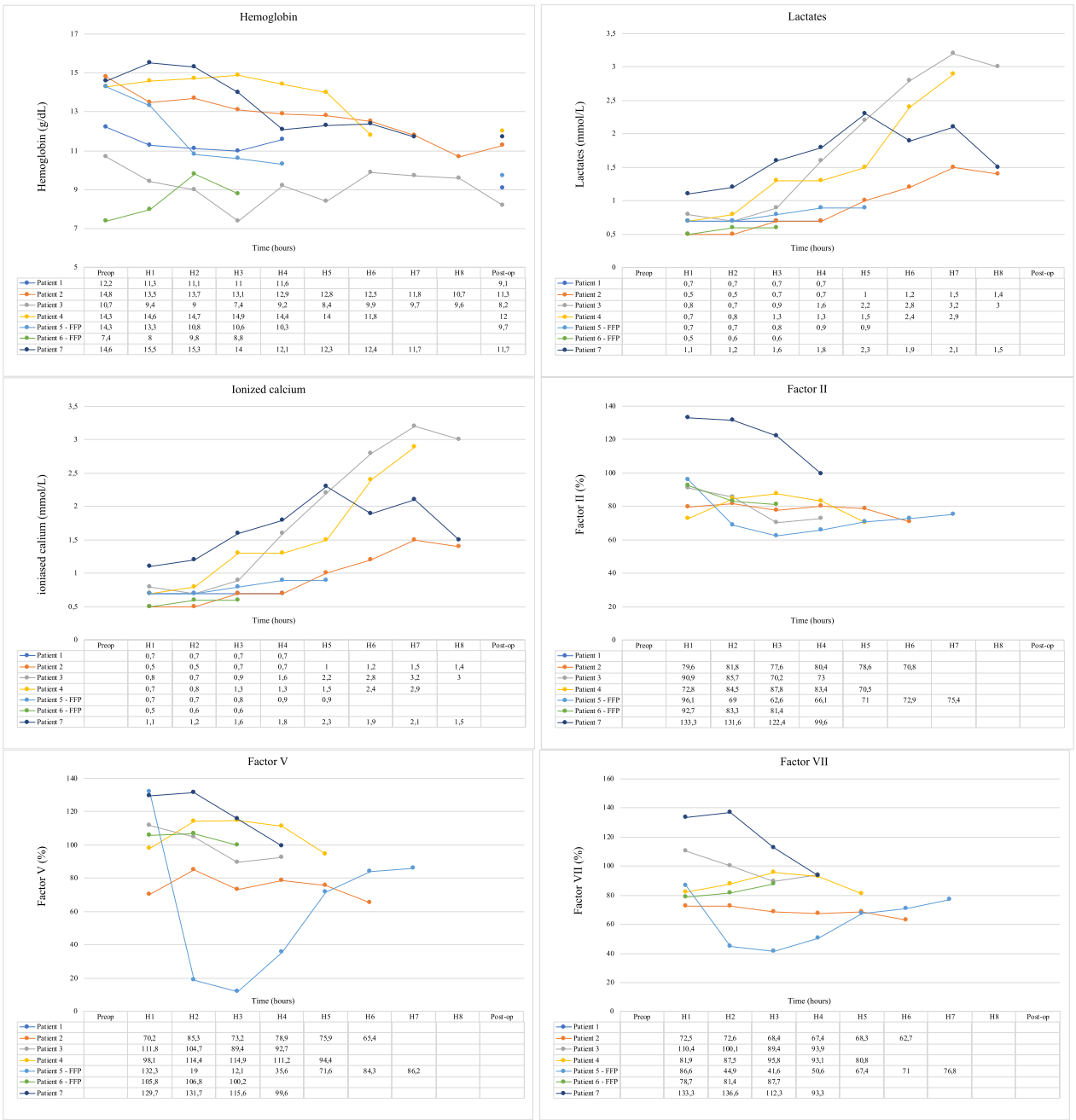


Fig. 1 continued

Discussion

Abnormalities in certain haemostatic parameters, such as low prothrombin time, high vWF:Ag, high plasma fibrinogen, and low Ca2 + levels, appear to be associated with difficulties in achieving effective haemostasis and may serve as indicators for the need for future FFP transfusion. In our case, the difficulty in obtaining satisfactory haemostasis was a notion described by the surgical team, which was not based on any metric scale or biological measurement. However, measurements of factors II, V, VII, VIII, IX, X, XI, XIII, protein C, and protein S do not seem to be associated with challenges in achieving appropriate haemostasis but rather with the need for transfusion. Notably, the administration of FFP appears to influence the levels of factors V, VII, X, and to a lesser extent factor II.

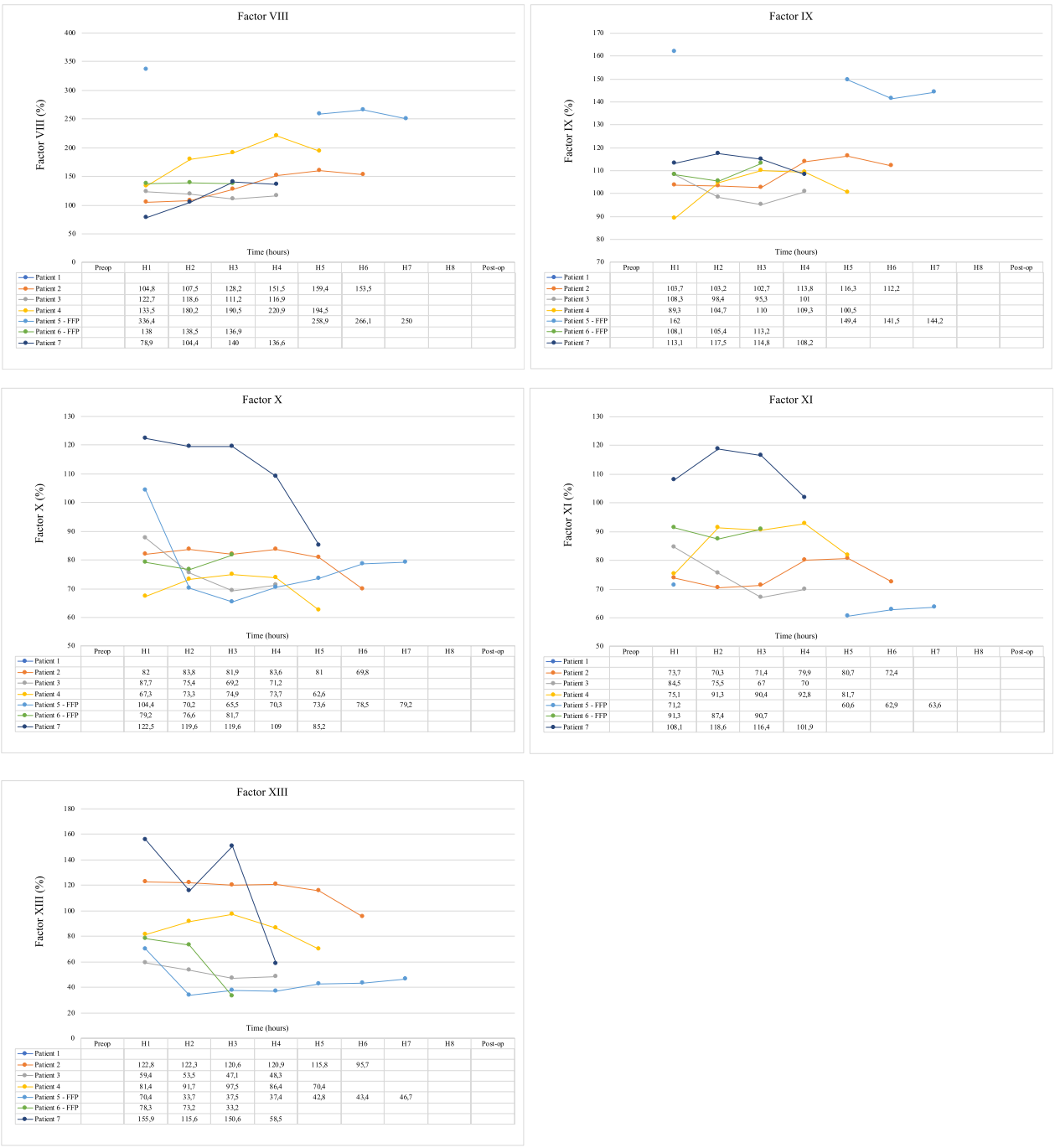
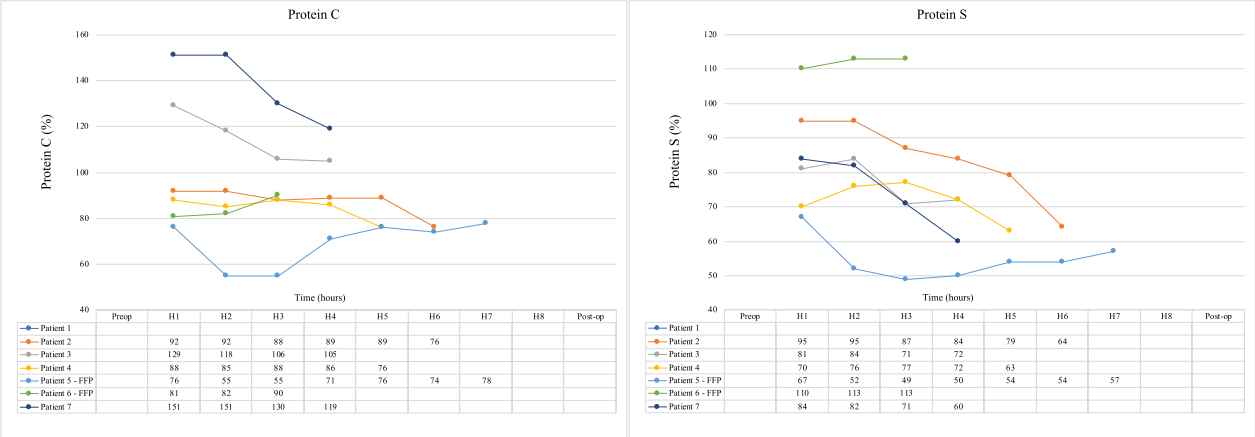


Fig. 1 continued

The use of a cell saver device was considered an integral component of managing blood loss and its potential impact on haemostasis. Cell savers aid in reducing allogeneic blood transfusions, yet they may influence the coagulation profile by reintroducing red blood cells with minimal clotting factors or platelets. This can contribute to the haemostatic challenges observed.

A key strength of our study lies in the frequent sampling intervals, with blood samples collected at least every hour during surgery and around transfusion events. Additionally, continuous monitoring of hemodynamic parameters at a 2-min interval allowed for



	Preop	H1	H2	H3	H4	H5	H6	H7	H8	Post-op
Patient 1										
Patient 2		92	92	88	89	89	76			
Patient 3		129	118	106	105					
Patient 4		88	85	88	86	76				
Patient 5 - FFP		76	55	55	71	76	74	78		
Patient 6 - FFP		81	82	90						
Patient 7		151	151	130	119					

	Preop	H1	H2	H3	H4	H5	H6	H7	H8	Post-op
Patient 1										
Patient 2		95	95	87	84	79	64			
Patient 3		81	84	71	72					
Patient 4		70	76	77	72	63				
Patient 5 - FFP		67	52	49	50	54	54	57		
Patient 6 - FFP		110	113	113						
Patient 7		84	82	71	60					

Fig. 1 continued

precise monitoring. To our knowledge, this is the first study to provide such detailed monitoring of FFP transfusion during surgery.

Hypocalcemia has been recognized as a known risk factor for bleeding, and both transfused patients in our study exhibited lower-than-expected calcium values. Previous studies have highlighted hypocalcemia in polytrauma patients, with evidence that hypocalcemia worsens over time. Our study contributes to the understanding that even mild hypocalcemia can lead to haemostatic disorders. However, there is currently no consensus on the optimal calcium dosage, although international societies recommend maintaining ionized calcium levels above 0.9 mmol/l [1, 5].

The fibrinogen plasma level is used as an indicator of heavy bleeding and uncontrolled bleeding when it is low and as a marker of inflammation when it is high [6–8]. Both transfused patients had higher fibrinogen plasma levels than the other patients. However, this elevation did not allow fibrinogen plasma levels to serve as an indicator of bleeding. This confirms that a patient's inflammatory state greatly impairs their clotting ability [9]. The role and influence of hyperfibrinogenaemia are described superficially in the current literature [8] and would merit further exploration.

Although coagulation factors were measured in our study, their dosage did not reveal significant deficiencies or marked increases indicative of active coagulopathy. Notably, this study is one of the few to examine factor XIII in the context of active haemorrhage, despite current European recommendations suggesting its inclusion in monitoring algorithms. However, the interest in factor XIII measurement was not confirmed by our small-scale study.

It is important to acknowledge the selection bias in our patient sample, which primarily included orthopaedic, digestive, and cardiac patients, with no inclusion of acute polytrauma patients. In addition, our population represents a small number of patients, making it impossible to draw firm conclusions for clinical practice. Similarly, the fact that only 2 patients were transfused, and in small quantities, meant that the effect of FFP could not be studied as expected, but only allowed us to draw up guidelines for the study of factors. Finally, the factor XII assay was not carried out due to a lack of role in coagulation. However, its monitoring could provide a better understanding of the evolution of the consumption of the various factors.

This study has a number of limitations, as described above. All the results should therefore be interpreted conditionally, taking these limitations into account. The results should be interpreted as guidelines for future research. For example, the question of the transfusion yield of FFP when the pre-transfusion samples are within normal limits must be interpreted with caution. Nevertheless, we believe that these results will enable certain teams to focus their work on certain coagulation factors more precisely. In addition, we have provided proof that hourly sampling in the context of haemorrhage is feasible and technically possible. These hourly samples were taken by the anaesthetist in charge of the patient without this interfering with the proper management of the patient, and no adverse events were reported.

Conclusions

The findings of this study are preliminary and do not permit definitive conclusions. However, the data may suggest that elevated fibrinogen levels, mild hypocalcaemia,

shortened prothrombin time, and increased levels of Von Willebrand factor antigen could probably serve as potential markers of haemostatic disorders. In evaluating transfusion efficacy, factors II, V, VII, and X emerge as probably relevant. This research offers a framework for more focused investigations in future studies. Moreover, we demonstrate the feasibility of repeated sampling, even in haemorrhagic conditions.

Abbreviations

DIC	Disseminated Intravascular Coagulation
TIC	Trauma Induced Coagulopathy
FFP	Fresh frozen plasma
RBC	Red blood cell
PTI	Prothrombin Time Index (%)
aPTT	Activated partial thromboplastin time (sec)
INR	International Normalized Ratio
TT	Thrombin time (sec)
vWF:Ag	Von Willebrand Antigen
vWF:RCof	Von Willebrand Ristocetin cofactor

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Author contributions
O. Duranteau: This author helped in protocol design, literature review, data collection, writing J. Decamps: This author helped in data collection P. Cauchie: This author helped in protocol design, data collection, advice on writing A. Daper: This author helped in advice on therapeutics, advice on writing B. Ickx: This author helped in advice on writing and protocol design, proofreading of the document T. Tuna: This author helped in advice on writing and protocol design, proofreading of the document.

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Availability of data and material
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Ethical approval for this study (Ethical Committee N°P2019-422) was obtained from the ethical committee of Erasme hospital, Anderlecht, Belgium (chairperson Prof J-M BOEYNAEMS) on October 19th, 2019.

Consent for publication
Written informed consent was obtained from all participating patients at least 48 h prior to surgery.

Competing interests
The authors declare no competing interests.

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