Maintaining plasma quality and safety in the state of ongoing epidemic – The role of pathogen reduction

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ABSTRACT

In the field of transfusion medicine, many pathogen reduction techniques (PRTs) are currently available, including those based on photochemical (PI) and photodynamic inactivation (PDI). This is particularly important in the face of emerging viral pathogens that may pose a threat to blood recipients, as in the case of the COVID-19 pandemic. However, PRTs have some limitations, primarily related to their adverse effects on coagulation factors, which should be considered before their intended use. A comprehensive search of PubMed, Wiley Online Library and Science Direct databases was conducted to identify original papers. As a result, ten studies evaluating fresh plasma and frozen-thawed plasma treated with different PI/ PDI methods and evaluating concentrations of coagulation factors and natural anticoagulants both before and after photochemical treatment were included in the review. The use of PI and PDI is associated with a significant decrease in the activity of all analysed coagulation factors, while the recovery of natural anticoagulants remains at a satisfactory level, variable for individual inactivation methods. In addition, the published evidence reviewed above does not unequivocally favour the implementation of PI/PDI either before freezing or after thawing as plasma products obtained with these two approaches seem to satisfy the existing quality criteria. Based on current evidence, if implemented responsibly and in accordance with the current guidelines, both PI and PDI can ensure satisfactory plasma quality and improve its safety.

1. Introduction

Fresh frozen plasma (FFP) plays a crucial role in everyday clinical practice. Despite the growing availability of novel pharmaceutical agents designed to control the disorders of hemostasis, FFP still remains a primary recommended option in case of acute bleeding and massive transfusion [1,2]. However, FFP obtained from the whole blood donation or by apheresis may be a source of infectious agents that can be transferred to the transfusion recipient. Apart from routine preventive measures, such as serological and molecular testing combined with a quarantine period, a number of pathogen reduction techniques (PRTs) are available nowadays to decrease the risk of transmission even further. Considering the emergence of new viruses, bacteria and other pathogens that might constitute a threat for blood recipients [3–5], PRT might be in fact the only technology that guarantees FFP safety, or at least reduces the risk of a blood-borne virus infection, during an outbreak of an infectious disease, as is the case with COVID-19. It should be noted,

though, that the transmission of SARS-CoV-2 virus through blood and blood components transfusion has not been documented so far [6,7]. There was also no development of COVID-19 disease in blood recipients who received blood or its component from a donor with confirmed infection with SARS- CoV- 2 in the post-donation period [8,9]. Nevertheless, the theoretical risk of TTI and TTD associated with SARS-CoV-2 virus does exist and should not be underestimated [10–13].

In this review, we focus on the consequences of implementing pathogen reduction techniques during plasma preparation, in particular techniques using photochemical (PI) and photodynamic inactivation (PDI), and analyze the potential use of these methods in the event of an epidemiological threat. Our considerations focus primarily on coagulation factors and natural anticoagulants, as well as on their concentration before and after using PI and PDI methods for both freshly obtained plasma and frozen- thawed plasma. We compare and summarize available research results to identify pathogen reduction strategies that can improve the quality and safety of FFP, especially through the prism of a

pandemic condition, which is a huge challenge for the field of transfusion medicine.

2. Material and methods

The main purpose of the review was to investigate the effect of PI and PDI on various coagulation factors and natural anticoagulants present in plasma. Simultaneously, we wanted to relate our observations to the current epidemiological situation and the role of PI and PDI in ensuring plasma safety and quality in the event of an epidemic of an emerging infectious disease, in particular caused by Coronaviridae viruses.

2.1. Search strategy

PubMed/ Wiley Online Library / Science Direct electronic databases were searched for original articles, using the following keywords: inactivation, pathogen reduction, fresh frozen plasma, thawed plasma, methylene blue, amotosalen, riboflavin, inactivation factor VIII, inactivation fibrinogen, 2019 novel coronavirus, COVID-19. The search was limited to the articles published in English. The period from 2000 to 2020 was adopted as the time criterion when searching for studies. Moreover, the reference lists of the most relevant articles were cross-checked for some additional publications.

2.2. Inclusion criteria

In our review, we included original studies that met the following inclusion criteria: 1) pathogen reduction of plasma by PI, 2) measurement of coagulation factor / natural anticoagulant activity before and after PI/ PDI implementation, expressed in units of the SI system, 3) PI/ PDI implemented to fresh plasma and / or frozen-thawed plasma.

In all the studies included in the review, the fibrinogen level was measured using the Clauss method and the level of coagulation factors was assessed by means of one-stage clotting assays. The studies included in the review (n = 10) are summarized in Table 1.

2.3. Data extraction

The following data were extracted from studies that met the inclusion criteria: number of plasma units tested, plasma preparation for research (split and pool vs single units analysis), fibrinogen measurement method, coagulation factors measurement method, natural anticoagulants measurement method, PI/ PDI method used, plasma sampling method, source of plasma (whole blood-derived or apheresis). Studies that did not report on any of these data were excluded from this review.

3. Photochemical and photodynamic inactivation characteristics

3.1. Technologies of PI

Different methods of pathogen reduction can be divided into two main groups: those used by the plasma fractionation industry and by individual blood centers. Methods applied during plasma fractionation are commonly reserved for pools of up to 2500 recovered plasma units, contributing to plasma-derived medicinal products safety. In contrast, systems based on PI or PDI, using photosensitising substances such as amotosalen / riboflavin / methylene blue (Table 2), facilitate preparation of individual plasma units with reduced pathogen load. Importantly, the inactivation procedure of the blood component can be carried out "on site" at any suitably equipped blood center.

3.2. Time point of PI

The period that elapses between ordering a plasma product by hospital unit and its transfusion to the patient is critical to the transfusion result. Unfortunately, the amount of time needed to prepare thawed plasma encompasses identification of the appropriate unit, thawing it with one of the available thawing systems, and often inactivating it if necessary. Thawing results in a decrease in the content of coagulation factors in plasma, which is a serious problem that may affect the clinical effect of transfusion. In accordance with the standards of the American Association of Blood Banks [14], a plasma product can be stored up to 24 h post-thawing unless labelled as "thawed plasma" which warrants up to 5-day storage. According to the UK Guidelines for the Blood Transfusion Services [15], after thawing, FFP should be transfused as soon as possible, but if a delay be unavoidable, it can be stored and used within 4 h if kept at a temperature of $22 \pm 2\,°C$ or a maximum of 120 h, if stored at $4 \pm 2\,°C$. In contrast, in case of an inevitable delay in transfusion, plasma that has been inactivated with methylene blue should be used within 4 h if stored at $22 \pm 2\,°C$, but if stored at $4 \pm 2\,°C$, storage time is only 24 h (compared to 120 h allowed for non-inactivated FFPs). However, account should be taken of the fact that prolonged storage after thawing will reduce the content of labile coagulation factors. Finally, the European Guide [16] recommends an "immediate" use of thawed plasma with a maximum storage time of up to 4 h if maintained at $22 \pm 2\,°C$ or 24 h if kept at $4 \pm 2\,°C$. When a major bleeding is considered, the use of thawed plasma is allowed by the European Guide for up to 5 days if stored at $4 \pm 2\,°C$, with the notion that the extended post-thawing storage might result in a decline in the content of labile coagulation factors.

According to the suppliers of the inactivation technology and the Council of Europe [16], PRT can be implemented both before and after freezing, which is crucial when considering plasma pool management policy in blood banks. The possibility of choosing the inactivation time point is all the more important as the activity of coagulation factors decreases with the time that elapses from the end of plasma donation to the start of the freezing procedure [17,18]. For this reason, shortening this period and implementing PI or PDI after freezing may be beneficial. However, it has been shown that freezing time is more important for the recovery of coagulation factors compared to freezing delay [19], so it is difficult to make unambiguous recommendations regarding the optimal time of PI application.

3.3. Reactive oxygen species and protein damage

The applicability of PRTs seems to raise some concerns, especially regarding the fluctuation of coagulation factors and clinical consequences thereof. Although it has been shown that plasma stored in a frozen state until the required quarantine period is reached maintains a stable level of coagulation factors compared to plasma that has been subjected to pathogen reduction [20], inactivation and its effect on coagulation factors still appear to be an area requiring further study. Undoubtedly, all PRTs have an adverse effect on both labile and stable coagulation factors, which is associated with exposure to ultraviolet A (UVA) or visible light. The reason for this is the activation of photosensitive molecules such as riboflavin (commonly referred to as vitamin B2), amotosalen (S-59 molecule) and methylene blue during exposure to ultraviolet A (as is the case with riboflavin or amotosalen systems) or visible light (used in techniques involving methylene blue). During photodynamic methods (involving riboflavin or methylene blue) the reactive oxygen species (ROS) are formed, which then attack the DNA strand thus preventing the replication of pathogens [21]. In contrast, amotosalen, used in the photochemical method, targets nucleic acids and intercalates or "docks" between nucleic acid base pairs. UVA illumination activates amotosalen, which results in permanent cross-linking between the helical strands. It is cross-linking that prevents further replication and is the cause of pathogen inactivation.

Unfortunately, ROS do not selectively attack the DNA or RNA material of the pathogen. They also affect the protein structure of coagulation factors, which become a kind of "bystander" victims. Studies in a

Table 1
Study characteristics.

Study	Number of plasma units collected for research	Source of plasma	Plasma preparation for research	Natural anticoagulants measurement method	Photochemical inactivation method used	Plasma sampling
Backholer, 2016	84	whole blood-derived	split and pool [b]	AT -chromogenic assay	AS-PCT	Plasma samples were collected from plasma pool after split at 3 time-points: before plasma freezing, after plasma thawing- before PI, after PI
				PC- chromogenic assay PS- antigen assay	MB	Samples were rapidly frozen and stored at −40 °C until assay
Cid, 2008	36	whole blood-derive	split and pool[b]	Natural anticoagulants not included in the study	AS-PCT	Plasma samples were collected from plasma pool after split at 2 time-points: before and after PI Plasma sample collected before PI was stored at room temperature (20–24 °C) for the duration of the photochemical treatment
					MB	After the PI was complete, the samples were stored at room temperature, then concurrently frozen at -30 °C and stored at -30 °C until assay all plasma samples were frozen within 24 h of blood donation.
Coene, 2014	120	whole blood-derived	split and pool [b]	AT -chromogenic assay	AS-PCT	Plasma samples were collected before and after PI
				PC- chromogenic assay	MB	Samples were blast frozen in an ultralow temperature freezer and stored at less than −80 °C until assay
				PS- antigen assay AT -chromogenic assay	RF-PRT	Plasma samples were collected before and after PI
Hornsey, 2001	40[a]	whole blood-derived	split and pool [b]	PC- chromogenic assay		Samples were rapidly frozen and stored at less than − 30 °C until assay
					MB	Samples were thawed at 37 C and assayed for FV and FVIII
				PS- not included in study		Aliquots from the thawed samples were refrozen, other assays were carried out within 1 month and included fibrinogen, FIX, FXI, AT, PC
Hornsey, 2009	20	whole blood-derived	single units analysis [c]	AT -chromogenic assay	RF-PRT	Plasma samples were collected before and after PI
				PC- chromogenic assay		Samples were frozen by placing multiple aliquots in cryotubes at −40 °C immediately after PI
				PS- antigen assay		Samples were stored at −40 °C until assay
Osselaer, 2008	12	obtained by apheresis	split and pool [b]	AT -chromogenic assay	AS-PCT	Plasma samples were collected from plasma pool after split at 3 time-points: after PI before plasma freezing, after plasma thawing and after 24 h of storage at 4 °C
				PC- chromogenic assay	MB	
				PS- antigen assay		Samples were frozen at -30 °C and stored at -30 °C until assay.
Rapaille, 2014	30	whole blood-derived and obtained by apheresis	single units analysis [c] for whole blood-derived split and pool [b] for apheresis plasma	Natural anticoagulants not included in the study	MB	Plasma samples were collected before and after PI
						Samples were snap-frozen and stored at a less than −70 °C until assay
Singh, 2006	91[d]	obtained by apheresis	single units analysis [c]	AT -chromogenic assay	AS-PCT	Plasma samples were collected before the addition of amotosalen (pre- PI) and after PI including CAD treatment (post-PI)
				PC- one- stage clotting assay PS- one- stage clotting assay		Samples were snap-frozen and stored at or below −65 °C until assay
Smith, 2010	20	obtained by apheresis	single units analysis [c]	AT -chromogenic assay	RF-PRT	Plasma samples were collected before and after PI
				PC- one- stage clotting assay PS- one- stage clotting assay		Samples were frozen concurrently, within 8 h of collection and stored at −70 °C until assay
Valensart, 2009	30	obtained by apheresis	single units analysis [c]	AT- chromogenic assay		Plasma samples were collected at 4 time-points: before plasma freezing, from thawed plasma before PI (previous to addition of AS), after PI (including CAD)
				PC- chromogenic assay	AS-PCT	After PI and 30 days of frozen storage one storage container was thawed for the last sampling Post- thawing samples were kept at room temperature throughout treatment process and frozen at same time as post-PI samples
				PS- antigen assay		All samples were snap-frozen and stored at a temperature below −65 °C until assay

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Abbreviations: AT: antithrombin, PC: Protein C, PS: Protein S, AS-PCT: amotosalen photochemical treatment, RF-PRT: riboflavin pathogen reduction technology, MB: methylene blue light treatment.

[a] 30 units treated with MB, 10 units used as a control; 10 MB units used for study, remaining MB-treated units were stored for further studies.

[b] Specific number of ABO-matched plasma units were pooled and mixed before being split into units of specific volume.

[c] Each plasma unit prepared from whole blood donations was treated separately.

[d] Different number of units tested for FII activity (n = 59), PC (n = 25), PS (n = 25), AT (n = 26).

Table 2
Characteristics of PI technologies.

Company	Inactivation system/ photosensitizer	Multiple/single donation	Inactivation of frozen-thawed plasma	Total plasma loss during inactivation	Illumination time	Maximum storage period (according to the technology provider)
TerumoBCT	Mirasol/ riboflavin	Single unit, up to 360 mL	yes	No loss	5−8 min	1 year from the date of collection
Cerus	Intercept/ amotosalen	Up to 3 pooled units of apheresis- or WB-derived plasma	yes	2−4% [a]	3−6 min	1 year from the date of collection storage below-18 °C
Macopharma	Theraflex MB- Plasma/ methylene blue	WB-derived single units/ Apheresis plasma if split into single 200-300 mL units	yes	7−15% [a]	15 min	3 years from the date of collection storage below -30 °C

[a] volume loss due to the filtration step required to remove amotosalen or methylene blue.

mouse model have shown that antioxidants such as butylated hydroxyanisole (BHA) can stimulate the secretion of functional wild-type FVIII both in vitro and in vivo [22]. In addition, Feys et al. [23] documented the protective effect of hypoxia demonstrating that degasification with pressurized inert nitrogen gas resulted in improved recovery of FVIII and fibrinogen during pathogen reduction with riboflavin/UV. Moreover, previous studies unequivocally demonstrated fibrinogen to be susceptible to ROS-mediated oxidative structural modification, which has a detrimental effect on its function [24–26].

3.4. Photochemical and photodynamic inactivation in the time of COVID-19 outbreak

At present, the entire world of science is centered around a pandemic caused by the SARS-CoV-2 virus, trying to develop some effective diagnostic methods and therapeutic management. Issues related to the safe acquisition and transfusion of blood and its components remain in the center of attention of researchers, as was the case during the epidemic states caused by other viruses from the Coronaviridae family. Experience gained during previous epidemics shows that in assessing the risk of transmitting an infectious agent by blood, serum viral load is of key importance [27–31]. The virus responsible for the development of COVID-19 is detected in serum and the viral load appears to vary depending on the phase of the infection [5]. In one of the first cohort studies (a group of 41 patients) Huang et al. analyzed RNAemia in patient plasma using the real time polymerase chain reaction (RT-PCR) method. It is worth noting that the target of the RT-PCR test in this case was the NP gene responsible for the synthesis of the nucleoprotein component of the capsid, which is present, among others, in influenza viruses. The presence of virus genetic material was demonstrated in only 15 % of patients (which corresponded to 6 patients of the study group) [5]. The intensive work of scientific centeres around the world in recent months has made it possible to learn about the virus genome and to develop more sensitive and specific tests to determine the genetic material of the virus [32–34].

Currently, leading global organizations dealing with the problems of transfusion medicine, including the Center for Disease Prevention and Control (CDC), Federal Drug Administration (FDA) and American Association of Blood Banks (AABB), constantly monitor the epidemiological situation associated with the COVID-19 pandemic. In its announcement of March 6, 2020, AABB emphasized that to date no cases of SARS-CoV-2 contamination by blood transfusion had been found. Moreover, no blood-borne infection associated with other members of the Coronaviridae family (SARS, Severe Acute Respiratory Syndrome Coronavirus, and MERS-CoV, Mideast Respiratory Syndrome), which have been the cause of pandemics in recent decades, has been reported [35]. Nevertheless, there is a theoretical risk that the genetic material of the SARS-CoV-2 virus may be transmitted through the blood, and there is a particular risk associated with asymptomatic individuals who may be potential donors of blood and its components. Another challenge for blood service during a pandemic is when the donor reports infection or contact with an infected person after donation. In such cases, post-thaw reduction of pathogens appears to be the only means to ensure safe plasma for transfusion. One of the first photosensitizing substances assessed during the ongoing COVID-19 pandemic was methylene blue. Technology using this substance has proven to be a safe method of reducing pathogens in the convalescent plasma [36].

It is worth recalling that the assessment of the inactivation efficiency is based on the calculation of the logarithmic reduction of the post- to pre-treatment viral load. A commonly accepted criterion for a method's effectiveness is a three to four log pathogen reduction, which corresponds to 99.9 % and 99.99 % reduction, respectively [37]. Commercially available PI and PDI methods have already been analyzed after the SARS and MERS virus epidemics - studies using these methods [38–42] are summarized in Table 3. The effectiveness of PI and PDI techniques has also been assessed in relation to platelet concentrates [43,44], but this issue goes beyond the scope of this review. We suspect, therefore, that the assessment of PI and PDI effectiveness in relation to the SARS-CoV-2 virus will soon become the subject of in-depth research.

4. Photochemical and photodynamic inactivation - effects on coagulation factors and natural anticoagulants

4.1. Factor VIII and fibrinogen

Fibrinogen (clotting factor I) and antihaemophilic factor A (clotting factor VIII) are members of the fibrinogen family. Fibrinogen provides ultimate strength and stability of the clot, as the formation of fibrin is the last step in the common pathway of hemostasis. Apart from its primary role as the precursor of fibrin, fibrinogen also has some other biological functions: i) it acts as a suppressor or activator of other molecules binding to their regulatory sites, ii) it interacts with platelets to engage them into the formed clot, and iii) it modulates leukocyte function via MAC-1 integrin, which goes far beyond hemostasis [45]. Factor VIII is an inactive molecule which upon activation to FVIIIa acts as a cofactor of FIXa during its conversion of FX to FXa in a vital step of the propagation phase of coagulation [46].

Because of their important roles described above, concentrations of

Table 3
Studies assessing the effect of photochemical inactivation on MERS and SARS viruses.

Photoactive compound	PI System	Mechanism of inactivation	Study, date	Mean log reduction of viral load
amotosalen	INTERCEPT™ (Cerus)	Amotosalen is exposed to UVA radiation, resulting in permanent bonds within DNA or RNA, which prevents pathogen replication.	Singh et al., 2005	SARS-CoV $\geq 5.5 \pm 0.1$
			Hindawi et al., 2017	MERS-CoV > 4.67 ± 0.25
				MERS-CoV ≥ 4.07 (pooled plasma)
riboflavin	Mirasol® (Therumo BCT)	Riboflavin reacts with guanine residues when exposed to ultraviolet light, generating free oxygen radicals that irreversibly modify nucleic acids, thereby preventing pathogen replication.	Keil et al., 2016	MERS-CoV $\geq 4.42 \pm 0.08$ (single-donor plasma)
methylene blue	THERAFLEX MB-Plasma (Macopharma)	Methylene blue, by adsorbing visible light, undergoes photoactivation and, as a result, produces free oxygen radicals that damage pathogenic nucleic acids.	Eickmann et al., 2018	MERS-CoV ~3.30
			Eickmann et al., 2020	SARS-CoV ≥ 3.1

fibrinogen and FVIII are measured at various steps of plasma preparation to ensure the best possible quality of the final product. As expected, all the studies included in the review [40,47–55] demonstrated that implementation of PI/ PDI, regardless of the method used, contributed to the loss of both FVIII and fibrinogen activity (presented in Table 4). When evaluated globally, the most effective recovery of FVIII activity was reported by *Rapaille et al. (2014),* when using the MB technique. In turn, the maximum loss of FVIII activity was reported by *Hornsey et al. (2009)* who used the riboflavin/UVA technique; however, the short time of riboflavin/UVA processing (presented in Table 2) needs to be considered while analyzing the applicability of this method to routine clinical practice. Regarding the fibrinogen recovery, the results of the source studies varied considerably, even for the same PI methods, hence, no definitive conclusions about the superiority of any can be formulated. Nevertheless, the recovery rates for both fresh plasma and frozen-thawed plasma exceeded 60 % of the pretreatment values, hence they satisfied the European Council's criteria [16].

Analyzing particular PI and PDI techniques in detail, we found many interesting relationships deserving commentary. For PDI using methylene blue, the relative loss ranged from $11 \pm 6\%$–25.8 ± 2.9 % for FVIII and from $8 \pm 4\%$–$36 \pm 7\%$ for fibrinogen. When the results were analyzed for fresh and frozen-thawed plasma separately, the mean % loss of FVIII was 20.36 (95 % CI: 13.04–27.68) and 18.40 (95 % CI: 14.33–22.47), respectively. In terms of fibrinogen concentration, the mean % loss was 20.66 (95 % CI: 7.72–33.60) for fresh plasma and 21.20 (95 % CI: 7.66–50.05) for frozen- thawed plasma. Analysis of PI with the use of amotosalen showed that the relative loss varied between $17 \pm 7\%$

and $27 \pm 7\%$ for FVIII and between $11 \pm 2\%$ and $28 \pm 5\%$ for fibrinogen. When analyzing FVIII, the mean % loss was 21.33 (95 % CI: 8.59–34.08) for fresh plasma and 23.10 (95 % CI: -13.75–59.95) for frozen- thawed plasma, while for fibrinogen the mean % loss was 18.67 (95 % CI: -2.75–40.08) for fresh plasma and 17.85 (95 % CI: -31.07–66.77) for frozen- thawed plasma. Finally, the relative loss upon pathogen reduction with riboflavin varied between 25 ± 16 % and 31.5 ± 3.5 % for FVIII activity and between 21.2 ± 4.5 % and $23 \pm 4\%$ for fibrinogen concentration. However, the limited amount of data in relation to the riboflavin method should be emphasized, which hampers reliable statistical analysis. Additionally, these values refer only to fresh plasma because the only study in which the inactivated component was thawed plasma [48] was limited to coagulation factors assessment before and after PDI and did not report a percentage change following PDI. A cumulative comparison of the % loss of FVIII and fibrinogen after PRT is presented in Figs. 1–4.

Unfortunately, even a thorough assessment does not suffice to formulate firm conclusions about the effects of various PI and PDI methods on the final product's quality. This results primarily from i) differences in the quantities of tested plasma units, ii) inconsistent result reporting, e.g. missing information on the relative (%) loss of coagulation factors in some studies, and iii) substantial heterogeneity of the results obtained with the same PI.

4.2. Factors II, VII, IX

Clotting factors II (prothrombin), VII (proconvertin) and IX (anti-hemophilic factor B) belong to the group of vitamin K-dependent proteins. Factor II is a key determinant of final clot strength both in the coagulation cascade concept, according to which it is activated to thrombin by prothrombinase complex, and according to the current theory based on initiation, amplification, propagation and stabilization phases, in which thrombin generation and up- and down-regulation are the primary objectives. Factor VII deficiency is the most common rare autosomal recessive bleeding disorder with a prevalence of approximately one per 500 000 [56]. Noticeably, FVII activity does not necessarily correlate with the bleeding tendency [57]. Factor IX, widely recognized because of its congenital defect leading to haemophilia B, a severe bleeding disorder, is activated to FIXa by TF/VIIa/Ca2+ complex and generates additional amounts of TF/FVIIa in a reverse feedback loop. Some domains in FIXa were shown to interact with FVIIIa, which results in a several-fold increase in the catalytic activity of the former to generate FXa [58]. We analyzed the concentrations and recovery rates of all the factors mentioned above (summarized in Table 5), depending on the applied PI/ PDI method and plasma type (fresh vs. frozen-thawed). While differences in the methodology of the source studies hinder formulation of any ultimate conclusions, the recovery rates for all vitamin K-dependent proteins in both fresh and frozen-thawed plasma were higher than 70 % (except frozen-thawed plasma treated with riboflavin/UV in the case of which no data were provided). Noticeably, the recovery rates for FVII in frozen-thawed plasma were quite high, exceeding 90 % and 93 % for amotosalen and methylene blue, respectively (no data for riboflavin treatment were available). However, the recovery rates for FIX in frozen-thawed plasma treated with amotosalen and methylene blue were only slightly lower, above 82 % and 87 %, respectively (again, no data for riboflavin treatment were available).

4.3. Factor V

Factor V (proaccelerin) is a component of the prothrombin complex, which is also involved in the inactivation of the activated factor VIII (FVIIIa), and which hence, influences the pro/anticoagulant balance. Deficiency of FV (Owren's disease, parahaemophilia) results in bruising and bleedings similar to those observed in hemophilia; the severity of the disease depends on the concentration of FV and eventual presence of its inhibitor [59]. Unless a concentrate of FV becomes commercially

Table 4
Levels of FVIII and fibrinogen following photochemical inactivation of plasma.

Component exposed to PI	Photosensitizing compound	Author, year	Number of plasma units tested	FVIII activity pre/post PI [a] (IU/mL)	FVIII loss (%)	Fibrinogen pre/post PI [b] (mg/dl)	Fibrinogen loss (%)
Fresh plasma	Methylene blue	Hornsey, 2000	40	1.07 ± 0.19/0.83 ± 0.16	25.8 ± 2.9	258 ± 26/197 ± 13	23.3 ± 4.0
		Cid, 2008 [c]	12	0.91 ± 0.28/0.71 ± 0.16	20 ± 10	320 ± 50/210 ± 50	36 ± 7
		Osselaer, 2008	24	1.22 ± 0.30 0.91 ± 0.23	25 ± 7	372 ± 57 294 ± 40	21 ± 5
		Rapaille, 2014	30 [e]	1.20 ± 0.30/1.08 ± 0.27	11 ± 6	258 ± 49/235 ± 40	8 ± 4
			30 [f]	1.04 ± 0.27/0.83 ± 0.24	20 ± 10	257 ± 38/219 ± 35	15 ± 7
		Singh, 2006	91	1.57 ± 0.35/1.15 ± 0.28	27 ± 7	290 ± 40/209 ± 36	28 ± 5
	Amotosalen	Cid, 2008 [c]	12	0.91 ± 0.28/0.70 ± 0.19	20 ± 19	320 ± 50/260 ± 50	17 ± 7
		Osselaer, 2008	24	1.22 ± 0.30/0.94 ± 0.27	17 ± 7	372 ± 57/333 ± 55	11 ± 2
	Riboflavin	Hornsey, 2009	20	1.28 ± 0.30/0.76 ± 0.17	315 ± 3,5	285 ± 52/195 ± 35	212 ± 4,5
		Smith, 2010	20	1.3 ± 0.5/1.0 ± 0.3	25 ± 16	345 ± 80/267 ± 64	23 ± 4
Frozen- thawed plasma	Methylene blue	Coene, 2014 [d]	120	1.03 ± 0.16/0.80 ± 0.14	N/A	268 ± 18/223 ± 18	N/A
		Rapaille, 2014	30 [e]	0.81 ± 0.16/0.67 ± 0.18	18 ± 10	269 ± 49/229 ± 48	15 ± 7
			30 [f]	1.04 ± 0.33/0.85 ± 0.27	17 ± 5	284 ± 66/244 ± 53	14 ± 4
		Backholer, 2016	84	0.76 ± 0.10/0.61 ± 0.08	20.2	260 ± 17/170 ± 15	34.6
		Valensart, 2009	30	0.78 ± 0.22/0.57 ± 0.16	26 ± 5	250 ± 49/212 ± 36	14 ± 12
	Amotosalen	Coene, 2014 [d]	120	1.04 ± 0.14/0.69 ± 0.10	N/A	272 ± 19/224 ± 19	N/A
		Backholer, 2016	84	0.73 ± 0.09/0.58 ± 0.08	20.2	262 ± 18/205 ± 17	21.7
	Riboflavin	Coene, 2014 [d]	120	1.03 ± 0.17/0.57 ± 0.11	N/A	271 ± 18/181 ± 15	N/A

[a] for the purposes of the review, the results of the measurement of FVIII activity from all studies were unified to IU/mL.

[b] for the purposes of the review, the results of the measurement of fibrinogen concentration from all studies were unified to mg/dl.

[c] for the purposes of the review, values of coagulation factors activity were rounded to two decimal numbers.
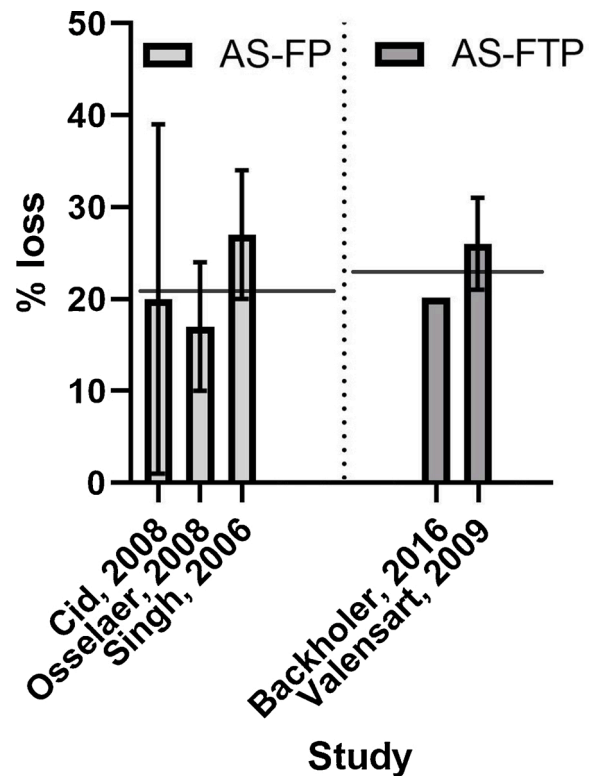
[d] the % change of coagulation factors activity was presented in the form of scatter plots.

[e] plasma obtained from whole blood donation.
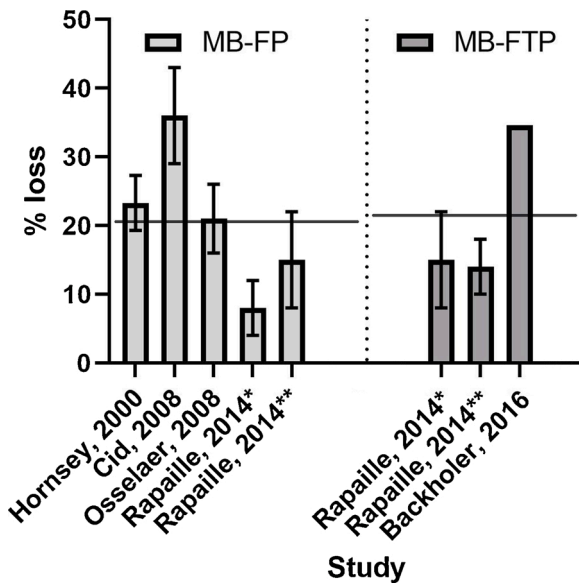
[f] plasma obtained from plasmapheresis.
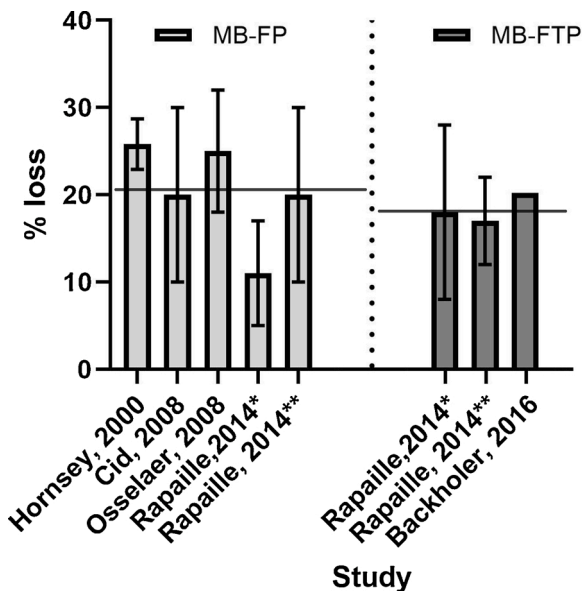
Fig. 1. FVIII loss (%) in methylene blue-treated plasma.
Note: Horizontal lines indicate mean value of FVIII loss.
Abbreviations: MB-FP; methylene blue-treated fresh plasma, MB-FT; Pmethylene blue-treated frozen-thawed plasma
*- plasma obtained from whole blood donation, **- plasma obtained from plasmapheresis

available, FFP is the only treatment in FV deficiency [60]. The recovery rates for FV (presented in Table 5) after application of various PI and PDI methods were similar, between 73 ± 8% (riboflavin) and 98 ± 6% (amotosalen) for fresh plasma and between 87.9 % (amotosalen) and 90.5 % (methylene blue) for frozen-thawed plasma (no data for
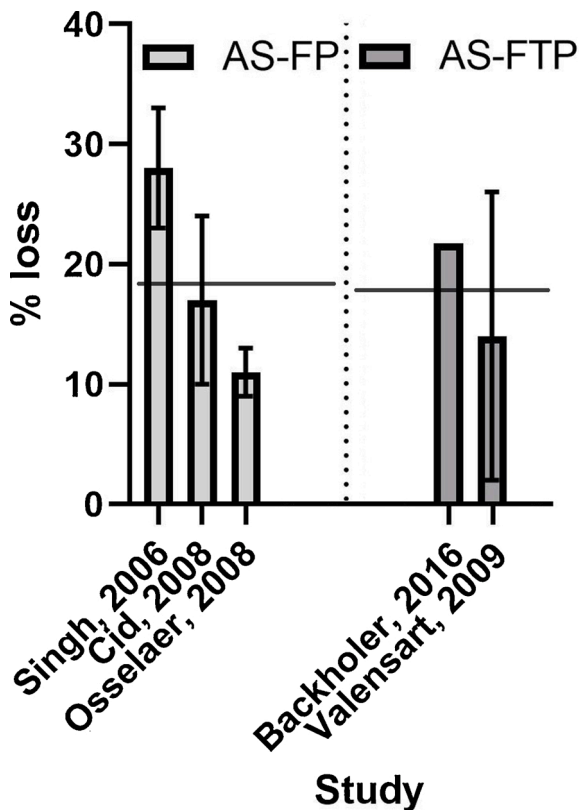

Fig. 2. FVIII loss (%) in amotosalen-treated plasma.
Note: Horizontal lines indicate mean value of FVIII loss.
Abbreviations: AS-FP: amotosalen-treated fresh plasma, AS-FTP: amotosalen-treated frozen-thawed plasma

Fig. 3. Fibrinogen loss (%) in methylene blue-treated plasma.
Note: Horizontal lines indicate mean value of fibrinogen loss.
Abbreviations: MB-FP: methylene blue-treated fresh plasma, MB-FTP: methylene blue-treated frozen-thawed plasma. *- plasma obtained from whole blood donation, **- plasma obtained from plasmapheresis.

Fig. 4. Fibrinogen loss (%) in amotosalen-treated plasma.
Note: Horizontal lines indicate mean value of fibrinogen loss.
Abbreviations: AS-FP: amotosalen-treated fresh plasma, AS-FTP: amotosalen-treated frozena-thawed plasma

riboflavin treatment of frozen-thawed plasma were available). The available evidence suggests that amotosalen is the most effective treatment in terms of FV preservation, and hence, regimens based on this

agent should be considered the "PI of choice" in plasma preparation for FV-deficient patients.

4.4. Factor XI

Factor XI, a member of the contact family factors, which circulates in the blood as a complex with high-molecular-weight kininogen [61], contributes to hemostasis by activating factor IX [62]. Deficiency of FXI (haemophilia C) can be inherited in an autosomal recessive or dominant pattern and results in bleeding, especially after surgical procedures [63]. Treatment options, especially in surgical patients, include plasma, FXI concentrate and recombinant activated factor VII (rFVIIa) [64]. The results of the included studies (summarized in Table 5) imply that the recovery rates for FXI were the lowest after application of either riboflavin treatment to fresh plasma (about 67 %) or MB-treatment to frozen-thawed plasma (61.4 %). In turn, markedly higher recovery rates were obtained after amotosalen treatment, ranging from $86 \pm 5\%$–$93 \pm 6\%$ and from $84 \pm 6\%$–88% in fresh and frozen-thawed plasma, respectively.

4.5. Natural anticoagulants

Natural anticoagulants, protein C (PC), protein S (PS) and antithrombin (AT), are essential to maintain a necessary balance between activation and inhibition within the coagulation system. Deficiency of these factors, whether inherited or occurring in certain diseases/ during pharmacotherapy, is associated with a significant increase in the risk of thrombus formation and resultant thromboembolism [65]. According to *Flamholz et al. (2000),* repeated transfusions of solvent/ detergent treated plasma with reduced activity of protein S may lead to depletion of its functional form in the recipients [66]. Analysis of the studies included in our review, which evaluated natural anticoagulants [40,48, 49,51,52,54], showed that AT, PC and PS levels remain within normal range after using PI and PDI, with recovery rates that vary depending on the PI method used (presented in Table 6). According to *Osselaer* et al. *(2008),* treatment with MB contributed to a more evident decrease in protein S concentration when compared to amotosalen treatment, yet it was MB, that better preserved AT III. In turn, *Smith* et al. *(2010)* highlighted the sensitivity of protein C to riboflavin-based PDI. *Coene* et al. *(2014)* conducted a head-to-head comparison of the effects of all the PRT methods on the natural coagulants contained in frozen-thawed plasma; regardless of the PRT used, the post-treatment loss of AT III, protein C and protein S was no higher than 10 %. However, the same study demonstrated that MB preserved natural anticoagulants more effectively than the other two reagents. Finally, *Valensart* et al. *(2009)* showed that treatment of frozen-thawed plasma contributed to a more significant decrease in protein C concentrations than in protein S and AT III levels. Unfortunately, none of the studies compared directly the recovery rates for the natural anticoagulants contained in frozen-thawed plasma and fresh plasma treated with PRT.

4.6. Cost-effectiveness of PI and PDI

Assessment of the importance of the implemented technology in modern transfusion medicine would be incomplete without considering the cost- effectiveness [67]. Therefore, below we will try to present the costs of implementing the methods based on PI and PDI in the management of plasma resources. At the outset, it is worth emphasizing that the cost-effectiveness of PRTs has already been assessed in in relation to the platelet concentrates, indicating this intervention as cost-effective [68–71]. Still, this is related to a number of factors, including a significant reduction in the risk of bacterial contamination of platelet concentrates and extending their storage period, which translates directly into lower losses of this component, and thus generates savings [71]. However, with regard to plasma, the situation is different. It is mainly related to the technological progress in the field of microbiological

Table 5
Levels of FII, FV, FVII, FIX, FXI following photochemical inactivation of plasma.

Component exposed to PI	Photosensitizing compound	Author, year	Number of plasma units tested	FII activity pre/post PI [a] (IU/mL)% recovery	FV activity pre/post PI [a] (IU/mL)% recovery	FVII activity pre/post PI [a] (IU/mL)% recovery	FIX activity pre/post PI [a] (IU/mL)% recovery	FXI activity pre/post PI [a] (IU/mL)% recovery
Fresh plasma		Hornsey, 2000	40	N/A	0.85 ± 0.07 / 0.76 ± 0.07 / 89.8 ± 3.9 %	N/A	0.95 ± 0.10 / 0.88 ± 0.10 / 88.9 ± 4.4 %	0.91 ± 0.11 / 0.84 ± 0.11 / 86.7 ± 7.1 %
	Methylene blue	Cid, 2008 [b]	12	0.98 ± 0.07 / 0.91 ± 0.06 / 93 ± 2	1.11 ± 0.14 / 1.00 ± 0.12 / 90 ± 4	1.27 ± 0.18 / 1.19 ± 0.16 / 94 ± 3	N/A	N/A
		Osselaer, 2008	24	1.19 ± 0.09 / 1.12 ± 0.10 / 94 ± 2	1.24 ± 0.11 / 1.15 ± 0.08 / 93 ± 4	1.04 ± 0.11 / 1.07 ± 0.10 / 103 ± 4	1.28 ± 0.15 / 1.15 ± 0.13 / 90 ± 4	1.14 ± 0.16 / 1.01 ± 0.19 / 88 ± 7
		Singh, 2006	91	0.96 ± 0.11 / 0.85 ± 0.11 / 88 ± 4%	1.30 ± 0.23 / 1.19 ± 0.19 / 92 ± 7%	1.23 ± 0.32 / 0.95 ± 0.20 / 78 ± 6%	1.08 ± 0.21 / 0.88 ± 0.16 / 82 ± 4%	1.03 ± 0.22 / 0.87 ± 0.18 / 86 ± 5%
	Amotosalen	Cid, 2008 [b]	12	0.98 ± 0.07 / 87.5 ± 6.9 / 89 ± 3	1.11 ± 0.14 / 1.05 ± 0.12 / 95 ± 3	1.27 ± 0.18 / 1.12 ± 0.18 / 88 ± 6	N/A	N/A
		Osselaer, 2008	24	1.19 ± 0.09 / 1.11 ± 0.09 / 93 ± 4	1.24 ± 0.11 / 1.21 ± 0.11 / 98 ± 6	1.04 ± 0.11 / 1.14 ± 0.14 / 109 ± 7	1.28 ± 0.15 / 1.14 ± 0.18 / 89 ± 5	1.14 ± 0.16 / 1.06 ± 0.16 / 93 ± 6
	Riboflavin	Hornsey, 2009	20	1.28 ± 0.20 / 0.95 ± 0.15 / 85.0 ± 3.7 %	1.08 ± 0.20 / 0.74 ± 0.15 / 78.9 ± 4.1 %	1.15 ± 0.21 / 0.79 ± 0.15 / 79.0 ± 4.2 %	1.24 ± 0.24 / 0.85 ± 0.19 / 79.0 ± 8.6 %	1.31 ± 0.33 / 0.77 ± 0.20 / 67.7 ± 3.9 %
		Smith, 2010	20	1.10 ± 0.20 / 0.90 ± 0.10 / 80 ± 6%	1.10 ± 0.20 / 0.80 ± 0.10 / 73 ± 8%	N/A	1.40 ± 0.40 / 1.00 ± 0.20 / 76 ± 6%	1.20 ± 0.20 / 0.80 ± 0.10 / 67 ± 5%
Frozen thawed plasma	Methylene blue	Backholer, 2016	84	0.98 ± 0.08 / 0.90 ± 0.07 / 82.1%	0.81 ± 0.06 / 0.73 ± 0.05 / 90.5 %	1.13 ± 0.13 / 1.08 ± 0.12 / 95.8 %	1.07 ± 0.09 / 0.95 ± 0.09 / 88.9 %	0.68 ± 0.06 / 0.42 ± 0.05 / 61.4 %
		Coene, 2014 [c]	120	0.92 ± 0.04 / 0.90 ± 0.05	1.02 ± 0.08 / 0.94 ± 0.08	N/A	1.20 ± 0.15 / 1.09 ± 0.12	1.30 ± 0.29 / 0.95 ± 0.22
		Coene, 2014 [c]	120	0.94 ± 0.05 / 0.87 ± 0.04	1.03 ± 0.08 / 0.98 ± 0.09	N/A	1.24 ± 0.14 / 1.06 ± 0.12	1.35 ± 0.26 / 1.19 ± 0.29
	Amotosalen	Valensart, 2009	30	0.99 ± 0.12 / 0.85 ± 0.09 / 86 ± 5%	0.93 ± 0.20 / 0.80 ± 0.15 / 87 ± 9%	1.04 ± 0.19 / 0.93 ± 0.18 / 90 ± 7%	0.87 ± 0.13 / 0.71 ± 0.10 / 82 ± 5%	0.89 ± 0.16 / 0.75 ± 0.12 / 84 ± 6%
		Backholer, 2016	84	0.98 ± 0.07 / 0.90 ± 0.07 / 81.5%	0.79 ± 0.06 / 0.77 ± 0.05 / 97.2 %	1.12 ± 0.14 / 1.05 ± 0.14 / 93.7 %	1.06 ± 0.08 / 0.92 ± 0.08 / 86.7 %	0.66 ± 0.06 / 0.58 ± 0.06 / 88 %
	Riboflavin	Coene, 2014 [c]	120	0.93 ± 0.04 / 0.82 ± 0.04	1.02 ± 0.08 / 0.72 ± 0.06	N/A	1.21 ± 0.13 / 0.91 ± 0.11	1.35 ± 0.28 / 0.81 ± 0.18

[a] for the purposes of the review, the results of the measurement of coagulation factors of all studies were unified to IU/mL.
[b] for the purposes of the review, values of coagulation factors activity were rounded to two decimal numbers.
[c] the % change of coagulation factors activity was presented in the form of scatter plots.

diagnostics and the widespread screening with the use of serological and molecular methods, which greatly improved the safety of plasma intended for treatment [72]. The fact that plasma is stored in a frozen state is also important, as it dramatically reduces the risk of microbial development [73]. In such a situation, subjecting the plasma to the PRT process does not significantly improve its microbiological safety, which results in unsatisfactory cost-effectiveness. This was demonstrated by Pereira et al. [74], whose analyzes showed that the average cost of transfusing plasma subjected to PRT compared to standard plasma was over 2 million dollars per QALY (quality-adjusted life-year) gained. An equally important analysis was also carried out by Babigumira et al. [75] who presented an interesting simulation model for methylene blue-plasma (MBP) and quarantine plasma (QP), which demonstrated that quarantine was less costly than MB treatment considering the production process alone. However, if the cost of QP was not reimbursed by sales of non-usable FFP for fractionation, MB treatment turned out to be much more cost-effective, even if WB collections with passive quarantine were considered. MB treatment was also more cost-effective if analyzed in a broader context, including potential extra costs related to residual risks of plasma transfusion-related adverse events and breakthrough infection [75].

It seems, however, that this issue may be presented somewhat differently in the context of pandemics such as SARS, MERS and finally COVID-19 emerging in the last century. When estimating the costs of medical intervention (in this case, PRT) in this exceptional situation, one should take into account the reduction of costs associated with the treatment of previously unknown diseases, which is often associated with the use of expensive, long-term therapy, which has already been analyzed in the context of COVID-19 [76]. In conclusion, we are convinced that analytical models aimed at improving health economics will be increasingly implemented in the field of transfusion medicine, especially in the face of new challenges such as infectious agents.

5. Conclusion

Despite being a milestone in improving blood and blood product safety, pathogen reduction of plasma continues to be an accessory treatment, rather than a self-sufficient procedure; this is primarily related to the high cost of PRTs, including PI and PDI methods. Consequently, obtaining the required quarantine period remains a leading strategy in the area of plasma management and pathogen transmission prevention.

The persisting demand for plasma products necessitates the optimal management of plasma resources, including its clinical use. Plasma distribution and storage are therefore indispensable elements of the activity of each blood center, which is associated with the search for an

Table 6
Levels of protein C, protein S and antithrombin III following photochemical inactivation of plasma.

Component exposed to PI	Photosensitizing compound	Author, year	Number of plasma units tested	Protein C pre/post PI [a] (IU/mL) % recovery	Protein S pre/post PI [a] (IU/mL) % recovery	Antithrombin III pre/post PI [a] (IU/mL) % recovery
Fresh plasma	Methylene blue	Hornsey, 2000 [b]	40	0.85 ± 0.04 0.89 ± 0.11	N/A	0.92 ± 0.07 0.96 ± 0.17
		Osselaer, 2008	24	1.11 ± 0.16 1.08 ± 0.16 97 ± 2	1.45 ± 0.31 1.13 ± 0.21 79 ± 10	1.06 ± 0.06 1.08 ± 0.06 102 ± 3
	Amotosalen	Osselaer, 2008	24	1.11 ± 0.16 1.07 ± 0.15 96 ± 3	1.45 ± 0.31 1.39 ± 0.28 96 ± 5	1.06 ± 0.06 1.05 ± 0.06 99 ± 4
		Singh, 2006	25	1.09 ± 0.15 1.02 ± 0.14 95 ± 9 %	1.09 ± 0.12 1.07 ± 0.12 98 ± 5 %	0.94 ± 0.05 0.91 ± 0.06 $96 \pm 3\%$
	Riboflavin	Smith, 2010	20	1.40 ± 0.50 1.10 ± 0.30 81 ± 11 %	1.10 ± 0.20 1.00 ± 0.20 $91 \pm 9\%$	1.00 ± 0.10 1.00 ± 0.10 $99 \pm 4\%$
Frozen-thawed plasma	Methylene blue	Coene, 2014 [c]	120	1.06 ± 0.06 1.02 ± 0.06	1.01 ± 0.08 0.97 ± 0.08	1.11 ± 0.05 1.12 ± 0.05
	Amotosalen	Valensart, 2009	30	1.05 ± 0.17 0.89 ± 0.14 $85 \pm 8\%$	1.24 ± 0.22 1.14 ± 0.19 $93 \pm 9\%$	0.90 ± 0.09 0.85 ± 0.09 $94 \pm 4\%$
		Coene, 2014 [c]	120	1.06 ± 0.06 0.96 ± 0.05	1.02 ± 0.08 0.96 ± 0.07	1.12 ± 0.05 1.05 ± 0.04
	Riboflavin	Coene, 2014 [c]	120	1.06 ± 0.06 0.81 ± 0.04	1.01 ± 0.08 0.87 ± 0.06	1.11 ± 0.05 0.94 ± 0.04

[a] for the purposes of the review, the results of the measurement of natural anticoagulants activity from all studies were unified to IU/mL.
[b] % recovery of factors not measured in the study.
[c] the % change of coagulation factors activity was presented in the form of scatter plots.

optimal strategy to minimize the waste of blood products without compromising their quality. As any wastage is unacceptable, implementation of PI or PDI might facilitate the management of plasma from one-time-only donors, in the case of which quarantine is not feasible. Since according to international regulations regarding fractionated plasma, such donations might be disqualified from further processing due to the incompleteness of epidemiological data, the only option to use them safely is the application of PI or PDI after the unit is thawed. However, the implementation of PI before freezing might interfere with quarantine and eventually generate unnecessary costs. In accordance with the recommendations of the PI and PDI technology providers and blood product manufacturing guidelines [16], the post-thawing application of PRT is an acceptable option, and the quality of the treated plasma remains within the reference ranges. The published evidence reviewed above does not unequivocally favor the implementation of PI/ PDI either before freezing or after thawing, as plasma products obtained with these two approaches seem to satisfy the existing quality criteria. Thus, the time point at which PI or PDI is applied should be adjusted to the actual demand, to increase the pool of plasma available for clinical use and to prevent its wastage.

Rational implementation of PI and PDI might reduce the cost of plasma management if adequately supported with the quarantine procedure. Nevertheless, every possible effort should be made to ensure effective, safe and cost-efficient use of the currently available PI and PDI methods, as new pathogens, including blood-borne ones, keep emerging [3–5]. This seems even more important for developing countries, especially in Africa and Asia where new viral infection outbreaks occur most often, and where the use of PI and PDI is sometimes the only chance to provide an essential and life-saving transfusion with the lowest possible blood-borne disease risk [77,78]. It is worth noting, however, that the emergence of a new infectious agent may also be a problem for the blood service in highly developed countries. This is mainly due to the reduced reporting of donors to blood centers, as seen during the ongoing COVID-19 pandemic on the example of Italy and the United States, and due to the risk of the infectious agent spreading in the population, including the blood donor population. In this situation, PRT may prove to be the only way to minimize the risk of blood infection and thus to provide safe blood components for clinical use.

To conclude, if implemented responsibly and in accordance with the current guidelines, both PI and PDI can ensure satisfactory plasma quality and improve its safety. Moreover, PI and PDI may be one of the necessary measures to achieve self-sufficiency of the national blood management systems, which is recommended by the leading world agencies dealing with the issue of blood management.

Author contributions

Study concept and design: TW and PR; search and analysis of included studies: TW, AR, BBR; drafting of the manuscript: TW and AR; critical revision of the manuscript for important intellectual content: AZ, LB, JP, PR; study supervision: TW.

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