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BRIEF REPORT

TRANSFUSION

Effect of prolonged storage on quality characteristics of recovered plasma: Is there an expiration date?

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Abstract

Background: Although there are guidelines on industrial manufacture of plasma-derived medicinal products, there are no clear recommendations about plasma intended for fractionation, as there is no expiry time and the effect of prolonged storage on the activity of coagulation factors is unknown.

Study and design methods: A total of 237 units of plasma stored at −30°C in the National Blood Transfusion Centre for 1 year (62 units), 5 years (75 units), and 10 years (100 units) were studied. The effect of storage time was investigated by determining the activity of clotting factors FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII using coagulometric methods and antithrombin III and fibrinogen with chromogenic assays, using System BCS^{R} > XP (Siemens Healthcare diagnostics Marburg, Germany). Albumin was measured by Medilyzer (BX, Medicon). ABO blood group was recorded and correlated with the levels of FVIII. Comparison of values between one and five, 1 and 10 and 5 and 10 years of storage was performed via the SAS for Windows 9.4 software platform (SAS Institute Inc., NC, U.S.A.).

Results: Albumin, AT III, fibrinogen, FIX, FXI, FXII, and FXIII remain rather stable even after 10 years of storage. Levels of FII, FV, FVII, FVIII, and FX decreased after 5 years of storage.

Discussion: Our study is in agreement with all the previous studies and concludes that there is a putative usability of recovered plasma and some of its coagulation factors after many years of storage at the recommended temperature.

KEYWORDS

coagulation, coagulation factors, fresh frozen plasma, plasma fractionation

1 | INTRODUCTION

Recovered plasma is plasma derived from single units of whole blood and intended for further manufacturing plasma-derived medicinal products (PDMPs) such as albumin, coagulation factors, and immunoglobulins.[1]

Coagulation factors are serine proteases (enzymes), except Tissue Factor (TF), FV, and FVIII (glycoproteins), and FXIII (transglutaminase). They circulate as inactive enzymes and are activated into serine proteases, which are the catalyst to activate more serine proteases, a

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process that leads to the final step of blood coagulation. The coagulation cascade is classically divided into three pathways (a) the intrinsic pathway which is activated through the exposure of endothelial collagen and begins with the activation of FXII (b) the extrinsic pathway which is activated when damaged endothelial cells release TF and activates FVII and (c) the common which begins with the activation of FX and leads to the formation of a stable fibrin clot.[2]

According to the European Pharmacopeia (EP) standards, when plasma, obtained by plasmapheresis or from whole blood, is intended for fractionation for recovery of non-labile proteins (fibrinogen, FII, FVII, FIX, FX, FXI, FXII, FXIII, albumin, immunoglobulins), it should be stored at −20°C or below as soon as possible and the latest within 24 and 72 h following donation respectively. For recovery of labile proteins (FV, FVIII), plasma should be stored within 24 h after collection in conditions that ensure a temperature of −25°C or below in the core of the units within 12 h of placing them into the freezer.[3,4]

Although FFP (fresh frozen plasma, plasma frozen within 8 h after collection) can be stored up to 36 months at −30°C,[5,6] in both EP and the part of the Code of Federal Regulations (CFR) on Source Plasma no expiration time is forecasted for recovered plasma and the effect of a prolonged storage time is unknown.[7–9]

Many factors affect the quality and stability of coagulation factors, such as freezing procedure, time from collection to freezing, temperature of storage, and freeze–thaw procedures.[10] The activity of coagulation factors is, also, affected by the ABO blood group since group O blood units have lower levels of FVIII:C compared to non-O blood units.[11,12]

If plasma units, intended for fractionation, are obtained from suitable donors and with the appropriate technique, the desired limit of 50 g/L of albumin and 70% of FVIII:C is achieved.[3] These are, also, the criteria for plasma intended for transfusion.[5]

The effect of the storage time on the stability of coagulation factors depends on the storage temperature. It has been shown that coagulation factors, including FVIII and FV, can remain acceptably stable after storage of 36 months at −30°C.[13]

When lower temperature was tested, studies have shown a stability of factors when stored at −74°C for 18 months[14] and up to 14 years when stored at –80°C.[15]

The different type of anticoagulants, the collection and plasma production procedures can also affect plasma quality, since they can lead to coagulation cascade activation and or physical or chemical degradation of the coagulation factors.[16]

The aim of our study was to investigate for the first time in the literature the effect of long storage time (5 and 10 years) on kinetics of several coagulation factors' degradation and the long-term factors' stability of recovered plasma. We retrospectively studied plasma units stored in National Blood Center in the context of an existing but currently inactive fractionation program to draw conclusions which would be useful for the pharmaceutical industry (manufacturing PDMPs and non-licensed products, e.g. test kit reagents).

2 | MATERIALS AND METHODS

A total of 237 units of recovered plasma were studied: 62 units after 1 year, 75 units after 5 years and 100 units after 10 years of storage at −30°C in the National Blood Donation Centre in Greece (NBDC). Plasma was collected from whole blood and was frozen within 12 h after collection. The study was approved by Aretaieion Hospital ethical committee (344/11-06-2021).

The plasma samples were thawed in a 37°C water bath for 15 min and measurements were performed immediately and completed within 2 h after thawing.

Clotting factors FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII were measured with coagulometric methods and antithrombin III and fibrinogen with chromogenic assays, using System BCS^R > XP (Siemens Healthcare diagnostics Marburg, Germany). Albumin was measured by Medilyzer (BX, Medicon).

Reverse ABO blood group was determined at 108 units and was correlated with the levels of FVIII.

Statistical analysis was performed via the SAS for Windows 9.4 software platform (SAS Institute Inc., NC, U.S.A). Comparisons between groups for the categorical parameters were performed by chi-square test. For the arithmetic parameters normality was not possible to be ensured for all measured factors (by Shapiro–Wilk test), thus the Mann Whitey *U* test was preferred. The significance level (*p*-value) was set <0.05 and all tests were two sided.

3 | RESULTS

The levels of coagulation factors are listed in Figure 1 and the percentage of units with normal/abnormal levels of coagulation factors are depicted in Table 1.

The normal ranges of measured factors are presented in Table 2.

Fibrinogen, FII, FV, FVII, FVIII, FX, and FXIII vary at a statistically significant level between five and 10 years of storage ($p = 0.0132$, $p < 0.0001$, $p = 0.0001$, $p = 0.0001$, $p = 0.0056$, $p < 0.0001$, and $p = 0.0001$, respectively). In contrast, there was no statistically significant change in albumin, AT III, FIX, FXI and FXII

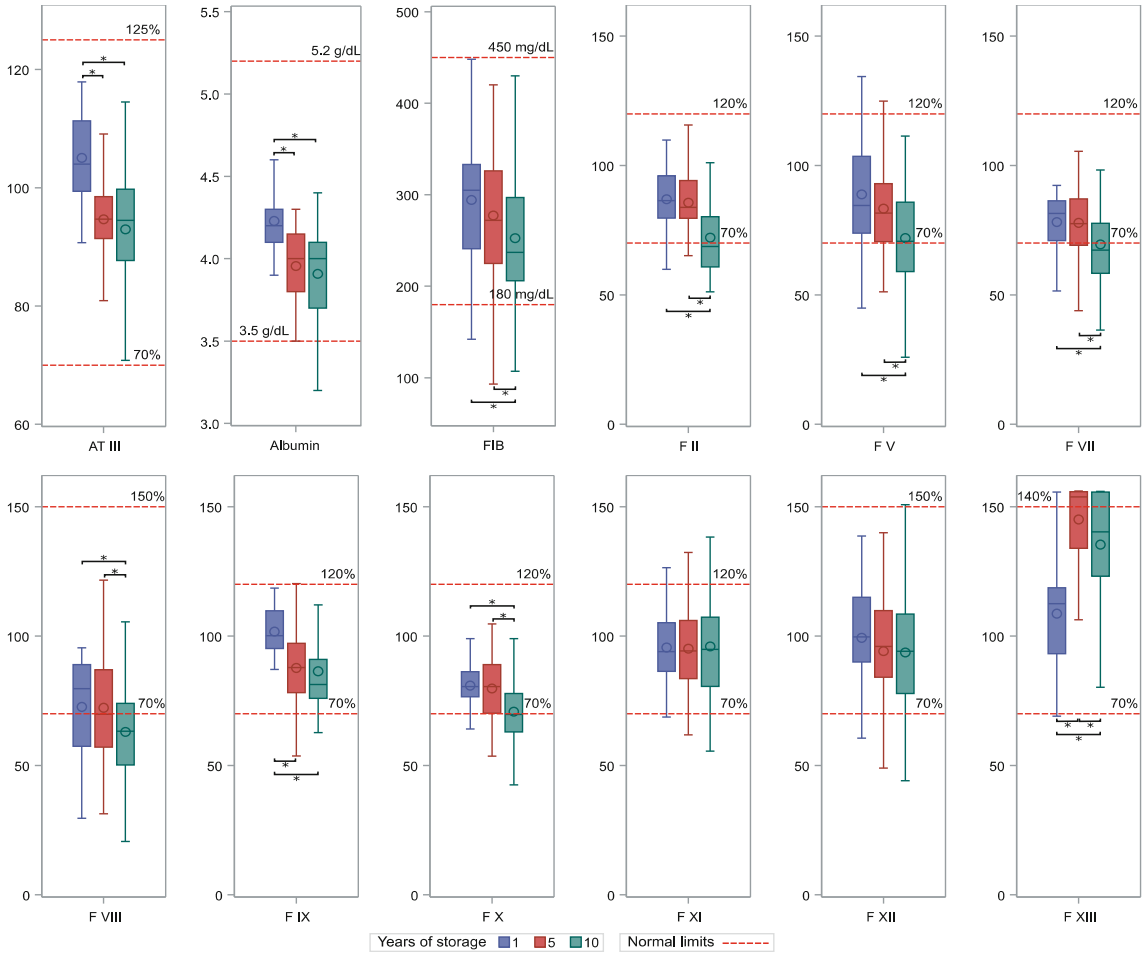

FIGURE 1 Box and whisker plots of plasma characteristics levels for each year period. Box limits indicate the Q1 and Q3 values, whisker limits indicate minimum and maximum values after outlier exclusion (outliers are not shown), lines within the boxes indicate median value and circles the mean value. Horizontal lines indicate the lower and upper normal limits. Asterisks indicate statistically significant difference between the corresponding years

($p = 0.0762$, $p = 0.5296$, $p = 0.0624$, $p = 0.8956$ and $p = 0.7873$, respectively).

More specifically, despite the relative reduction of ATIII levels, especially during the first 5 years of storage (105.1% ± 7.4% vs 94.7% ± 7.4%, $p < 0.05$), after five and 10 years in 99% and 96% of units, respectively, ATIII levels remained within normal range ($p > 0.05$).

Although the absolute value of albumin decreased from 1 to 5 years of storage (4.2 ± 0.2% vs. 4.0 ± 0.3%, $p < 0.0001$), albumin seems to be stable, since all units stored for 1 year, 97.2% of units stored for 5 years and 95% of the units stored for 10 years had normal albumin levels ($p \geq 0.05$ for all comparisons).

Fibrinogen seems to be equally stable. The absolute values did not differ after storage and the percentage of units with normal levels was 93.5%, 93.3% and 92% after 1, 5 and 10 years of storage, respectively ($p \geq 0.05$ for all comparisons).

FII seems to be stable for up to 5 years of storage, but there is a significant reduction from 5 to 10 years depicted both in the absolute levels (85.7% ± 11.8% vs 72.2% ± 14.2%, $p < 0.05$), as well as in the percentage of normal units (89.3% vs. 46%, $p < 0.0001$).

FV was stable between one and 5 years ($p = 0.324$), however after 10 years of storage the mean level decreased more than 15% (83.3% ± 16.9% vs. 72.0% ± 18.9%, $p < 0.0001$). This was reflected to the percentage of normal plasma units after five and 10 years of storage (77.3% vs. 52%, $p = 0.0006$). The odds ratio for low FV was approximately 3% when comparing either 1 or 5 to 10 years.

There was no difference in FVII levels between 1 and 5 years of storage (72.6% ± 18.0% vs. 72.3% ± 21.2%), but FVII decreased significantly between 5 and 10 years of storage (63.0% ± 19.4%, $p < 0.0001$). This is reflected to the percentage of units that have normal levels of FVII during storage (75.8%,73.3% and 41% of plasma units stored for 1, 5 and 10 years, respectively).

The mean levels of FVIII at 1, 5 and 10 years of storage were 72.6%, 72.3% and 63%, respectively (the pair

TABLE 1 Number and percentage of cases within the normal/abnormal range: 1 year vs. 5 years (p1), 1 year vs. 10 years (p2), 5 years vs. 10 years (p3)

Storage time Plasma characteristic	1 year ($N = 62$) Normal/abnormal (N, %)	5 years ($N = 75$) Normal/abnormal (N, %)	10 years ($N = 100$) Normal/abnormal (N, %)	p1	p2	p3
AT III (%)	31/0 (100%/0%)	74/1 (98.7%/1.33%)	96/4 (96%/4%)	0.5183	0.2581	0.2947
Albumin (g/dl)	31/0 (100%/0%)	70/2 (97.2%/2.8%)	95/5 (95%/5%)	0.3487	0.2043	0.4668
FIB (mg/dl)	29/2 (93.5%/6.5%)	70/5 (93.3%/6.67%)	92/8 (92%/8%)	0.9677	0.7767	0.7392
FACTOR II (%)	57/5 (91.9%/8.1%)	67/8 (89.3%/10.7%)	46/54 (46%/54%)	0.6050	<0.0001	<0.0001
FACTOR V (%)	24/7 (77.4%/22.58%)	58/17 (77.3%/22.67%)	52/48 (52%/48%)	0.9923	0.0122	0.0006
FACTOR VII (%)	47/15 (75.8%/24.2%)	55/20 (73.3%/26.7%)	41/59 (41%/59%)	0.7411	<0.0001	<0.0001
FACTOR VIII (%)	32/30 (51.6%/48.4%)	37/38 (49.3%/50.67%)	32/68 (32%/68%)	0.7905	0.0131	0.0202
FACTOR IX (%)	31/0 (100%/0%)	67/8 (89.3%/10.67%)	86/14 (86%/14%)	0.0586	0.0275	0.5104
FACTOR X (%)	29/2 (93.5%/6.45%)	57/18 (76%/24%)	49/51 (49%/51%)	0.0357	<0.0001	0.0003
FACTOR XI (%)	28/3 (90.3%/9.68%)	68/7 (90.7%/9.33%)	82/18 (82%/18%)	0.9560	0.2698	0.1049
FACTOR XII (%)	28/3 (90.3%/9.68%)	62/13 (82.7%/17.33%)	82/18 (82%/18%)	0.3166	0.2698	0.9090
FACTOR XIII (%)	28/3 (90.3%/9.68%)	20/55 (26.7%/73.33%)	48/52 (48%/52%)	<0.0001	<0.0001	0.0042

TABLE 2 Normal ranges of measured coagulation factors

Factor	Normal range
Fibrinogen	1.8–4.5 g/L
II	70–120%
V	70–120%
VII	70–120%
VIII	70–150%
IX	70–120%
X	70–120%
XI	70–120%
XII	70–150%
XIII	70–140%
Antithrombin III	70–125%
Albumin	3.5–5.2 g/dl

comparisons were significant for more than 5 years of storage, $p < 0.05$). Similarly the percentage of plasma units with normal levels of FVIII was 51.6%, 49.3% and 32% for 1, 5 and 10 years of storage, respectively. Comparison between 1 and 5 years was proved not significant ($p = 0.7905$), while between 5 and 10 years the difference was significant ($p = 0.0202$).

FIX decreased from 1 to 5 years (101.7% ± 9.0% vs 87.7% ± 13.9%, $p < 0.0001$), but then seemed to be stable for up to 10 years of storage (86.4% ± 17.8%, $p < 0.05$). All plasma units stored for 1 year, 89.3% and 86% of the units stored for 5 and 10 years, respectively, were within normal range ($p > 0.05$).

Measurements of FX for 1 and 5 years were similar (80.9% ± 8.0% vs 79.8% ± 13.8%, $p = 0.7128$). However, there was an important drop in the values after 10 years (70.8% ± 11.1%, $p < 0.0001$). At 1, 5 and 10 years of storage the percentage of units within normal range for factor X was 93.5%, 76% and 49%, respectively (for all pair comparisons $p < 0.05$). Additionally, the odds for a unit to be abnormal are 15 times higher if the unit is stored for 10 years compared to 1 year (OR = 15.1. 95% CI: 3.4–66.7, $p < 0.0001$).

FXI and FXII can be considered rather stable irrelevant of storage years either as a numerical value or as the percentage of units with normal levels of these coagulation factors. For FXI the measurements were 95.6% ± 13.9%, 95.1% ± 15.3% and 96.0% ± 21.8% and for FXII 99.3% ± 21.6%, 94.2% ± 21.4% and 93.7% ± 22.8% for 1, 5 and 10 years of storage, respectively ($p > 0.05$ for all pair comparisons). Similarly, 90.3%, 90.7% and 82% of the units had normal FXI levels and 90.3%, 82.7% and 82% had normal FXII levels over the years ($p > 0.05$ in all pair comparisons).

As far as reverse blood grouping was concerned, 65 of the units were determined as non-O blood group and 43 as O-blood group. In blood group O units FVIII was lower than non-O type during all years of storage (mean 57.0% ± 17.9% vs. 69.0% ± 17.9% respectively, $p = 0.0005$).

4 | DISCUSSION

EP has established the storage conditions, as well as the quality recommendations of plasma intended for

fractionation.[3] However, there is no expiration date of recovered plasma and the effect of prolonged storage on quality characteristics of recovered plasma is unknown.

This is the first time in the literature that a study evaluates the activity of several coagulation factors on samples of recovered plasma stored at −30°C for 5 and 10 years.

Our study shows that AT III, albumin, fibrinogen, and FIX, FXI, FXII, FXIII remain rather stable even after 10 years of storage, while for FII, FV, FVII, FVIII, and FX a decrease was observed after 5 years of storage with FV and FVIII being more sensitive in that degradation.

In contrast to the study that examined FV, FVIII and fibrinogen after 14 years of storage at −80°C,[15] our study showed that a degradation of labile factors (FV, FVIII) after more than 5 years of storage does occur. This could be explained by the difference in storage temperature (−30°C vs −80°C).

Our results are in agreement with most studies that have found that the activity of most coagulation factors can remain satisfactory stable after up to 36 months of storage at different temperatures.[13,17,18]

Illert et al. examined plasma units derived from CPD whole blood after a storage period of 37 months at −40°C,[18] while Kotitschke et al. examined CPD and apheresis plasma after storage at −20°C for 2 years or at −25°C, −30°C, or −40°C for 3 years.[13]

In contrast, another study showed a significant activity loss of labile factors after 18 months of storage at −74°C, a difference that can be attributed to the fact that 3 ml aliquots of plasma derived from plasmapheresis were studied.[14]

A limitation of our study was the inability to have the basic levels of coagulation factors before freezing, since the study design was retrospective and assessed plasma collected from different blood banks and stored in the Blood Center for a fractionation program. Such data could be more informative on coagulation factors' kinetics taking into account the inter-individual variability mainly during the first year of storage.

In conclusion, our study highlights the putative usability of recovered plasma for manufacturing PDMPs and/or non-transfusable products, for example, test kit reagents even after many years of storage at the recommended temperature.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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