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Stability of plasma proteins and factors in Chinese universal pooled plasma

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Abstract

Objective: This study aimed to determine the precision dose of Chinese universal pooled plasma (CUPP) developed by our laboratory, and the stability of plasma proteins and factors.

Methods: A total of 100 single fresh-frozen plasma (FFP) units were selected to test plasma proteins, including total protein, albumin, fibrinogen, factor V, factor VIII, antithrombin-III, and protein C. Different pooling protocols with 20, 40, 60, 80, and 100 units were used to optimize the number of pooled units. The pooled plasma was then used to further evaluate the optimal storage conditions and duration at 22°C, 4°C, and −20°C.

Results: There were considerable differences in plasma protein levels among single units of FFP. After different pooling protocols, the mean value of plasma proteins did not significantly change. However, with a larger number of pooled samples, plasma proteins were more stable with a smaller standard deviation. Acceptable storage for CUPP was achieved with storage for 1 day at 22°C, 4 days at 4°C, and 3 months at −20°C.

Conclusion: A uniform level of plasma proteins and factors in CUPP appears to support establishment of a precise dose of plasma.

Keywords

Stability, Han Chinese population, pooled plasma, protein, storage duration, coagulation factor, universal

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Introduction

Fresh-frozen plasma (FFP) from a single donor is used to supply coagulation factors. These coagulation factors are used to rectify plasma protein levels in coagulopathic patients who have a deficiency in coagulation factors or a coagulation disorder that is either hereditary or acquired.[1–3] In the China Guidelines of Clinical Blood Transfusion, there are only two standards of plasma proteins, as follows: total protein (TP) levels > 50 g/L and factor VIII levels > 0.7 IU/mL for single FFP units.[4] A primary concern is that there are individual differences in the concentration of plasma proteins and factors in single unit of FFP from different donors. Moreover, preparation of FFP *in vitro*, which involves the effects of centrifugal forces and freezing practices, may further affect retention of plasma proteins, especially unstable factors. This leads to even greater variability when comparing with reference levels *in vivo*. Therefore, plasma protein levels in a single unit of FFP are ambiguous and difficult to unify and quantify. This has hindered the development of precise plasma transfusion for several years.

Pooled plasma is a possible strategy to address this issue, such as the Octaplas® LG. Pooled plasma has the advantage of having more stable and easily quantifiable protein levels compared with single FFP, although some factors slightly decrease through storage of 6 days at 2°C to 6°C.[5] We developed a type of pooled plasma called Chinese universal pooled plasma (CUPP) for the Han Chinese population to address the issue of ABO universal transfusion and precise quantification of the protein dose with an optimal pooling rate of blood groups of A:B:AB = 6:2.5:1.5.[6,7] We also established a flow-based device that was effectively applied to inactivate viruses in pooled plasma by flow treatment to meet the guidelines of virus inactivation for blood products.[8] An initial standard of protein levels for CUPP based on the prepared rate of CUPP needs to be established. However, the optimal pooling number which can stabilize protein levels and be convenient for uniformity compared with a single unit of plasma and the stability of plasma proteins under different storage conditions still need to be investigated.

Therefore, in this study we investigated the differences in levels of the main plasma functional proteins in single FFP units in a Chinese regional population. We also examined stability of plasma proteins with different pooling protocols and the optimal storage conditions and storage time.

Materials and methods

Selection of plasma samples

A total of 100 units of single FFP with 60 units in group A, 25 units in group B, and 15 units in group AB were randomly selected from donors in the Clinical Blood Transfusion Center, Chinese PLA General Hospital. All units of plasma were prepared as FFP by a standard processing based on the practice guidelines in China.[4] Before use, FFP was thawed in a shaking water bath (MultiTemp III; GE Healthcare Europe GmbH, Freiburg, Germany) at 37°C. The study protocol was approved by the Institutional Ethics Committee of Chinese PLA General Hospital.

Plasma pooling protocol

One hundred units of single FFP were thawed at the same time. The pooling operation was performed in 30 minutes at room temperature after single FFP thawing. The pooling protocol was based on the proportion of A:B:AB = 6:2.5:1.5 resulting from the preparation rate of CUPP. The minimum pooling number was 20 units of plasma involving 12 units of group A

plasma, 5 units of group B plasma, and 3 units of group AB plasma. Plasma samples with a volume of 1 mL were extracted from each unit of FFP. Pooling protocols included 20, 40, 60, 80, and 100 units of pooling, and were pooled for 10 minutes. After pooling, 1 mL of plasma sample was randomly extracted in each pooling protocol five times (Figure 1). Plasma proteins in these samples were measured within 30 minutes.

Pooled plasma storage

Pooled plasma with an optimal pooling number was divided into several aliquots of 2 mL and stored at 4 ± 2°C for 4 days, 22 ± 2°C for 4 days, and frozen at -20 ± 2°C for 3 months. After samples were stored at 4°C for 1, 2, 3, and 4 days, 22°C for 1, 2, 3, and 4 days, or −20°C for 1, 2, and 3 months, plasma samples were taken out and immediately thawed for measurement of plasma proteins within 30 minutes.

Measurement of variable plasma factors

One hundred units of thawed single FFP, pooled plasma from different pooling protocols, and pooled plasma from different storage periods at different storage temperatures were measured for plasma proteins. Plasma proteins included TP, albumin (Alb), fibrinogen (Fg), factor V (FV), factor VIII (FVIII), antithrombin-III (anti-III), and protein C (PC). All of the protein assays were performed within 30 minutes after thawing plasma. TP and Alb levels were measured on a fully automatic biochemistry analyzer (cobas c 701/702; Roche, Mannheim, Germany) using a colorimetric method with a TP test kit (Roche) and Alb test kit (Roche), respectively. Fg activity tests were performed on a coagulation analyzer (STA Compact; Diagnostica Stago, Asnieres, France) with the STA Compact using the Clauss clotting method and a Fg kit (STA-fibrinogen 5 kit; Diagnostica Stago). FVIII, FV, anti-III, and PC levels were measured by the Instrumentation Laboratory coagulation system (Instrumentation Laboratory Company, Bedford, MA, USA) using an activated partial thromboplastin time assay with an FVIII-deficient plasma kit (HemosIL; Instrumentation Laboratory Company), a prothrombin time assay with an FV-deficient plasma kit (HemosIL; Instrumentation Laboratory Company), an automated chromogenic assay with a liquid antithrombin kit (HemosIL; Instrumentation Laboratory Company),

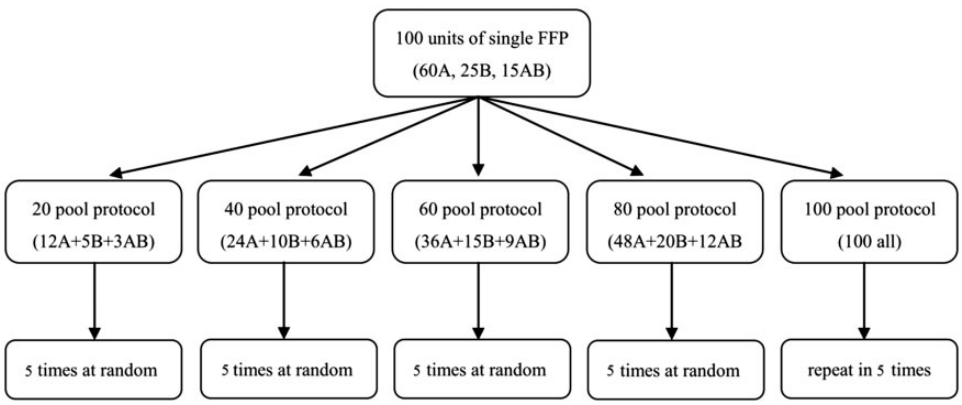

Figure 1. Different pooling protocols based on the prepared rate of universal plasma.
Equal aliquots of 1 mL were extracted from each unit of single fresh-frozen plasma to pool together.

and an automated chromogenic assay with a PC kit (HemosIL, Instrumentation Laboratory Company).

Statistical analysis

The experiments related to plasma protein assays were each performed three times. The results are presented as the arithmetic mean ± standard deviation (SD). The SD was used to quantify the extent of dispersion and stability. Multiple comparisons (Student's t-tests) were used to analyze the mean differences between the results of different groups and the control group (0 days). A P value < 0.05 (two-tailed) was considered statistically significant. SPSS for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis

Results

Content of plasma proteins and factors in 100 units of single FFP

We examined TP, Alb, Fg, FV, FVIII, anti-III, and PC levels among 100 units of single FFP. Plasma protein levels were significantly different ($P < 0.05$) with a wide range of protein concentrations in each single FFP. The protein with the maximum fluctuation in levels was FVIII (0.34–1.36 IU/mL), that with the minimum fluctuation in levels was TP, and Alb showed the highest levels (Table 1).

Changes in plasma proteins and factors in different protocols

We examined plasma protein levels from different pooling protocols compared with individual units. With a larger number of pooled samples, plasma proteins were more stable with a lower SD value. Generally, in six types of plasma proteins, the SD value tended to be smaller in the 40 units of pooling protocol compared with the 20 units of pooling protocol (Table 2). Figure 2 shows the change in mean value and SD of TP, Fg from coagulation factors, and PC from coagulation inhibitors in different pooling protocols.

Stability of plasma proteins and factors at 22°C storage

We examined plasma protein levels for pooled plasma in the 40 units of pooling protocol stored at 22°C from 1 to 4 days. We observed different changing trends in different plasma proteins stored at room temperature at 22°C. With prolonged storage, TP, Alb, Fg, anti-III, and PC levels significantly increased over time with a maximum increase in TP by 115% at 4 days (all $P < 0.05$ compared with 0 days). However, FV and FVIII levels were significantly reduced over time, with a minimum decrease in FVIII levels by 27% at 4 days (all $P < 0.05$ compared with 0 days) (Table 3).

Table 1. Protein and factor levels in 100 units of single fresh-frozen plasma.

	n	Mean ± standard deviation	Range
Total protein (g/L)	100	55.13 ± 3.77	45.8–69.8
Albumin (g/L)	100	36.45 ± 2.21	31.9–43.9
Fibrinogen (g/L)	100	2.15 ± 0.42	1.32–3.84
Factor V (IU/mL)	100	0.82 ± 0.14	0.48–1.39
Factor VIII (IU/mL)	100	0.64 ± 0.19	0.34–1.36
Antithrombin-III (IU/mL)	100	1.29 ± 0.13	1.00–1.61
Protein C (IU/mL)	100	1.06 ± 0.21	0.70–1.77

Data are presented as the mean standard ± deviation, n = 3 for each protein.

Table 2. Changes in plasma protein levels in different pooling protocols compared with individuals.

	Individuals	20 units of pooling	40 units of pooling	60 units of pooling	80 units of pooling	100 units of pooling
Total protein (g/L)	55.13 ± 3.77	55.72 ± 1.43	55.63 ± 0.25	54.23 ± 0.61	55.10 ± 0.56	57.67 ± 0.47
Albumin (g/L)	36.45 ± 2.21	36.60 ± 0.95	36.67 ± 0.51	36.23 ± 0.35	36.13 ± 0.15	37.83 ± 0.15
Fibrinogen (g/L)	2.15 ± 0.42	2.06 ± 0.29	2.09 ± 0.11	1.99 ± 0.09	2.06 ± 0.06	2.40 ± 0.01
Factor V (IU/mL)	0.82 ± 0.14	0.78 ± 0.05	0.80 ± 0.02	0.84 ± 0.03	0.86 ± 0.03	0.85 ± 0.02
Factor VIII (IU/mL)	0.64 ± 0.19	0.60 ± 0.11	0.67 ± 0.04	0.60 ± 0.08	0.60 ± 0.09	0.68 ± 0.02
Antithrombin-III (IU/mL)	1.29 ± 0.13	1.26 ± 0.05	1.23 ± 0.03	1.13 ± 0.02	1.17 ± 0.02	1.26 ± 0.03
Protein C (IU/mL)	1.06 ± 0.21	1.04 ± 0.04	1.03 ± 0.01	0.94 ± 0.02	0.98 ± 0.02	1.05 ± 0.00

Data are presented as the mean ± standard deviation, n = 3 for each protein.
*$P < 0.05$ versus individuals.

Stability of plasma proteins and factors at 4°C storage

We examined plasma protein levels for pooled plasma from the 40 units of pooling protocol stored at 4°C from 1 to 4 days. Levels of all plasma proteins, except for FV, showed slight significant decreases over time, including TP and Alb (both $P < 0.05$ compared with 0 days), and Fg levels appeared to decrease, but this was not significant. Anti-III, FVIII, and PC levels were significantly decreased from days 1 to 4 compared with 0 days (all $P < 0.05$). FV showed a slight significant increase during storage, with the highest increase by 16% at 4 days ($P < 0.05$ compared with 0 days). With regard to stable factors, Fg was more resistant to storage conditions of 4°C compared with the other proteins, although this difference was not significant (Table 4).

Stability of plasma proteins and factors at −20°C storage

We examined the change in plasma protein levels for pooled plasma in the 40 units of pooling protocol stored at −20°C from 1 to 3 months. All plasma protein levels showed a varying amount of decrease from 0 days, with a maximum decrease by 37% for FVIII and a minimum decrease by 6% for TP after 3 months of storage at −20°C. With the exception of Fg, all of the plasma proteins were significantly decreased when stored for 1 month or longer compared with 0 days (all $P < 0.05$) (Table 5).

Discussion

Previous studies have shown that plasma proteins in pooled plasma, such as solvent/detergent-inactivated pooled plasma, are more stable and can be quantified compared with single units of plsama.[5,9] Moreover, a previous study showed that there was no significant difference in clinical outcomes between transfusion with solvent/detergent plasma and FFP under routine clinical conditions.[10] For CUPP, studying the stability of plasma proteins in pooled plasma to establish a standard for precise dosing and accomplishing precise transfusion is important for clinical plasma transfusion in China.

In our study, the values of all plasma proteins had a wide distribution among single units of FFP (Table 1). A total of 100 single units of FFP all originated from our center with distinct regional characteristics. Therefore, our results only partly depict the differences between single units of FFP. FVIII was considered as the most unstable factor and had a wide range,

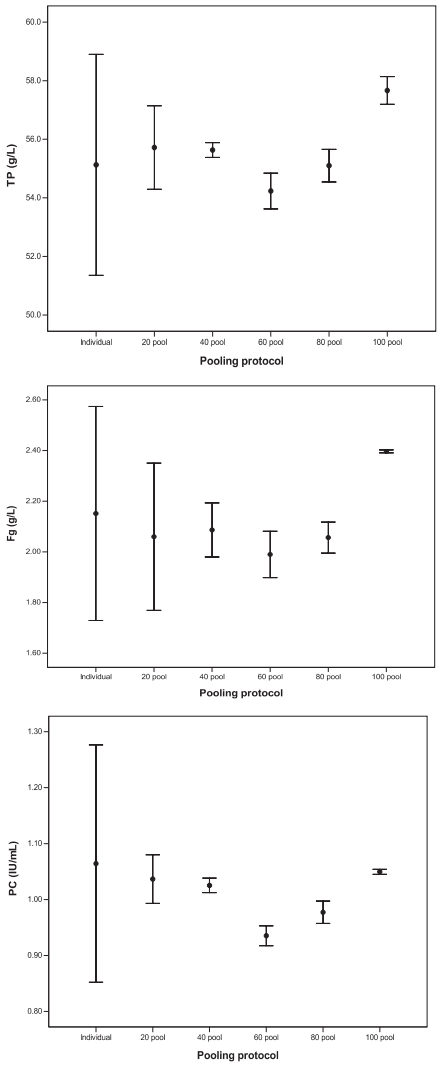

Figure 2. Changes in levels of plasma proteins, such as TP, Fg, and PC, in different pooling protocols.
There was no significant difference in the arithmetic mean between the results from different pooling protocols and individuals. However, with a larger number of pooled samples, levels of plasma proteins or factors, such as TP, Fg, and PC, tended to be stable with a decrease in standard deviation value. TP = total protein; Fg = fibrinogen; PC = protein C.

with the highest value of 1.36 IU/mL to the lowest of 0.34 IU/mL. However, TP and Alb levels were relatively stable or concentrated because of their characteristics. This significant individual difference in single units of FFP could potentially result in imprecise dosing of plasma and a failure to meet the requirement of precise plasma transfusion. The use of CUPP could address this problem. Table 2 and Figure 2 show that a larger pooling number was associated with more stable and consistent levels of plasma proteins with a lower SD compared with individual units. An example of this finding is as follows: the SD values of Fg with 20, 40, 60, and 80 units of pooling were 1/1.5, 1/3.8, 1/4.7, and 1/7 times the results of Fg with individual units, respectively; and the SD values of FVIII with 20, 40, 60, and 80 units of pooling were 1/1.7, 1/4.8, 1/2.4, and 1/2.1 times the results of FVIII with individual units, respectively. Different plasma proteins responded differently to pooling in each pooling protocol. However, we conclude that plasma protein levels tended to be stable at the beginning of the 40 units of pooling protocol, which represents the minimum optimized pooling number.

We also evaluated the stability of plasma proteins and factors to 4 days of storage at 22°C. The China Guidelines of Clinical Blood Transfusion suggest that FFP should be used to transfuse patients at room temperature within 6 hours after thawing.[4] However, some research has demonstrated the stability of Fg and other factors in thawed cryoprecipitate that is stored at 20°C to 24°C. This finding suggests that the shelf life can be safely extended to 24 hours.[11] We showed that the majority of plasma protein activity increased in pooled plasma stored at 22°C from 1 to 4 days, although FV and FVIII showed reduced activity (Table 3). This increase in values was possibly caused by

Table 3. Changes in plasma protein levels for the 40 units of pooling protocol at 22°C storage.

		1 day	2 days	3 days	4 days
	0 days	Value/change (%)	Value/change (%)	Value/change (%)	Value/change (%)
Total protein (g/L)	55.63 ± 0.25	62.80 ± 0.17 (+13)*	74.47 ± 0.59 (+34)*	84.70 ± 1.05 (+52)*	119.57 ± 4.00 (+115)*
Albumin (g/L)	36.67 ± 0.51	41.01 ± 0.06 (+12)*	48.23 ± 0.42 (+32)*	55.67 ± 0.46 (+52)*	76.13 ± 1.70 (+108)*
Fibrinogen (g/L)	2.09 ± 0.11	2.14 ± 0.13 (+2)	2.24 ± 0.14 (+7)	2.54 ± 0.23 (+22)	3.85 ± 0.45 (+84)*
Factor V (IU/mL)	0.80 ± 0.02	0.45 ± 0.01 (−44)*	0.13 ± 0.01 (−84)*	0.03 ± 0.00 (−96)*	0.06 ± 0.01 (−93)*
Factor VIII (IU/mL)	0.67 ± 0.04	0.48 ± 0.02 (−28)*	0.49 ± 0.04 (−27)*	0.50 ± 0.02 (−25)*	0.49 ± 0.15 (−27)*
Antithrombin-III (IU/mL)	1.23 ± 0.03	1.32 ± 0.04 (+7)	1.67 ± 0.07 (+36)*	1.84 ± 0.19 (+50)*	2.00 ± 0.04 (+63)*
Protein C (IU/mL)	1.03 ± 0.01	1.15 ± 0.04 (+12)	1.44 ± 0.06 (+40)	1.67 ± 0.33 (+62)*	2.02 ± 0.10 (+96)*

Data are presented as the mean ± standard deviation, n = 3 for each protein.
*$P < 0.05$ versus 0 days.

Table 4. Changes in plasma protein levels for the 40 units of pooling protocol at 4°C storage.

		1 day	2 days	3 days	4 days
	0 days	Value/change (%)	Value/change (%)	Value/change (%)	Value/change (%)
Total protein (g/L)	55.63 ± 0.25	53.47 ± 0.38 (−4)*	56.70 ± 0.35 (+2)*	52.53 ± 0.15 (−6)*	56.87 ± 0.32 (+2)*
Albumin (g/L)	36.67 ± 0.51	35.63 ± 0.76 (−3)	37.20 ± 0.17 (+1)	34.80 ± 0.35 (−5)*	37.60 ± 0.10 (+3)
Fibrinogen (g/L)	2.09 ± 0.11	1.99 ± 0.05 (−5)	1.93 ± 0.08 (−8)	1.93 ± 0.06 (−8)	1.97 ± 0.06 (−6)
Factor V (IU/mL)	0.80 ± 0.02	0.88 ± 0.07 (+1)	0.85 ± 0.04 (+6)	0.77 ± 0.01 (−4)	0.93 ± 0.05 (+16)*
Factor VIII (IU/mL)	0.67 ± 0.04	0.50 ± 0.05 (−25)*	0.50 ± 0.04 (−25)*	0.43 ± 0.04 (−36)*	0.43 ± 0.05 (−36)*
Antithrombin-III (IU/mL)	1.23 ± 0.03	1.08 ± 0.03 (−12)*	1.07 ± 0.02 (−13)*	1.07 ± 0.04 (−13)*	1.05 ± 0.01 (−15)*
Protein C (IU/mL)	1.03 ± 0.01	0.90 ± 0.02 (−13)*	0.92 ± 0.03 (−11)*	0.92 ± 0.04 (−11)*	0.94 ± 0.03 (−9)*

Data are presented as the mean ± standard deviation, n = 3 for each protein.
*$P < 0.05$ versus 0 days.

Table 5. Values and changes in plasma proteins for the 40 units of pooling protocol at −20°C storage

		1 month	2 months	3 months
	0 days	Value/change (%)	Value/change (%)	Value/change (%)
Total protein (g/L)	55.63 ± 0.25	52.57 ± 0.25 (−6)*	52.07 ± 0.81 (−6)*	52.00 ± 0.36 (−6)
Albumin (g/L)	36.67 ± 0.51	32.77 ± 0.42 (−11)*	34.10 ± 0.56 (−7)*	33.97 ± 0.60 (−7)
Fibrinogen (g/L)	2.09 ± 0.11	1.94 ± 0.14 (−7)	1.95 ± 0.13 (−7)	1.96 ± 0.12 (−6)
Factor V (IU/mL)	0.80 ± 0.02	0.71 ± 0.04 (−11)*	0.68 ± 0.07 (−15)*	0.70 ± 0.04 (−13)
Factor VIII (IU/mL)	0.67 ± 0.04	0.48 ± 0.03 (−28)*	0.56 ± 0.01 (−16)*	0.42 ± 0.03 (−37)*
Antithrombin-III (IU/mL)	1.23 ± 0.03	0.91 ± 0.03 (−26)*	1.08 ± 0.04 (−12)*	1.02 ± 0.08 (−17)*
Protein C (IU/mL)	1.03 ± 0.01	0.87 ± 0.02 (−16)*	0.90 ± 0.03 (−13)*	0.91 ± 0.03 (−12)*

Data are presented as the mean ± SD, n = 3 for each protein.
*$P < 0.05$ versus 0 days.

protein degradation or changes in bioequivalence.[12] When pooled plasma was stored at 22°C for 1 day, there were no significant changes in Fg, anti-III, and PC levels compared with 0 days, but TP, Alb, FV, and FVIII levels were significantly changed by 1 day. With a prolonged storage time, there were even greater differences in more proteins. Therefore, storage at 22°C may be best for short-term preservation for emergency use rather than long-term preservation to maintain plasma protein levels.

As a rule, the optimal storage condition is 4°C for blood products, except for platelet concentrations. In particular, thawed plasma fully maintains the level of plasma proteins, including coagulation factors and inhibitors. Some studies have suggested that the expiration date for thawed plasma should be extended beyond 5 days, even to 10 days in 1°C to −6°C storage.[13–16] In our study, the levels of all plasma proteins slightly declined without an increase in pooled plasma during storage at 4°C from 1 to 4 days (Table 4). FVIII was labile, and the mean activity significantly declined from 1 to 4 days, with a minimum reduction by 25% at 1 day to a maximum reduction by 36% at 4 days. In contrast, another labile factor, FV, maintained a relatively higher retention level during the same storage conditions. The retention level of Fg was similar to that of FV, with no significant changes during storage to day 4. TP levels showed significant changes during storage, but these slight changes are still acceptable. In our study, storage at 4°C and then at 22°C were considered as a method of moderate to short-term preservation for our pooled plasma.

Repeated freezing and thawing is not permitted by the China Guidelines because of the influence of such action on coagulation factors. However, previous studies have reported that levels of prothrombin, FVII, FIX, FX, and Fg remain stable and adequate for transfusion in twice-thawed and refrozen FFP.[17] Furthermore, FFP can be safely used as a source of vitamin K-dependent clotting factors and Fg. The effect of freezing and thawing plasma proteins of our pooled plasma twice is not clear. We evaluated the effect of freezing and thawing on plasma proteins in pooled plasma during −20°C storage for 3 months. We found that all plasma protein levels, except for Fg, significantly declined from 1 to 3 months when stored at −20°C (Table 5). FVIII levels were decreased by 28% in 1 month and by 37% in 3 months.

The coagulation inhibitors anti-III and PC were also significantly decreased by 10% from 1 to 3 months compared with 0 days. However, consistent with previous reports, there was no significant loss in Fg levels.[17] Although there were massive losses of some proteins in twice-thawed and refrozen pooled plasma, our data still suggest the feasibility and availability for our pooled plasma stored at −20°C in long-term preservation.

There are limitations in our study that should be considered. All test values for proteins may have been affected by the detection method. Additionally, data were only considered as a scientific reference rather than a standard of products without affecting our overall conclusion. We intend on investigating the regularity and feasibility of our CUPP. Larger scale experiments will be conducted as a next step in demonstrating standardization of universal-activated pooled plasma products. We expect to establish a concept of the "active dose for plasma", which refers to the average active dose or level of main functional plasma proteins in each unit of CUPP with approximately 100 mL. Although some proteins in CUPP after storage fail to meet the China Guideline, such as FVIII (<0.5 IU/mL), explicit dose standards for plasma proteins in CUPP will be convenient for plasma transfusion, not only for improving treatment efficacy, but also for reducing the amount of wasted plasma.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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