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ORIGINAL ARTICLE

Coagulation parameters of thawed fresh-frozen plasma during storage at different temperatures

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SUMMARY. Once thawed, fresh-frozen plasma (FFP) should be used, according to guidelines, within 24 h. In hospital practice, this may be associated with wastage. This study has been performed to investigate the coagulation levels of thawed quarantine FFP as used in the Netherlands. Five units of quarantine FFP, obtained by plasmapheresis, were thawed and by sterile docking divided into satellite bags (SB). SB 2–4 were stored at room temperature (RT) for, respectively, 1, 3 and 6 h and SB 5–9 at 4 °C for 6, 12 and 24 h and 1 and 2 weeks. At each time point, activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen, factor V (FV), factor VIII (FVIII) and ADAMTS13 activity were measured. During storage at RT for up to 6 h, no major differences were found in the levels of FV, PT, fibrinogen and ADAMTS13 activity. FVIII activity showed a decrease of 16% and the APTT was prolonged by 6%. During storage at 4 °C for 2 weeks, FV and FVIII were reduced by 35 and 45%, respectively. The APTT and PT were prolonged by 17 and 15%, respectively. Fibrinogen was decreased by 8%. No change in ADAMTS13 activity was found. FFP stored at RT for 6 h or at 4 °C for 2 weeks can provide sufficient support for adequate haemostasis except for patients with a known deficiency for FVIII and can be used for plasmapheresis in patients with thrombotic thrombocytopenic purpura (TTP).

Key words: coagulation factors, storage of plasma.

The purpose of administering fresh-frozen plasma (FFP) to patients is to replace deficient or dysfunctional coagulation factors (Bianco, 1999). The Dutch guidelines cite the following indications: blood loss or expected massive blood loss in combination with combined coagulation factor deficiencies, isolated factor V (FV) deficiency, thrombotic thrombocytopenic purpura (TTP) and the antagonizing of fibrinolytics (CBO, 2004). The main issue in storing FFP is the preservation of functional coagulation factors in the frozen state as well as after thawing. In citrated plasma stored at a temperature between −18 and −24 °C, all coagulation proteins are stable for 3 months (Counts *et al.*, 1979). When stored at −30 °C for 12 months, antithrombin was minimally affected (Omran *et al.*, 1996). A storage temperature of −74 °C will extend the storage period to 18 months and for some coagulation proteins even to 24 months (Woodhams *et al.*, 2001). European guidelines allow 24 months of storage of FFP at below −25 ° (Council of Europe, 2005).

FFP is considered adequate for transfusion immediately after thawing (Klein, 1996). According to the present Dutch guidelines, FFP should be used, after thawing and storage at room temperature (RT), within 6 h; after storage at 1–6 °C within 24 h (CBO, 2004). A great disadvantage of thawed FFP in stock is this reduced storage time. Wastage of thawed plasma in many clinics is still large, e.g. more than 10% in our hospital, in particular due to wrong estimations for expected demands in major surgery. Once thawed after request, FFP is not always transfused immediately and kept for a period of time at RT. Usually these FFPs are disposed, which may not however be necessary. Several contradictory reports have been

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published as to what temperature FFPs can be stored before they are no longer suitable for transfusion. Smak Gregoor *et al.* (1993) showed that all haemostatic components in FFP and even in cryosupernatant plasma (CSP) were sufficient to provide adequate haemostasis (except FVIII in CSP) when stored for 28 days at 4 °C. Nilsson *et al.* (1983) collected evidence that plasma stored at 1–6 °C can be transfused for up to 35 days after thawing. O'Neill *et al.* (1999) reported that there was no significant change in the level of coagulation factors in whole blood stored at RT (22 °C) for 24 h, except for FVIII, which decreased by 13% after 8-h storage and by another 15–20% after 24-h storage.

Currently, quarantine FFP is used in the Netherlands, which implies a prolonged storage interval, often more than 9 months, in the frozen state prior to release to hospitals. In order to investigate the optimal storage condition of thawed quarantine FFP, we studied the level of coagulation parameters after storage of the thawed FFP at RT for 6 h and at 4 °C for up to 2 weeks.

MATERIALS AND METHODS

Obtaining plasma, storage and sampling

Quarantine FFP, obtained by plasmapheresis, was provided by the regional blood bank of the Dutch blood supply organization Sanquin after storage interval of 6–10 months. Five units of approximately 325 mL FFP (mother bags, MB) of blood group O were thawed in a 37 °C water bath with shaking until a temperature of <10 °C. The donors had given written permission to use part of their blood for research purposes. Plasma was transferred to satellite bags (SB), by coupling the MB to SB with a sterile docking device [Terumo Sterile Tubing Welder SC-201 (TSCD), Terumo Corp., Tokyo, Japan] (Fig. 1).

Immediately after thawing, plasma was transferred to four SB: 21 mL in SB 1 and 14 mL in SB 2–4. The MB was stored at 1–6 °C, SB 1–4 at RT. At time 0, 7 mL of plasma (SB 0) was drawn from SB 1 and immediately analysed for coagulation parameters. The residual plasma from SB 1 was quick frozen in 1 mL aliquots and stored at −52 °C. After 1, 3 and 6-h storage at RT, respectively, 1 mL aliquots from SB 2, 3 and 4 were stored at −52 °C. Freezing was carried out by depositing the aliquots of plasma in the −52 °C freezer.

After 6-h storage at 1–6 °C, a second set of four SB (SB 5–8) containing 14 mL were removed from the MB by sterile docking. SB 5 was filled, sampled and stored at −52 °C. After 12 h, 24 h and 1 week, the same procedure was repeated for SB 6, 7 and 8, respectively. After 2 weeks at 1–6 °C, the MB (SB 9) was sampled and stored at −52 °C. In order to reduce the interassay variance, all samples were thawed simultaneously in a water bath of 37 °C for 10 min, just before measurement of coagulation factors.

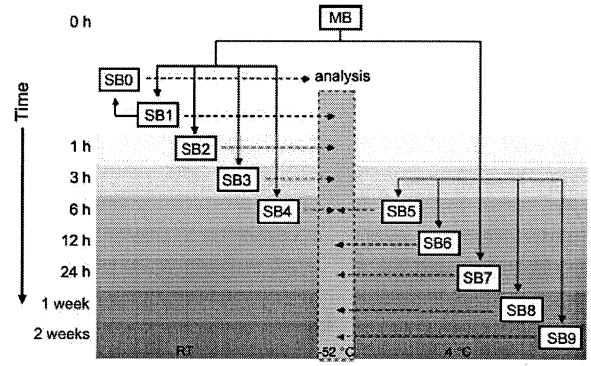

Fig. 1. Plasma storage and sample. MB, mother bag; SB, satellite bag; RT, room temperature.

Laboratory procedures

Activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen and FV were measured on an automated coagulation analyzer Electra 1800C (Medical Laboratory Automation, Inc., Pleasantville, NY, USA), FVIII was measured on an automated coagulation analyzer STA-R® (Diagnostica Stago, Asnières sur Seine, France). APTT and PT were measured using Cephotest (Nycomed Pharma, Oslo, Norway) and Recombiplastin (Instrumentation Laboratory, Breda, the Netherlands), respectively. Fibrinogen was measured according to the Clauss method using thrombin (Fibriquik; bioMérieux, Boxtel, the Netherlands) calibrated against a fibrinogen standard (STA Unicalibrator; Diagnostica Stago). FV:C was measured in a one-stage PT-based clotting assay using FV-deficient plasma and Recombiplastin (Instrumentation Laboratory). Plasma FVIII:C was measured in a one-stage APTT-based clotting assay using home-made immunodepleted FVIII-deficient plasma and automated APTT (bioMérieux). ADAMTS13 activity was measured using the FRETS-VWF73 assay (Peptide Institute Inc., Louisville, KY, USA). Results for FV:C and ADAMTS13 were expressed as percentage of an in-house normal plasma pool. Results for FVIII:C were expressed as international units (IU) with reference to the normal plasma pool calibrated against the 4th WHO international standard FVIII/VWF plasma (97/586) (NIBSC, Potters Bar, UK).

RESULTS

The baseline coagulation parameters in the five FFP 'mother' units analysed immediately after thawing of the FFP unit (SB 0; Fig. 1) are given in Table 1.

To correct for the possible effects of refreezing of aliquots at −52 °C and re-thawing prior to analysis of samples (which appeared to yield only a small loss of activity), we used as baseline the results of the SB 1 sample that was immediately removed from the MB, refrozen and measured at the same time as all subsequent samples for further comparisons (SB 1; Fig. 1). Changes in the coagulation parameters during storage are expressed as percentage of baseline (SB 1) values in order to normalize for the differences in coagulation parameters between the five plasma units. There were no significant changes for FV, PT and fibrinogen during storage at RT for up to 6 h. FVIII showed a decrease in activity of 16% and the APTT was prolonged by 6% after storage at RT for 6 h (data not shown). Figure 2 shows the changes in coagulation parameters during storage at 4 °C for 2 weeks. By the end of 2 weeks, FV and FVIII were reduced by 35 and 45%, respectively. In contrast to the gradual decrease in FV, the activity of FVIII decreased mainly in the first 24 h and remained nearly stable afterwards. The APTT and PT were prolonged by 17 and 15%, respectively, and fibrinogen decreased by 8%.

The ADAMTS13 activity measurements are summarized in Table 2. The changes in time were considerably less than the variation between the 5 units immediately after (re-)thawing.

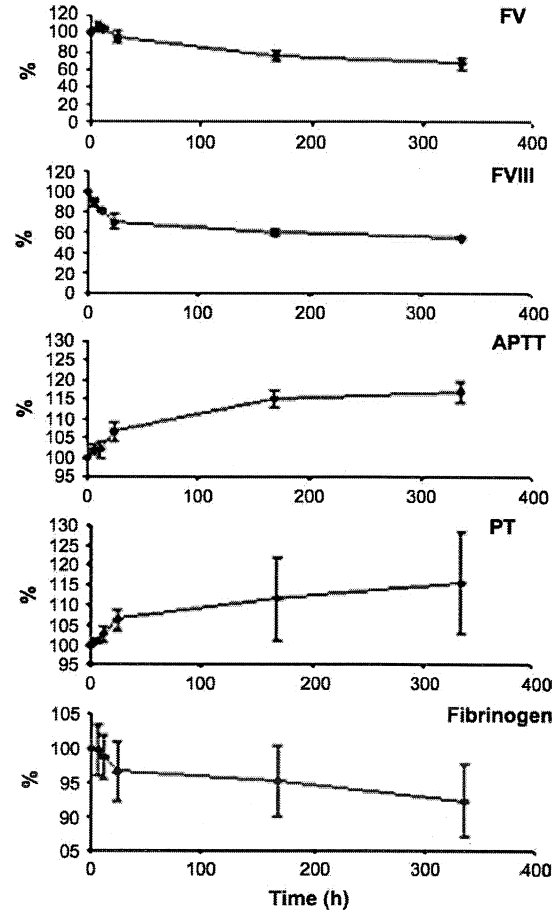

Fig. 2. Coagulation parameters during storage at 4 °C for 14 days (SB 5–8), expressed as a percentage of values (±SD) obtained for SB 1 (baseline sample measured after storage at −52 °C).

DISCUSSION

In the past, several reports were published on the storage time and -temperature of thawed FFPs before they are no longer adequate for transfusion. Different results were due to the various study designs; variability in original FFP source, thawing temperature, storage period, storage temperature and the coagulation factors measured (Heil *et al.*, 1998; Downes *et al.*, 2001; Buchta *et al.*, 2004; Suontaka *et al.*, 2005).

Consequently, we felt that these reported results might not be directly applicable to conditions in the hospital as far as to advising on the storage conditions and storage interval of FFP after thawing. For this purpose, we evaluated the level of coagulation

Table 1. Baseline coagulation parameters of the five plasma units

Plasma unit	FV (%)	FVIII (IU mL^{-1})	APTT (s)	PT (s)	Fibrinogen (g L^{-1})
1	131	1·13	25·1	11·6	3·3
2	92	0·94	26·5	11·4	3·9
3	95	0·71	26·7	12·2	2·9
4	110	0·95	27·0	11·1	5·1
5	106	0·79	27·8	12·3	3·2
Mean (SD)	107 (15)	0·9 (0·2)	26·6 (1·0)	11·7 (0·5)	3·7 (0·9)

SD, standard deviation.

Table 2. ADAMTS13 activity

Temperature	Time	Mean (SD)
RT	0 h	95 (9·2)
RT	6 h	95 (7·3)
4 °C	6 h	90 (11·2)
4 °C	2 weeks	94 (11)

Values are expressed as percentage of normal pool plasma (100%).

parameters of thawed FFP during storage at RT for up to 6 h and storage at 4 °C for 2 weeks. Blood group O plasma, which is known to have about 20% lower FVIII activity than blood group A plasma, was used to assess the 'at-worst' storage degradation. We found minor changes of coagulation parameters during storage for 6 h at RT, but more outspoken changes while storing for up to 2 weeks at 4 °C.

Do the levels of FV and FVIII after storage under these conditions still meet the criteria for adequate support of haemostasis in patients? In the 11th edition of the European Guide to the preparation and storage of blood components, the required properties of FFP are described as follows: 'plasma should contain normal levels of stable coagulation factors, ≥70 international equivalents of FVIII per 100 mL and at least similar quantities of the other labile coagulation factors. Quality control demands FVIII above 70% of the value of the freshly collected plasma unit' (Council of Europe, 2005). This value seems to apply to FFPs from all blood groups. Our results show that blood group O FFPs stored at RT for 6 h or at 4 °C for 2 weeks do not meet these criteria. However, they do meet the criteria of the American Association of Blood Banks (Klein, 1996). The FVIII level at the end of the storage interval has decreased to 55% after 2 weeks, this corresponds with 0·50 IU mL^{-1} in FFPs only thawed once. As we tested blood group O products, this value is expected to be higher and approximately 0·60 IU mL^{-1} in FFP derived from non-O donors. The fast decline of FVIII is probably of no consequence for the patient transfused with FFP. O'Neill reports that a decrease of FVIII in fresh plasma of 28–33% would still be sufficient to support haemostasis in situations where all coagulation factors were decreased (O'Neill *et al.*, 1999). In addition, endogenous FVIII may be produced in a higher concentration in previously healthy individuals under the stress of bleeding, surgery, etc. (Jern *et al.*, 1989). We conclude, therefore, that the storage conditions examined in this report are sufficient to keep FFPs adequate for transfusion for 6 h at RT or for 2 weeks at 4 °C except for the rare patient with FVIII deficiency.

Apart from other options such as refreezing (Ben Tal *et al.*, 2003) which we did not investigate, fluid storage for more than 6 h raises the possibility of re-issuing non-transfused plasma units, provided that there is a continual monitoring of the temperature of the cooled unit during transport to and from the ward. This will be investigated using temperature recording 'chip-cards' in the actual conditions of our hospital. Moreover, the remarkable stability of the ADAMTS13 activity allows for an easier logistics of plasmapheresis for thrombotic thrombocytopenic purpura.

The microbial safety of issuing and certainly re-issuing thawed plasma units stored for up to 2 weeks at 4 °C is of course less than FFP stored <24 h at 4 °C. The risk is, however, similar or less for red cells stored at 4 °C for up to 35 or 42 days. We issued, in our service, over 60 000 plasma units after fluid storage for up to 2 weeks at 4 °C without one reported case of bacterial contamination.

Finally, we want to stress that we are not advocating the routine use of liquid-stored plasma units because these products just contain the minimally adequate dose. In concert with others, we are of the opinion that creeping downwards is undesired. Our conclusions should rather be considered against the even more undesired alternative of disposal.

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