Storage of thawed plasma for a liquid plasma bank: impact of temperature and methylene blue pathogen inactivation

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BACKGROUND: Rapid transfusion of fresh-frozen plasma (FFP) is desired for treating coagulopathies, but thawing and issuing of FFP takes more than 40 minutes. Liquid storage of plasma is a potential solution but uncertainties exist regarding clotting factor stability. We assessed different storage conditions of thawed FFP and plasma treated by methylene blue plus light (MB/light) for pathogen inactivation.

STUDY DESIGN AND METHODS: Fifty thawed apheresis plasma samples (approx. 750 mL) were divided into three subunits and either stored for 7 days at 4°C, at room temperature (RT), and at 4°C after MB/light treatment. Clotting factor activities (Factor [F] II, FV, FVII through FXIII, fibrinogen, antithrombin, von Willebrand factor antigen, Protein C and S) were assessed after thawing and on Days 3, 5, and 7. Changes were classified as "minor" (activities within the reference range) and "major" (activities outside the reference range).

RESULTS: FFP storage at 4°C revealed major changes for FVIII (median [range], 56% [33%-114%]) and Protein S (51% [20%-88%]). Changes were more pronounced when plasma was stored at RT (FVIII, 59% [37%-123%]; FVII, 69% [42%-125%]; Protein S, 20% [10%-35%]). MB/light treatment of thawed FFP resulted in minor changes. However, further storage for 7 days at 4°C revealed major decreases for FVIII (47% [12%-91%]) and Protein S (49% [18%-95%]) and increases for FVII (150% [48%-285%]) and FX (126% [62%-206%]).

CONCLUSION: Storage of liquid plasma at 4°C for 7 days is feasible for FFP as is MB/light treatment of thawed plasma. In contrast, storage of thawed plasma for 7 days at RT or after MB/light treatment at 4°C affects clotting factor stability substantially and is not recommended.

Fresh-frozen plasma (FFP) is frequently transfused to treat severe coagulopathies after acute trauma or major surgery. Rapid issuing of FFP is desired in emergency situations, but FFP must be kept frozen to preserve clotting factor activities (–18°C in the United States; –30°C in Europe) and must be thawed before transfusion, which takes approximately 30 to 40 minutes.

Recently, we showed that lyophilized pathogen-inactivated plasma is one option to achieve rapid plasma provision in emergencies without the need for a cold chain,[1] but lyophilized plasma is currently not available at many centers. Another option is to store thawed FFP in liquid plasma banks at 1 to 6°C, allowing rapid issuing in case of a bleeding emergency. In the United States, the Food and Drug Administration approved storage of plasma after thawing for 24 hours (21 CFR 640.120, as of December 28, 2010); it is accepted (although not approved) to extend this storage for up to 5 days at 1 to 6°C.[2,3] Storage of thawed plasma at room temperature (RT) would reduce cold chain requirements of currently

ABBREVIATIONS: MB/light = methylene blue plus light; QH = quarantine hold; RT = room temperature; TFPI = tissue factor pathway inhibitor; VWF-Ag = von Willebrand factor antigen.

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applied liquid plasma banks and would facilitate a plasma depot in the emergency room.

Quarantine hold (QH) of FFP is used in several countries to decrease the risk of pathogen transmission.[4] Release of QH-plasma for treatment requires at least 4 months and a follow-up donation testing negative for blood-borne pathogens such as human immunodeficiency virus, hepatitis B virus, and hepatitis C virus and their corresponding antibodies. QH may lead to problems in case of shortages or an increased demand of therapeutic plasma. Despite sufficient supply of frozen plasma, these products are not available until QH has expired.

Methylene blue plus light (MB/light) pathogen inactivation might be an option to replenish the available plasma stock by allowing premature release of FFP from QH while preserving safety from pathogens. MB/light treatment is well established for pathogen inactivation of single plasma units in Europe.[5] In case of plasma shortages, MB/light could easily be applied to inactivate pathogens in thawed plasma and to cease QH. However, it is unclear whether stored MB/light-treated thawed plasma is stable at 4°C for several days during liquid storage. This study was conducted to define optimal conditions for the storage of liquid plasma, by evaluating thawed FFP and thawed MB/light-treated plasma with regard to clotting factor stability over a 7-day storage period.

MATERIALS AND METHODS

Plasmapheresis

Fifty consenting healthy donors suitable for blood donation according to the German hemotherapy guidelines[6] underwent plasmapheresis (approx. 750 mL). All procedures were performed on apheresis devices employing collection and harness sets in 4% sodium citrate (PCS2, Haemonetics, Braintree, MA) according to the manufacturer's instructions. After apheresis the plasma bags were deep-frozen (plasma freezer, HOF, Lohra, Germany) within 6 hours and stored at –30°C.

Sample preparation

Bags of frozen apheresis plasma (n = 50) were thawed at 37°C within 1 hour using an approved thawing device (Plasmatherm Barkey, Leopoldshoehe, Germany). Subsequently, apheresis plasma samples were divided into three subunits each, resulting in three bioequivalent plasma units of approximately 220 mL volume. Subunits I and II were transferred into Compoflex transfer bags (Fresenius Kabi, Bad Homburg, Germany) via sterile docking (Terumo TSCD-II, Terumo Corp., Tokyo, Japan). Subunit I was stored at 4°C and Subunit II at RT (20-24°C), respectively.

Subunit III was transferred into a plasma bag system (Theraflex MB, Macopharma, Mouvaux, France). MB/light treatment was performed according to the manufacturer's instructions with a plasma illumination system (Macotronic B2, Macopharma, Mouvaux, France; peak wavelength 627 ± 10 nm, 120 J/cm^2). Afterward, MB was removed from plasma by an integrated filter (PLAS4 and Blueflex filtration, Macopharma) and Subunit III was kept at 4°C. All three subunits were stored for 7 days with aliquots for coagulation studies obtained by sterile docking of a sample bag on Days 0, 3, 5, and 7; immediately transferred into 3-mL glass tubes (Vacutainer, Becton Dickinson, Belliver Industrial Estate, Plymouth, UK) and assessed for clotting factor activities within 1 hour after sampling.

Clotting factor assays

Clotting factor activities were measured by standard assays: Factor (F)II, FV, FVII, FVIII, F IX, FX, FXI, FXII, and Protein S using clotting-based tests with factor-deficient plasma (BCS-XP analyzer, Siemens Healthcare Diagnostics, Eschborn, Germany); FXIII, antithrombin, and Protein C using a chromogenic assay (Berichrom chromogenic assay, Siemens Healthcare Diagnostics); fibrinogen by the method of Clauss (Multifibren U, Siemens Healthcare Diagnostics); and von Willebrand factor antigen (VWF-Ag) by an immunoturbidimetric assay (VWF-Ag reagent, Siemens Healthcare Diagnostics).

Sterility testing

Bacterial and fungal growth were tested in all plasma units after 7 days of storage by incubating 10 mL of each bag under aerobic and anaerobic conditions at 36°C for 7 days (Bactec Plus Aerob/F, Bactec Lytic/10 Anaerob/F, Bactec 9240, BD, Heidelberg, Germany).

Statistical analysis

Statistical comparisons were performed between the baseline values of thawed plasma and of MB/light-treated thawed plasma on Day 0 to identify changes attributable to this pathogen inactivation procedure. In addition, baseline values of clotting factor activities were compared with those after storage of untreated thawed plasma at 4°C, at RT, and after MB/light treatment and storage at 4°C. For all statistical comparisons the Wilcoxon signed-rank test was applied. A p value of less than 0.05 was considered significant.

Changes in clotting factor activities were classified as: 1) *major* changes, defined as those leading to median values outside of the reference range of the respective clotting factor; and 2) *minor* changes, defined as those resulting in more than 10% decreases (or increases) in median factor activities, but with the median value remaining within the reference range. All changes in clot-

ting factor activities were determined as absolute changes between the baseline values obtained from thawed plasma on Day 0 and after 7 days of storage in the respective subunit, that is, a median value of 81% for Protein S activity on Day 0 that declined to a median value of 20% Protein S activity on Day 7 is reported as a decline by 61% in Protein S activity.

RESULTS

Fifty apheresis-derived plasma products (ABO blood group A, n = 15; blood group O, n = 24; blood group B, n = 10; blood group AB, n = 1) were thawed after a mean deep-freeze storage time of 256 days (range, 14-408 days). The mean volume of plasma products was 741 mL (range, 455-845 mL). Each plasma bag was divided into three subunits for storage under three conditions: 1) liquid storage at 4°C (n = 50; mean, 219 ± 27 mL); 2) storage at 22°C (n = 50; mean, 218 ± 26 mL; and 3) MB/light treatment after thawing and storage at 4°C (n = 50; mean, 210 ± 6 mL). All units were stored for 7 days.

Figure 1 shows baseline values of thawed plasma on Day 0, values obtained directly after MB/light treatment of thawed plasma (Day 0), values after 7 days of storage of thawed plasma at 4°C and RT, and values after 7 days storage of MB/light-treated plasma at 4°C. The numerical values of the respective plasma preparations for Days 0, 3, 5, and 7 after MB/light treatment for clotting factor activities are provided in Tables S1 to S3 (available as supporting information in the online version of this paper).

Storage of thawed plasma at 4°C and at RT

Storage of thawed plasma at 4°C for 7 days revealed major changes for only two factors, FVIII and Protein S (Fig. 1; details of clotting factor activities on Days 0, 3, 5, and 7 are given in Table S1): FVIII (decrease, –49%, i.e., median activity 105% before storage falling to median activity of 56% after storage; $p < 0.0001$) and Protein S (decrease, –27%, i.e., median activity 78% before storage falling to median activity 51% after storage; $p < 0.0001$). Minor changes were observed for FV (decrease, –19%, i.e., median activity 110% before storage falling to median activity 91% after storage; $p < 0.0001$), FVII (decrease, –15% i.e., median activity 106% before storage falling to median 91% activity after storage; $p = 0.0439$), and VWF-Ag (decrease, –11%, i.e., median activity 114% before storage falling to median activity 103% after storage; $p < 0.0001$).

In contrast, the storage for 7 days at RT affected activities of all clotting factors and inhibitors except protein C (Fig. 1; details of clotting factor activities on Days 0, 3, 5, and 7 are given in Table S2). Major changes were observed for Protein S (decrease, –61%, i.e., median activity 81% before storage falling to median activity 20% after storage; $p < 0.0001$), FVIII (decrease, –44%, i.e., median activity 104% before storage falling to median activity 59% after storage; $p < 0.0001$), and FVII (decrease, –38%, i.e., median activity 107% before storage falling to median activity 69% after storage; $p < 0.0001$). Minor changes were seen for activities of FV (decrease, –38%, i.e., median activity 109% before storage to median activity 71% after storage; $p < 0.0001$), FX (decrease, –28%, i.e., median activity 119% before storage falling to median activity 91% after storage; $p < 0.0001$), and F IX (decrease, –13%, i.e., median activity 99% before storage falling to median activity 86% after storage; $p < 0.001$). In contrast, FXII activity displayed a minor increase (increase, +14%; median activity 99% before storage rising to median activity 113% after storage; $p < 0.0001$). No fungal or bacterial growth occurred in either group, 4°C or RT.

MB/light treatment of thawed plasma and storage at 4°C

Treatment with MB/light immediately affected most of the clotting factor activities compared to thawed plasma. All observed changes, however, did not lead to alterations beyond the reference range and were thus classified as minor (Fig. 1; details of clotting factor activities before and after MB/light treatment are given in Table S3). The most pronounced decreases were detected in activities of FVIII (decrease, –23%, i.e., median activity 105% before MB/light-treatment falling to median activity 82% after MB/light treatment; $p < 0.0001$), FV (decrease, –17%, i.e., median activity 110% before MB/light-treatment falling to median activity 93% after MB/light-treatment; $p < 0.0001$), FXI (decrease, –13%, i.e., median activity 95% before MB/light-treatment falling to median activity 82% after MB/light-treatment; $p < 0.0001$), F IX (decrease, –11%, i.e., median activity 96% MB/light treatment falling to median activity 85% after MB/light treatment; $p = 0.0011$), and FX (decrease, –11%, i.e., median activity 118% before MB/light treatment falling to median activity 107% after MB/light treatment; $p = 0.003$).

MB/light-treated thawed plasma showed a more pronounced decrease in clotting factor activities over 7 days of storage at 4°C compared to non–MB/light-treated thawed plasma (Fig. 1). Major changes were detected for FVIII (decrease, –58.5%,%, i.e., median activity 105% before MB/light treatment falling to median activity 47% after MB/light treatment and storage; $p < 0.0001$) and Protein S (decrease, –29.5%, i.e., median activity 78% before MB/light treatment falling to median activity 49% after MB/light treatment and storage; $p < 0.0001$). Interestingly, median activity of FVII increased during storage (increase, 44.5%, i.e., median activity 106% before MB/light treatment rising to median activity 150% after MB/light treatment and storage; $p < 0.0001$). Also, FX activity increased over 7 days of storage (increase, 19%,

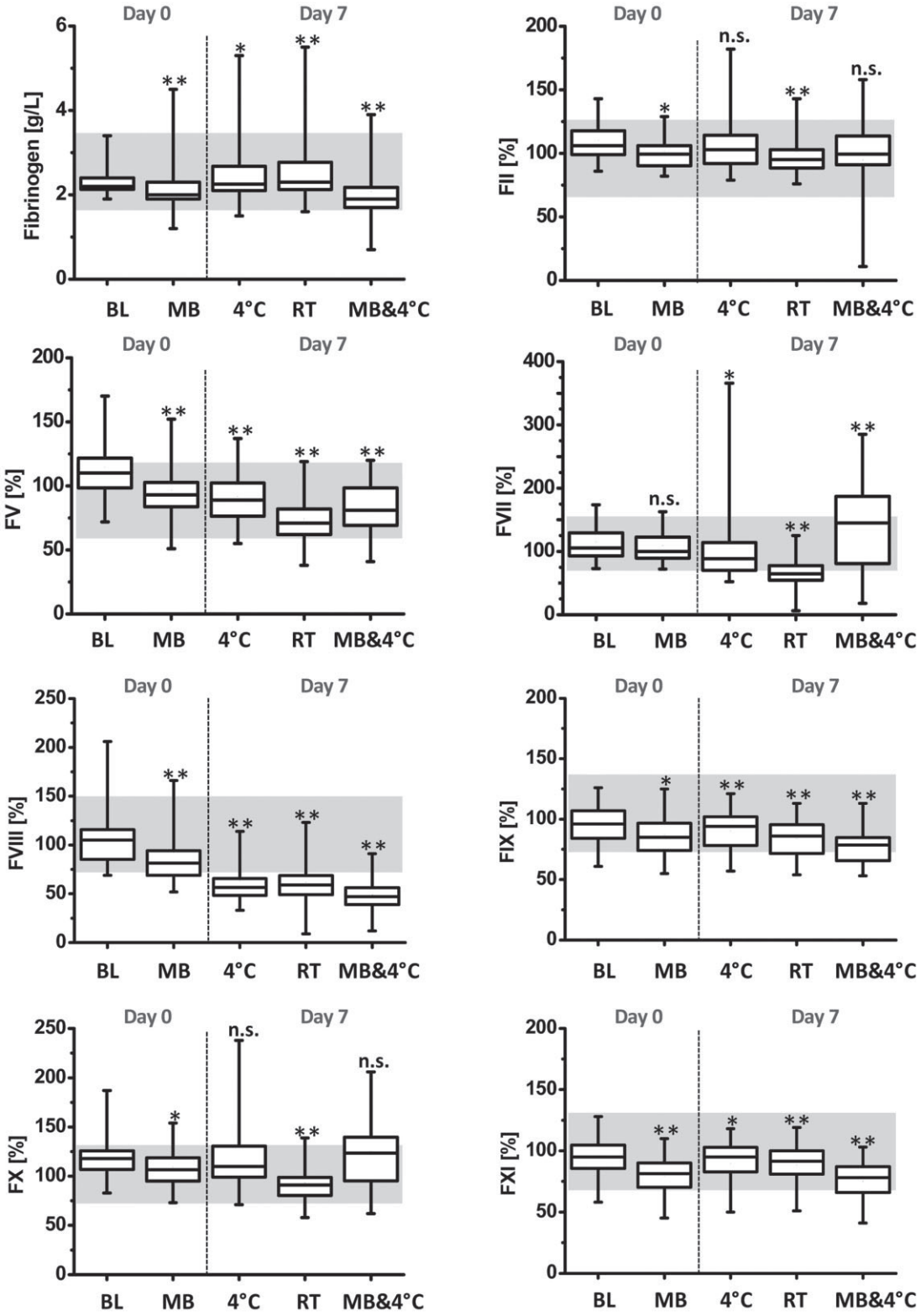

Fig. 1. Profiles of clotting factors and inhibitors. Displayed are boxplots of values of clotting factors and inhibitors in thawed plasma obtained directly after thawing on Day 0 (baseline [BL]), after MB/light treatment on Day 0 (MB), after 7 days of plasma storage at 4°C, at RT, and after MB/light treatment and storage at 4°C (MB&4°C) over 7 days. Clotting factor activities are given as median activities, 25% and 75% quartiles with minimum-maximum intervals. The gray-shaded area indicates the reference range. *Significance with $p < 0.05$; **$p < 0.01$; n.s. = not significant.

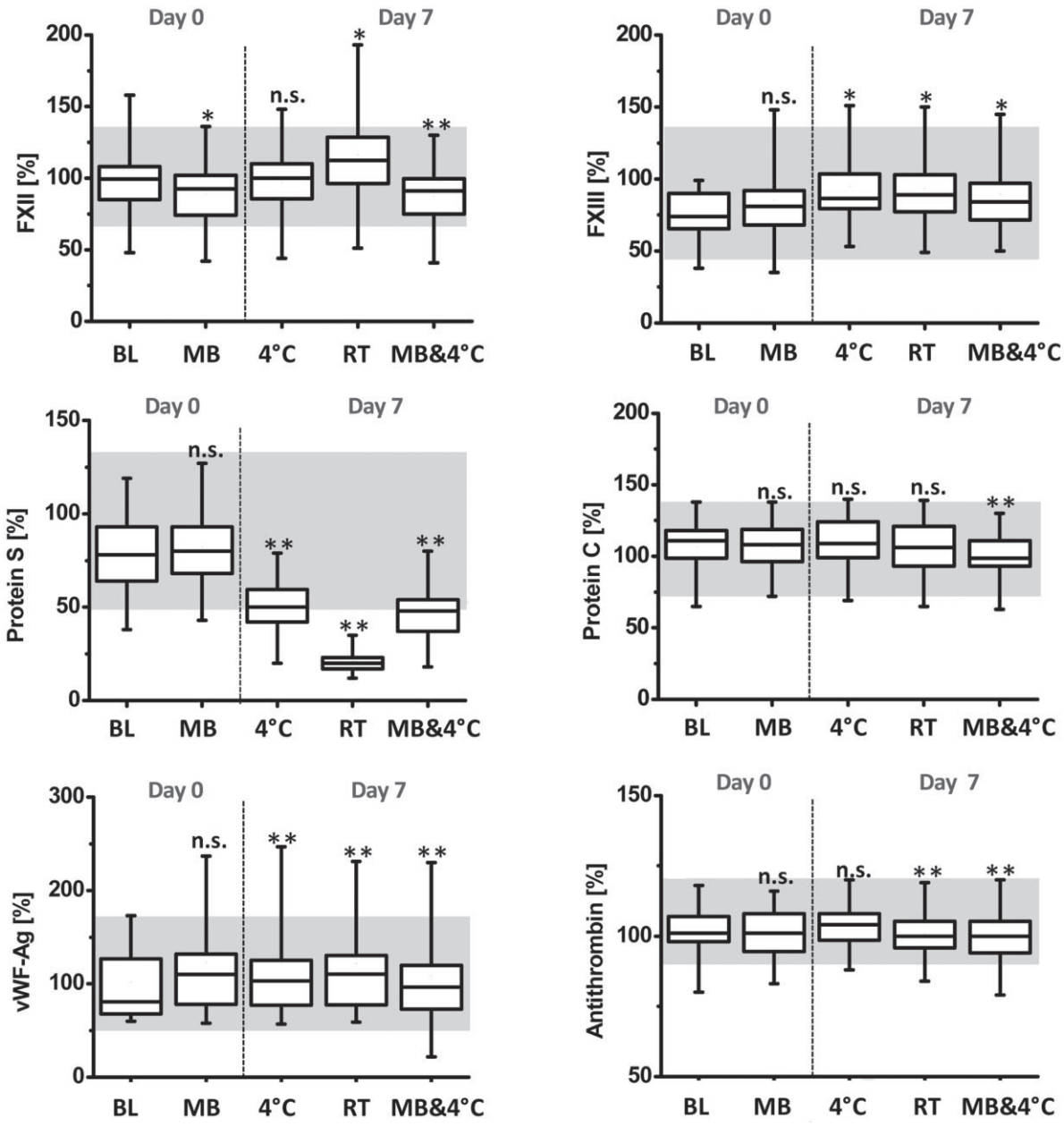

Fig. 1. ***Continued.***

i.e., median activity 107% before MB/light treatment rising to median activity 126% after MB/light treatment and storage; $p < 0.0001$; Fig. 1; details of clotting factor activities on Days 0, 3, 5, and 7 are given in Table S3). In none of the plasma bags was any fungal or bacterial growth detected.

DISCUSSION

In this study we show that thawed plasma stored at 4°C for 7 days maintained activities of all clotting factors, with the exception of FVIII and Protein S, thus presumably sufficient for treating all but FVIII-deficient patients. In line with previous studies,[3,7-16] this underscores the feasibility of a liquid plasma bank, which is already employed in clinical practice in some countries,[14,17,18] while in other medical jurisdictions, for example, in Germany, plasma currently may only be stored for a maximum of 6 hours after thawing.[19] We further demonstrate that RT storage of thawed plasma causes significant reductions in numerous clotting factor activities, with FVIII, Protein S, and FVII being most vulnerable under this storage condition, which cannot therefore be recommended from our perspective.

We also show that MB/light treatment of thawed plasma resulted only in minor changes in clotting factor activities, which were most pronounced for FVIII and FV. This is comparable with previous studies showing the effect of MB/light treatment on fresh plasma.[20,21] Garwood and coworkers[22] demonstrated that the impact of MB/light treatment is even less when applied to fresh plasma compared to frozen-thawed plasma. However, as factor activities in our study remained within the reference range when MB/light treatment was applied directly after thawing, we consider MB/light pathogen inactivation of frozen-thawed plasma as a possible option to replenish stocks of therapeutic plasma within hours in the case of an increased demand and shortage of quarantined FFP. This would greatly increase flexibility of plasma supply for blood banks that primarily supply quarantine-stored plasma.

In contrast, MB/light-treated thawed plasma shows more pronounced changes in clotting factor activities compared to nontreated thawed plasma when stored at 4°C. Previous studies reported tolerable measures of clotting factors[23] and thrombin generation[24] after 24 hours storage of frozen-thawed MB/light-treated plasma. Our study indicates that extension of storage of MB/light-treated plasma for up to 7 days at 4°C should not be recommended. An interesting and unexpected finding of our studies on extended storage of MB/light-treated plasma was the increase in the procoagulant factor activities of FVII and FX. To our knowledge, this has not been reported previously. We hypothesize that MB/light treatment affects tissue factor pathway inhibitor (TFPI) because both activated FVII and FX are inhibited by TFPI by forming complexes with these factors.[25-27] When TFPI is affected by MB treatment, the altered TFPI can no longer neutralize FVIIa and FXa and consequently their activities increase. However, further studies are needed to examine the possible molecular mechanisms of this observation.

While a liquid plasma bank has obvious logistical advantages, storage of thawed plasma might increase the risk of microbial growth in these products. However, based on the experience with routine storage of red blood cells stored under similar conditions for much longer periods, the risk of bacterial growth at 4°C likely is acceptably low.

Some limitations of our study should be considered. The study is an in vitro study and does not provide data about the clinical efficacies of stored thawed plasma products investigated. This is especially relevant for pathogen-inactivated plasma, as some clinical trials suggest that MB/light treatment might reduce efficacy of FFP,[28] while other studies, however, reported safe and efficient use of MB/light-treated plasma products.[5] A more technical limitation is the interpretation of the changes in VWF values. We determined VWF by an antigen assay. The increase of VWF-Ag observed in our study might be a result of VWF degradation and could therefore indicate a loss rather than an increase in biologic activity. However, as the overall changes in VWF were moderate, it is unlikely that this has a major biologic impact. Finally, it should be noted that storage conditions slightly differ among countries. Especially in the United States, plasma is kept frozen at –18°C, while in our study, –30°C was used. Because plasma quality does not appear to differ if stored at –20 or –40°C,[29] our findings likely will be applicable to US blood bank conditions.

In conclusion, this study shows two ways to improve the logistics of plasma provision, thereby allowing a more rapid and flexible plasma supply. First, it provides evidence that liquid plasma storage at 4°C for 7 days is feasible, without major declines in clotting factor activities except for FVIII. This should facilitate the establishment of a liquid plasma bank, permitting the rapid release of therapeutic plasma in emergencies without delay due to time for thawing. In addition, we show that in times of plasma shortages—but with availability of quarantined FFP—therapeutic plasma can be recaptured from frozen plasma before the end of quarantine storage through application of MB/light pathogen inactivation. In contrast, prolonged storage of thawed plasma at RT or prolonged storage of thawed MB/light-treated plasma leads to more pronounced changes in clotting factor activities, and therefore these two approaches cannot be recommended.

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AUTHORSHIP CONTRIBUTION

TT, SK, and KS designed the study and provided the study material; CW, MN, and KZ performed measurements; TT, SK, GH, TEW, AG, and KS analyzed results; AW performed statistical analysis; GH and KS made the figures and tables; and TT, SK, TEW, AG, and KS wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

AG has a consultant contract with MacoPharma, Germany. The other authors declare no competing financial interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Storage of thawed plasma at 4°C.
Table S2. Storage of thawed plasma at room temperature.
Table S3. Freeze thawed plasma before and after MB/light treatment and after storage at 4°C.

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