Cold-stored whole blood in a Norwegian emergency helicopter service: an observational study on storage conditions and product quality

Christopher Bjerkvig[1,2,3] *Joar Sivertsen*[4] *Hanne Braathen*[4] *Turid Helen Felli Lunde*[4] *Geir Strandenes*[4,5] *Jörg Assmus*[6] *Tor Hervig*[3,4] *Andrew Cap*[7] *Einar K. Kristoffersen*[3,4] *Theodor Fosse*[1,2,3] *and Torunn Oveland Apelseth*[4,8]

BACKGROUND: Increasing numbers of emergency medical service agencies and hospitals are developing the capability to administer blood products to patients with hemorrhagic shock. Cold-stored whole blood (WB) is the only single product available to prehospital providers who aim to deliver a balanced resuscitation strategy. However, there are no data on the safety and in vitro characteristics of prehospital stored WB. This study aimed to describe the effects on in vitro quality of storing WB at remote helicopter bases in thermal insulating containers.

STUDY DESIGN AND METHODS: We conducted a two-armed single-center study. Twenty units (test) were stored in airtight thermal insulating containers, and 20 units (controls) were stored according to routine procedures in the Haukeland University Hospital Blood Bank. Storage conditions were continuously monitored during emergency medical services missions and throughout remote and blood bank storage. Hematologic and metabolic variables, viscoelastic properties, and platelet (PLT) aggregation were measured on Days 1, 8, 14, and 21.

RESULTS: Storage conditions complied with the EU guidelines throughout remote and in-hospital storage for 21 days. There were no significant differences in PLT aggregation, viscoelastic properties, and hematology variables between the two groups. Minor significantly lower pH, glucose, and base excess and higher lactate were observed after storage in airtight containers.

CONCLUSION: Forward cold storage of WB is safe and complies with EU standards. No difference is observed in hemostatic properties. Minor differences in metabolic variables may be related to the anaerobic conditions within the thermal box.

ABBREVIATIONS: aPTT = activated partial thromboplastin time; BE = base excess; CWB = cold whole blood; DCR = damage control resuscitation; LTA = light transmission aggregometry; MA = maximum achieved clot strength; HEMS = helicopter emergency medical services; PRP = platelet-rich plasma; TRAP = thrombin receptor-activating peptide; WB = whole blood.

From the [1]Department of Anaesthesia and Intensive Care, the [4]Department of Immunology and Transfusion Medicine, the [6]Department of Research and Development, and the [8]Department of Clinical Biochemistry and Pharmacology, Haukeland University Hospital; the [2]Norwegian Naval Special Operations Commando, Norwegian Armed Forces; the [3]Institute of Clinical Sciences, University of Bergen, Bergen; [5]Department of War Surgery and Emergency Medicine, Norwegian Armed Forces Medical Services, Oslo, Norway; and the [7]U.S. Army Institute of Surgical Research, Fort Sam Houston, Texas.

Address reprint requests to: Christopher Bjerkvig, Department of Anaesthesia and Intensive Care, Haukeland University Hospital, P.O. Box 1400, Bergen 5021, Norway; e-mail: christopher.bjerkvig@helse-bergen.no.

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During the past two decades there has been a paradigm shift in resuscitation strategies for major hemorrhage and hemorrhagic shock.[1–3] Damage control resuscitation (DCR) strategies now favor the early transfusion of blood components containing red blood cells (RBCs), platelets (PLTs), and plasma, contradicting the previous policies favoring the use of clear fluids. Although definitive evidence concerning transfusion ratios is lacking, there is a strong trend to aim for physiologic ratios of blood components in the initial phase of resuscitation. Simultaneously, there is a clear vision that the prehospital phase should be as short as possible even if blood components are available at an early stage.[4–6] Balanced blood product replacement aims at addressing both shock and coagulopathy, which underscores the rationale for starting this approach in the prehospital phase of care. A growing number of prehospital emergency services in Europe and the United States have implemented this strategy.[7] Although DCR principles address trauma, early prehospital transfusion of blood products may also benefit patients who suffer from hemorrhagic shock due to medical conditions.[8] It is also common for helicopter emergency medical services (HEMS) to respond to rural clinics and hospitals with limited transfusion capabilities for stabilization and subsequent transport of bleeding patients. Prehospital transfusion programs may be of value in these circumstances as well.

Owing to the logistics and limitations associated with transfusion in the prehospital arena, DCR based on component therapy may not be feasible. At present, cold-stored RBCs and lyophilized plasma are the components most widely used in the prehospital environment.[9] Hence, the PLT supply is the weak link to achieve what may be considered currently a "gold standard" DCR. In most circumstances it is logistically impossible to supply room temperature–stored agitated PLTs for forward resuscitation. Even if cold-stored PLTs are considered as an alternative, the component-based strategy suffers from the fact that the 1:1:1 ratio yields a dilute mixture because of the RBC additive solutions.[10] Second, it is a great logistic challenge for the prehospital provider to deliver three different products simultaneously in a time-sensitive emergency. Recent experience from the war theaters in Iraq and Afghanistan suggest that whole blood (WB) resuscitation for trauma victims may be a viable option in this context.[11–15]

At present in excess of 30 HEMS in the United States and Norway carry cold-stored low-titer group O WB to patients. Further, several military units have implemented WB in war theaters over the past 6 years.[13,16,17]

The storage conditions for cold WB (CWB) in the prehospital environment differ from in-hospital CWB storage. Prehospital CWB is usually stored in closed isothermal containers. Impaired gas-exchange conditions raise concerns about the potential reduction in hemostatic function due to impaired PLT function in the product.

The problem with adhering to storage standards may be a reason why some emergency medical services no longer carry blood. The lack of data on prehospital storage conditions and data on the hemostatic function of prehospital stored WB is the rationale for conducting this study.[18]

In our study we wanted to assess how forward cold storage of WB in a civilian emergency helicopter service would affect the product quality. Our primary objective was to evaluate if the storage conditions support blood that complies with EU standards for up to 21 days of storage.[19] The secondary objective was to assess product in vitro quality and hemostatic function of WB during storage for up to 21 days in an approved airtight thermal insulating storage container.

MATERIALS AND METHODS

Study design and ethics

This is a prospective single-center, two-armed observational study of forward storage of WB in airtight thermal insulating storage containers. The study was conducted at the Department of Immunology and Transfusion Medicine, Haukeland University Hospital (Bergen, Norway), and at the Norwegian Air Ambulance HEMS base Grønneviksøren (Bergen, Norway), a location 2 km from Haukeland University Hospital, from May to October 2017. According to routine procedures for the Bergen HEMS prehospital blood program, WB units in the test group (HEMS group) were stored continuously in an airtight approved container at the HEMS base for 1 week before being returned to the blood bank for continuous total storage of 21 days in the thermal container. The control group units were stored according to routine procedures without agitation in a temperature-controlled refrigerator at 4 ± 2^{o}C in the blood bank the entire study period. Sampling from the test units was performed immediately before and after transfer to the base. The test units were included in the study from the regular inventory at the HEMS base if not used at the HEMS base within 7 days of storage. There were no patient interventions. The study was approved by the regional ethics committee (REK ID 2017/157). Informed written consent was obtained from all donors of WB.

Bergen HEMS

The emergency helicopter service in Bergen, Norway, responds to both medical and trauma cases in a 60:40 ratio 24 hr/day 365 days/year. The HEMS is staffed with an experienced prehospital anesthesiologist, a HEMS rescue paramedic, and a pilot. Emergency response is carried out by helicopter or by a rapid response car. Bergen HEMS implemented routinely cold-stored, leukoreduced low-titer group O WB for remote hemostatic resuscitation in December 2015. Also, lyophilized plasma is available in the service.

Collection and preparation of WB units

Whole blood was collected from regular blood donors using the 450-mL WB-SP CPD collection set (BB*LGQ456E6, Terumo BCT). The WB units were rested for 2 hours followed by a gravitational filtration through the inline PLT-sparing, leukoreducing filter, allowing for a PLT content of more than 90% of the unit before filtration. The residual white blood cell (WBC) concentration was less than 1×10^6/unit, in line with requirements for cellular blood components. To minimize risk of hemolytic transfusion reactions, all blood donors had blood group O. The titers of IgM anti-A and anti-B were below 256 and all units were correspondingly labeled "low titer." Two CWB units were dispatched to the HEMS service each week.

Cold chain maintenance and forward monitoring

The test group WB units (n = 20) were transferred to an approved primed portable thermal container (Crēdo Duracube HD with Golden Hour inner container, Pelican BioThermal) together with a data logger that recorded ambient temperature, three-axis acceleration, humidity, light, and barometric pressure (MSR 145 mini datalogger, MSR Electronics GMBH). According to local routines, the container was then placed in a regular refrigerator at the HEMS base. The units were carried on missions in the container for 7 days before returned to the Department of Immunology and Transfusion Medicine at Haukeland University Hospital and then stored in the same container for an additional 14 days up to a total of 21 days. The container was only brought on HEMS missions involving calls to patients with potential bleeding. HEMS crew members recorded the duration of the mission and how long the thermal box was outside the refrigerator. These records were compared to the data logger recordings inside the box. Control group WB units (n = 20) were stored without agitation in a conventional approved, monitored refrigerator (2-6°C) in the blood bank according to routine.

Laboratory investigations

Samples were collected on Days 1, 8, 14, and 21 by sterile transfer of approximately 25 mL to a transfer bag (Teruflex, BB*T015CM, Terumo BCT), which was then used to further aliquot to the appropriate sample tubes.

Leukoreduction status was verified by counting residual WBCs using the WBC counting kit and flow cytometer (BD LeucoCount and a FACSCanto II, respectively (BD Biosciences;). Hematologic variables (hemoglobin [Hb], hematocrit [Hct], RBC count, PLT count, and mean PLT volume) were quantified with a hematology analyzer (Cell-Dyn Sapphire, Abbott Diagnostics). To evaluate hemolysis, Hb in plasma was measured on a plasma/low Hb photometer (HemoCue, HemoCue AB) and percent hemolysis wad calculated as $((\text{plasma-Hb}/10) \times (100 - \text{Hct}))/\text{Hb}$.

Blood gas variables (pH, pO_2, pCO_2, and base excess [BE]) were analyzed on a blood gas analyzer (ABL825 FLEX, Radiometer Medical ApS). Sodium and potassium, glucose, and lactate levels were measured on a chemistry analyzer (Cobas 8000, ISE/c702, Roche Diagnostics GmbH).

The effects of storage on coagulation were studied by measuring activated partial thromboplastin time (aPTT), prothrombin time/international normalized ratio (INR), Factor (F)VIII and fibrinogen using a hemostasis analyzer (STA-R Evolution/STA-R Max; STA-Liquid Fib/STA-Fibrinogen 5, STA-Deficient VIII, STA-SPA+, STA-PTT Automate 5, STA-CaCl2 and STA-Unicalibrator, Stago S.A.S.).

The hemostatic function was evaluated by kaolin-initiated thromboelastography on a thromboelastograph (TEG 5000, Haemonetics Corporation) and quantified as time to start of clot formation, clot formation speed (angle), and maximum achieved clot strength (MA).

To quantify PLT aggregation, we performed multiplate impedance aggregometry (Roche Diagnostics GmbH) with 6.5 μmol/L adenosine diphosphate (ADP), and light transmission aggregometry (LTA; Chrono-log Model 700, Chrono-log Corporation) with 10 μmol/L ADP and 30 μmol/L thrombin receptor-activating peptide 6 (TRAP-6, Roche Diagnostics GmbH).

Samples for impedance aggregometry were analyzed using the manufacturer's procedure for citrated samples, including partial recalcification with 3 mmol/L $CaCl_2$. PLT-rich plasma (PRP) for LTA was prepared by $200 \times g$ centrifugation for 10 minutes. The remaining material was further centrifuged at $1500 \times g$ for 15 minutes to yield PLT-poor plasma. PLT count in PRP was verified to be in the 150×10^9 to 600×10^9/L range.[20] PRP and PLT-poor plasma was visually inspected for hemolysis and lipemia and incubated at 37°C for 3 minutes before performing LTA at 37°C with 1000 RPM stirring. Samples were run in two parallels, and the mean was reported.

Platelet activation and adhesion were also investigated in 8 control units and 6 HEMS units by use of flow cytometry. Samples were stimulated with 2,8 μL ADPtest and 1,7 μL TRAPtest. A premade mix of monoclonal mouse anti-human antibodies were thereafter added without further incubation (BD Bioscience) with PerCP CD61 (Clone EUU-PL 7 F12, Cat. No. 347408), APC CD42b (Cat. No. 551061), and PE CD62P (Cat. No. 561921). Thirty minutes of incubation in the dark at room temperature was followed by a RBC lysing step where we added 465 μL of lysis buffer (Dako EasyLyse, Ref. No. S2364, Agilent). After 2 mL of flow cytometry sheath fluid (FACSFlow, Cat. No. 342003, BD Biosciences) was added, the samples were run on a flow cytometer using its software (BD FACSCanto II and FACSDiva, Version 8.0.1, respectively, BD Biosciences). We gated the PLTs using forward scatter height versus area to avoid doublets and side scatter versus forward scatter to narrow the gating. The same gating was used

on all samples, regardless of age. Results reported as age of percent parent of CD61-positive cells for the activation markers.

Statistical analysis

The effect of forward storage in an airtight container at the HEMS base on each of the outcome variables was investigated using a linear mixed-effect model with storage time, study group, and their interaction as predictors. In this model, we used simple contrasts to investigate whether the outcome variables changed from Day 1 to 8, Day 1 to 14, and Day 1 to 21. Additionally, we used the interaction between storage time and study group to describe whether this level of change was different between the blood stored at the blood bank and at the HEMS base. The p value reported in the text and figures are for this interaction. Potential differences in age and sex distribution between the two groups were examined using independent-sample t tests. A p value of less than 0.05 was considered significant. Results were presented as mean (95% confidence interval [CI]). All analyses were performed with computer software (R version 3.6.0 with the NLME package version 3.1-140, The R Foundation for Statistical Computing).

We found no previous studies that could be used for sample size calculation. With the general assumption of normality for outcome measures enabling the use of a two-sided t-test, setting the mean to 1 and a standard deviation to 0.5 with a significance level of 0.05 and a power of 0.8, we found that we needed a minimum of 17 individual units in each group. Based on this we chose to include 20 WB units in each study group.

RESULTS

Quality requirements

A total of 40 CWB units were monitored and tested, 20 in the HEMS group and 20 in the control group. During storage at the HEMS base, there were no recorded breaches of temperature limits (1-10°C). Table 1 show the number of HEMS missions in which CWB were carried and the total duration of storage outside of the refrigerator. The blood was carried on a mean (95% CI) number of 4.1 (2.86-5.34) missions. The mean (95% CI) duration of the missions was 339 (209-469) minutes.

All units in the study complied with the requirements set forth in the "Guide to the Preparation, Use and Quality Assurance of Blood Components."[19] The hemolysis at the end of storage was below the EU requirement of 0.8% of the RBC mass in all units throughout storage in both groups. The Hb level was above the EU requirements of 43 g/unit in all observed units until the end of storage (Table S1, available as supporting information in the online version of this paper).

TABLE 1. Number and duration of HEMS missions where WB units were carried*

WB unit	Number of missions	Mission duration (min)
1 & 2	3	120
3 & 4	6	600
5 & 6	3	180
7 & 8	6	360
9 & 10	6	420
11 & 12	2	180
13 & 14	4	300
15 & 16	3	360
17 & 18	6	660
19 & 20	2	210

* There were no recorded temperature breaches.

Changes during 21-day storage

Hematology

There was no significant difference in change during storage regarding PLT count, mean PLT volume, or number of RBCs between the two groups (Fig. 1A, Table S1). We found a statistically significant difference in the change of Hb levels from Day 1 to Day 21 between the two groups ($p < 0.05$; Fig. 1B).

Clinical chemistry

We found no significant difference in change in potassium between the two groups during 21 days of storage (Fig. 1C).

Metabolism

When investigating how the metabolic markers changed in the two groups, we found that the glucose concentration declined more in the HEMS group on Days 8, 14, and 21 ($p < 0.05$) compared to the control group (Fig. 1D). The same was evident in regard to pH and BE (Fig. 1E and Table S1). Conversely, we found that the lactate increased significantly in the HEMS group compared to the control group (Fig. 1F).

Hemostatic properties

There was no significant difference in change in fibrinogen, international normalized ratio and FVIII levels (Figs. 1G and 1H and Table S1). However, we found a reduction in aPTT on Day 21 in the test group (Fig. 1I). When investigating the change in hemostatic capacity by thromboelastography, we found no difference in change during storage regarding time to start of clot formation and K between the two groups (Fig. 1J and Table S1). However, we found that the TEG angle declined less in the HEMS group compared to the control group on Days 8, 14, and 21 ($p < 0.05$; Fig. 1K). This was also observed in regard to TEG MA, where the TEG MA declined less in the HEMS group (Fig. 1L). As discussed later, we also observed significant differences between the two groups at baseline.

When investigating the PLT aggregation capacity by the use of the multiplate analyzer with ADP test, we found no

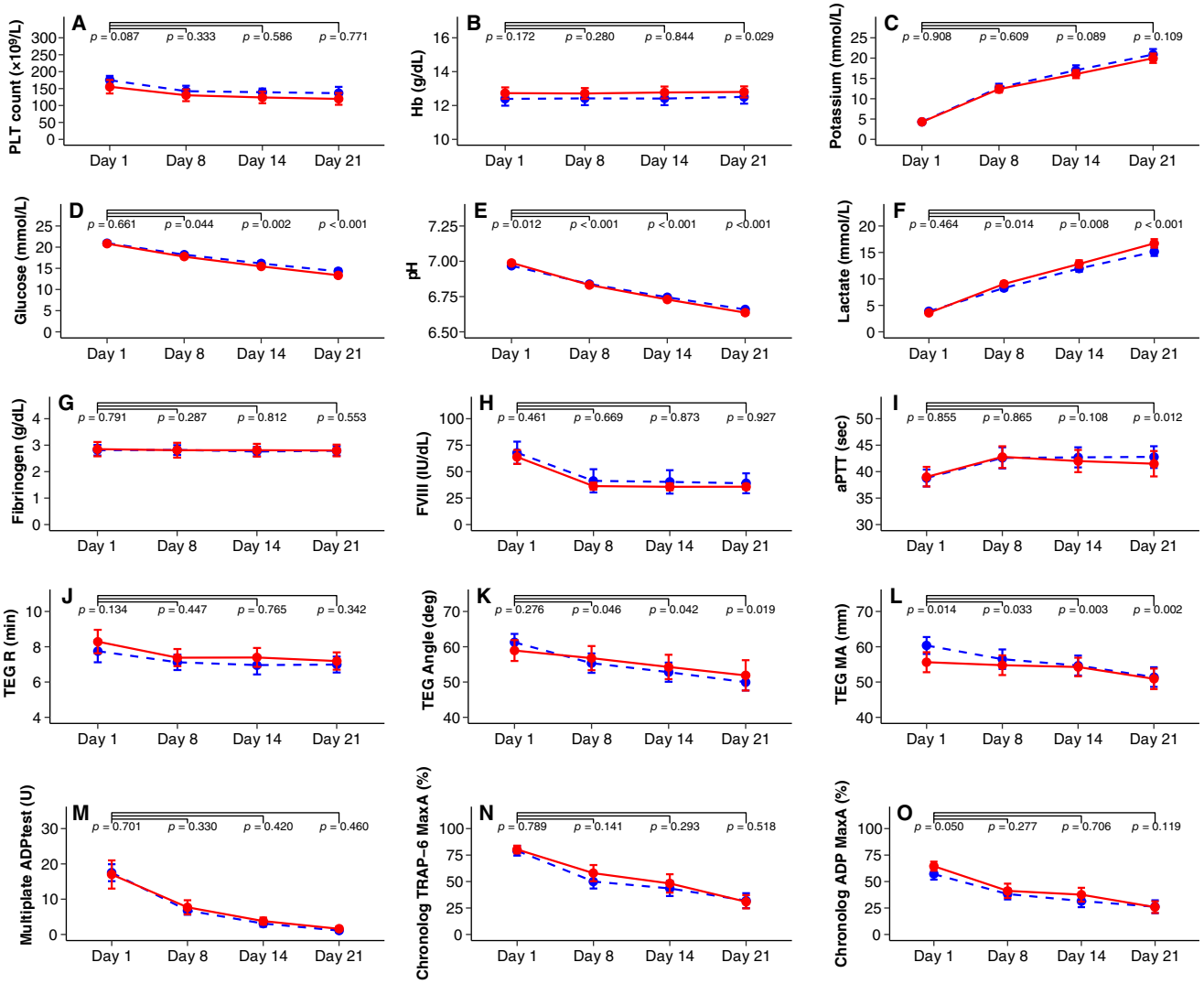

Fig. 1. Mean and 95% CIs of the variables during 21 days of storage. (------) Control group; (——) HEMS group. A linear mixed-effects model with storage time, study group, and their interaction as predictors was fitted. The p value shown represents the interaction between storage time and study group and signifies whether there was a significant difference between the two groups in how the variable changed from Day 1 to 8, Day 1 to 14, and Day 1 to 21. [Color figure can be viewed at wileyonlinelibrary.com]

significant difference during storage between the two groups (Fig. 1M). There were also no differences between the two groups when utilizing LTA to assess maximum aggregation with TRAP and ADP as stimulants (Figs. 1N and 1O). The spontaneous aggregation was comparable in the two groups (Table S1). Finally, in regard to PLT function, we found no significant difference between the two groups when investigating ADP- and TRAP-stimulated expression of CD62P and CD42b by flow cytometry (Table S1).

Additional findings

We found a statistically significant difference in pH, BE, and TEG MA on Day 1, indicating that there was a difference between the two groups at baseline in these variables. There were no significant differences between the groups in the distribution of donor ages (HEMS 36.2 [30.3-42.1] versus control 42.6 [36.1-49.1]; p = 0.134) and sex (HEMS 15 male, five female vs. control nine male, 11 female; p = 0.053).

DISCUSSION

This study evaluated changes in hematology, clinical chemistry, metabolism, and hemostatic variables of CWB stored in an airtight thermal box forward at a HEMS base, for up to 21 days, compared to changes of CWB stored according to routine practice in the blood bank. All CWB units complied

with the requirements set forth in the "Guide to the Preparation, Use and Quality Assurance of Blood Components" throughout storage for 21 days, regardless of storage conditions. The hemolysis grade was below 0.8% of the total RBC mass, and that the Hb content was above 43 g/unit.[19]

The storage of CWB at the HEMS base revealed no safety concerns concerning temperature limits (1-10°C). This is important as increased temperature may compromise the product quality.[21–23] Thus, the crew must be vigilant in monitoring the operability of the refrigerator and to ensure that there has not been breach of temperature limits before administering the blood in the field. Temperature monitoring is an essential part of quality control in transfusion medicine. One of the main disadvantages of the present storage container is that the crew must open the box to read the logger. Future designs should consider providing an external temperature reading on the thermal box to improve safety.[24]

The number of missions varied from two to six, and the total duration of missions varied from 120 to 660 minutes for the blood units in the HEMS group. In addition to changes in temperature, the blood properties could potentially be influenced by changes in pressure and vibration during missions. Due to the low number in each group, we were not able to adjust for differences in the amount and duration of missions in the results. However, if these factors were substantial, we would expect the variance of the results in the HEMS group to be generally larger than that in the control group (Table S1). Generally, for all our outcome measures small CIs were observed in both groups.

We found a significant difference in how the Hb levels had changed in the two groups at 21 days of storage. However, the difference between the two groups is without clinical significance as the difference in change of Hb concentration between the two groups was only 0.05 g/dL.

Prehospital CWB is stored in an airtight thermal box and placed inside a conventional refrigerator at the HEMS base. The changes in metabolic variables during storage and comparison between the two groups suggest that there are minor alterations in gas exchange conditions for CWB stored in airtight thermal insulated storage containers. We observed slightly higher lactate levels and lower BE and pH levels in CWB stored forward. The clinical impact of these values is uncertain and probably without importance. There was a significant difference in how aPTT had changed at 21 days, with less increase in the HEMS group on Day 21. The difference was minimal and for the benefit of the HEMS group, however, probably without clinical significance.

Viscoelastic hemostatic capacity as measured by TEG was present in both groups for the entire 21-day storage period. As mentioned, we found that the TEG angle had declined less in the HEMS group. The TEG angle measures the speed at which fibrin builds up and cross-links. Hence, the angle assesses the rate of clot formation. This process is propagated by thrombin formation and is dependent on PLTs and fibrinogen. We did not find that there was a significant difference in these measurements on Day 1, nor any difference in how these variables changed. Based on this, it seems unlikely that the difference in the TEG angle can be explained by PLT and fibrinogen effects. Further, there was also a significant difference in the TEG MA evolution during storage. Again, it declined less in the HEMS group. The TEG MA represents in simple terms the ultimate strength of the fibrin clot, dependent on PLT function and fibrin levels. We did not find significant differences when evaluating PLT count and function using LTA and multiplate or flow cytometry.

When it comes to the hemostatic properties of CWB in general, these results show that viscoelastic hemostatic properties are to a great extent maintained for up to 21 days. The final MA in both groups was approximately 50, whereas the plasma fibrin contribution to clot strength alone typically yields an MA of 10.[25] This indicates that the PLTs in CWB retain considerable ability to catalyze clot formation and contraction throughout storage duration. The PLT function as measured by PLT aggregation and agonist-induced glycoprotein expression was preserved for at least up to 8 days of storage, after this we observed a decline in response. This is consistent with previous studies.[26–28]

We found significant differences in pH, BE, and TEG MA, between the two groups on Day 1. We performed secondary analysis to see if the differences were related to the distributions of sex and age between the groups but did not find a statistical relationship. However, examining the data, although not significant, the mean PLT count was 175 in the control group versus 155 in the HEMS group. The TEG MA is affected by PLT count, and this may explain this finding. Regarding pH and BE, although there were significant differences at baseline, the actual values are without clinical significance (mean pH 6.97 in control group vs. 6.99 in HEMS group). Further, these findings should not interfere with secondary objective results as the statistical design takes into account differences at baseline.

We conclude that storage of CWB in thermal containers in a prehospital emergency service is feasible. The logistics and maintenance of the thermal conditions are easy and safe. The product quality is within EU regulations for up to 21 days of storage. The hemostatic properties of CWB stored prehospital are similar to those of CWB stored in the blood bank. The PLT function in CWB deteriorates after 8 days of storage but is still clinically useful for up to 21 days. These findings support the practice of extending storage in resource-poor environments and thus reducing waste.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

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

Table S1. Mean and 95% confidence intervals for all measured variables. A linear mixed effect model with storage time, study group and their interaction as predictors was fitted. For time, simple contrasts were used. p_{Day} signifies whether there was a statistically significant change from Day 1 to the specified storage day. $p_{Interaction}$ signifies whether this change was statistically different between the two study groups. The $p_{Interaction}$ given for Day 1 indicates whether the baseline measurement was significantly different in the two groups.