

Coagulation function of stored whole blood is preserved for 14 days in austere conditions: A ROTEM feasibility study during a Norwegian antipiracy mission and comparison to equal ratio reconstituted blood

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BACKGROUND:	Formulation of a medical preparedness plan for treating severely bleeding casualties during naval deployment is a significant challenge because of territory covered during most missions. The aim of this study was to evaluate the concept of “walking blood bank” as a supportable plan for supplying safe blood and blood products.
METHODS:	In 2013, the Royal Norwegian Navy conducted antipiracy operations from a frigate, beginning in the Gulf of Aden and ending in the Indian Ocean. Crews were on 24-hour emergency alert in preparation for an enemy assault on the frigate. Under an approved command protocol, a “walking blood bank,” using crew blood donations, was established for use on board and on missions conducted in rigid-hulled inflatable boats, during which freeze-dried plasma and leukoreduced, group O low anti-A/anti-B titer, cold-stored whole blood were stored in Golden Hour Boxes. Data demonstrating the ability to collect, store, and provide whole blood were collected to establish feasibility of implementing a whole blood–focused remote damage-control resuscitation program aboard a naval vessel. In addition, ROTEM data were collected to demonstrate feasibility of performing this analysis on a large naval vessel and to also measure hemostatic efficacy of cold-stored leukoreduced whole blood (CWB) stored during a period of 14 days. ROTEM data on CWB was compared with reconstituted whole blood.
RESULTS:	Drills simulating massive transfusion activation were conducted, in which 2 U of warm fresh whole blood with platelet sparing leukoreduction were produced in 40 minutes, followed by collection of two additional units at 15-minute increments. The ROTEM machine performed well during ship-rolling, as shown by the overlapping calculated and measured mechanical piston movements measured by the ROTEM device. Error messages were recorded in 4 (1.5%) of 267 tests. CWB yielded reproducible ROTEM results demonstrating preserved fibrinogen function and platelet function for at least 3.5 weeks and 2 weeks, respectively. The frequency of ROTEM tests were as follows: EXTEM (n = 88), INTEM (n = 85), FIBTEM (n = 82), and APTEM (n = 12). CWB results were grouped. Compared with Days 0 to 2, EXTEM maximum clot firmness was significantly reduced, beginning on Days 10 to 14; however, results through that date remained within reference ranges and were comparable with the EXTEM maximum clot firmness for the reconstituted whole blood samples containing Day 5 room temperature–stored platelets.
CONCLUSION:	A “walking blood bank” can provide a balanced transfusion product to support damage-control resuscitation/remote damage-control resuscitation aboard a frigate in the absence of conventional blood bank products. ROTEM analysis is feasible to monitor damage-control resuscitation and blood product quality. ROTEM analysis was possible in challenging operational conditions. (<i>J Trauma Acute Care Surg.</i> 2015;78: S31–S38. Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.)
LEVEL OF EVIDENCE:	Therapeutic study, level V.
KEY WORDS:	Whole blood transfusion; austere environments; reconstituted whole blood; remote damage-control resuscitation; damage-control resuscitation.

Formulation of a medical preparedness plan for treating severely bleeding casualties during naval deployment is a significant challenge because of the sheer size of territory

covered during most missions. Tertiary hospital care is largely unavailable; thus, ships require a logistically supportable plan for supplying safe blood and blood products. Remote

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damage-control resuscitation (RDCR) has been defined as the prehospital application of damage-control resuscitation (DCR) principles. RDCR is distinguished from DCR because of the differences in capabilities and limitations of gaining optimal management strategies in the prehospital environment compared with in-hospital environment. The current and evolving standard for RDCR in both military and civilian settings for hemorrhagic shock is to administer low-volume hemostatic blood products delivered as close to the point of injury as possible.^{1,2} Emphasis is placed on supplying a balanced resuscitation, which delivers whole blood (WB) functionality, to include both oxygen-carrying capacity and coagulation.³ RDCR encompasses all these principles with the additional constraint that the patient might experience delays in reaching definitive surgical control.^{4,5} In 2013, the Royal Norwegian Navy conducted antipiracy operations from a frigate, beginning in the Gulf of Aden and ending in the Indian Ocean. Crews were on 24-hour emergency alert in preparation for an enemy assault on the frigate. Because prolonged transfer times to definitive surgical care were expected during this mission, a “walking blood bank” was established by ensuring that ABO blood grouping and transfusion-transmitted disease (TTD) testing was up to date for all on board. A small surgical team, responsible for the blood supply and emergency care, underwent an extensive training program before deployment.

The purpose of both RDCR and DCR, in addition to maintaining oxygen-carrying capacity, is to prevent the metabolic consequences of hemorrhagic shock by early prevention of oxygen debt accumulation and control of blood loss with balanced components in a 1:1:1 ratio (red blood cells, plasma, and platelets) or WB to sustain coagulation and avert acute coagulopathy of trauma. In addition, evidence is accumulating that severe trauma is associated with platelet dysfunction and early platelet transfusion is linked to survival benefits in retrospective studies.⁶⁻⁹ Providing those platelets can be difficult in austere environments because they are stored at room temperature under constant agitation, with a shelf-life of only 5 days (United States) or 7 days (Europe). Given the size of the territory covered by the antipiracy mission and the relatively low number of severe hemorrhage cases expected, supplying blood products by air, particularly in the case of platelets, would have been prohibitively expensive. Due to logistical constraints with supplying blood components, we concluded that the only feasible option open to the prehospital team was the use of freeze-dried plasma and cold-stored leukoreduced whole blood (CWB) as the primary resuscitating fluid for RDCR, and CWB, warm fresh whole blood (WFWB), or both for the surgical team DCR in the operating room.

The objectives of this study were to (1) establish whether ROTEM analysis is feasible on a naval vessel on an extended mission as determined by piston movement analysis, error message frequency, and result variability and (2) compare the clotting properties of cold-stored WB obtained from a walking blood bank in a deployed setting to those of standard-of-care blood product components obtained from a civilian blood bank. The hypotheses were that (1) ROTEM analysis is feasible and yields reasonably consistent results on a vessel in the open ocean and (2) stored WB obtained from a walking blood bank on a naval vessel demonstrates clotting properties that are

similar to those of reconstituted WB (RWB) from standard-of-care components obtained from an accredited blood bank.

MATERIALS AND METHODS

From May to December 2013, the Royal Norwegian Navy conducted antipiracy operations from a frigate in the Gulf of Aden, along the coast of Somalia (the Somali basin), the coast of Puntland, and in the Indian Ocean. Under an approved command protocol, combat medics took Golden Hour Boxes packed with 2 U of freeze-dried plasma and 2 U of leukoreduced, group O low anti-A/anti-B titer, cold-stored WB on missions conducted in rigid-hulled inflatable boats (RHIBs). If called upon to resuscitate a wounded crew member, combat medics would have implemented a “plasma first” protocol (NORNAVSOCRDCR protocol).¹⁰

Establishing the Walking Blood Bank

In late 2012, to prepare for the mission, the Royal Norwegian Naval Medical Services approached the local department of immunology and transfusion medicine requesting blood product solutions for the frigate KNM Nansen during its upcoming antipiracy campaign. Given regulatory, legal, and resource constraints, the Royal Norwegian Naval Medical Services liaised with the local civilian blood bank in the development of a “walking blood bank,” for which the chief of the blood bank would assume medical responsibility and establish standard operating procedures (SOPs) to be executed by the captain and crew while at sea. Transferring all donor selection and screening requirements from the civilian setting to the frigate donor pool was not a feasible proposition. A history of travel on the coast of Africa defers a donor in Norway; thus, a reevaluation of civilian procedures and a new risk-benefit analysis, accounting for TTD and other transfusion reaction probabilities, was conducted to create a SOP reflective of mission realities and based on a legal principle of necessity.

Recruitment

Predeployment selection of donors from voluntary members of the two alternate crews to be deployed was made at the local blood bank by civilian health care staff. Standard procedures were used for collecting donor information and screening donors, for blood grouping, for determination of antibody titers, and for infectious disease screening. Group O donors were categorized as high-titer (anti-A/B \geq 100 IgMor400 IgG antibody titers) or low-titer donors.¹¹ Of 143 volunteers, none tested positive for TTDs in two separate samples, and screening for irregular blood type antibodies was weakly positive for one potential donor, and he was excluded. Phlebotomists were recruited among the drafted military personnel normally working in the frigate mess, and they participated in a 3-day intensive theoretical and practical course together with the nurses and doctors responsible for the walking blood bank SOP on the frigate. The course was held by staff from the local civilian blood bank.

During deployment, donors were selected from the predeployment registry, and names, blood type and titers, and upon presentation to the donation site were asked to identify themselves, fill out a simplified donor selection form, and participate in a donor interview. The process was completed

when the donor signed the selection form, which tracked TTD screening and blood grouping results. That same form doubled as a transfusion form and was used to record the recipient's information and blood group results. Immediately before donation, the donors were asked to verify the information on the form and the blood collection bag.

Only group A and O donors were included as active donors. Procedures are discussed in the following sections.

WB Collection and Storage Procedures

The walking blood bank SOP instructions mandated that every 10th day, 2 U of low-titer group O blood was collected and stored for use through Day 10 but not discarded until Day 25. In this way, a small store of blood was always available, of which 2 U were no older than Day 10. Leukocyte reduction using a platelet-sparing filter (TerumoBCT, IMUFLEX WB-SP, Lakewood, CO) was standard. This quadruple blood bag system is equipped with anticoagulant citrate phosphate dextrose (CPD) and is designed to produce three leukoreduced blood components: red blood cells, platelets, and plasma. However, on this deployment, fractionation was not performed, but rather, CWB was stored in the intermediary bag with connection ports for blood transfusion.

All stored CWB was group O low titer. For patients with known identity and blood group A or group O, type-specific blood was chosen. For group B and AB recipients, universal group O low titer was chosen because of the low number of group B and AB donors. We considered it safe to start with 2 U of group O low titer and continue with type-specific WB. For patients with unknown blood type (typically hostile combatants), group O low-titer WB was chosen first and then group O high titer, if necessary. Rhesus D titers were not measured because all special operation military personnel (and, presumably, hostiles) were male. The donors were placed in a half-supine position on a dining table for phlebotomy procedures. Capillary blood samples were obtained just before donation for confirmatory blood grouping using Eldon cards (cat. no. 2511-1, Eldon Biologicals, Gentofte, Denmark), and for TTD testing (MultiploHBc/HIV/HCV, MedMira Inc, Halifax, Canada). Two staff members confirmed the identity of the donated unit, the group and screen results, as well as the information from the registry list.

After collection, CWB was rested for 2 hours, followed by gravitational filtration for leukocyte reduction. The CWB bag was then transferred to a cooler containing two temperature sensors with data loggers (LIBEROTi1-S, Elpro-Buchs AG, Buchs, Switzerland), and the cooler was placed in the kitchen refrigerator at 2°C to 6°C. Two units of CWB and two units of freeze-dried plasma routinely accompanied Special Forces military personnel in RHIBs on missions for up to 14 hours, stored in a Golden Hour Box (Minnesota Thermal Sciences, Plymouth, MN) also containing a temperature data logger. Leukocyte-reduced WFWB with platelet sparing was produced by gravitational filtration omitting the 2-hour rest because of the need for rapidly supplying the resuscitation team with WFWB.

RWB and Other Comparisons

Samples of RWB for comparison with CWB were generated using (1) packed red blood cells (pRBCs) of similar age

to that of CWB at the end of storage (Days 21–27: end of storage refers to the fact that some of the CWB on board was stored until Day 27 and ROTEM tested, even though it was not intended for use after it expired on Day 21 of storage); (2) thawed plasma (FFP) components at Day 5; and (3) platelet apheresis concentrates at Day 5. The recorded volume on the component bag was used to determine a proportional aliquot size (1/1,000th of the original bag volume), where possible. Because the clinical blood bank that provided the components does not routinely record pRBC volumes, pRBC aliquots were estimated using the specific gravity and the pRBC component weight minus the bag weight. Multiple combinations of component aliquots and ½ aliquots were used to create eight distinct samples of RWB. Because the frigate did not have the capacity to perform CWB aggregometry studies, previously published refrigerated stored WB results from our laboratory were used for comparison with the RWB results, and citations were provided.

Laboratory Analysis

The frigate carried minimal laboratory equipment but was furnished with a rotational thromboelastogram (ROTEM, TEM International GmbH, München, Germany). From June 20 to December 5, 2013, 20 U were collected. Functional testing of CWB was performed regularly during storage for up to 25 days. After the bags were thoroughly mixed, samples were extracted in a sterile fashion by carefully stripping the blood bag flexible tube endings and welding a 10-cm length of tubing using a sealer (Hematron III, Fenwal, Lake Zurich, IL). Samples were analyzed using the following tests: INTEM with contact activation assessing the intrinsic pathways, with greater sensitivity for the depletion of factors over time, such as factor VIII; EXTEM using tissue factor activation for fast assessment, primarily of the extrinsic pathway; FIBTEM using tissue factor activation with platelet inhibition to isolate the fibrinogen contribution; and APTEM assessing hyperfibrinolysis (using reagents: IntemS, ExtemS, FibtemS, and AptemS, respectively, TEM International GmbH).

RWB was evaluated with INTEM, EXTEM, and FIBTEM rotational thromboelastometry (Intem, Extem, and Fibtem, respectively, TEM International GmbH) as described earlier, with the exception that single use reagents were not used. In addition, platelet aggregation was measured with impedance aggregometry (Multiplate multiple electrode aggregometer, Verum Diagnostica GmbH, Munich, Germany) using the following platelet agonists: adenosine diphosphate (ADP), arachidonic acid (ASPI), collagen (COL), ristocetin (RISTO), and a thrombin receptor agonist, thrombin receptor activating peptide or TRAP (ADPtest, ASPItest, COLtest, RISTOtest, and TRAPtest, respectively, TEM International GmbH).

Statistical Analysis

Descriptive statistics, independent sample *t* tests, and repeated-measures analysis of variance were performed using SPSS version 22.0 (IBM Corp., Armonk, NY) for the data generated during the frigate mission. Statistical significance was set at $p < 0.05$. RWB data were within published reference ranges and analyzed on a single day; thus, statistical comparisons were not performed. Box plot graphs for the frigate

TABLE 1. Donor Blood Types and Sample Sizes

	Crew N	Crew A
O low	13	24
O high	14	13
A	35	38
Total	67	75

mission data were generated with SPSS version 22.0 and with GraphPad Prism version 5.01 (GraphPad Software, Inc., La Jolla, CA).

RESULTS

A frigate is a mid-sized vessel with a crew of approximately 160. The number and types of donors recruited for the walking blood bank are listed in Table 1. Implementation of the walking blood bank SOP was feasible and safe. During on-board training and drills, performed once a week, two teams

of two phlebotomists per donor successfully performed blood collection procedures, and there were no adverse events. Selection form and interview findings resulted in several deferrals due to sexual activity or different infectious diseases incompatible with being a donor, leading to a decrease in the donor pool over time (e.g., crew on shore leave). The group O low-titer donor numbers were most affected (assumed random), particularly in the case of “Crew N.”

Drills simulating massive transfusion activation were conducted, in which 2 U of WFWB with platelet-sparing leukoreduction were produced in 40 minutes, followed by collection of two additional units at 15-minute increments. The blood bags could not be weighed because of malfunction of the digital scales; thus, the contents were estimated during phlebotomy and, after filtration, found to be within $\pm 10\%$ of 450 mL. Determining blood type with Eldon cards confirmed previously established blood types for all personnel. The TTD rapid tests frequently failed because of pipetting errors and required a repeat test to be performed. Blood bag labeling, donor identification, and the donor/transfusion form procedures were adequate. No out-of-range



Figure 1. Control data of mechanical piston movement in the ROTEM machine during Sea State 3 with 4-m waves and 12-degree rolling of the ship. Red lines (actual rotation of the pistons) exactly overlay the calculated black lines.

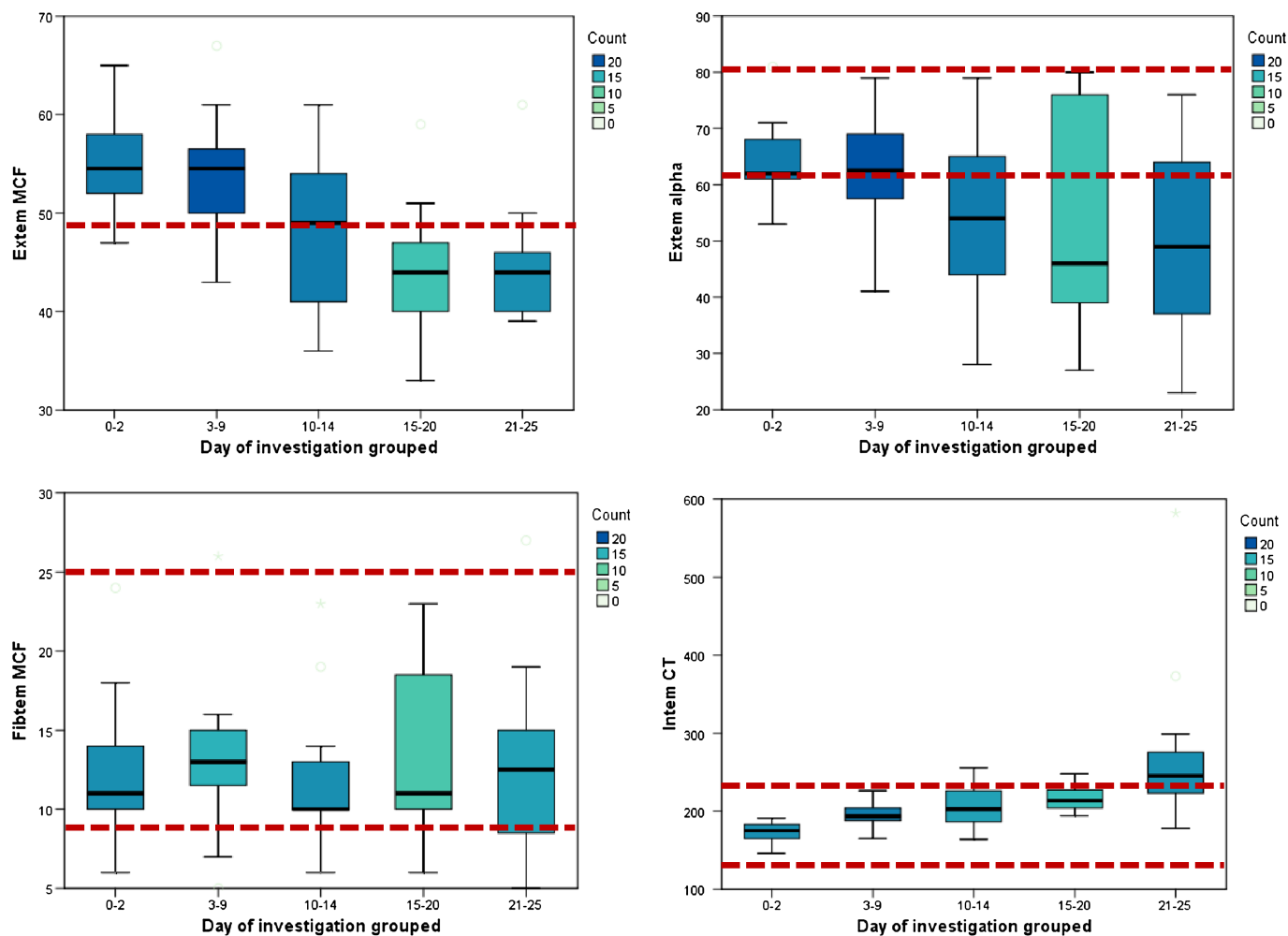


Figure 2. Results are shown as minimum, first quartile, median, third quartile, and maximum. Outliers marked with \circ or $*$. Dashed lines indicate published reference ranges, and only the lower limit is shown if the upper limit is beyond the graph axis.¹²

temperatures were recorded in the 9-month period despite one instance of refrigerator failure and RHIB expeditions, occurring twice per week on average, in which blood was stored in Golden Hour Boxes for up to 14 hours.

Investigations of WB Quality During Storage

The ROTEM machine performed well during ship-rolling, as shown by the overlapping calculated and measured mechanical piston movements measured by the ROTEM device

TABLE 2. Comparison of Rotational Thrombelastography Results of Stored Whole Blood from Start of Storage (Day 0–2) through the End of Storage

	Day of Investigation (Grouped)									
	0–2		3–9		10–14		15–20		21–25	
	n		n	n		n		n		n
EXTEM										
MCF, mm	18	55.6 (4.7)	20	53.6 (5.7)	18	48.7 (7.5)*	13	44.2 (6.5)*	17	44.4 (5.4)*
α , degree	18	63.7 (6.6)	12	62.2 (8.9)	18	54.5 (14.4)*	13	51.3 (19.6)*	18	50.0 (17.8)*
INTEM										
CT, s	17	173 (12)	18	195 (15)*	16	207 (24)*	14	216 (17)*	17	269 (92)*
FIBTEM										
MCF, mm	17	12.5 (4.0)	15	13.1 (4.7)	17	11.4 (4.3)	12	13.3 (5.4)	16	12.8 (5.6)

* $p < 0.05$ when compared with Day 0 to 2 (independent-samples t test. SPSS version 22.0)

Results shown as mean (SD). Before analysis, tests warning screen errors, preliminary results, or sampling errors were removed.

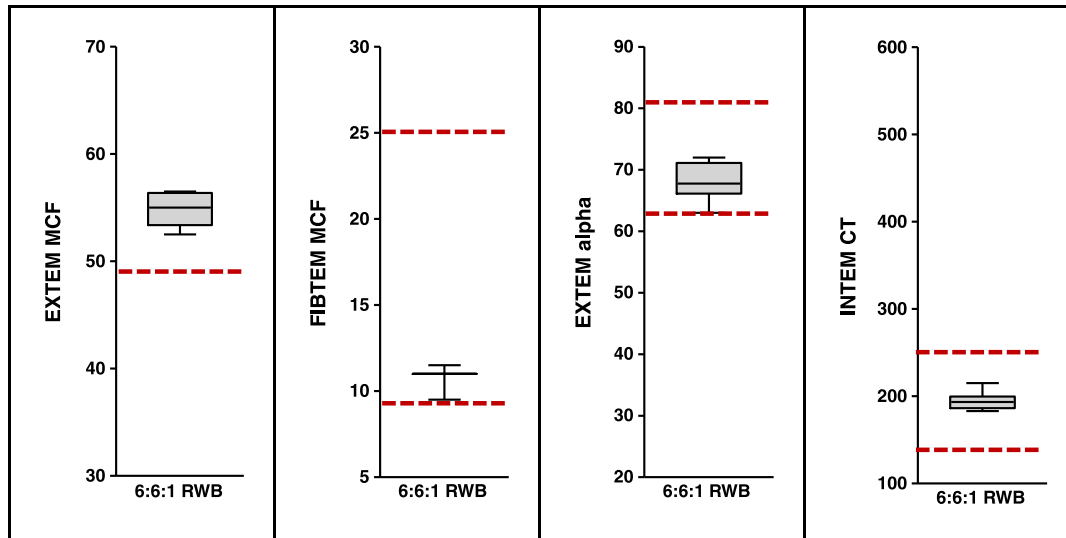


Figure 3. ROTEM results for RWB using components in equal ratios (6:6:1). Results are shown as minimum, first quartile, median, third quartile, and maximum. Data do not contain outliers. Dashed lines indicate published reference ranges, and only the lower limit is shown if the upper limit is beyond the graph axis.¹²

(Fig. 1). Error messages were recorded in 4 (1.5%) of 267 tests. The first error message reported a significant mechanical shock or vibration and/or dried sample still in place in holder, and the remaining three indicated that measurement was influenced by drying of the sample, temporary malfunction of the axis, obstacle in the optical path, and/or worn axis ball bearings.

The frequency of ROTEM tests were as follows: EXTEM (n = 88), INTEM (n = 85), FIBTEM (n = 82), and APTEM (n = 12). Because of operational needs that prevented the crew from keeping to an exact schedule of sample analysis, CWB results were grouped into bins rather than being reported as discrete time points (Fig. 2 and Table 2, excluding APTEM). Compared with Days 0 to 2, EXTEM maximum clot firmness (MCF) was significantly reduced, beginning on Days 10 to 14; however, results through that date remained within reference ranges¹² and were comparable with the EXTEM MCF for the RWB samples containing Day 5 platelets (Fig. 3). Even after Day 14 when CWB median values fell below reference ranges, results stabilized, demonstrating that viable platelets were present throughout. This was confirmed by comparison of the FIBTEM

and EXTEM MCFs, which showed that the platelet contribution only fell from approximately 80% of clot strength at baseline to approximately 70% on Days 21 to 25 (Fig. 2). The CWB FIBTEM MCF data further demonstrated that the fibrinogen contribution to clot strength did not significantly change throughout the storage period. Similarly, EXTEM α angle decreased significantly over time compared with Days 0 to 2, but the functional deficit was less than 50% even on Days 21 to 25 (Fig. 2). INTEM clotting time (CT) increased significantly over time compared with Days 0 to 2, demonstrating that the plasma in WB degraded over time, but results remained within reference ranges through Days 15 to 20, and excursions on Days 21 to 25 were consistent with preservation of considerable coagulation function (Fig. 2). FIBTEM MCF, EXTEM α angle, and INTEM CT were within reference ranges for all RWB samples (Fig. 3). No hyperfibrinolysis was observed in the CWB samples at any time point (data not shown); thus, fibrinolysis was not assessed in RWB.

Direct measurement of platelet aggregation could not be performed during this deployment; however, data for refrigerated

TABLE 3. Comparison of Impedance Aggregometry From Equal Ratio RWB Versus Healthy Subject Fresh WB on the Day of Collection (Day 1) and at Day 5

	Impedance Aggregometry (AUC, U)				
	ADP	COL	TRAP	ASPI	RISTO
Published Reference Ranges for WB	36.0–101.0	24.0–79.0	75.0–137.0	42.0–100.0	27.0–124.0
Healthy Subject WB Day 1	44.8 (4.1)	35.8 (2.7)	83.4 (5.8)	37.3 (5.4)	43.9 (9.1)
Refrigerated Stored WB Day 5	30.8 (6.7)	17.6 (5.2)	43.3 (6.9)	31.9 (6.1)	30.9 (7.7)
Refrigerated WB Day 10	16.7 (4.6)	10.3 (3.2)	25.8 (4.1)	15.9 (3.7)	18.5 (3.0)
6:6:1 RWB with Day 5 Platelets*	0.08 (0.2)	0.00	4.7 (2.1)	0.00	0.00

*Stored at 22°C

Average platelet count for 6:6:1 RWB samples was $95 (2) \times 10^3/\mu\text{L}$. For comparison, published reference ranges¹⁴ are shown in Row 1, and stored WB values from healthy subjects¹³ previously published by our laboratory are shown in Rows 2 to 4.

6:6:1 RWB, reconstituted WB created from equal ratio components; ASPI, arachidonic acid; AUC, area under the curve; COL, collagen; RISTO, ristocetin; TRAP, thrombin receptor activating peptide; U, arbitrary units.

WB have been previously published¹³ and are shown in Table 3. Briefly, platelet aggregation in our healthy donor population was slightly low compared with published reference ranges¹⁴ and decreased significantly over time. Even so, platelets maintained approximately 30% to 50% of the baseline response to agonists on Day 10. In comparison, after 5 days of storage as components, platelets from the RWB samples were almost completely unresponsive to agonists (Table 3).

DISCUSSION

This study demonstrated that a walking blood bank SOP can be effectively implemented in the austere environment of a frigate on an antipiracy mission. The predeployment training sessions were adequate to prepare military personnel, who were then able to maintain a small store of CWB. Experiences from performed drills showed that more WB could be procured on short notice, enough to sustain one to three casualties. However, filtration is time consuming and, in extreme situations, should be omitted. This will require that the Terumo BCT Imuflex WB-SP collection kit be amended to include access ports in the collection bag. The TTD rapid test used should be improved, and a new version has since come on the market (MedmiraInc). During the course of a long deployment, the walking blood bank would not be sustainable because of the continual loss of donors. A mechanism for replenishing donors is necessary to support long missions. In addition, it is clear that predeployment recruitment can be improved, as less than half of the crew members elected to participate. Moreover, the walking blood bank SOP could have included specific transfusion procedures as well as disposal instructions for expired blood.

The goals of CWB thromboelastic point-of-care monitoring were twofold: (1) to investigate whether WB ROTEM evaluation could be conducted in austere conditions such as a navy vessel in open seas and (2) to evaluate the quality of cold-stored WB prepared for transfusion under those conditions. Given that the ship carried an antipiracy Special Operations Unit at risk of severe injury, a surgical unit that required blood support and some measure of coagulative function, and a walking blood bank, these aims were considered relevant to the mission.

The value of point-of-care monitoring in patients with severe hemorrhage is well established. Although uncertainties remain, well-trained operators are able to produce reliable results in an expedient fashion that can help direct and monitor resuscitation efforts. Several instruments are available for point-of-care monitoring; however, we found no reports evaluating the quality of point-of-care devices under rough conditions at sea; thus, we based our selection on instrument specifications, a technical evaluation of the test procedure, and on expert user experience. Based on these analyses, the ROTEM analyzer seemed to be a viable candidate for deployment in austere environments, and this has been substantiated by our study results. As we demonstrated in Fig. 1, the ROTEM instrument performed well during weather condition-related rotational ship movement. Error warnings appeared only four times during the course of the study.

Importantly, the study also demonstrated that CWB provides viable platelets and hemostatic function in the absence of conventional blood banking support at least through Day 14

and possibly longer. There were significant changes in EXTEM and INTEM parameters over time, indicating that there was some decline in CWB quality during storage; however, the remaining function may be sufficient to support hemostasis when WFWB supplies have been exhausted. Furthermore, FIBTEM MCF was stable throughout the storage period, indicating that the fibrinogen level was sufficient and that its contribution to the clot strength was preserved. Jobses et al.¹⁵ and our laboratory¹³ reported preserved thrombelastographic function of refrigerated WB through Days 31 and 21, respectively; however, those study results were compared with published reference ranges determined from samples drawn from WB bags containing CPD or CPDA-1, which are lower than reported values from citrated blood collection tube samples. We did not find similar studies assessing ROTEM results from WB bags, which may explain why baseline results in this study were at the low end of published reference ranges.

Prospective clinical studies are needed to determine the efficacy of Day 21 to 25 WB for DCR; however, the results of this study confirm that CWB retains considerable thromboelastic capacity during storage, which is similar to that of RWB from components within their shelf-life. It is therefore not unreasonable to assume that, in the absence of WFWB or other standard blood products, CWB can sustain both oxygen-carrying capacity and hemostatic function better than other available options during deployment. This is further supported by comparison of previously reported platelet aggregometry results with our findings in RWB from stored components. Whereas the latter demonstrated almost no response to aggregometry, we previously showed that significant platelet aggregometry is preserved in WB even at Day 10 (Table 3).

This study had limitations. As detailed in the Materials and Methods section, procedures and study time points had to be adjusted in response to operational needs. Donors for the stored WB studies were healthy, young military personnel, whereas the health status and age of donors for the reconstituted blood from components were unknown. Comparisons were made to previously reported literature, in which leukoreduction was not performed, and the baseline was labeled Day 1, not Day 0, among other possible methodological differences. Despite these limitations, this study contributes important findings. Rotational thromboelastometry is possible in a challenging environment, and CWB demonstrates preserved *in vitro* clot formation for at least 2 weeks.

CONCLUSION

The walking blood bank concept described in this study is feasible and provides a balanced transfusion product, which can support DCR/RDCR aboard a frigate. ROTEM analysis is possible during rough seas and in challenging operational conditions. Cold-stored WB yields reproducible ROTEM results demonstrating preserved fibrinogen function and platelet function for at least 3.5 weeks and 2 weeks, respectively.

AUTHORSHIP

G.S. initiated and designed the project and drafted the manuscript, to which all authors contributed. I.A. contributed to data collection and analysis (ROTEM on board the frigate). T.O.A. conducted the statistical analysis and advised on other aspects of the analysis. T.A.H., A.P.C., and

E.K.K. contributed to the design and provided critiques of the manuscript. M.C.H. contributed to data collection and analysis. H.F.P. contributed to the design, data analysis, data interpretation, and critical revision of the manuscript.

DISCLOSURE

The authors declare no conflicts of interest.

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