# Safety profile of uncrossmatched, cold-stored, low-titer, group O+ whole blood in civilian trauma patients

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**BACKGROUND:** The use of cold-stored low-titer group O whole blood (LTOWB) for civilian trauma patients is gaining popularity. However, hemolysis might occur among non–group O recipients. This study evaluated the serologic safety of transfusing up to 4 units of LTOWB.

**STUDY DESIGN AND METHODS:** Hypotensive male and at least 50-year-old female trauma patients who received leukoreduced, uncrossmatched, group O+, low-titer (<50 anti-A and anti-B), platelet-replete whole blood during initial resuscitation were included in this prospective, observational study. Biochemical markers of hemolysis were measured on the day of LTOWB receipt (Day 0) and over the next 2 days. Blood product administration in the first 24 hours of admission and reported transfusion-associated adverse events were also reviewed.

**RESULTS:** There were 102 non–group O and 70 group O recipients of 1 to 4 LTOWB units analyzed. The non–group O recipients received a median volume of 600 mL (range, 300-4100 mL) of ABO-incompatible plasma, including the contribution from the LTOWB units. There were no significant differences in median haptoglobin, lactate dehydrogenase, total bilirubin, creatinine, or potassium levels at any time point between the non–group O and group O recipients. There were also no differences in these markers between the subset of 23 non–group O and 14 group O recipients who received 3 or 4 LTOWB units. No transfusion-associated adverse events were reported.

**CONCLUSIONS:** Administration of up to 4 units of LTOWB in civilian trauma resuscitation was not associated with clinical or biochemical evidence of hemolysis. Six units per trauma patient are now permitted at these institutions.

The use of cold-stored low-titer group O whole blood (LTOWB) as a therapeutic modality in damage control resuscitation of the civilian trauma patient has for the past several decades been generally avoided in favor of component therapy. Recently, however, there has been increased interest in returning to the use of cold-stored LTOWB in the civilian trauma setting.[1-4] LTOWB provides all of the components of blood (red blood cells [RBCs], platelets [PLTs], and plasma, which includes fibrinogen) in a single convenient package, thereby providing balanced resuscitation that simultaneously addresses oxygen debt and coagulopathy while simplifying the logistics of blood product

**ABBREVIATION:** LTOWB = low-titer group O whole blood.

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Data on some of the patients in this study have been previously published in Seheult JN, Triulzi DJ, Alarcon LH, Sperry JL, Murdock A, Yazer MH. Measurement of hemolysis markers following transfusion of uncrossmatched, low-titer, group O+ whole blood in civilian trauma patients: initial experience at a level 1 trauma center. Transfus Med 2017;27:30-35, and Yazer MH, Jackson B, Sperry J, Alarcon L, Triulzi DJ, A Murdock. Initial safety and feasibility of cold stored uncrossmatched whole blood transfusion in civilian trauma patients. J Trauma Acute Care Surg 2016;81:21-26

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resuscitation.[5-7] Recently, the AABB changed Standard 5.15.1 in the 31st edition of the *Standards for Blood Banks and Transfusion Services* to permit the transfusion of LTOWB provided that the RBC component must be ABO compatible with, but not necessarily identical to, the recipient.[8] This change permits the use of LTOWB in a manner that is analogous to how group O uncrossmatched RBCs are provided to a recipient of unknown ABO group in an emergency. However, the possibility of causing a hemolytic transfusion reaction after the administration of potentially ABO-incompatible plasma in the LTOWB unit to a non–group O recipient has been a potential barrier to the widespread adoption of this therapy.[9]

The University of Pittsburgh Medical Center implemented a program for using cold-stored LTOWB for the initial resuscitation of trauma patients beginning in December 2014. The program started at one of this system's Level 1 trauma hospitals and has recently expanded to include both of its Level 1 adult trauma hospitals. Preliminary evidence from one hospital in this health care system has demonstrated that receipt of small quantities of cold-stored LTOWB, up to 2 units per patient, was not associated with increased mortality rates compared to injured patients who received only component therapy.[10] Furthermore, the absence of hemolysis or reported hemolytic transfusion reactions amongst the non–group O recipients of up to 2 units of cold-stored LTOWB has also been demonstrated.[11] This latter finding was not unexpected given the experience with transfusing small quantities of incompatible plasma in PLT concentrates[12] and even larger quantities of group A plasma to civilian trauma patients of unknown ABO group,[13] and it led to an increase in the maximum number of cold-stored LTOWB units that could be transfused to 4 units. This report describes the ongoing monitoring of civilian trauma recipients of cold-stored LTOWB who are now eligible to be transfused with larger quantities of cold-stored LTOWB during their initial resuscitation than had been previously reported at this health care institution.

## MATERIALS AND METHODS

Group O+ whole blood (WB) units were obtained from the local Food and Drug Administration (FDA)-licensed blood center, which collects WB only from male donors to comply with the AABB/FDA transfusion-related acute lung injury risk mitigation requirement.[8] Briefly, approximately 500 mL of WB is collected in citrate phosphate dextrose (CPD) solution, using an FDA-approved collection system that leukoreduces the WB while sparing the PLTs (Imuflex WB-SP, Terumo BCT). WB collected and processed with this system can be stored between 1 and 6°C for up to 21 days. Isohemagglutinin titers were determined using a room temperature immediate spin test to detect primarily immunoglobulin (Ig)M antibodies. Only the units with low titers (<50) of both anti-A and anti-B were used as LTOWB. Units with high-titer antibody(-ies) were processed into RBC units. To perform the titers, 0.1 mL of the plasma from the WB unit was added to 4.9 mL of saline. This mixture was then added to a 3% suspension of commercially available $A_1$ and B reagent RBCs (Biorad) in tube and the agglutination strength was interpreted after being immediately centrifuged (i.e., no incubation period). The anti-A and anti-B titers were determined on every WB unit, even if it came from a repeat donor, as it is thought that the titers can change depending on the donor's exposure to A- and B-like substances.[14,15]

Uncrossmatched, cold-stored LTOWB units were stored in the emergency department's monitored refrigerator at a Level 1 trauma center and were available for immediate use before the recipient's ABO group became known. Initially the units were manually rocked at each nursing shift change, but this practice was stopped when the data indicated that this manipulation was not necessary to maintain PLT function.[16] When the cold-stored LTOWB program began in December 2014, hypotensive, male trauma patients were eligible to receive a maximum of 2 cold-stored LTOWB units during their initial resuscitation. At that time, female trauma patients were not eligible for cold-stored LTOWB transfusion due to limitations in availability of D– cold-stored LTOWB. As the cold-stored LTOWB units were kept in the emergency department's refrigerator, these units were intended to be the first blood products administered to eligible patients at the discretion of the trauma team. Conventional laboratory testing, as well as thromboelastography testing, was ordered as soon as possible after admission and the results used to guide subsequent transfusion therapy with conventional blood components, which includes AS-1 and AS-3 RBC units, and thawed plasma, including low-titer group A plasma, at this trauma center.

The hospital's transfusion committee approved an increase in the maximum number of cold-stored LTOWB units to 4 units per trauma patient in April 2016 when it became clear from analyzing the patients' clinical courses and the biochemical markers of hemolysis that the non–group O patients did not have demonstrable evidence of hemolysis compared to the group O recipients.[11] At that time, female trauma patients over the age of 50 were also made eligible to receive cold-stored LTOWB, which is consistent with the hospital's policy for providing D+ cellular blood products to D– patients. In September 2017, a second Level 1 trauma hospital in this health care system that was supplied by the same blood center began using cold-stored LTOWB; this hospital followed the same policies and protocols for the resuscitation and posttransfusion monitoring of trauma patients as the first hospital because both transfusion services are managed by the same centralized transfusion service. This second hospital

began its cold-stored LTOWB program using a maximum of 4 units of cold-stored LTOWB per patient.

If the cold-stored LTOWB units were not transfused initially by Day 10 (later extended to Day 14), they were reclaimed by the blood bank and were available to be manufactured into RBC units that could be transfused up to Day 21. The PLT-rich plasma supernatant was discarded when the cold-stored LTOWB unit was processed into an RBC unit.

This prospective, observational study collected biochemical and clinical data on a cohort of cold-stored LTOWB recipients at these two hospitals between December 24, 2014, and February 4, 2018. As part of the cold-stored LTOWB recipient protocol, the following biochemical markers of hemolysis were measured on the day of cold-stored LTOWB transfusion (Day 0; these samples were typically drawn shortly after the cold-stored LTOWB administration) and for the next 2 days: haptoglobin concentration, lactate dehydrogenase (LDH) activity, total bilirubin concentration, creatinine concentration, and serum potassium concentration. If a patient had multiple measurements of a variable on the same day, an average of that day's values was calculated. To be included in this study, trauma patients had to have received at least 1 unit of cold-stored LTOWB; survived for at least 24 hours after admission; and had at least one measurement of haptoglobin, total bilirubin, and/or LDH on Days 0, 1, and/or 2.

The total number of blood products transfused to each cold-stored LTOWB recipient during the first 24 hours of admission was collected from the blood bank's electronic database. The numbers of PLT and cryoprecipitate units are presented as individual units obtained from WB; the typical adult dose of PLTs and cryoprecipitate at this institution consists of 4 or 5 units in a pool. Transfused apheresis PLTs were converted to WB PLT equivalents by multiplying by 5. The average volume of plasma contained in each product was calculated from a random sampling of between 8 and 12 units supplied by the blood center: 300 mL of plasma per cold-stored LTOWB unit, 240 mL of plasma per plasma unit, and 70 mL of plasma per WB PLT unit. Information on any suspected transfusion reactions reported by the clinical teams within 2 days of cold-stored LTOWB transfusion, and the results of any direct antiglobulin tests (DAT) that were performed within 2 days of cold-stored LTOWB transfusion were also obtained from the blood bank's electronic database. The University of Pittsburgh's Quality Improvement Review Committee approved this data collection protocol.

Tests of normality were performed for continuous variables and the appropriate descriptive statistics were calculated. The Mann-Whitney U test was used to compare the mean rank of continuous variables between both groups and quantile regression was used to test for the equality of medians between both groups, while chi-square test or the Fisher's exact test, where appropriate, was used to compare the differences between dichotomous variables (StataCorp). Tests of hypotheses were considered significant if the p value was less than 0.05.

## RESULTS

### All eligible cold-stored LTOWB recipients

Overall, 269 trauma patients received at least 1 unit of cold-stored LTOWB during the study period; 97 of these recipients were excluded because they did not survive for at least 24 hours after admission and/or did not have at least one measurement of haptoglobin, total bilirubin, and/or LDH on Days 0, 1, and/or 2. This analysis included the remaining 102 non–group O and 70 group O cold-stored LTOWB recipients. Eight of the cold-stored LTOWB recipients (all non–group O) were female. There were no significant differences in the demographic or clinical variables between the non–group O and group O patients (Table 1), nor were there any significant differences between these two groups in terms of the median quantities of RBCs, PLTs, plasma, or cryoprecipitate units transfused in the first 24 hours after admission (Table 2). The ratios of transfused PLTs:RBCs and plasma:RBCs within the first 24 hours were also not significantly different.

The non–group O patients received a median volume of 600 mL (range, 300-4100 mL) of ABO-incompatible plasma in total, including the contribution from the cold-stored LTOWB units (Table 2). There were no significant differences in median haptoglobin, LDH, total bilirubin, creatinine, or potassium levels on Days 0, 1, and 2 between the non–group O and group O recipients (Fig. 1). With the exception of the elevated median LDH values for both non–group O and group O cold-stored LTOWB recipients on Days 0, 1, and 2, and the below normal median haptoglobin concentrations for both non–group O and group O recipients on Day 0, the median values for the other biochemical markers of hemolysis were within their respective reference ranges at the three time points measured. Two group O patients had a polyspecific DAT performed on samples collected within 2 days of cold-stored LTOWB receipt; both patients' polyspecific DATs were positive; and one of the patients had a negative monospecific IgG DAT that required no additional serologic investigation according to the blood bank's standard protocols, while the other patient had a positive IgG DAT with anti-D identified in the eluate that was also detectable in his serum on the day of the cold-stored LTOWB transfusion. On Days 0 and 1, this latter patient had decreased haptoglobin (<5.8 and 12.2 mg/dL, respectively; reference range, 36-195 mg/dL), elevated total bilirubin (4.9 and 4.7 mg/dL, respectively; reference range, 0.3-1.5 mg/dL), and elevated LDH levels (837 and 426 IU/mL, respectively; reference range, 0-171 IU/mL), and normal creatinine and serum potassium levels. Furthermore, there was no visible evidence of

**TABLE 1. Demographics of the non–group O and group O cold-stored LTOWB recipients in this study***

| Variables | All recipients (1-4 units) | | | Recipients of 3 or 4 units | | |
|---|---|---|---|---|---|---|
| | Non–group O (n = 102) | Group O (n = 70) | p value† | Non–group O (n = 23) | Group O (n = 14) | p value† |
| Demographic variable | | | | | | |
| Age (years) | 38 (26-58) | 41 (26-60) | 0.69 | 39 (32-55) | 35 (26-59) | 0.41 |
| Injury Severity Score‡ | 22 (14-29) | 22 (14-34) | 0.75 | 22 (17-27) | 22 (17-33) | 0.92 |
| Admission systolic blood pressure (mmHg) | 103 (88-122) | 95 (80-120) | 0.16 | 100 (82-118) | 91 (72-96) | 0.15 |
| Admission heart rate (beats/min) | 112 (90-134) | 104 (89-119) | 0.09 | 116 (92-141) | 121 (89-140) | 1.00 |
| Admission Glasgow Coma Scale score | 15 (3-15) | 15 (3-15) | 0.32 | 14 (3-15) | 15 (10-15) | 0.42 |
| Hospital length of stay (days)§ | 14.0 (7.0-26.0) | 14.0 (5.0-21.0) | 0.28 | 15.0 (7.0-26.0) | 15.0 (8.0-19.0) | 0.55 |
| ICU length of stay (days)§ | 5.0 (3.0-13.0) | 5.0 (2.0-11.0) | 0.36 | 8.0 (3.0-16.0) | 4.5 (4.0-7.0) | 0.20 |
| ICU-free days§ | 6.5 (2.0-14.0) | 5.0 (2.0-12.0) | 0.61 | 6.0 (4.0-14.0) | 6.0 (3.0-16.0) | 0.91 |
| Days on ventilator§ | 3.0 (0-8.0) | 2.0 (0-7.0) | 0.35 | 4.0 (1.0-11.0) | 3.0 (0-6.0) | 0.28 |
| Ventilator-free days§ | 9.0 (3.0-16.0) | 8.0 (3.0-18.0) | 0.57 | 8.0 (4.0-15.0) | 8.5 (4.0-18.0) | 0.71 |
| In-hospital mortality, ratio (%)§ | 13/102 (12.8) | 9/69 (13.0) | 0.95 | 3/23 (13.0) | 3/14 (21.4) | 0.50 |
| Post-ED destination | | | | | | |
| ICU | 22 (21.6) | 19 (27.2) | 0.40 | 5 (21.7) | 3 (21.4) | 1.00 |
| Operating room | 74 (72.5) | 46 (65.7) | 0.34 | 15 (65.2) | 11 (78.6) | 0.48 |
| Interventional radiology | 4 (3.9) | 1 (1.4) | 0.65 | 3 (13.1) | 0 (0) | 0.28 |
| Hospital ward | 2 (2.0) | 3 (4.3) | 0.40 | 0 (0) | 0 (0) | |
| Discharged from ED | 0 (0) | 1 (1.4) | 0.41 | 0 (0) | 0 (0) | |
| Discharge location§ | | | | | | |
| Home | 34 (33.3) | 30 (43.5) | 0.18 | 8 (34.8) | 8 (57.2) | 0.18 |
| Rehabilitation or skilled nursing facility center | 42 (41.2) | 22 (31.9) | 0.22 | 10 (43.5) | 2 (14.3) | 0.08 |
| Other‖ | 13 (12.7) | 8 (11.6) | 0.82 | 2 (8.7) | 1 (7.1) | 1.00 |

* Data are reported as median (IQR) or number (%) unless otherwise reported. The "other" category for discharge location destination includes transfers to hospice and to other hospitals. The p values compare the variables between the group O vs. non–group O recipients for either all cold-stored LTOWB recipients or those who received 3 or 4 cold-stored LTOWB units.

† The Mann-Whitney U test was used to compare the mean rank of continuous variables between both groups, while chi-square test or the Fisher's exact test, where appropriate, was used to compare the differences between dichotomous variables.

‡ Injury Severity Score (ISS) is a validated score to assess trauma severity, which combines the highest severity scores in each of the three most severely injured ISS body regions. A major trauma is defined as an ISS greater than 15.[26]

§ Data are missing for one group O recipient of 2 units of cold-stored LTOWB; this patient was still in hospital at the time of data analysis.

‖ Includes patients discharged to hospice, long-term care, a legal authority, another hospital, and a psychiatric unit, as well as patients who self-discharged against medical advice.

ED = emergency department; ICU = intensive care unit; IQR = interquartile range.

hemolysis in the plasma portion of this patient's sample when the DAT was performed. None of the cold-stored LTOWB recipients had a suspected transfusion reaction of any kind reported to the blood bank on the day of cold-stored LTOWB receipt or over the following 2 days.

### Recipients of 3 or 4 units of cold-stored LTOWB

A subset of recipients (23 non–group O and 14 group O) received 3 or 4 units of cold-stored LTOWB. Two of these cold-stored LTOWB recipients (both non–group O) were female. There were no significant differences in the demographic or clinical variables between these patients (Table 1), nor were there any significant differences in the median quantities of RBCs, PLTs, plasma, or cryoprecipitate units transfused in the first 24 hours after admission between both groups of recipients (Table 2). The ratios of transfused PLTs:RBCs and plasma:RBCs within the first 24 hours were also not significantly different.

The non–group O recipients received a median volume of 1200 mL (range, 900-2520 mL) of ABO-incompatible plasma in total, including the contribution from the cold-stored LTOWB units (Table 2). No significant differences in median haptoglobin, LDH, total bilirubin, creatinine, or serum potassium levels on Days 0, 1, and 2 after transfusion of cold-stored LTOWB were observed between the non–group O and group O recipients (Fig. 2). With the exception of the elevated median LDH values for both non–group O and group O cold-stored LTOWB recipients on Days 0, 1, and 2, and the below normal median haptoglobin concentrations for both non–group O and group O recipients on Day 0, the median values for the other biochemical markers of hemolysis were within their respective reference ranges at the three time points measured.

### Rate of high-titer WB donors at this blood center and WB unit utilization

Over a 2-year period, 3710 WB units underwent antibody titer testing at the blood center; 748 of 3710 (20.2%) were found to have anti-A and/or anti-B titers that were more than 50 and were available to be processed into RBC

**TABLE 2. Blood product transfusions for the non–group O and group O cold-stored LTOWB recipients in the 24 hours of admission***

| | All recipients (1-4 units) | | | Recipients of 3 or 4 units | | |
|---|---|---|---|---|---|---|
| Variables | Non–group O (n = 102) | Group O (n = 70) | p value† | Non–group O (n = 23) | Group O (n = 14) | p value† |
| Number of blood products transfused in first 24 hr of admission‡ | | | | | | |
| WB | 2.0 (1.0-2.0) | 1.5 (1.0-2.0) | 0.33 | 4.0 (3.0-4.0) | 4.0 (4.0-4.0) | 0.75 |
| RBCs | 2.0 (0-5.0) | 1.0 (0-4.0) | 0.52 | 2.0 (0-8.0) | 4.0 (2.0-9.0) | 0.32 |
| Plasma | 0 (0-8.0) | 0 (0-6.0) | 0.42 | 3.0 (0-8.0) | 6.0 (0-13.0) | 0.43 |
| PLTs§ | 0 (0-5.0) | 0 (0-4.0) | 0.35 | 3.0 (0-5.0) | 4.0 (0-5.0) | 0.85 |
| Cryoprecipitate§ | 0 (0-0) | 0 (0-0) | 0.31 | 0 (0-4.0) | 0 (0-4.0) | 0.84 |
| Patients who received at least one additional product in first 24 hr‖ | | | | | | |
| Received only WB | 35 (34.3) | 25 (35.7) | 0.85 | 6 (26.1) | 2 (14.3) | 0.68 |
| RBCs | 62 (60.8) | 41 (58.6) | 0.77 | 15 (65.2) | 11 (78.6) | 0.48 |
| Plasma | 47 (46.1) | 30 (42.9) | 0.68 | 14 (60.9) | 9 (64.3) | 0.84 |
| PLTs | 41 (40.2) | 24 (34.3) | 0.43 | 14 (60.9) | 8 (57.1) | 0.82 |
| Cryoprecipitate | 23 (22.6) | 11 (15.7) | 0.27 | 6 (26.1) | 4 (28.6) | 1.00 |
| Blood product ratios¶ | | | | | | |
| Plasma:RBCs | 1.00 (0.81-1.23) | 1.00 (0.73-1.29) | 0.82 | 1.00 (0.86-1.36) | 1.11 (1.00-1.33) | 0.66 |
| PLTs:RBCs | 1.00 (0.54-1.00) | 1.00 (0.62-1.00) | 0.77 | 1.00 (0.73-1.00) | 0.67 (0.60-1.00) | 0.24 |
| Number of incompatible plasma-containing products transfused** | 2 (1-6, 1-52) | | | 4 (4-9, 3-18) | | |
| Volume (mL) of incompatible plasma transfused** | 600 (300-1200, 300-4100) | | | 1200 (1200-1550, 900-2520) | | |

* Data are reported as median (IQR), number (%), or median (IQR, range). The p values compare the variables between the group O vs. non–group O recipients for either all cold-stored LTOWB recipients or those who received 3 or 4 cold-stored LTOWB units.
† The Mann-Whitney U test was used to compare the mean rank of continuous variables between both groups, while chi-squared test or the Fisher's exact test, where appropriate, was used to compare the differences between dichotomous variables.
‡ The RBC, plasma, PLT, and cryoprecipitate contribution from the cold-stored LTOWB was not included in the median number of individual blood products transfused.
§ Individual WB-derived units.
‖ The fraction of patients who received at least one additional product includes any patient who received at least one other blood component in addition to the cold-stored LTOWB during the first 24 hours of their admission; for example, 62 of 102 (60.8%) of the non–group O patients received at least 1 RBC unit after receipt of cold-stored LTOWB.
¶ The RBC, plasma, and PLT contribution from each unit of cold-stored LTOWB was included along with any additional blood components received in these ratios.
** Includes the volume of incompatible plasma in the transfused cold-stored LTOWB unit(s), PLT concentrates, and low-titer A plasma units.
IQR = interquartile range.

units. Of the 2962 LTOWB units that were available for transfusion, 712 of 2962 (24%) were transfused to trauma patients at all of the hospitals with trauma centers supplied by this blood center (i.e., not only the patients at the two hospitals included in this study). Thus, 2250 LTOWB units were not transfused as such and were available to be processed into RBC units. Including the 748 WB units that had a high titer and were thus not available to be transfused as LTOWB, there were a total of 2998 units that could have been processed into an RBC unit. Of these, 2548 of 2998 (85%) were transfused as RBCs. The remaining 450 of 3710 units (12% of the total number of WB units that were titered) were not processed into RBC units and expired as LTOWB in the blood bank or were processed into RBC units and expired in the blood bank before they could be transfused or were issued to patients and became wasted due to improper storage or handling on the ward.

## DISCUSSION

This study extends the safety profile of cold-stored LTOWB transfusion during civilian trauma resuscitation when transfused in larger quantities and to a larger recipient cohort than previously reported.[11] There were no significant differences in the levels of the biochemical markers of hemolysis on the day of cold-stored LTOWB transfusion and during the 2 days after the administration of up to 4 units of cold-stored LTOWB between the non–group O and group O recipients. There were also no suspected transfusion reactions reported to the blood bank. Based on these observations, it appears to be safe to administer up to 4 units of this product to civilian trauma patients.

As cold-stored LTOWB is often administered before the recipient's ABO group is known, one potential risk of transfusing this product is hemolysis if the recipient turns out to be non–group O. One way of mitigating this risk is

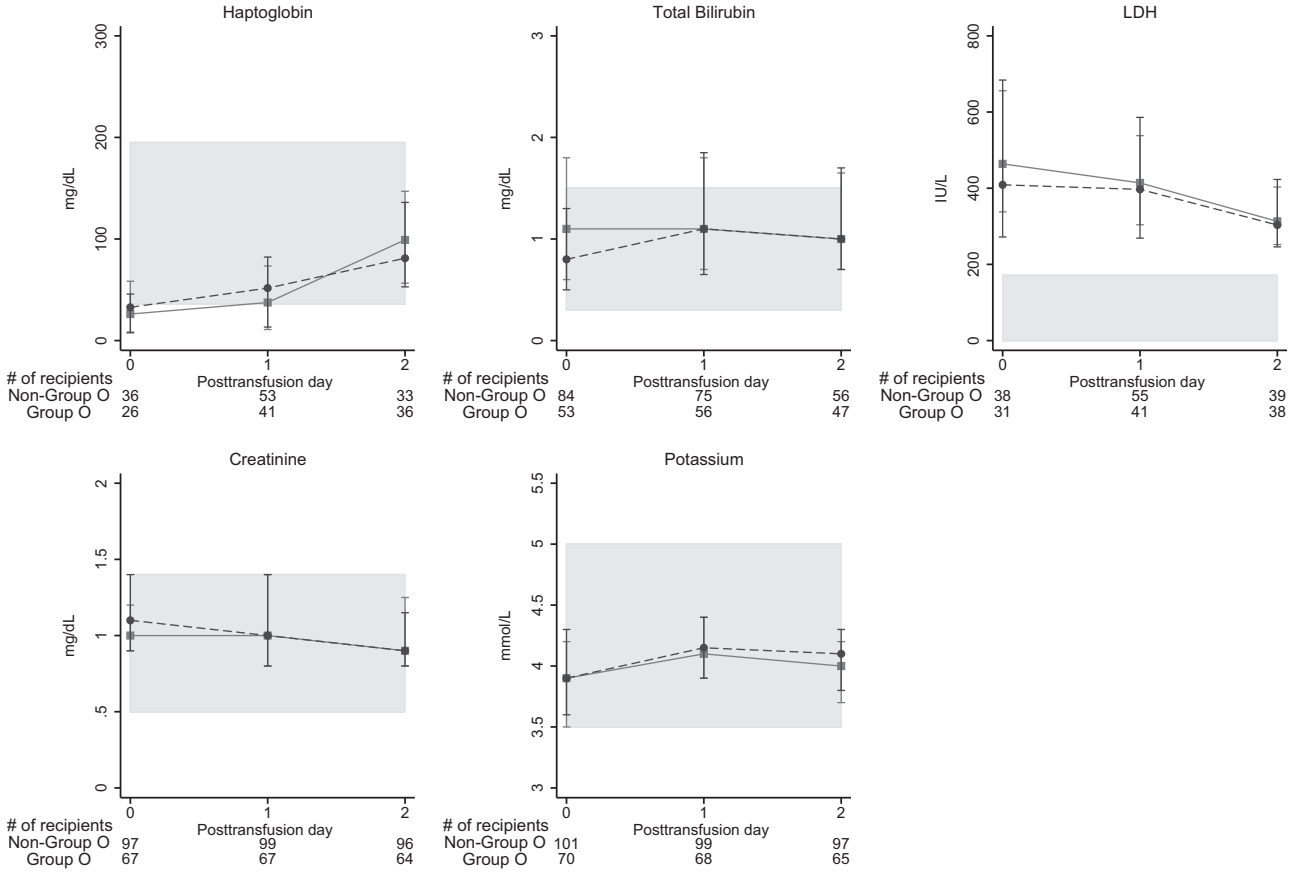


**Fig. 1. Biochemical markers of hemolysis in the non–group O (■, 1-4 units, n = 102) and group O (●, 1-4 units, n = 70) recipients of at least 1 unit of cold-stored LTOWB. These data are shown as the median and the interquartile range. The gray-shaded areas represent the laboratory's reference ranges for each analyte. The numbers below each figure reflect the number of patients on whom the biochemical variable had been assayed at each time point. There were no significant differences between the non–group O and group O cold-stored LTOWB recipients for any of the analytes measured at any time point.**

to use WB donors who have a "low titer" of both anti-A and anti-B.[17] At this institution, a critical titer of less than 50 for both anti-A and anti-B was chosen largely based on its experience with transfusing group A plasma with an anti-B titer of less than 50. However, there currently is no uniform agreement on the methods used to perform the titer or on a critical antibody titer threshold above which the risk of hemolysis becomes unacceptably high.[9] The published data, and past clinical experience in both civilian and military trauma resuscitation, support anti-A and anti-B titers below 100 to 200 (IgM saline titer method) and 250 to 400 (antihuman globulin titer technique) as reasonably safe when group O WB and PLT units are transfused in an incompatible manner.[4,14,17-20] Other institutions use different critical titer thresholds such as less than 200 at the Mayo Clinic and less than 256 at US Army medical centers.[4] Furthermore, other centers are already transfusing more than 4 units of cold-stored LTOWB to trauma patients, such as at the San Antonio Military Medical Center in Texas where there is no limit on the quantity of cold-stored LTOWB that can be transfused to a trauma patient so long as the patient's attending anesthesiologist and the blood bank physician agree that it is appropriate to continue transfusing this product.[4] It will be interesting to see the biochemical markers of hemolysis and outcomes data for patients at all of the institutions where larger quantities of cold-stored LTOWB are being transfused, as the safety data in this study are limited to recipients of up to 4 units of cold-stored LTOWB.

The main limitation of this study is the method by which hemolysis was assayed. The biochemical markers of hemolysis, LDH, bilirubin, and haptoglobin are sensitive for hemolysis, but they are not specific for hemolysis as LDH and bilirubin can be liberated from tissues other than RBCs in a patient with traumatic injuries; it is also known that haptoglobin decreases as the volume of WB and RBCs transfused in trauma patients increases, which is likely caused by the passive accumulation of free hemoglobin in the WB or RBC unit that occurs as it ages.[21] The amount of hemolysis observed during rapid infusion of

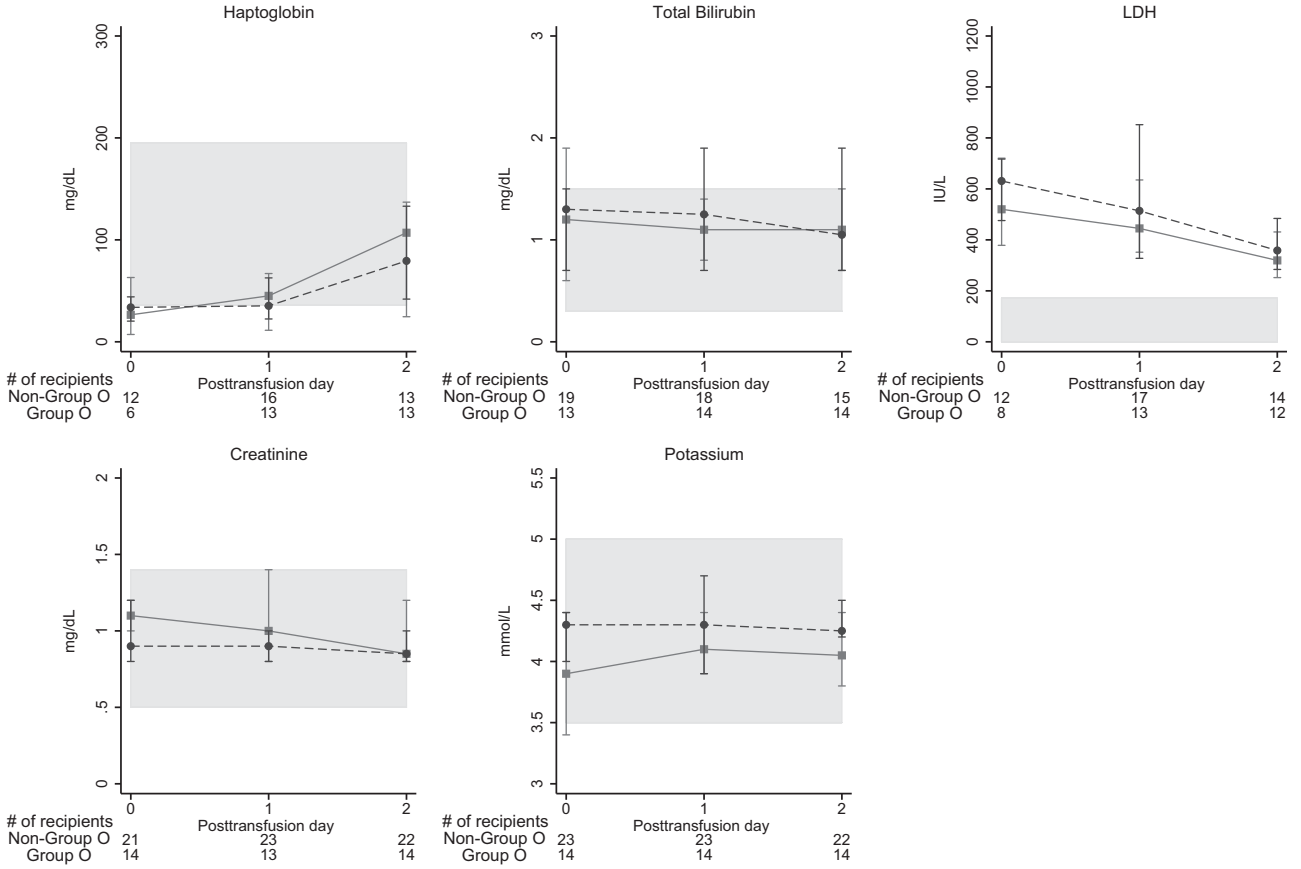


**Fig. 2. Biochemical markers of hemolysis in a subset of non–group O (■, 3 or 4 units, n = 23) and group O (●, 3 or 4 units, n = 14) recipients of between 3 and 4 units of cold-stored LTOWB. These data are shown as the median and the interquartile range. The gray-shaded areas represent the laboratory's reference ranges for each analyte. The numbers below each figure reflect the number of patients on whom the biochemical variable had been assayed at each time point. There were no significant differences between the non–group O and group O cold-stored LTOWB recipients for any of the analytes measured at any time point.**

large quantities of RBCs, such as in the resuscitation of a trauma patient, varies from 0.05% to 4%,[22,23] but this is usually only associated with a small, transient reduction in serum haptoglobin levels.[24] During severe hemolytic reactions, however, the haptoglobin concentration is usually undetectable.[25] In this study, the median haptoglobin concentrations for both the non–group O and group O patients were only slightly below the reference range on Day 0 and increased to be within the reference range by Day 2. This trend, combined with the fact that the median concentrations of all three of the biochemical markers of hemolysis at all three time points in the non–group O recipients was not significantly different from that of the group O recipients, suggests that substantial hemolysis was not occurring in the non–group O recipients. Furthermore, that the hospital and intensive care unit length of stays, as well as the rate of in-hospital mortality, did not differ between the group O and non–group O recipients also suggests that while some degree of low-level hemolysis might have been occurring among the non–group O recipients, it was not severe enough to affect these important clinical variables. It is unfortunate that not every laboratory analyte was measured on every patient and that the number of recipients of 3 or 4 cold-stored LTOWB units is relatively small. However, the absence of any significant difference in the biochemical markers of hemolysis and the very similar appearing trends in the median concentrations of these markers over time suggest that the serologic safety of transfusing up to 4 units of cold-stored LTOWB has been demonstrated.

Furthermore, due to the passive reporting of suspected transfusion reactions to the blood bank by the clinical services, it is possible that the signs and symptoms of a transfusion-associated adverse event might have been overlooked or ascribed to the patient's underlying disease and not reported to the blood bank. However, given the heightened awareness of the potential for hemolysis in these cold-stored LTOWB recipients, it is unlikely that gross evidence for hemolysis, such as unexpected hypotension and hemoglobinuria, would have gone unreported.

From the operational perspective of these blood banks, the approximately 20% WB donor high-titer rate can at times be a burden on the PLT inventory as these hospitals rely heavily on WB-derived PLTs, and when a high-titer unit is detected, the plasma and PLT components must be discarded to process the WB into an RBC unit according to FDA and AABB requirements. Furthermore, the 12% wastage or expiration rate of the unused cold-stored LTOWB units is high. Nevertheless, these blood banks remain committed to supplying cold-stored LTOWB to these hospitals.

This study demonstrated that the transfusion of up to 4 units of uncrossmatched, cold-stored LTOWB to civilian trauma patients did not cause hemolysis or lead to reports of suspected transfusion reactions. Based on these data, the maximum number of cold-stored LTOWB units that can be transfused to these patients at these two institutions will be increased to six, and the possibility of expanding the use of cold-stored LTOWB to all massively bleeding patients will be explored. Both of these changes might help to increase the amount of cold-stored LTOWB transfused at these hospitals thereby lowering the wastage or expiration rates of the unused units. As larger quantities of cold-stored LTOWB are now being transfused at these hospitals, future studies will be undertaken to determine the efficacy of cold-stored LTOWB in terms of potentially reducing the number of blood products transfused and improving survival among recipients of this product.

### CONFLICT OF INTEREST

MHY has given paid lectures for Terumo, the manufacturer of the WB collection kit used in this report. The other authors have disclosed no conflicts of interest.

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