The rebirth of the cool: a narrative review of the clinical outcomes of cold stored low titer group O whole blood recipients compared to conventional component recipients in trauma

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ABSTRACT
There has been renewed interest in the use of low titer group O whole blood (LTOWB) for the resuscitation of civilian casualties. LTOWB offers several advantages over conventional components such as providing balanced resuscitation in one bag that contains less additive/preservative solution than an equivalent volume of conventional components, is easier and faster to transfuse than multiple components, avoids blood product ratio confusion, contains cold stored platelets, and reduces donor exposures. The resurgence in its use in the resuscitation of civilian trauma patients has led to the publication of an increasing number of studies on its use, primarily amongst adult recipients but also in pediatric patients. These studies have indicated that hemolysis does not occur amongst adult and pediatric non-group O recipients of a modest quantity of LTOWB. The published studies to date on mortality have shown conflicting results with some demonstrating a reduction following LTOWB transfusion while most others have not shown a reduction; there have not been any studies to date that have found significantly increased overall mortality amongst LTOWB recipients. Similarly, when other clinical outcomes, such as venous thromboembolism, sepsis, hospital or intensive care unit lengths of stay are evaluated, LTOWB recipients have not demonstrated worse outcomes compared to conventional component recipients. While definitive proof of the trends in these morbidity and mortality outcomes awaits confirmation in randomized controlled trials, the evidence to date indicates the safety of transfusing LTOWB to injured civilians.

Introduction

Although conventional blood components are manufactured from whole blood (WB), the whole is both greater and lesser than the sum of its parts. WB is ‘greater’ than components in that it has many advantages over using an equivalent quantity of conventional components. Perhaps most importantly, the use of WB will greatly simplify the logistics of the resuscitation by providing balanced resuscitation in one bag instead of up to three bags that all have to be separately procured from the blood bank and stored under different conditions [1]. This is especially cumbersome in the pre-hospital setting with restricted storage space in helicopters and ground ambulances, and when intravenous access to the patient is often limited thereby reducing the speed by which the patient can be resuscitated with individual components [2].

Whole blood units, which for civilian use are stored in the refrigerator for a maximum of either 21 or 35 days depending on the solution into which they are collected, also contain PLTs if prepared using a PLT-sparing leukoreduction (LR) filter, or if the WB is not leukoreduced at all. Cold stored PLTs have demonstrated superior in vitro hemostatic properties compared to room temperature PLTs [3–5], suggesting that they might be primed to promote coagulation once transfused. In addition, the use of WB effectively permits the storage of PLTs for the entire shelf life of the WB unit. This is very convenient as remote emergency facilities, such as at ski or diving resorts, that would not normally stock PLTs due to concerns over wastage because of the short shelf life would be able to provide a source of PLTs during a patient’s resuscitation by using WB. Indeed, given that many hospitals in the US do not regularly stock PLTs, LTOWB could also be of benefit to bleeding patients at these hospitals [6]. The cold storage of the PLTs also likely reduces their risk of bacterial contamination compared to conventional room temperature storage.

Over the last decade, balanced transfusion of RBCs, plasma and PLTs has become the standard of care for initial resuscitation of massively bleeding patients [7]. However, adhering to a 1:1:1 ratio upon initial presentation of a severely injured trauma patient is difficult.
Avoiding this issue of ‘ratio confusion’ is an attractive benefit of LTOWB and may improve outcomes.

Another reason why WB is ‘greater’ than the sum of its parts is the safety advantage that it confers over conventional products, especially RBCs. It is known that the emergency department is one of the sites in the hospital where pre-transfusion testing samples are most frequently miscollected [8], i.e. the blood in the tube does not come from the patient whose name is on the label affixed to the tube. This sort of collection error can lead to ABO mistransfusions, an important cause of transfusion-related death [9,10]. Rather than switching the patient to ABO-type specific RBCs, with the inherent risk of mistransfusing a unit during a hectic resuscitation, using group O WB provides RBCs that are universally compatible. Since the group O WB units that are issued when the patient’s ABO group is unknown must contain low titers of anti-A and anti-B [11,12], a unit of low titer group O WB (LTOWB) is essentially a universal donor product.

WB is also ‘lesser’ than components in that there is less saline-based fluid in a unit of WB compared to one unit each of RBC + plasma + PLT [13]. WB contains 70 ml of CPD, whereas the combination of one unit each of RBC, plasma, and PLTs contains 180 ml of CPD and additive solution. During a resuscitation where many blood products are transfused, this volume difference can become substantial. Also, WB exposes the recipient to fewer donors compared to using three conventional components from different donors.

Given these advantages over conventional components, it is not surprising that the use of LTOWB is increasing in the US [14–19] and around the world [20–22] for the resuscitation of bleeding civilian trauma patients. At the same time, data are accumulating about the clinical outcomes of patients who were resuscitated with LTOWB, although ongoing randomized trials have yet to be reported (see below) [23]. This paper will review the data on the serological safety, i.e. the chance that hemolysis will occur when cold stored LTOWB is transfused to a non-group O recipient, and the morbidity and mortality clinical outcomes of pediatric and adult civilian trauma patients who were resuscitated with LTOWB compared to those resuscitated exclusively with conventional components (Table 1).

**Adult trauma patients**

**Serological safety**

Serological safety refers to the probability that hemolysis, i.e. the premature destruction of RBCs in circulation, will ensue when a patient is transfused with ABO-incompatible plasma. Historically, the main risk for hemolysis came from transfusing ABO-incompatible PLTs [24], although the literature on the early civilian experience of transfusing WB before a low titer requirement was instated for recipients of unknown ABO group also describes several cases of hemolysis [25]. Group O plasma contains anti-A and anti-B, which can bind to the corresponding antigens on the RBCs of recipients who are non-group O and cause the destruction of the RBCs by activating complement or stimulating macrophage phagocytosis. WB that is administered before the recipient’s ABO group is known, as would be the case in the pre-hospital transfusion setting, must be group O so as to be compatible with the recipient’s anti-A and/or anti-B and it also must contain a low titer of both anti-A and anti-B [12]. However, because ‘low titer’ does not mean zero titer of these antibodies, the risk of hemolysis amongst non-group O recipients of LTOWB still theoretically exists.

In an early report of a civilian LTOWB program, the mean (SD) plasma haptoglobin concentration that was measured on the day following the transfusion of LTOWB in 7 non-group O trauma patients was 25.1 (9.3) mg/dl, which was slightly below the laboratory’s reference range of 30–200 mg/dl. The number of LTOWB units transfused to these 7 patients was not reported, although the median (range) quantity of incompatible plasma transfused to the entire cohort of LTOWB recipients (n=47) was 1000 (200–10,400) ml. There were not any transfusion reactions reported to the blood bank on the 47 LTOWB recipients in this study. The titer threshold at this institution at the time of these transfusions was <50 [26]. Several other studies from the same institution using the same LTOWB titer threshold did not find significant differences in an assortment of biochemical markers such as lactate dehydrogenase (LDH), total bilirubin, haptoglobin, creatinine, and potassium concentrations between injured group O and non-O LTOWB recipients [27–29]. It should be noted that most of the recipients in these studies received a median of between approximately 1–4 units with few patients receiving >6 units, so the demonstration of serological safety from these studies applies only to those receiving a modest dose of LTOWB with a titer <50.

Several other studies have also investigated the serological safety of LTOWB. Williams et al. investigated several of the biochemical markers of hemolysis amongst injured civilian recipients of 1–2 LTOWB units [30]. The titer threshold was <256. The authors found that the median 24-hour bilirubin concentration was actually significantly lower amongst the 198 LTOWB recipients compared to the 152 component therapy patients who did not receive LTOWB during their resuscitation [median (IQR) 0.7 (0.5–1.1) vs 1.1 (0.7–2.5), respectively, p=0.014]. There were not any differences in the other measured hemolysis markers (LDH, haptoglobin, creatinine, and potassium concentrations) at any of the 4 study time points. Similarly,
in a study of injured civilian patients who received a median (IQR) of 6.5 (3–11) LTOWB units during their resuscitation (titer threshold <200), there were not any differences in any of the biochemical markers of hemolysis (potassium, creatinine, hemoglobin, and total bilirubin concentrations) that were measured at 5 different time points during the hospitalization between the 42 LTOWB recipients compared to the 83 conventional component recipients [31]. However, the authors of both of these studies did not stratify the LTOWB recipients by their ABO group, so it was not possible to compare the hemolysis markers between the group O LTOWB recipients who would not have been at risk for hemolysis vs. non-group O LTOWB recipients who could have hemolyzed.

Lastly, in a case report featuring a 69-year old male who was struck by a car and received 38 units of LTOWB (titer threshold <200), 13 units of RBCs, 12 units of plasma, and 2 doses each of PLTs and cryoprecipitate in his first 12 h of admission, no clinical evidence of a hemolytic reaction was reported and his bilirubin concentration was normal during and immediately after his resuscitation [32]. The patient was reported to have been group O, but the report did not detail when the sample for pre-transfusion testing was procured during the resuscitation, thus, his true ABO group might have been obscured [33] by the 51 (presumably) group O RBC unit-equivalents that he received; if his autologous group was O, he would not have been at risk for hemolysis from the plasma in the LTOWB.

A final note on incompatible plasma transfusion from any source: two studies have evaluated the mortality risk amongst trauma patients who received incompatible plasma transfusions during their resuscitation [34,35]. Neither study found an increased risk of mortality amongst the recipients of incompatible plasma compared with the recipients of compatible plasma [34,35]. However, the authors of one of these studies did not stratify the LTOWB recipients by their ABO group, so it was not possible to compare the hemolysis markers between the group O LTOWB recipients who would not have been at risk for hemolysis vs. non-group O LTOWB recipients who could have hemolyzed.

Clinical outcomes

1. Mortality

As shown in Table 2, there have been multiple investigations into the effect of LTOWB transfusion on mortality in injured civilian adult patients compared to those who received conventional components during their resuscitation. Most studies did not reveal
Table 2. Mortality and non-mortality clinical outcomes between the LTOWB and conventional component groups in the studies analyzed. Studies that only analyzed biochemical markers of hemolysis are not included. A significant p-value indicates a significant difference in favor of the LTOWB group unless indicated otherwise. Blank cells indicate that the study did not evaluate this parameter. The italicized studies are of pediatric patients. ARDS = acute respiratory distress syndrome, CCT = conventional component therapy, ICU = intensive care unit, IQR = interquartile range, LOS = length of stay, LTOWB = low titer group O whole blood, MODS = multi-organ dysfunction score, NS = not significant, PLT = platelet, RBC = red blood cell.

| Reference Year | Mortality benefit (LTOWB vs CCT) | Other Clinical outcomes |
|----------------|---------------------------------|------------------------|
|                | ED 6-hour 24-hour 28-day 30-day | Overall/in-hospital     |
| 44* 2013       | p=0.83                          | p=0.26                 | NS for all comparisons |
| 26 2016        | p=0.74                          | p=0.27                 | NS for all comparisons |
| 13 2018        | p=0.33                          | p=0.24                 | NS for all comparisons |
| 40 2019        | p=0.039                         | p>0.05                 | NS for all comparisons |
| 37 2020        | p=0.006**                       | p=0.011***             | p=0.013*** |
| 31 2020        | p=0.8                           | p=0.2                  | NS for all MODS comparisons |
| 30 2020        | p=0.047***                      | p=0.059~               | NS for all clinical outcomes #
| 39 2021        | p=0.04~                         | p=0.22                 | Significantly lower ARDS and ventilator days in overall cohort of LTOWB patients |
| 55 2021        | p=0.45~                         | p=0.05~                | NS for all comparisons #
| 42^ 2021       | p=1.00                          | p=0.43                 | Significantly faster time to receive LTOWB than a single unit of RBC, plasma, and PLT |
| 1 2018         | p=0.835                         | p=0.43                 | NS for all mortality comparisons #
| 48 2020        | p=0.40                          | p=0.60                 | NS for all comparisons except for significantly shorter ICU LOS and ICU-free days, both p=0.03
| 47 2021        | p=0.60                          | p=0.502                | p=0.021 for Ventilation days, NS for other outcomes |
| 46 2021        | p=0.546                         | p=0.502                | NS for all comparisons |

*Intent-to-treat analysis, see text for other analyses; platelet-depleted WB, patients were supplemented with conventional PLTs after 6 WB units.
**Logistic regression analysis, see text for details.
^Also fewer complications and shorter hospital LOS for LTOWB recipients stratified by blunt and penetrating injuries.
***Multivariate analysis.
~Adjusted mortality analysis; see text for survival analysis.
^Comparisons are for the propensity score match analysis; see text for coarsened exact match analysis.

a survival benefit to receipt of LTOWB, which is not surprising given the relatively small number of patients studied, the generally low number of LTOWB units administered, and the approximately 24% hemorrhagic mortality rate in adult trauma at 30 days [36]. However, there were several studies that showed an association between LTOWB transfusion and improved survival in this population.

Hanna et al. reviewed the American College of Surgeons Trauma Quality Improvement Program (TQIP) database between 2015–2016 and analyzed the outcomes of injured adults ≥18 years of age who received LTOWB during their resuscitation compared to recipients of conventional components [37]. It should be noted that the AABB Standards at that time required that WB transfusions be ABO-identical to the recipient, thus few institutions in the US were using this blood product for trauma resuscitation, and those that were using it tended to use small quantities of the product per patient [11]. This analysis found 280 LTOWB recipients; 95% of these patients received 1 unit of LTOWB and 5% received 2 units. Despite this relatively small volume of LTOWB, the univariate and multivariate analyses revealed significantly reduced 24-hour and in-hospital mortality for the LTOWB recipients compared to those who only received conventional components. A sub-analysis also showed that LTOWB receipt was associated with significantly lower 24-hour and in-hospital mortality amongst those with penetrating injuries, but only with lower 24-hour mortality amongst those with blunt injuries.

Shea et al. performed a single center prospective observational study of 44 adult LTOWB recipients and 42 conventional component recipients and found in their adjusted model that LTOWB receipt was associated with a significantly lower odds ratio (OR) of 24-hour mortality but not 28-day mortality [38]. LTOWB transfusion was associated with a significantly higher OR of both 24-hour and 28-day survival. The median (IQR) volume of LTOWB transfused was 23.6 (11.9–45.2) ml/kg, or approximately 3.8 units in an 80 kg person. In a multivariate logistic regression model, this study also found that LTOWB recipients with a mean clot firmness (MCF) ≤60 mm had improved 24-hour and 28-day survival compared to recipients of conventional components with a similar clotting derangement.

Williams et al. analyzed 198 injured adults who received LTOWB either in the pre-hospital or in-hospital phases of the resuscitation [30]; 25 patients received LTOWB in both phases. All LTOWB recipients in the pre-hospital phase received a maximum of 2 units, while 91% of those who received LTOWB while in the hospital received a maximum of 2 units. While
there was no difference in survival at 30-days in their univariate analysis between the LTOWB and conventional components groups, a multivariate regression analysis found that LTOWB transfusion was significantly associated with improved 30-day survival.

A single-center, prospective observational study, where the patients were effectively randomized to receive LTOWB or conventional components based on the even- or odd-nature of the calendar day on which they were injured, analyzed 73 LTOWB recipients and 180 conventional components recipients [39]. This year-long study found higher mortality in the ED amongst the entire LTOWB group compared to the entire conventional (components group 4.1% vs. 0.6%, respectively, \(p=0.04\)) although the differences in OR, ICU and overall mortality were not significantly different between these 2 groups, and LTOWB receipt was not associated with mortality in their multivariate Cox regression analysis (HR=1.25, \(p=0.55\)). Conversely, Hazelton et al. found higher ED mortality amongst 182 conventional component recipients compared to 91 LTOWB recipients in their propensity-score matched study (8.8% vs. 2.2%, \(p=0.039\)) [40]. This mortality difference was no longer apparent at both 24-hours and 30-days (\(p>0.05\)).

Lastly, a review of the American National Trauma Data Bank in 2009 found that amongst 1745 injured adults between 18–45 years of age who had an injury severity score (ISS) >25 and received a transfusion during their resuscitation, receipt of components (n=1662) was associated in a multivariate analysis with a 3-times higher rate of death than the WB recipients (n=83) [41]. The quantity of blood products transfused was not recorded in the database. The WB transfused in this study would likely have been ABO-specific as the regulations did not specify the need for LTOWB when the recipient’s ABO group was unknown, and civilian trauma resuscitation protocols have evolved significantly since 2009. Nevertheless, WB was associated with reduced mortality compared to components.

It is important to note that none of these studies were randomized controlled trials (RCT) that were specifically powered to detect a mortality difference at a specific time point. There are several prospective RCTs comparing the use of LTOWB to conventional component therapy in massively bleeding trauma patients that are in various stages of completion. The results of a recently completed single center pilot study of LTOWB transfusion to injured patients who were transported to the hospital by helicopter, the Pragmatic Prehospital Group O Whole Blood Early Resuscitation Trial (PPOWER; clinicaltrials.gov identifier: NCT03477006), are expected in late 2021. This US pilot trial’s primary outcome was 28-day all-cause mortality in patients who received two units of LTOWB in the pre-hospital setting along with up to four more LTOWB units once the patient arrived at the hospital compared to those who received the standard of care for pre-hospital resuscitation followed by fixed ratio conventional component resuscitation in the hospital. The standard of care could have been either saline or RBCs alone depending on the practice at each helicopter base. A more definitive multicenter trial to be performed across the US, known as Type O Whole Blood and Assessment of Age During Prehospital Resuscitation Trial (TOWAR; clinicaltrials.gov identifier: NCT04684719), will examine 30-day mortality as its primary outcome with shorter mortality time points (such as 3-, 6- and 24-hour) as well as some of its secondary outcomes. Its design will be based on the PPOWER study, and it is expected to start accruing patients in mid-2021. It is expected to last for approximately 4 years. These trials should yield definitive answers about the potential mortality benefit of LTOWB transfusion in trauma patients who are able to receive LTOWB starting in the pre-hospital phase of the resuscitation. In the meantime, the results of these observational trials suggest that the receipt of LTOWB does not result in increased overall mortality amongst recipients; in fact in some studies mortality was reduced compared to conventional component recipients, and given the substantial logistical benefits of LTOWB, its continued use appears to be safe from this perspective in injured patients.

2. Non-mortality clinical outcomes

Several studies have investigated non-mortality clinical outcomes amongst injured recipients of LTOWB compared to conventional components recipients (Table 2). Most of these studies have found that there were no differences in any of the non-mortality clinical outcome parameters, i.e. receipt of LTOWB does not lead to increased morbidity compared to receipt of conventional components. For example, Yazer et al. performed both a propensity-score matching and a coarsened exact match study of transfused trauma patients [42]; there were 92 and 34 patients in both the LTOWB and conventional component groups in these analyses, respectively. In the propensity-score match study the median (IQR) volume of LTOWB transfused was 4 (3–6) units, while in the coarsened exact match study the volume was 4 (3–8) units. Both studies analyzed multiple clinical parameters such as ventilator free days, several permutations on the multiorgan dysfunction score (MODS), kidney injury, sepsis, venous and arterial thromboembolism, as well as other clinical outcomes. The only significant difference between the LTOWB and conventional component recipients in both analyses was a lower delta MODS amongst the LTOWB recipients in the coarsened exact match compared to the conventional component recipients, otherwise there were no differences between the recipient groups. Similarly, Shea et al. found no significant differences in the total MODS
score or in its individual organ-system components between LTOWB and conventional component patients [38].

However, some studies found differences in some parameters between these 2 groups of recipients. For example, Hanna et al. in the multivariate analysis of their TQIP database study found that receipt of LTOWB [median (IQR) 1(1–1)] unit] was associated with a significant reduction in complications (p=0.013), which collectively included such as acute kidney injury (AKI), acute respiratory distress syndrome (ARDS), deep venous thrombosis (DVT), pulmonary embolism (PE), myocardial infarction, cardiac arrest, unplanned intubation, pneumonia, sepsis, and cerebrovascular accident compared to the conventional component group [37]. This relationship persisted when patients were stratified by the mechanism of injury, i.e. blunt or penetrating, for the complications that were studied in this subgroup analysis (AKI, ARDS, DVT, PE). They also found a shorter length of hospital stay amongst LTOWB recipients in this subgroup analysis.

The single center year long-study by Duchesne et al. found fewer ventilator days amongst the overall LTOWB cohort compared to the overall conventional therapy cohort [39], as well when the patients were stratified by injury mechanism. This study also found that 0% of LTOWB recipients developed ARDS whereas 6.1% of the control group had this complication (p=0.03). There were no other differences between the two groups identified in the other morbidity parameters they analyzed.

Like the mortality outcomes, it is likely that these observational studies were not sufficiently powered to detect differences in these clinical outcomes, so the definitive evidence for the safety for LTOWB transfusion will have to wait until the appropriate large, multicenter randomized trials are performed. One such study that might contribute important data on the safety of LTOWB transfusion is in an advanced stage of planning in France; known as the STORHM trial (Sang Total pour la Réanimation des Hémorragies Massives), it will employ a non-inferiority design to compare LTOWB to conventional blood components transfused in a 1:1:1 ratio in severely bleeding trauma patients [43]. The primary endpoint will be a thromboelastograph parameter (maximum amplitude, MA) assessed at the sixth hour after admission. Secondary endpoints will include early and overall mortality, lactate clearance (a reflection of the effectiveness of resuscitation) and organ failure at 24 h post-admission. This trial will begin recruiting 200 patients at six French trauma centers in the summer of 2021. However, like the mortality outcomes described above, it is reassuring that the trends in morbidity outcomes in the studies that have been reported to date are at least neutral, i.e. LTOWB is not often associated with a high rate of adverse non-mortality clinical outcomes, and in at least a few studies, these outcomes favor the transfusion of LTOWB to injured civilians.

3. Transfused blood product volumes

Many of the studies on the outcomes following LTOWB transfusion reported on the quantity of blood products transfused (Table 3). One such study was an RCT of modified WB [44]; the authors were required to use leukoreduced WB but did not have access to a PLT-sparing leukoreduction filter, so every 6th unit of WB or RBC that was transfused was supplemented with a conventional room temperature apheresis PLT. In the intent to treat analysis there were 55 modified WB patients and 52 who received conventional components. In this analysis there was not a significant difference in the quantity of blood products transfused between these two groups at 24-hours. The same trends at 24-hours were found in the per-protocol analysis of 36 and 41 patients, respectively. However, when the patients with severe traumatic brain injury were excluded, there were significant reductions in the median quantity of RBCs and plasma units transfused, as well as the median total number of blood products transfused in 24 h, amongst the modified WB group (n=33) compared to the conventional component recipients (n=34). It was not clear how many modified WB units were transfused in this study as this number was not reported separately from the total number of individual components.

Duchesne et al. also found significant reductions in the number of RBC and plasma units, but not PLT units, transfused in the first 24 h of admission amongst the LTOWB recipients although it is not clear if the RBC and plasma components from the LTOWB were enumerated amongst the individual RBC and plasma units that were also transfused to some LTOWB recipients [39]. Gallaher et al. indicated that each unit of LTOWB was counted along with the individual blood components and similarly found that amongst the LTOWB recipients [31], there were significantly fewer RBC and plasma units, but not PLT units, transfused compared to the conventional component recipients at 24-hours. The LTOWB patients in this study received a median (IQR) of 6.5 (3–11) LTOWB units. Similarly, Williams et al. found significantly fewer RBC units were transfused to the LTOWB recipients when they were in the ED compared to the conventional component group [30], and fewer RBC and plasma units were transfused to the LTOWB recipients after they left the ED. In fact, the LTOWB recipients received significantly fewer total products after they left the ED compared to the control group and this relationship was also significant in their multivariate analysis. Interestingly, Yazer et al. [26] reported that the LTOWB recipients actually received significantly higher total volumes of plasma and PLT compared to the
Table 3. Volume of blood products transfused between the LTOWB and conventional component groups in the studies analyzed. A significant p-value indicates a significant difference in favor of the LTOWB group unless indicated otherwise. Blank cells indicate that the study did not evaluate this parameter. The italicized studies are of pediatric patients. ED = emergency department, PLT = platelet, RBC = red blood cell.

| Reference | Year | Reduced blood transfusion volumes (LTOWB vs CCT) | RBC | Plasma | PLT |
|-----------|------|--------------------------------------------------|-----|--------|-----|
| 44*       | 2013 | 24 h: p=0.78                                    | 24 h: p=0.71 | 24 h: p=0.41 | 24 h: 0.61 |
| 26        | 2016 | 24 h: p=0.078                                   | 24 h: p=0.012** | 24 h: p=0.001** | 24 h: p<0.01** |
| 13        | 2018 | 24 h: p=1.00                                    | 24 h: p=0.011* | 24 h: p=1.00 | 24 h: p<0.01** |
| 40        | 2019 | 4 h: p=0.061, 24 h: 0.269                       | 4 h: p=0.503, 24 h: p=0.107 | 4 h: p=0.322, 24 h: p=0.078 |
| 37        | 2020 | 24 h: p=0.542                                   | 24 h: p=0.791 | 24 h: p=0.169 |
| 31        | 2020 | 24 h: p=0.003                                   | 24 h: p=0.01 | 24 h: p=0.20 | 24 h: p=0.19 |
| 30        | 2020 | ED RBC: p=0.04, post-ED:                        | ED RBC: p=0.005, post-ED: | ED RBC: p=0.216, post-ED: | ED: p=0.001 |
|           |      | p=0.001                                         | p=0.018 | p=0.231 |
| 38        | 2020 | 24 h: p<0.001                                   | 24 h: p=0.04 | 24 h: p=0.96 | 72 h: p=0.093 |
| 39        | 2021 | 24 h: p<0.001                                   | 24 h: p=0.001 | 24 h: p=0.06*** | 4 h: p<0.001, 24 h: p<0.001 |
| 55        | 2021 | 4 h: p=0.04, 24 h: p=0.77***                    | 4 h: p=0.50, 24 h: p=0.17*** | 4 h: p<0.10, 24 h: p=0.06*** | 4 h: p<0.001, 24 h: p<0.001 |
| 42~       | 2021 | 24 h: p=0.251                                   | 24 h: p=0.639 | 24 h: p=0.035 |
| 48        | 2020 | 24 h: p=0.01                                    | 24 h: p=0.04 | 24 h: p=0.03 | 24 h: p=0.08 |
| 47        | 2021 | 24 h: p=0.12                                    | 24 h: p=0.84 | 24 h: p=0.63 | 24 h: p=0.74 |
| 46~       | 2021 | 4 h: p=0.008, 24 h: p<0.001                      | 4 h: p<0.001, 24 h: p<0.001 | 4 h: p<0.001, 24 h: p<0.001 | 4 h: p<0.013, 24 h: p<0.001 |

*Intent-to-treat analysis, see text for other analyses; platelet-depleted WB, patients were supplemented with conventional PLTs after 6 WB units.

**Significantly fewer units were transfused in the conventional component group.

***Comparing LTOWB + CCT to CCT.

~Comparing all 3 groups.

~Comparisons are for the propensity score match analysis; similar trends were observed for the coarsened exact match analysis.

~~~These p-values are for the median (IQR) values; the trends were identical when the data were presented as mean (SD).

conventional components group, perhaps because the LTOWB units were counted amongst the individual components in this study. Similarly, Seheult et al. [13] found that there was a significantly higher total volume of plasma and PLT components transfused to LTOWB recipients in the first 24-hours of admission compared to the conventional component controls, again perhaps because the LTOWB was enumerated along with the individual blood components.

It is important to note that in order for these differences in transfusion volumes to be meaningful, the decision to transfuse should have been consistently made based on well-adhered-to institutional guidelines that direct the administration of blood products during trauma resuscitation. If the transfusion decisions were based on the clinical team’s perception of bleeding, as they often appropriately are during trauma surgery, then an element of subjectivity is introduced because the perception of bleeding and the need to correct it with blood products can vary between clinical teams. If a reduction is significant but small in an absolute sense, such as a median difference in a certain blood component of only 1 or 2 units between the LTOWB and conventional component groups, perhaps the generalizability of this reduction is limited. This is another outcome that is perhaps best studied in the setting of a clinical trial where the transfusion guidelines are standardized and where assistance to follow these guidelines is available.

### Pediatric trauma patients

The use of LTOWB has not been as extensively studied in pediatric trauma patients as it has been in adults (Table 1). However, there are some data demonstrating its safety and efficacy in this population.

#### Serological safety

Two studies from the same institution have evaluated the serological safety of administering LTOWB. The first study included 18 injured children and found that between the group O (n=8) and non-group O LTOWB (n=10) recipients, there were no differences in the reticulocyte count, and creatinine and potassium concentrations on the day of the transfusion and on the following day [1]. The median (IQR) volume of LTOWB transfusion was 15 (9–23) ml/kg, or roughly 1.5 units of LTOWB. A more comprehensive analysis was performed amongst 21 group O and 26 non-group O injured children who received a median of 16 (10–25) ml/kg, or roughly 1.3 units for the non-group O recipients whose median weight was 40 kg [45]. This study found no statistically significant differences in any of the biochemical markers of hemolysis that were measured including LDH, total bilirubin, haptoglobin, creatinine and potassium concentrations, and the reticulocyte count between these two groups on the day of the transfusion or on the ensuing two days. The LTOWB’s antibody titer threshold in both of these studies was <50.

#### Clinical outcomes

1. **Mortality**

   There have been three studies that have evaluated clinical outcomes of injured pediatric patients who were resuscitated with LTOWB compared to those
who received conventional components (Table 2). One study analyzed the aforementioned TQIP database through the end of 2017 [46]. Patients between the ages of 1–17 years who were injured and who received any volume of LTOWB (with or without conventional components) were included, with several exceptions such as patients with burns or those in transfer between institutions. The authors performed a propensity-matched study between 135 LTOWB recipients and 270 matched patients who received only conventional components during their resuscitation, and found that there were not any differences between the groups in terms of 24-hour and in-hospital mortality. The median (IQR) dose of LTOWB was 19 (11–31) ml/kg, or roughly 1.7 units per median 44.7 kg LTOWB recipient, and the titer threshold was not provided although it might have differed between the institutions that contributed to the database.

A second propensity-score matched study performed at a single center matched 36 injured pediatric LTOWB recipients to 36 injured patients who received only conventional components during their resuscitation and similarly found that there were not any differences in in-hospital mortality between the groups [47]. This study also found that there were not any differences in the mechanisms of death between the two groups, either hemorrhage or traumatic head injury. The median (IQR) dose of LTOWB was 15 (9–23) ml/kg, or roughly 1 unit per median 32.0 kg LTOWB recipient, and the titer threshold was <50. A third propensity-score matched study from the same center also did not find differences in mortality between 28 LTOWB and 28 conventional component recipients; in this study the median (IQR) dose of LTOWB was 15 (10–22) ml/kg [48].

2. Non-mortality clinical outcomes

None of these three studies in pediatric patients found a significant difference in the hospital length of stay between the LTOWB and conventional components groups (Table 2). However, in the TQIP propensity-score matched study [46], the LTOWB patients had significantly fewer median number of days on the ventilator compared to the conventional component group, while neither of the single center propensity-score matched studies found a significant difference in this parameter, nor in the number of ventilator-free days at day 28, between the groups [47,48]. The lack of difference might have been due to the smaller number of patients in both arms of these studies; in one study, the 36 LTOWB recipients spent a median (IQR) of 1 (0–4) day on the ventilator while the conventional component therapy patients were ventilated for a median (IQR) of 4 (0–10) days, p=0.42 [47]. In the other single center study, the median (IQR) number of days on the ventilator for the 28 patients were 2 (0–5) and 1 (0–6), respectively, p=0.8 [48]. The former single center study found a significantly lower ICU LOS amongst the LTOWB recipients, [median (IQR): 3 (2–8) days vs 6 (3–14) days, respectively, p=0.03] [47], while the latter propensity matched study did not find a difference in this parameter. Both of these studies found no significant differences between LTOWB recipients and conventional component patients in terms of functional disability [47,48].

The TQIP propensity-score matched study did not find differences between the two groups in terms of the incidence of acute kidney injury, ARDS, venous thromboembolism, and sepsis; one of the single center studies also did not find differences in these parameters although ARDS was not specifically reported in this study [47]. This study also did not find differences between the two groups in several other clinical parameters including transfusion reactions, and Pediatric Logistic Organ Dysfunction (PELOD-2) scores on days 3 and 7. Furthermore, no differences in a variety of other organ failure scores were noted between the two groups [47].

However, other clinical benefits of receiving LTOWB early in pediatric trauma resuscitation compared to conventional components have been demonstrated. Leeper et al. demonstrated that the median (IQR) time from arrival at the ED until the initiation of LTOWB in 18 injured patients at a Level 1 pediatric specialty hospital was 15 (14–77) minutes, while the time taken for receipt of one unit each of RBC, plasma, and PLT in 73 injured patients resuscitated with conventional components was 303 (129–741) minutes, p<0.001 [1]. As the provision of balanced blood product transfusion early in the resuscitation is important, this study highlighted a practical benefit of using LTOWB that would likely also be of benefit to adult patients. Lastly, one of the propensity score-matched studies from the same authors showed that receipt of LTOWB in 28 injured children resulted in a faster correction of the base deficit compared to 28 propensity-score matched patients who were resuscitated with conventional components [median (IQR) 2 (1–2.5) hours vs 6 (2–24) hours, respectively; p < 0.001] [48].

3. Transfused blood product volumes

In the TQIP propensity-score matched study, the pediatric LTOWB patients received significantly fewer median and mean ml/kg of RBC, plasma, and PLT transfusions as well as fewer total blood products at both 4 and 24 h after admission (Table 3) [46]. However, one of the single center propensity-score matched studies did not find a significant difference in the median ml/kg volume of individual products, or the 24-hour total ml/kg volume of all blood products, transfused [47]. The other single-center propensity-score matched study of 28 LTOWB and 28 conventional component patients found that there was a significantly lower volume (in ml/kg) of RBCs, plasma, and PLTs transfused to the LTOWB recipients, but there was
not a difference in the 24-hour total quantity of blood products transfused [48].

While further studies on pediatric LTOWB recipients are needed to confirm the findings of these studies, it would appear that transfusing modest doses of LTOWB with a conservative titer threshold is serologically safe amongst non-group O pediatric trauma recipients. That none of these studies demonstrated a mortality benefit to LTOWB transfusion is not surprising given the relatively small number of patients studied in spite of the relatively high 28-day pediatric mortality rate in trauma (36.1%) [49]. That none of these studies demonstrated a higher incidence of adverse outcomes amongst the LTOWB recipients compared to component recipients is encouraging.

Conclusion
The literature to date on mortality changes following resuscitation with LTOWB in adult and pediatric trauma patients were not powered to detect a difference between it and conventional component therapy recipients. RCTs on the effect of LTOWB on mortality are years away from completion. However, it is important to consider LTOWB in the context of a modern trauma resuscitation – today we are more aware of the physiology of trauma and have updated our approach to trauma patients accordingly; tranexamic acid is commonly used [50], blood pressure targets (90–110 mmHg SBP, >110 mmHg for patients with brain injury) have evolved to reflect tissue perfusion requirements [51], care is taken to avoid hypocalcemia [52], blood products are provided early in the resuscitation and in a balanced ratio [36,53], at least initially, and the area of damage control resuscitation continues to be studied and refined [54,55]. LTOWB is a part of the larger process of resuscitating a trauma patient and so its effect should be analyzed in light of all of the other advances in the field, i.e. it is an important part, but not the only part, of the resuscitation. If it turns out that patient morbidity and/or mortality is not improved (but not worsened) by using LTOWB but the logistics of the resuscitation are simplified, or if using LTOWB allows the clinical team to focus on other aspects of the resuscitation that are proven to reduce mortality, these are worthwhile endpoints that justify using LTOWB.

Disclosure statement
MHY has received travel reimbursement and has given paid lectures for Terumo BCT
JBH has the following disclosures:
1. Arsenal Medical Consultant
2. Cellphire Consultant
3. Safeguard Consultant
4. Decisio Health Co-founder and Board of Directors
5. QinFlow Board of Directors
6. Zibrio Board of Directors
7. Co-inventor of the Junctional Emergency Tourniquet Tool, receiving royalties from UT Health
The other authors report no relevant conflict of interest

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