SHED PLEURAL BLOOD FROM TRAUMATIC HEMOTHORAX CONTAINS ELEVATED LEVELS OF PRO-INFLAMMATORY CYTOKINES

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ABSTRACT—Purpose: The autotransfusion of unwashed (or unprocessed) shed hemothorax blood (USHB) in trauma patients is widely assumed to be beneficial; however, the inflammatory potential of shed pleural blood has not been thoroughly studied. Since previous studies have documented marked changes in coagulation function of shed pleural blood, we hypothesized that its level of inflammatory cytokines would be elevated. Methods: A prospective observational study of trauma patients in whom cytokine levels from USHB were compared to venous samples from healthy volunteers was conducted. Differences between the cytokine content of patient-derived samples were compared to those from healthy subjects. Results: There was a statistically significant increase in pro-inflammatory cytokines (IL-6, IL-8, TNFα, GM-CSF), a pro-inflammatory Th-1 cytokine (IFNγ), and anti-inflammatory Th-2 cytokines (IL-4 and IL-10) in shed pleural blood over four hours when compared with samples from healthy controls (P < 0.05). Cytokine levels in USHB are approximately 10- to 100-fold higher compared with healthy control venous samples. Conclusions: USHB, even collected within the accepted four-hour window, contains significantly elevated cytokine levels, suggesting the potential for deleterious effects from autotransfusion. Randomized trials are needed to determine the safety and efficacy of autotransfusion in trauma patients.

KEYWORDS—Autotransfusion, hemothorax, immune, inflammation, pleural blood, trauma

INTRODUCTION

Traumatic injuries and burns are frequently complicated by the systemic inflammatory response syndrome (SIRS) (1). The immunopathologic mechanisms important in trauma and burn patient outcomes, and their links to changes in coagulation function, are poorly understood at present. Hemorrhage is the leading cause of preventable death in combat and is associated with coagulopathy (2). Thus, specific changes in the patient’s immunoinflammatory profile (as defined by immune cell phenotypes and plasma cytokines), and its interaction with coagulation status early after injury, may be related to the development of subsequent complications such as multiorgan failure.

The frequent presentation of coagulopathy in combat trauma patients requiring transfusions has been a critical challenge correlated with increased mortality. Excessive crystalloid use may exacerbate coagulopathy and recently published data demonstrate an association of better outcomes with early transfusion of balanced blood components. High transfusion ratios of fresh frozen plasma and platelets (PLT), to red blood cells were associated with higher survival but not decreased blood requirements (3). Additionally, hemostatic resuscitation incorporating platelets and plasma within the first 6 h is associated with improved 24-h and 30-day survival in combat casualties requiring massive transfusion (4). Most recently, a large randomized trial showed that a high transfusion ratio resulted in improved early hemostasis, though not improved mortality (5). In the light of the benefits of early blood-based resuscitation, autotransfusion has become an attractive option in combat casualties with significant blood loss due to the logistical challenges of supplying blood products in the deployed setting and the possibility of minimizing alloergic blood transfusions (6–9). The American Association of Blood Banks guidelines and standards for perioperative autologous blood collection and administration permit use of hemothorax blood, if autotransfused within four hours of collection (10). This strategy has been adopted based on the assumption that autotransfused blood is a readily available and safe source of red blood cells. Our group previously examined the composition of shed pleural blood and found that HCT, PLT count, and coagulation factor levels were markedly abnormal and lower than those found in the subject’s own venous blood (11). This was accompanied by prolonged International Normalized Ratio, activated Partial Thromboplastin Time, and thrombin time, as well as very low levels of fibrinogen, factors V and VIII, and elevated levels of D-dimer (11). Those data indicate that we also need to examine previous assumptions regarding the comparability between the patient blood collected for autotransfusion from a hemothorax collecting unit versus that...
obtained by the blood bank under optimal conditions with regard to immunologically mediated adverse events following trauma. We hypothesized that shed pleural blood has elevated levels of inflammatory cytokines compared with venous blood from trauma patients.

**PATIENTS AND METHODS**

**Subject selection**

This manuscript presents data from two separate protocols. The samples analyzed in both studies were collected from May 2010 to January 2013. A prospective study involved the descriptive evaluation of trauma patients who underwent tube thoracostomy at the University Hospital, San Antonio, TX and was approved by The University of Texas Health Science Center at San Antonio Institutional Review Board (IRB). Informed consent was obtained from trauma patients who met the following inclusion criteria: admitted to the intensive care unit with blunt or penetrating trauma; an anticipated stay of 72 h or greater; at least 18 years of age; and a diagnosis of hemothorax producing more than 50 mL of shed blood within the first four hours of thoracostomy tube placement. Exclusion criteria included the following criteria: age <18 years, pregnancy, prisoner, and placement of tube thoracostomy outside the facility. Demographics, Injury Severity Score (ISS), and the calculated probability of survival using Trauma Score Injury Severity Score (TRISS) were obtained from the hospital trauma database (Digital Innovations, Forest Hill, Md). Venous samples from healthy volunteers (USHB-C) for comparison to the shed pleural blood were drawn at the US Army Institute of Surgical Research (USAISR), San Antonio, Texas under a US Army Medical Research and Materiel Command IRB-approved protocol after informed consent was obtained. Samples from the two institutions were processed in the same way and cytokine analysis performed concurrently on the same 96-well plate (see below for full details).

Demographics, ISS, and TRISS were obtained from the hospital database (Essentris, Clinicomp Intl., San Diego, Calif). The TRISS was calculated using the first set of vital signs in the data set. Emergency department vital signs were not collected for this protocol.

Volunteers were at least 18 years of age and in good health based upon observation and self-reporting. Women known to be pregnant were excluded due to the potential for pregnancy-induced changes in immune profiles.

**Sample collection and analysis**

Unprocessed shed hemothorax blood (USHB) was collected from trauma subjects in a commercially available collection unit (OCEAN 2050 or OASIS 3650; Atrium Medical Corporation, Hudson, NH) at 1-h intervals up to four hours after thoracostomy tube placement. USHB samples were obtained from the thoracostomy drain via a sterile syringe and allocated into tubes containing NaHeparin (BD Vacutainer Heparin Tubes, BD). USHB samples were kept at 4°C until centrifugation, which occurred within 24 h of collection, and was performed at 3,000 × g for 15 min, and then repeated again. USHB plasma was stored at –80°C until further analysis. USHB-C samples were collected in NaHeparin Vacutainer tubes (BD, Franklin Lakes, NJ), centrifuged and stored at –80°C in a similar fashion.

Heparinized samples were analyzed using the Bio-Plex Pro Human Cytokine 8-plex Assay #M50-000007A (BioRad Laboratories, Hercules, CA) to measure interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, granulocyte macrophage-colony stimulating factor (GM-CSF), interferon gamma (IFNγ), and tumor necrosis factor (TNFα) and citrated samples were analyzed using the Bio-Plex Human Th1 Cytokine 11-plex Assay #LN000000TU (BioRad Laboratories). Assays were run using a Bio-Plex 200 System and Bio-Plex Manager 6.0 Software.

**Statistics**

USHB cytokine levels were compared to USHB-C and analyzed for changes over time. Data are expressed as mean ± SEM. Comparisons within and between groups were analyzed using ANOVA (SigmaPlot 11.0; Systat Software Incorporated, San Jose, CA). Further analysis employed the Dunn method, with healthy volunteer plasma serving as the control group for multiple comparisons of each time point. A P value <0.05 was considered to be statistically significant for all analyses.

**RESULTS**

**Subject populations**

Subjects enrolled were as follows: USHB (n = 62) and USHB-C (n = 4). Time points were incomplete for 33 subjects in the USHB group and, of these, 27 patients had a single USHB sample collected at the four-hour time point. This was accounted for in the analysis which included all 62 USHB subjects in each cytokine assay. The USHB demographics are listed in Table 1. None of the patients in the USHB group demonstrated exsanguinating hemothorax at the time of chest tube placement and no patient in the USHB group actually received an autotransfusion.

**Inflammatory cytokine levels in shed pleural blood (USHB)**

Inflammatory cytokines, which included IL-6, IL-8, GM-CSF, and TNFα, were analyzed in the USHB at 1 through 4-h time points and compared to the samples from USHB-C (Fig. 1). There was a statistically significant difference at all time points as compared with USHB-C. IL-8 levels in USHB-C were negligible (~4 pg/mL). In contrast, GM-CSF levels were significantly greater than control values at 2 h (~3-fold greater), and levels remained relatively constant. TNFα showed at pattern of increase similar to that of GM-CSF, again remaining relatively constant over time. The median volume in the collection unit at the time of collection was 460 mL with a minimum of 140 mL and a maximum of 2470 mL.

**Th-1 cytokine levels in shed pleural blood (USHB)**

The Th-1 cytokines that promote cell-mediated immunity including IL-2 and IFNγ were also analyzed (Fig. 2). IL-2 did not increase to a statistically significant extent in the USHB over the four-hour collection period as compared with control values. However, IFNγ levels were significantly elevated at the 4-h time point and were approximately 3-fold greater than that of USHB-C blood. The increase was also significant when comparing the four-hour to the 1-h sample (229 pg/mL) time point in the USHB.

**Th-2 cytokine levels in shed pleural blood (USHB)**

Anti-inflammatory Th-2 cytokines that include IL-4 and IL-10 also demonstrated a significant increase at each time point compared with controls (Fig. 3). IL-4 levels were negligible in USHB-C but were elevated at 1 to 3 h in USHB. IL-4 was also significantly elevated when comparing the four-hour to the 1-h time point. IL-10 levels were much greater than those observed for IL-4, and were similarly elevated in USHB compared with USHB-C. An approximate 10-fold increase in IL-10 was observed as early as 1 h, compared with USHB-C. This difference was maintained over the 4-h study period.
DISCUSSION

In our study, we found that inflammatory and Th-1 cytokines were profoundly elevated in unprocessed shed hemothorax blood (USHB) as early as 1 h after tube thoracostomy placement. Anti-inflammatory Th-2 cytokine levels were also elevated in USHB, a concerning finding, given that high levels of anti-inflammatory cytokines, such as IL-10, have been associated with a counter anti-inflammatory response syndrome (CARS) and immune suppression (12, 13). Thus, autotransfusion of USHB within the accepted four-hour window could have potentially increased the circulating cytokine burden and aggravated the trauma-induced inflammatory and immune complications.

While the current study herein compared USHB to the blood of healthy volunteers, it is important to compare USHB with stored blood products. Interestingly, others have also shown that stored blood products can contain significant levels of pro-inflammatory cytokines. Benson et al. (14) showed elevated

![Graphs showing cytokine levels](https://via.placeholder.com/150)

**Fig. 1.** Inflammatory cytokines in unprocessed shed hemothorax blood (USHB) compared with plasma collected from healthy volunteers (USHB-C). USHB was collected at 1 to 4 h after thoracostomy and concentrations of IL-6, IL-8, GM-CSF, and TNF-α were determined by Bioplex assay as described in the Patients and Methods. Data are mean ± SEM for 8 in the USHB-C group and 40, 39, 38, and 62 for 1, 2, 3, and 4 h, respectively, in the USHB group. *P < 0.05 as compared with control. GM-CSF indicates granulocyte macrophage-colony stimulating factor; IL-6, interleukin 6; TNF-α, tumor necrosis factor α.

**Fig. 2.** Th-1 cytokines in unprocessed shed hemothorax blood (USHB) compared with plasma collected from healthy volunteers (USHB-C). USHB was collected at 1 to 4 h after thoracostomy and concentrations of IL-2 and IFN-γ were determined by Bioplex assay as described in the Patients and Methods. Data are mean ± SEM for 8 in the USHB-C group and 40, 39, 38, and 62 for 1, 2, 3, and 4 h, respectively, in the USHB group. *P < 0.05 as compared with control. †P < 0.05 as compared with 1 h. IFN-γ indicates interferon gamma; IL-2, interleukin 2.

**Fig. 3.** Anti-inflammatory Th-2 cytokines in unprocessed shed hemothorax blood (USHB) compared with plasma collected from healthy volunteers (USHB-C). USHB was collected at 1 to 4 h after thoracostomy and concentrations of IL-4 and IL-10 were determined by Bioplex assay as described in the Patients and Methods. Data are mean ± SEM for 8 in the USHB-C group and 40, 39, 38, and 62 for 1, 2, 3, and 4 h, respectively, in the USHB group. *P < 0.05 as compared with control. †P < 0.05 as compared with 1 h.
levels of MCP-1 and Rantes in the plasma fraction of aged red cells (28–42 days). Interestingly similar to our findings with USHB, TNFα levels were negligible. Studies by Tasaki et al. (15) showed elevated levels of IL-1β, IL-6, and IL-8 in autologous blood stored for 5 weeks, but in contrast to USHB the levels were only in the 1 to 10 pg/mL range. Storage duration also affects whole blood cytokine concentrations. IL-1β and IL-8 have been shown to increase steadily, whereas IL-6 and TNFα are maintained at a mean low level plateau in stored blood (16). However, these changes were in lower ranges, whereas our findings herein showed a change of 3 to 10,000-fold increase in the USHB samples. Addition of packed red cells, fresh frozen plasma, and platelet concentrates to venous blood from healthy volunteers has been shown to result in a dose-dependent increase in TNFα and IL-10 release (17). This marked difference in cytokine load between stored blood and USHB strongly suggests that the transfusion of USHB is likely to have deleterious inflammatory effects. An association between transfusion and poor clinical results has been established (18). Receipt of blood products within the first 24 h after injury is an independent predictor of mortality, SIRS, ICU admission, and ICU LOS (19). The cytokine content of blood products is of concern, as this can add to the existing elevated cytokine levels in circulating blood as a result of trauma and exacerbate inflammatory complications, such as SIRS and CARS. Under normal conditions, these two components of the inflammatory response (SIRS and CARS) are tightly regulated, resulting in immune homeostasis; however, major injury can disrupt the balance between SIRS and CARS, leading to MODS. Critically injured patients requiring massive transfusion are at high risk of developing ARDS and MODS (20).

The current findings herein show that USHB contains markedly higher levels of cytokines, such as IL-6, IL-8, and IL-10, than blood from uninjured volunteers, which is assumed to be similar to blood products from a blood bank. It is likely that the immense cytokine content of the USHB is in part a result of de novo synthesis in the pleural compartment due to interaction of immune cells with the thoracic tissues, rather than a direct reflection of circulating levels. In this regard, previous studies have shown that white blood cells, hematocrit, and hemoglobin levels are comparable between patient plasma and USHB and within normal ranges (21), suggesting that circulating cytokine levels are likely to be closer to normal than what was observed in USHB. Interestingly, unwashed salvaged blood in other conditions independent of trauma has been shown to contain a wide range of inflammatory mediators including cytokines (22). For example, drainage blood from orthopedic surgery has shown 10 to 20-fold elevations in IL-1β and TNFα over that of circulating blood (23). Others have also observed marked increases in drainage blood content of IL-6 and IL-8 after knee arthroplasty (24, 25). Dalen et al. (26) have shown a 750-fold elevation in drainage blood IL-8 levels after knee or hip replacement, which is consistent with our observations for IL-8 in USHB. More recently, a study by Islam et al. (27) has shown elevated levels of a wide range cytokines and chemokines in salvaged blood from arthroplasty and suggests that such blood can reverse post-traumatic immunosuppression. Thus, the elevated levels of various inflammatory cytokines in USHB are similar to those observed from surgical wound sites and not apparently unique to the trauma patient.

The guidelines as presented by The Society of Thoracic Surgeons and The Society of Cardiovascular Anesthesiologists for peri-operative blood transfusion in cardiac surgery support institution-specific protocols screening for high-risk patients, the use drugs to minimize blood loss, devices to conserve blood and algorithms for blood transfusion (28). While these guidelines may be applicable to the controlled setting of cardiac surgery, they are only in part applicable to the chaotic setting of the trauma bay with the hemorrhaging patient or austere setting of the battlefield. Only limited studies with trauma patients undergoing abdominal or thoracic trauma surgery have examined the use of cell salvage; a technique where the individual’s own blood is removed from the body, filtered, washed, and returned intravenously (29). Bowley et al. (30) found in a small randomized controlled trial for patients with penetrating abdominal trauma that cell salvage resulted in a significant reduction in the use of allogeneic blood products with no difference in postoperative infection or mortality between the control group and those that received cell salvage blood. Recent findings by Rhee et al. (31) in a retrospective chart review suggest that the autologous transfusion of shed blood collected through chest tubes for hemothorax is safe. These findings should be interpreted with caution as a number of limitations exist including the retrospective nature of the study, the fact that the analysis was limited to only two centers and that 45% of the patients who received autologous autotransfusion of the shed blood from chest tubes also received autologous autotransfusion from the abdominal cavity, in which the blood was washed. Nonetheless, these findings suggest that the use of cell salvage may be safe, but further evaluation as to complications and outcomes, and comparison to USHB, is needed.

The study herein has several limitations. Our study is limited by the lack of analysis of circulating cytokine values in the USHB subjects. We hypothesize that the levels of many proinflammatory cytokines in USHB will be higher than that seen in the circulation. Previous published studies (32, 33) examining circulating cytokine levels in trauma patients within 4 h of admission showed markedly lower levels than what we observed in the USHB. In these studies IL-6 and IL-8 levels were in the 25 to 100 pg/mL range. USHB levels of these cytokines were in the range of 10,000 and 400 pg/mL for IL-6 and IL-8, respectively. Importantly, these circulating cytokine levels were collected at times comparable to USHB. In contrast, TNFα, GM-CSF, IL-10, and IFNγ levels were comparable to USHB (5–25 pg/mL). The comparison of USHB samples with published values of plasma from trauma patients suggests that USHB cytokine values are markedly greater, but future studies will be needed to verify this concept. In addition, the potential role of the drainage device, placement of the chest tube, and injury location and severity in cytokine release have not been evaluated. Nonetheless, the data herein taken together with the previously published coagulation data raise questions about the role of hemothorax autotransfusion in trauma resuscitation.

Continued work is needed to further elucidate the clinical impact of autologous salvage products from the thoracic cavity, given that previous studies demonstrated decreases in platelets,
fibrinogen, and other factor levels (11, 34, 35). In the light of the findings of our previous study and those of others, hemo-
thorax autotransfusate cannot be considered a hemostatic prod-
uct (11). In summary, our study demonstrates that salvaged blood from the thoracic cavity, if autotransfused, has the potential to increase the cytokine load for the patient, possibly exacerbating pathophysiological consequences, such as ALI, ARDS, and end-organ injury, as elevated circulating levels of IL-6 and IL-8 early after injury have been associated with multiple organ failure (36). Based on these observations and our previous findings, we conclude that use of autologous hemothorax blood in trauma resuscitation should undergo further study in randomized trials, perhaps in comparison with washed, cell saver blood, to determine the safety and efficacy of this practice in a patient population known to be susceptible to immunoinflammatory complications.

REFERENCES

6. Brown CV, Foulkrod KH, Sadler HT, Richards EK, Biggan DP, Czysz C, Manuel R, Moldawer LL: Sepsis syndromes: understanding pathophysiological consequences, such as ALI, ARDS, and end-organ injury, as elevated circulating levels of IL-6 and IL-8 early after injury have been associated with multiple organ failure (36). Based on these observations and our previous findings, we conclude that use of autologous hemothorax blood in trauma resuscitation should undergo further study in randomized trials, perhaps in comparison with washed, cell saver blood, to determine the safety and efficacy of this practice in a patient population known to be susceptible to immunoinflammatory complications.

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