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An experimental model of hemothorax autotransfusion: impact on coagulation

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Abstract

BACKGROUND: Traumatic hemothorax (HTX) has been demonstrated to predictably contain low fibrinogen, low hematocrit, and low platelet counts. When analyzed on its own, shed HTX demonstrates coagulopathy. However, when mixed with normal pooled plasma (NPP) at physiologically relevant dilutions, HTX demonstrates accelerated coagulation. We hypothesize that when HTX is mixed with a patient’s own plasma, the mixture will demonstrate hypercoagulability. The accelerated coagulation of this mixture would have important implications for the autotransfusion of HTX as a method of resuscitating a trauma patient.

METHODS: Adult trauma patients from whom greater than 140 mL of HTX was evacuated within 1 hour of tube thoracostomy were included. HTX was sampled at 1 hour after evacuation, and a portion of the sample was centrifuged and stored as frozen plasma for later analysis. The remainder of the sample was analyzed (coagulation, hematology, electrolytes), and values were compared with concurrent venous values extracted via chart review. A citrate tube containing the patient’s venous blood was additionally spun down and frozen for subsequent mixing study analysis. Coagulation was further evaluated by mixing serial dilutions of the previously frozen HTX with NPP. Additionally, the previously frozen HTX was mixed in serial dilutions with the previously frozen sample of patient plasma (PTP).

RESULTS: Subjects (10) were enrolled based on inclusion criteria and collection of a discarded venous sample. In HTX samples analyzed alone, no thrombus was formed in any coagulation test (activated partial thromboplastin time [aPTT] > 180). The median aPTT value of PTP alone was 25.5. In 1-hour specimens mixed at a clinically relevant dilution of 1:4, HTX mixed with NPP had a median aPTT value of 26.0, whereas HTX mixed with PTP had a median aPTT value of 21.7. Thus, the mixture of HTX + PTP demonstrated a statistically significantly lower aPTT than the mixture of HTX + NPP ($P = 0.01$). Additionally, the mixture of HTX and PTP shows a statistically significantly lower aPTT value than PTP alone ($P = 0.03$), indicating a hypercoagulable state.

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The safety and efficacy of autotransfusion of hemothorax (HTX) has been investigated in recent years, primarily because of the risks and challenges presented by allogeneic transfusion. Recent evidence has shown that the use of packed red blood cell (RBC) units is directly related to increased 30-day mortality, acute respiratory distress syndrome, nosocomial infection rates, and hospital length of stay. Additionally, banked RBCs have been shown to have a “storage lesion” including a reduction in 2,3-diphosphoglycerate that impairs oxygen delivery when compared with fresh RBCs. The use of banked blood products also puts a strain on blood banks and presents a challenge to facilities that are without the resources to maintain a large blood bank.

HTX blood is an attractive source of RBCs in a trauma patient, as it is an immediately available and compatible blood supply. However, our prior research has shown that traumatic HTX is a reasonable source of RBC mass. HTX blood invariably demonstrated coagulopathy as measured by international normalized ratio and activated partial thromboplastin time (aPTT), which appears to be because of decreased levels of fibrinogen as well as depletion of other important components of the clotting cascade such as Factor V. Other work with unwashed shed blood in the settings of cardiothoracic surgery and orthopedics mirror these findings and have also found evidence of activated anticoagulation factors, fibrin degradation products, and elevated concentration of inflammatory mediators.

Additionally, our previous work sought to determine if the characteristics of HTX change over time. This information is pertinent to the practice of autotransfusion, as it could modify how long after drain placement HTX would remain a viable agent for resuscitation. We discovered that the hematologic characteristics of traumatic HTX are unchanged at hours 1, 2, 3, and 4 post drain placement.

Finally, in an attempt to determine the cause of coagulopathy demonstrated by shed pleural blood, in our previous work we performed mixing studies at serial dilutions with normal pooled plasma (NPP). We discovered that not only does HTX blood form a clot when mixed with NPP, but the mixture demonstrates a paradoxical hypercoagulability relative to the NPP.

The purpose of this study was to create an in vitro model of HTX autotransfusion to determine the likelihood of inducing a hypercoagulable state in a trauma patient. We hypothesized that when autotransfusion is simulated by mixing a patient’s own plasma with HTX blood, the mixture will demonstrate hypercoagulability.

**Patients and Methods**

**Patient selection**

This study was conducted during a 15-month period at University Hospital, San Antonio, TX, an American College of Surgeons-verified level 1 trauma center. The study was approved by the UTHSCSA institutional review board. Subjects were considered for enrollment if greater than 140 mL of HTX was drained in the first hour after thoracostomy. Subjects less than 18 years old, pregnant, known prisoners at the time of thoracostomy, or with tube thoracostomy performed at an outside institution were excluded from enrollment. Evacuated HTX was collected from either an autotransfusable collecting unit (Oasis 2050 ATS; Atrium Medical Corporation, Hudson, NH) or a non-autotransfusable collecting unit (Oasis 3650 ATS; Atrium Medical Corporation), with the type of collection device determined by nonstudy providers.

**Sample collection**

At 1 hour after chest tube placement, a sterile 50 cc syringe was used to withdraw 35 cc of evacuated HTX from the thoracostomy collection chamber needleless access port. After removal from the thoracostomy collection chamber, the evacuated HTX was transferred into laboratory collection tubes containing either no preservative or one of the following: sodium citrate (3.2% for a 1:9 anticoagulant to blood ratio), lithium heparin, or ethylenediaminetetraacetic acid (EDTA). Of the 35 cc collected, half was sent to the hospital core laboratory for coagulation, hematology, and electrolyte profile analysis. A citrate tube containing HTX blood was spun twice at 3,000g for 15 minutes and plasma supernatant was frozen for subsequent mixing study analysis. No samples collected in EDTA or heparin were stored for use in mixing studies. Additionally, a citrate tube containing patient venous blood collected at the time of admission was spun down and frozen for subsequent mixing study analysis. Concurrent venous blood values from the Emergency Center were obtained from the subjects’ charts at the time point closest to tube thoracostomy. This venous sample was then compared with the evacuated HTX sample.
Mixing studies

Frozen citrated evacuated HTX plasma and patient plasma were thawed at 37°C. Patient plasma was mixed with evacuated HTX plasma at a 1:4 dilution. NPP was also mixed with evacuated HTX plasma at a 1:4 dilution. Dilutions were performed by hand using a precision micropipette. The 1:4 dilution was selected as it simulates a clinically relevant transfusion of 1,250 mL in a patient with 5 L circulating blood volume. These mixtures were analyzed for aPTT (SynthASil; Instrumentation Laboratories, Orangeburg, NY) using an ACL TOP (Instrumentation Laboratories, Bedford, MD), with NPP (Precision Biologic, Dartmouth, NS) run alone to act as a control.

Data analysis

Data are expressed as mean ± standard error of the mean. Comparisons between groups were analyzed using ANOVA with SigmaPlot 11.0 software (Systat Software Incorporated, San Jose, CA). A P value of less than .05 was considered to be statistically significant for all analyses.

Results

Patient population

Ten patients were enrolled over a 15-month period and 100% were men. Median age was 36 (20 to 63) years with 2 patients lacking identifiable name and age on admission. The mechanism of injury distribution was as follows: 4 gunshot wounds, 3 stab wounds, 1 motor vehicle collision, 1 fall, and 1 assault. The requirement to be enrolled into this study was a chest tube output greater than or equal to 140 mL in the first hour. Of the 10 subjects included in this study, only 2 had a total output of greater than 1,000 mL. None of the patients demonstrated exsanguinating HTX at the time of chest tube insertion and no patient actually received an autotransfusion.

Hematology

Evacuated HTX white blood cell count was shown to be depleted when compared with patient venous laboratory values (Table 1). However, the white blood cell still fell within normal physiologic range. Platelet count of the evacuated HTX was shown to be significantly reduced compared with venous laboratory values. The mean platelet count of the evacuated HTX was 16% of the platelet count of the patient venous sample. Hemoglobin and hematocrit values of the evacuated HTX were not significantly different from the values of the patient venous sample.

Chemistry

The measured mean electrolyte concentrations of the pleural blood were found to be within physiologic range (Table 2). Evacuated HTX potassium, anion gap, and blood urea nitrogen were shown to be significantly elevated relative to the patient plasma values. Sodium, chloride, carbon dioxide, and calcium levels were shown to be comparable between the 2 samples.
Coagulation

International normalized ratio and aPTT were shown to be immeasurably high in all HTX samples (Table 3). Fibrinogen levels were undetectable, and D-Dimer was immeasurably high in all samples. Factor V was immeasurably low in all samples.

Mixing studies

The 1:4 mixture of HTX with NPP demonstrated accelerated coagulation relative to the HTX blood as well as the NPP alone (Fig. 1). However, the mixture did not demonstrate acceleration of coagulation relative to the patient venous laboratory values. Conversely, the 1:4 mixture of HTX with patient plasma demonstrated significant hypercoagulability relative not only to the HTX alone, but also to the patient venous coagulation values ($P = 0.03$) as well as the 1:4 mixture of HTX with NPP ($P = 0.01$).

Comments

One of the objectives in this study was to replicate the analysis of the composition of HTX and demonstrate consistency with our findings in previous studies. The leukopenia and thrombocytopenia of the HTX samples have been consistent across all 3 studies. Our previous studies demonstrated a mild degree of anemia of the HTX sample relative to the venous sample that was not shown in this study, likely because of small sample size as the values are similar. As demonstrated in our previous studies, all chemistry values fall within normal range. Finally, consistent with our previous work, evacuated HTX was shown to invariably demonstrate coagulopathy, defibrination, and elevated levels of D-Dimer.

Another objective in this study was to create an in vitro model of HTX autotransfusion to analyze the possible induction of a hypercoagulable state in a trauma patient. Our mixing studies focused on the clinically relevant dilution of 1:4 HTX mixed with patient plasma (70 kg male, 5 L total blood volume, 1,250 mL autotransfused HTX). We also used a HTX sample collected 1 hour post evacuation, as this is the likely clinical time period in which autotransfusion would be necessary. NPP mixed with HTX was used as a control sample. The mixing studies showed a paradoxical hypercoagulability of the 1:4 mixture of HTX with patient plasma relative to patient plasma alone. We theorize that this acceleration in coagulation is because of activation of coagulation factors in evacuated HTX, resulting in the consumption of fibrinogen, creating plasma in the collection chamber that is rich in activated coagulation factors but devoid of a substrate on which to act. The mixture with patient plasma allows for replacement of the missing fibrinogen and subsequent acceleration of coagulation in the sample. The fact that the mixture with patient plasma further accelerated coagulation relative to the mixture with NPP indicates that some element of the plasma itself differs from the NPP. The mean aPTT of NPP alone was 33.6, whereas the mean aPTT of the venous samples collected was 26.7. This suggests that the difference in coagulation in the mixing studies was because of the original accelerated coagulation of patient plasma relative to the NPP. Based on our previous work in which we performed mixing studies with HTX blood and NPP at multiple different dilutions, we believe that these results would be consistent throughout different transfusion ratios.

In conclusion, HTX is incoagulable on its own, but demonstrates hypercoagulability when mixed with patient plasma at a clinically relevant dilution. This suggests that
autotransfusion of HTX may result in hypercoagulability. Therefore, although HTX is an attractive source of RBCs, we cannot recommend autotransfusion of HTX when banked blood is available.

References


Discussion

Discussant: Dr Randeep Jawa (Stony Brook, NY). Ms. Harrison and colleagues from UT San Antonio have furthered their previous work on clotting characteristics of hemothorax blood. In a study of 10 patients with traumatic hemothorax, they found that no clot was formed in isolated hemothorax specimens, mixing of hemothorax blood with NPP demonstrated accelerated coagulation as compared to hemothorax blood, and a 1:4 mixture of hemothorax blood with patient plasma demonstrated a lower PTT value than that in hemothorax blood, patient plasma alone, and a mixture of hemothorax blood with pooled plasma. Their work certainly challenges commonly held beliefs regarding the beneficial effects of autotransfusion.

I have several questions and comments for the authors. First, what was the average volume of hemothorax in these patients? Did most of these patients have massive hemothorax at time of chest tube insertion? Theoretically, patients with exsanguinating hemorrhage may demonstrate a different coagulation profile in shed blood than those with much smaller volumes.

Second, what concentration or dilution of citrate was used for the hemothorax blood?

Third, was the full storage solution CPD (citrate, phosphate, dextran) or ACD-A solution used for hemothorax samples, venous samples, and pooled plasma?

Fourth, what was done with hemothorax blood that was stored with EDTA or heparin? Were the hypercoagulable findings replicated with these storage methods?

Fifth, have these findings been evaluated by thromboelastography? This may further support the concept that the hypercoagulable state has definite physiologic consequences. Finally, what is the current policy about autotransfusion of pleural blood at UTSA?

Hannah B. Harrison: To begin with, you asked about the average volume of hemothorax, and whether or not these patients demonstrated massive hemothorax. The requirement to be enrolled into this study was a chest tube output greater than or equal to 140 milliliters in the first hour. Of the 10 subjects included in this study, only 2 had a total output of greater than 1 liter. You were interested in the difference in coagulation profiles relative to the volume of hemothorax. While we did not directly correlate these two variables, the aPTT of the subjects ranged between 20 and 35, with a median value of 25.5.

To address your questions regarding storage and collection of the hemothorax specimen: blood samples for a coagulation testing were collected into 3.2% sodium citrate just as routine coagulation samples are collected. The anticoagulant to blood ratio was 1:9 just as it is in normal clinical coagulation samples. The hemothorax/autotransfusion system does not include any anticoagulant. Hemothorax was collected into EDTA for CBC data at the time of collection. If we stored EDTA samples, they were not used for additional testing.

You further asked about evaluation of our findings using thromboelastography. We have been looking into that with our partners at the Institute for Surgical Research. We know that when hemothorax is mixed with NPP as well as with the patient plasma, it demonstrates hypercoagulability; however, we don’t really know anything about the sturdiness or quality of this clot and what the repercussions would be if this were actually autotransfused. This is part of our continued ongoing investigation.

With regard to the current policy at University of Texas Health Science Center at San Antonio; there is currently no official policy in place. However, because we have such a well-stored blood bank at our disposal, we tend to not use autotransfusion at this time, especially with the concerns for hypercoagulability demonstrated by our research.