Red Blood Cells Accelerate the Onset of Clot Formation in Polytrauma and Hemorrhagic Shock

Nicholas J. Spoerke, MD, Philbert Y. Van, MD, Jerome A. Differding, MPH, Karen A. Zink, MD, S. David Cho, MD, Patrick J. Muller, BS, Z. Ayhan Karahan, MD, Jill L. Sondeen, PhD, John B. Holcomb, MD, and Martin A. Schreiber, MD

Background: Hemorrhage and coagulopathy are major contributors to death after trauma. The contribution of red blood cells (RBCs) in correcting coagulopathy is poorly understood. Current methods of measuring coagulopathy may fail to accurately characterize in vivo clotting. We aimed to determine the effect of RBCs on clotting parameters by comparing resuscitation regimens containing RBCs and plasma with those containing plasma alone.

Methods: Thirty-two Yorkshire swine were anesthetized, subjected to a complex model of polytrauma and hemorrhagic shock, and resuscitated with either fresh frozen plasma, lyophilized plasma (LP), or 1:1 ratios of fresh frozen plasma:packed RBC (PRBC) or LP:PRBC. Activated clotting time, prothrombin time, partial thromboplastin time, and thrombelastography (TEG) were performed at 1 hour, 2 hours, 3 hours, and 4 hours after resuscitation.

Results: Animals treated with 1:1 LP:PRBC had less blood loss than the other groups ($p < 0.05$). The activated clotting time was shorter in the 1:1 groups when compared with the pure plasma groups at all time points ($p < 0.05$). The 1:1 groups had shorter TEG *R* times (time to onset of clotting) at 1 hour, 3 hours, and 4 hours compared with pure plasma groups ($p < 0.05$). Other TEG parameters did not differ between groups. Partial thromboplastin time was shorter in the pure plasma groups than the 1:1 groups at all time points ($p < 0.05$).

Conclusions: Whole blood assays reveal that RBCs accelerate the onset of clot formation. Coagulation assays using spun plasma underestimate the effect of RBCs on clotting and do not completely characterize clot formation.

Key Words: Coagulopathy, Polytrauma, Lyophilized plasma, Accelerated clot formation.

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From the Division of Trauma, Critical Care, and Acute Care Surgery (N.J.S., P.Y.V., J.A.D., K.A.Z., S.D.C., P.J.M., Z.A.K., M.A.S.), Department of Surgery, Oregon Health & Science University, Portland, Oregon; Combat Casualty Care Research (J.L.S.), United States Army Institute of Surgical Research, Fort Sam Houston, Texas; and Division of Acute Care Surgery (J.B.H.), Department of Surgery, University of Texas Health Science Center at Houston, Houston, Texas.
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Address for reprints: Martin A. Schreiber, MD, Division of Trauma, Critical Care, and Acute Care Surgery, Department of Surgery, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Mail Code L-611, Portland, OR 97239-3098; email: schreibm@ohsu.edu.

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Hemorrhage and coagulopathy are major contributors to death after trauma.[1] Recent studies have shown that a transfusion ratio of plasma to red blood cells (RBCs) approaching 1:1 is associated with improved survival in those needing massive transfusion.[2–4] Fresh frozen plasma (FFP) must be gradually thawed using specially designed equipment or a warm water bath, and its utility as a resuscitation fluid is limited in far-forward combat environments, civilian prehospital, and hospital care environments.[5] Lyophilized plasma (LP) is a powdered form of plasma that can be stored at room temperature, easily transported, and quickly reconstituted.[6] We have previously shown that LP maintains 86% coagulation factor activity, and the use of LP in a 1:1 ratio with packed RBC (PRBC) results in significantly less blood loss after liver injury when compared with 1:1 ratios of FFP to PRBC, FFP alone, and LP alone.[7]

The observed decrease in blood loss was not adequately explained by the results of standard coagulation tests that revealed an increased activated partial thromboplastin time (PTT) in the 1:1 LP to PRBC group and no difference in prothrombin time (PT) between groups. The contribution of RBCs in correcting coagulopathy is poorly understood. As the scientific community unravels an increasingly complex and intertwined picture of coagulation and fibrinolysis, the traditional concepts of the intrinsic and extrinsic clotting pathways are proving to be overly simplistic. Furthermore, the acute coagulopathy of trauma introduces yet another degree of complexity in the clotting scheme.[1,8] Current methods of measuring coagulopathy may fail to accurately characterize in vivo clotting.[8–10] We hypothesized that resuscitation regimens, which included RBCs, accelerate clot formation, and this effect could be most accurately and completely measured by whole blood coagulation assays.

MATERIALS AND METHODS

Blood Product Preparation and Hemorrhagic Shock Model

All procedures involving animals were performed in accordance with the guidelines of the Institutional Animal Care and Use Committees at Oregon Health & Science University and the United States Army Institute of Surgical Research. RBCs and FFP were collected and stored, and LP was produced as described in our previous work.[7] A well-validated lethal triad (acidosis, coagulopathy, and hypother-

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mia) model of severe injury and hemorrhagic shock described by Cho et al.[11] (Fig. 1) was used for this study. The details of the polytrauma and hemorrhage model in this study were described in the primary article by Spoerke et al.[7] Briefly, animals were anesthetized, and arterial and venous lines were placed. Next, a severe soft tissue injury and femur fracture was induced with a Schermer Bolt Gun (Karl Schermer, Ettlinger, Germany). Animals were made hypothermic with lavaged saline, and 60% of their blood volume was removed by controlled hemorrhage. After 30 minutes of hemorrhagic shock, normal saline was infused in a volume equal to a 3:1 ratio of the controlled hemorrhage. Animals then received a grade V liver injury and underwent 30 seconds of uncontrolled hemorrhage. The study animals were then randomized to receive one of four resuscitation fluids: FFP, LP, 1:1 FFP:PRBC, or 1:1 LP:PRBC. The liver injury was performed by a single surgeon who was blinded to the intervention. To confirm that a standard injury was performed, each animal had a poststudy hepatectomy to analyze vessel injury.

Laboratory Assays

Platelets counts were determined by a reference laboratory (IDEXX VetConnect, Westbrook, ME). PT, PTT, and fibrinogen were measured using the BCS Coagulation System (Dade Behring, Marburg, Germany). Activated clotting time (ACT) was measured using a Hemochron 801 (International Technidyne, Edison, NJ). Ionized calcium levels were measured with the iSTAT (Abbott Laboratories, Princeton, NJ).

Thrombelastography

Citrated samples were collected and analyzed for coagulation parameters using a kaolin-activated thromboelastogram (TEG; Hemoscope Corporation, Niles, IL). TEG is an excellent test for interpreting overall coagulation by rapidly assessing the interaction of platelets with the protein coagulation cascade from the initial platelet-fibrin interaction, through platelet aggregation, clot strengthening, fibrin cross-linking, to eventual clot lysis. A TEG tracing can provide information on clotting factor activity, platelet function, and significant fibrinolysis in 30 minutes to 50 minutes. A fixed quantity of blood (340 μL) is placed in a kaolin cup with 20 μL of calcium chloride, which in turn is placed in a carriage assembly. A pin affixed to a torsion wire is lowered into the cup, which begins to oscillate at a fixed rate and amplitude. As the cup moves and the clotting is initiated, the blood begins to adhere to the pin, which in turn deflects the torsion wire. The deflection of the torsion wire increases as the clot strengthens. Eventually, maximum amplitude (MA) is

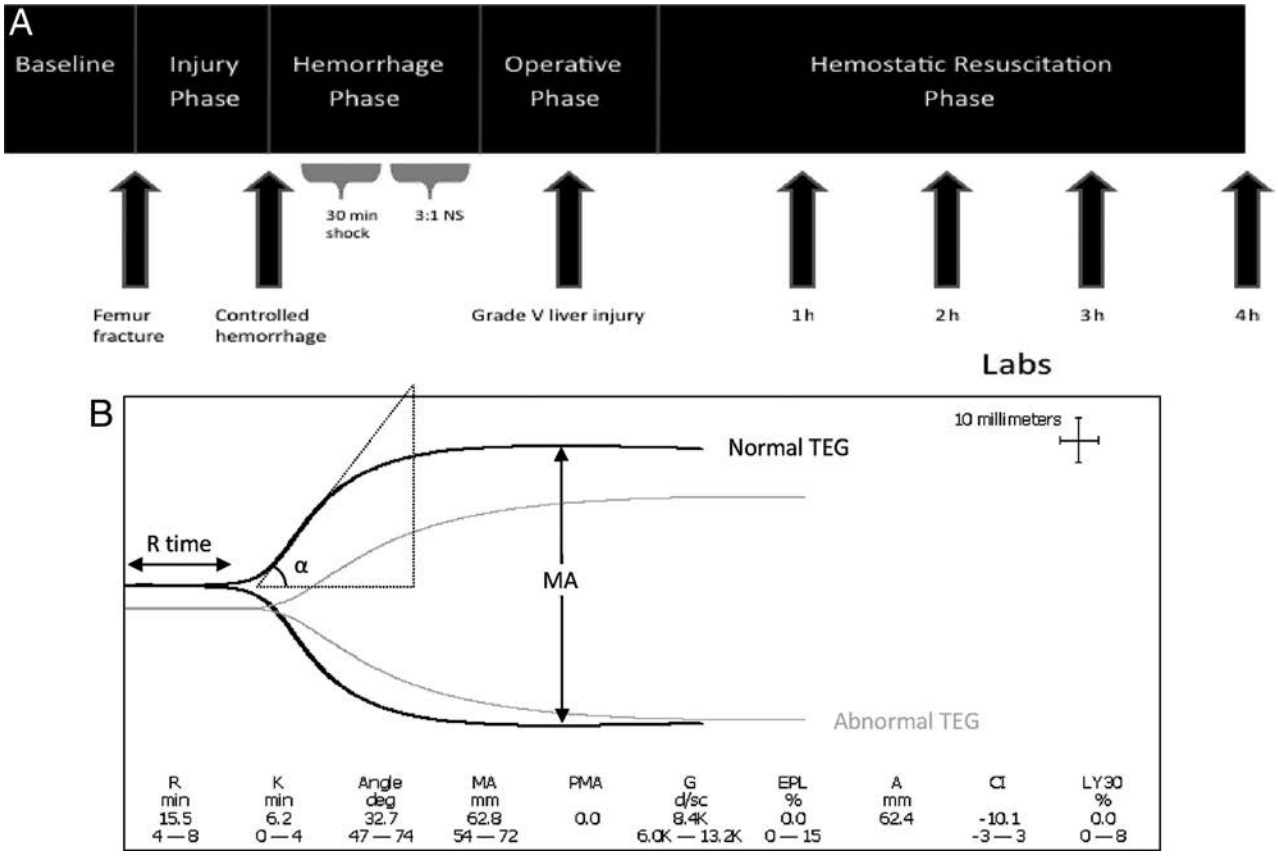

Figure 1. (*A*) Hemorrhagic shock and polytrauma model. (*B*) Normal and abnormal TEG tracings. "R time" represents time to clot formation. The "*á*" angle denotes rate of clot strengthening and "MA" shows maximal clot strength. The abnormal TEG has a prolonged R time (increased time to clot formation), decreased α angle (slower rate of clot strengthening), and a smaller MA (decreased clot strength) consistent with a hypocoagulable state.

reached, after which fibrinolysis decreases adhesion to the pin, which moves less and less. The time until the initial clot formation, represented by the split in the tracing, is the *R* time (minutes) and represents the activity of the soluble coagulation factors (i.e., the "intrinsic" and "extrinsic" pathways). The rate at which the clot strengthens is depicted by the angle made by the tracing, denoted by the α angle (degrees). This primarily represents the activity of fibrinogen. The MA of the tracing is the overall clot strength (mm) and is a measure of platelet activity. As fibrinolysis occurs, the deflection of the pin decreases, which decreases the overall amplitude of the tracing. Figure 1, B depicts a normal and abnormal TEG tracing. The abnormal TEG has a prolonged *R* time (increased time to clot formation), decreased α angle (slower rate of clot strengthening), and a smaller MA (decreased clot strength) consistent with a hypocoagulable state.

Statistical Analysis

Data were analyzed using SPSS software version 16.0 (SPSS, Chicago, IL). Variables were assessed for normal distribution. Comparisons between groups at the various time points were analyzed using independent samples Student's *t* tests. Paired samples Student's *t* tests were used to compare same-group samples across various time points. The Bonferroni adjustment was used with significance denoted as $p < 0.05$.

RESULTS

No animals died during the study. As described in our previous work,[7] the animals treated with a 1:1 ratio of LP to PRBC had significantly less blood loss than the other resuscitation groups. Animals randomized to the 1:1 groups (FFP to PRBC and LP to PRBC) had a significantly higher hematocrit than the pure plasma groups (FFP and LP) at all postliver injury time points (Fig. 2). This figure also shows the hemodilutional effect of normal saline resuscitation after controlled hemorrhage with a decrease in hematocrit in all resuscitation groups from the prenormal saline to the preliver injury time point.

Platelet counts were higher in the 1:1 plasma to PRBC ratio groups after liver injury (Fig. 3). Specifically, the 1:1 FFP to PRBC group had a significantly higher platelet count when compared with FFP alone at 1 hour postliver injury. At all time points after liver injury, the platelet count in the 1:1 LP to PRBC group was significantly greater than the FFP group. When comparing the platelet count between 1:1 LP to PRBC and LP alone, the 1:1 ratio group had a higher platelet count only at 1 hour, 2 hours, and 4 hours after grade V liver injury.

The mean time to clot formation (TEG *R* time) was significantly shorter in the 1:1 groups at 1 hour, 3 hours, and 4 hours after liver injury when compared with the pure plasma groups (Table 1 and Fig. 4). The 1:1 FFP to PRBC groups had a shorter mean *R* time at 1 hour and 3 hours postliver injury when compared with FFP alone. At 1 hour and 4 hours postliver injury, the 1:1 LP to PRBC group mean *R* times were shorter than the FFP group. Both 1:1 LP to PRBC and FFP to PRBC mean *R* time values were significantly shorter when compared with the LP group at 3 hours and 4 hours. Other TEG values measuring rate of fibrin cross-linking, clot strength, and fibrinolysis (α, MA, and percent lysis at 30 minutes) were not significantly different between groups at any time point.

ACT was significantly shorter in the 1:1 groups when compared with pure plasma alone after liver injury (Table 1 and Fig. 5). The 1:1 FFP to PRBC and LP to PRBC groups had significantly shorter ACTs when compared with the LP

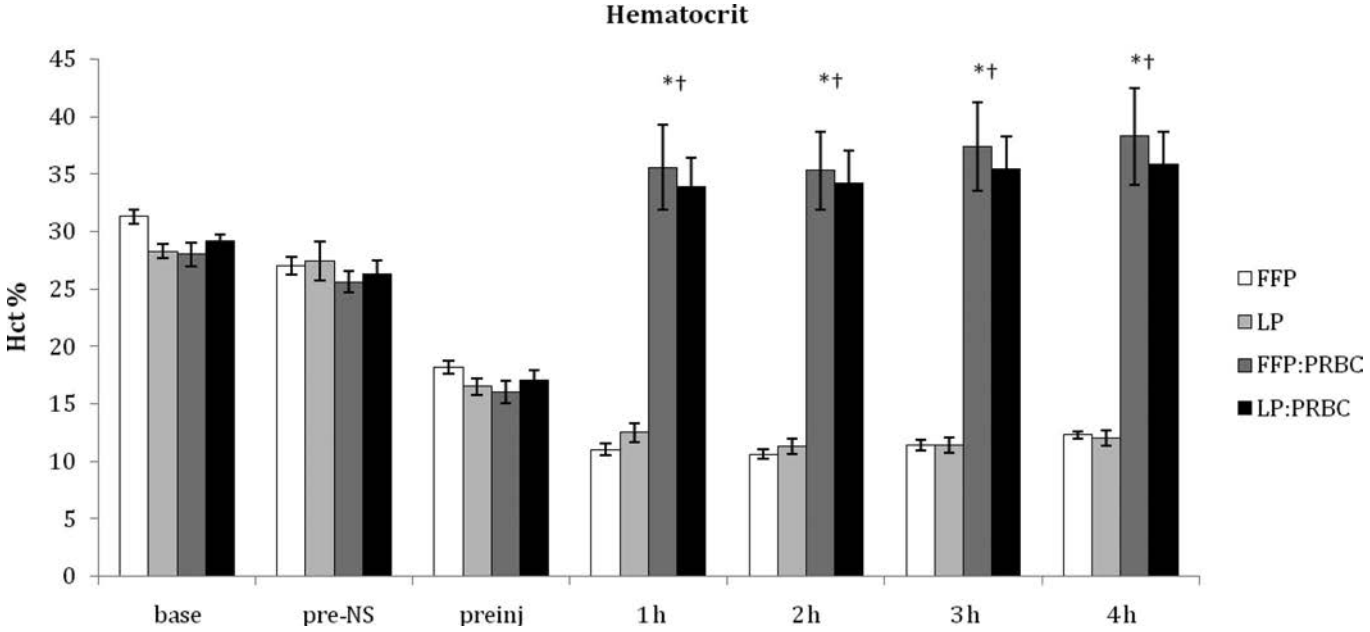

Figure 2. Hematocrit. Values are mean (SEM). *FFP:PRBC group higher than FFP and LP. †LP:PRBC group higher than FFP and LP. (All comparisons $p < 0.05$). The pre-NS is the time point before 3:1 resuscitation with normal saline. The preinj is the time point before liver injury.

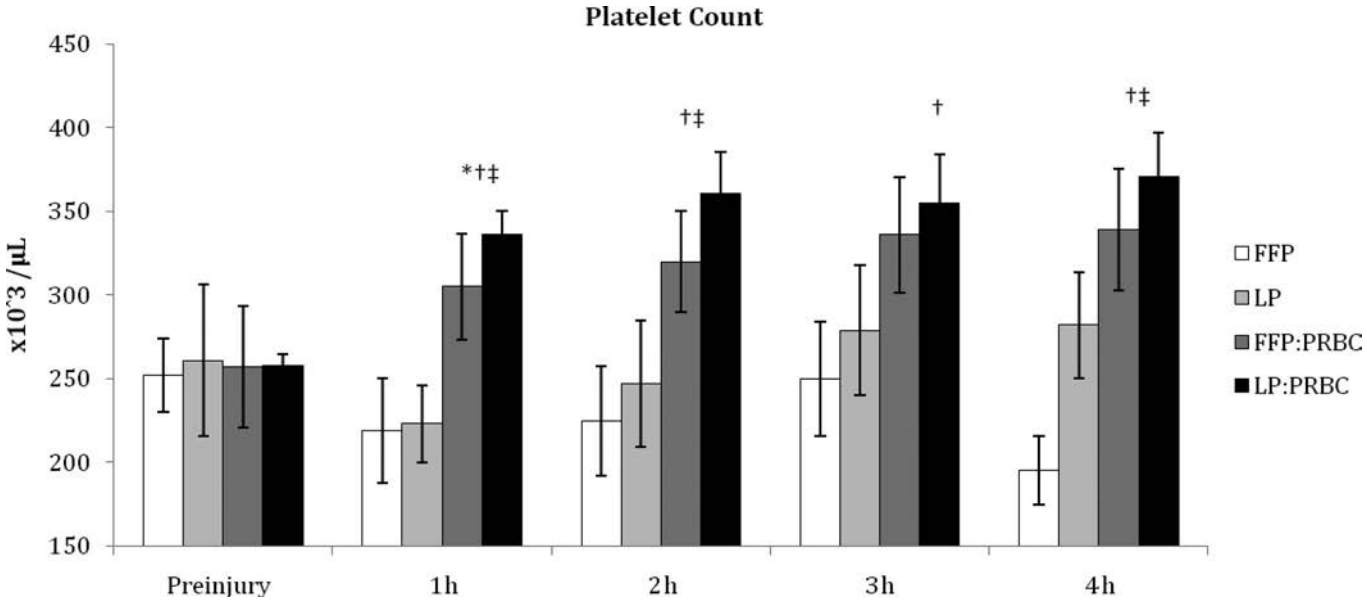

Figure 3. Platelets. Values are mean (SEM). *FFP:PRBC greater than FFP. †LP:PRBC greater than FFP. ‡LP:PRBC greater than LP. (All comparisons $p < 0.05$.)

TABLE 1. Coagulation Parameter Comparisons

Time (h)	Parameter	FFP	LP	FFP:PRBC	LP:PRBC	$p < 0.05$
1	ACT (s)	128	131	105	107	*†‡§
	R time (min)	7.5	6.2	5.4	5.2	*†
2	ACT (s)	123	122	104	98	†‡§
3	ACT (s)	117	118	96	94	*†‡§
	R time (min)	5.7	5.9	4.0	4.4	*‡§
4	ACT (s)	120	114	95	96	*†‡§
	R time (min)	5.5	5.7	4.2	3.6	†‡§

* FFP:PRBC < FFP.
† LP:PRBC < FFP.
‡ FFP:PRBC < LP.
§ LP:PRBC < LP.

group at all time points after liver injury. The 1:1 LP to PRBC group had shorter ACTs when compared with the FFP group at all time points postliver injury. The only time point at which the 1:1 FFP to PRBC group did not have a significantly shorter ACT was at 2 hours postliver injury.

No significant differences in PT, fibrinogen, and ionized calcium levels were observed between the fluid groups across any of the time points. However, the 1:1 groups had significantly prolonged PTT values at all time points after liver injury (Fig. 6). The 1:1 FFP to PRBC and LP to PRBC groups had significantly increased PTT values when compared with FFP alone at 1 hour, 2 hours, 3 hours, and 4 hours postliver injury. The PTT values at 2 hours, 3 hours, and 4 hours were increased in the 1:1 LP to PRBC group compared with the LP group. The PTT was increased in the 1:1 LP to PRBC group when compared with the LP group at 4 hours.

DISCUSSION

Current Advanced Trauma Life Support guidelines recommend the use of up to 2 L of warmed isotonic electrolyte solutions for initial resuscitation. RBC replacement is only considered if there is no response to initial resuscitation. These guidelines are still in place despite recognition that 25% of trauma patients develop an acute coagulopathy of trauma that is exacerbated with crystalloid resuscitation alone. The results of this study suggest that the earlier use of RBC replacement may benefit the trauma patient in ways other than volume expansion and improved oxygen delivery. Our results suggest that early incorporation of RBCs in resuscitation could accelerate clot formation and may improve outcomes in trauma patients with coagulopathy. As discussed previously, several retrospective studies have shown an association between improved survival and a high ratio of FFP to PRBC (1:1) in the setting of massive transfusion. Therefore, we chose to include 1:1 FFP to PRBC and 1:1 LP:PRBC in this study.

The 1:1 ratio groups had significantly higher hematocrit values than the pure plasma product groups. These data confirm that resuscitation was effective in increasing the red cell volume of the experimental animals. The hemodilutional effect of crystalloid resuscitation can be seen in Figure 2, with a decrease across all groups from pre- to postnormal saline infusion.

Despite higher platelet counts in the 1:1 ratio group, platelets are unlikely to have contributed to the acceleration of clotting in a clinically significant manner. If the increased platelet count affected clot strength, this should have been measurable by TEG and manifested by an increased MA value for the 1:1 ratio groups. In addition, platelets transfused were a component of the PRBCs, which were stored at 4°C for up to 24 hours. Although not destroying the platelets, the storage of platelets at 4°C results in their irreversible inactivation, thus negating their contribution to clot formation.[12]

Two independent measures of coagulation, TEG, and ACT, showed that the time to onset of clotting was significantly shorter in the 1:1 ratio of plasma product to PRBC groups. Both TEG and ACT are coagulation assays that use

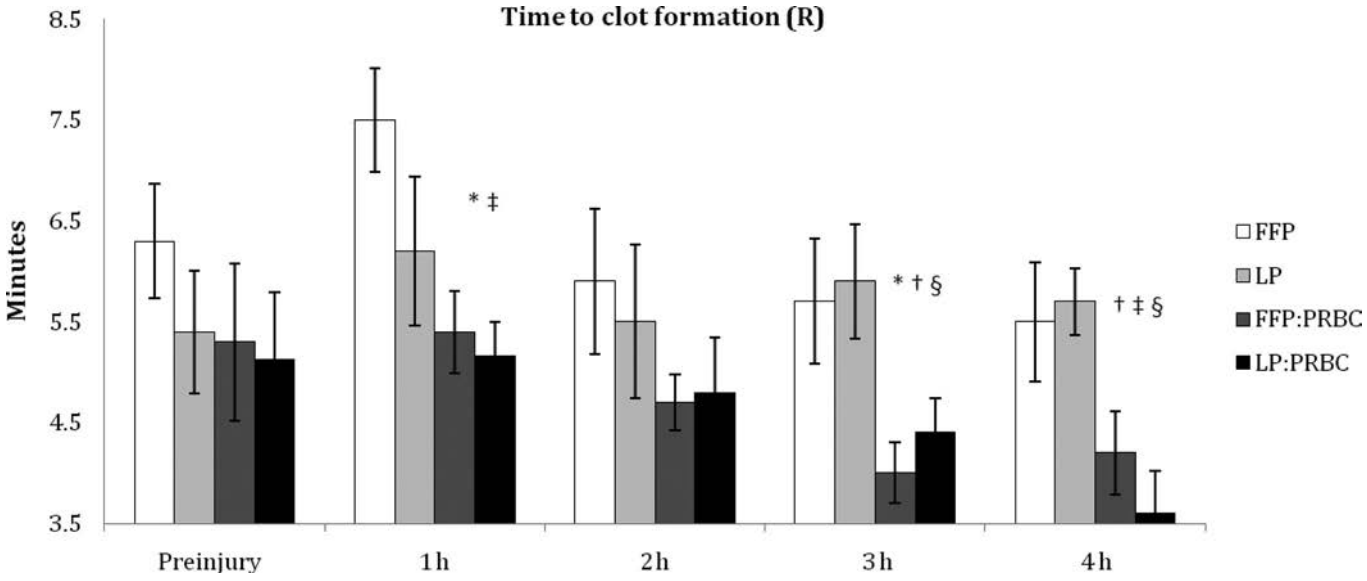

Figure 4. Time to clot formation (R). Values are mean (SEM). *FFP:PRBC lower than FFP. †FFP:PRBC lower than LP. ‡LP:PRBC lower than FFP. §LP:PRBC lower than LP. (All comparisons $p < 0.05$.)

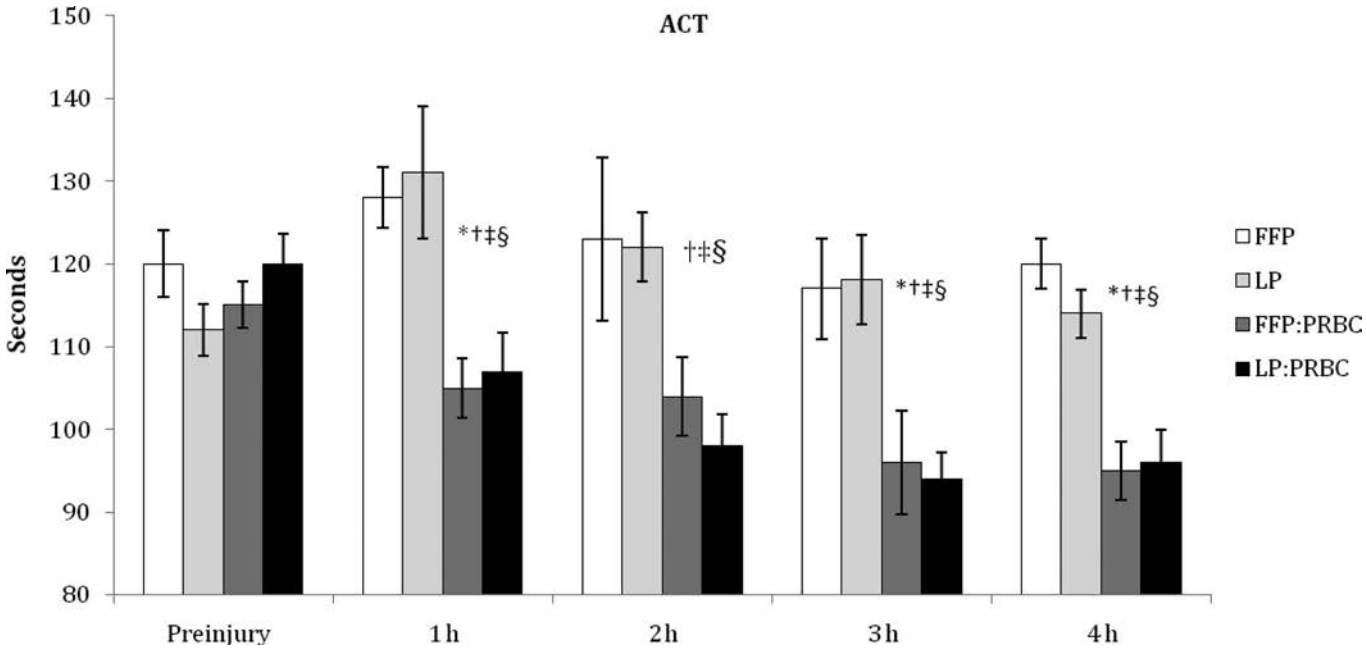

Figure 5. Activated clotting time. Values are mean (SEM). *FFP:PRBC lower than FFP. †FFP:PRBC lower than LP. ‡LP:PRBC lower than FFP. §LP:PRBC lower than LP. (All comparisons $p < 0.05$.)

whole blood and take into consideration the contribution of cellular elements of blood that contribute to clot formation. Several mechanisms could account for the acceleration of clot formation in the presence of RBCs. RBCs are crucial participants in clot formation. Cell membranes from RBCs help to activate factor IX and X in the coagulation cascade,[13] and RBCs enhance platelet reactivity by modulating their thromboxane production.[14] In addition, the formation of RBC rouleaux in the central portion of a blood vessel contributes to the biomechanical margination of platelets to the periphery, enhancing the concentration of platelets near the endothelium.[14,15] As previously discussed, the 1:1 LP to PRBC group demonstrated the lowest blood loss after grade V liver injury.[7] The combination of the RBC effects on clot formation with the superior coagulation factor activity of LP may have synergistic effects on hemostasis and may explain the decreased blood loss compared with 1:1 FFP to PRBC, LP alone, and FFP alone.

The absence of significant differences in PT, fibrinogen, and ionized calcium levels between fluid groups at

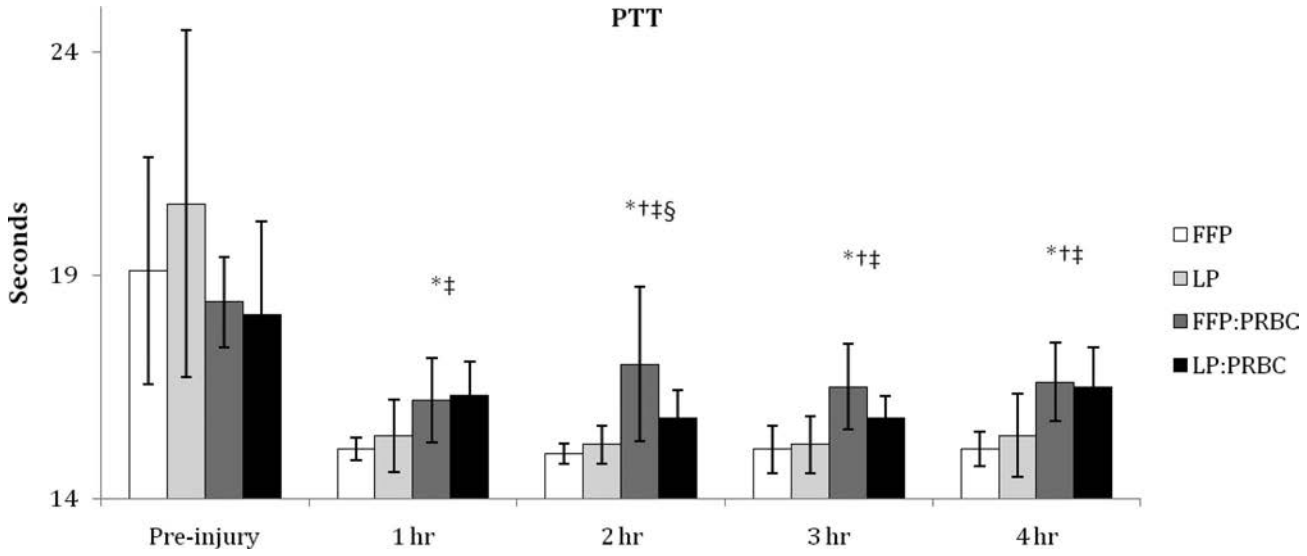

Figure 6. Activated partial thromboplastin time. Values are mean (SEM). *FFP:PRBC greater than FFP. †FFP:PRBC greater than LP. ‡LP:PRBC greater than FFP. §LP:PRBC greater than LP. (All comparisons $p < 0.05$.)

postinjury time points emphasizes the inadequacy of these tests in assessing coagulation status in hemorrhagic shock and polytrauma. Surprisingly, the PTT was prolonged in 1:1 ratio groups when compared with the FFP and LP groups at postliver injury time points. This result does not seem to be clinically significant as the PTT results are not in congruence with other clinical measures such as the blood loss and the whole blood assays (TEG and ACT). Standard coagulation assays that use plasma do not account for the contribution of RBCs to clot formation.

A weakness of this study was the lack of PRBC and whole blood experimental arms. The data generated from a PRBC-only arm would help to determine the relative effects of RBCs on acceleration of clot formation, and the inclusion of a whole blood arm would have clarified the effects of all clotting-related elements in blood. Eugster and Reinhart[15] have shown significantly shorter in vitro clotting times with hematocrits of 50% compared with 20%, although these times did depend on the relative platelet count. The optimal hematocrit at which clot formation occurs is unknown and is likely highly dependent on platelet count, clotting factor concentration and activity, and in vivo environmental factors. Further study in this area is certainly warranted.

In conclusion, our study suggests that the coagulation process involves a highly coupled, interdependent relationship between clotting factors and the concentration and function of RBCs. In addition, coagulation is a dynamic process that is best characterized using whole blood analysis systems such as TEG.

REFERENCES

1. Hess JR, Brohi K, Dutton RP, et al. The coagulopathy of trauma: a review of mechanisms. *J Trauma.* 2008;65:748–754.
2. Holcomb JB, Wade CE, Michalek JE, et al. Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. *Ann Surg.* 2008;248:447–458.
3. Sperry JL, Ochoa JB, Gunn SR, et al; Inflammation the Host Response to Injury Investigators. An FFP:PRBC transfusion ratio ≥1:1.5 is associated with a lower risk of mortality after massive transfusion. *J Trauma.* 2008;65:986–993.
4. Maegele M, Lefering R, Paffrath T, Tjardes T, Simanski C, Bouillon B; Working Group on Polytrauma of the German Society of Trauma Surgery (DGU). Red-blood-cell to plasma ratios transfused during massive transfusion are associated with mortality in severe multiple injury: a retrospective analysis from the Trauma Registry of the Deutsche Gesellschaft für Unfallchirurgie. *Vox Sang.* 2008;95:112–119.
5. Champion H. Combat fluid resuscitation: introduction and overview of conferences. *J Trauma.* 2003;54:S7–S12.
6. Shuja F, Shults C, Duggan M, et al. Development and testing of freeze-dried plasma for the treatment of trauma-associated coagulopathy. *J Trauma.* 2008;65:975–985.
7. Spoerke N, Zink K, Cho SD, et al. Lyophilized plasma for resuscitation in a swine model of severe injury. *Arch Surg.* 2009;144:829–834.
8. Brohi K, Cohen MJ, Davenport RA. Acute coagulopathy of trauma: mechanism, identification and effect. *Curr Opin Crit Care.* 2007;13:680–685.
9. Hardy JF, de Moerloose P, Samama CM. The coagulopathy of massive transfusion. *Vox Sang.* 2005;89:123–127.
10. Kashuk JL, Moore EE, Johnson JL, et al. Postinjury life threatening coagulopathy: is 1:1 fresh frozen plasma: packed red blood cells the answer? *J Trauma.* 2008;65:261–271.
11. Cho SD, Holcomb JB, Tieu BH, et al. Reproducibility of an animal model simulating complex combat-related injury in a multiple-institution format. *Shock.* 2009;31:87–96.
12. Murphy M, Vassallo R, Murphy S. Preservation and clinical use of platelets. In: Kaushansky K, Lichtman MA, Beutler E, et al, eds. *Williams Hematology.* 7th ed. New York, NY: McGraw-Hill; 2006:2175–2190.
13. Kaibara M, Iwata H, Ujiie H, Himeno R, Kaibara M. Rheological analyses of coagulation of blood from different individuals with special reference to procoagulant activity of erythrocytes. *Blood Coagul Fibrinolysis.* 2005;16:355–363.
14. Reinhart WH, Zehnder L, Schulzki T. Stored erythrocytes have less capacity than normal erythrocytes to support primary haemostasis. *Thromb Haemost.* 2009;101:720–723.
15. Eugster M, Reinhart WH. The influence of the haematocrit on primary haemostasis in vitro. *Thromb Haemost.* 2005;94:1213–1218.

DISCUSSION

Dr. Timothy Pritts (Cincinnati, Ohio): Thank you very much. I would like to congratulate Dr. Spoerke and his

coworkers for an outstanding paper and presentation. This topic continues to be central to all who care for the injured. This study and the group's ongoing work continues to advance our knowledge about the pathophysiology and treatment of coagulopathy in trauma patients.

In the present study, the investigators utilize their well-characterized and well validated model of polytrauma and shock in swine in order to evaluate the effect of elements of damage control resuscitation on coagulopathy. They demonstrated that the presence of red blood cells as part of the one-to-one resuscitation strategy is a key component for clot formation. The studies are well done and the manuscript is straightforward and well written.

Like most well-done experiments, this generates a few more questions than it answers. I have a few questions. First, can you speculate a little bit more of the mechanism for your findings? The technique that you used to separate whole blood concentrates platelets into the packed red blood cell component. Are you certain that they are inactivated? Are the increased amounts of platelets in the packed red blood cell component potentially at all responsible for your findings?

Secondly, if the platelets are not the mechanism for accelerated clot formation, what do you think is responsible? Is it simply the presence of a higher hematocrit? Is there a threshold or target hematocrit for transfusion that we should be using in order to optimize clot formation in the acutely bleeding patient?

Third, you utilized a one-to-one ratio of packed red blood cells to lyophilized plasma or FFP. While the use of this ratio is grounded in clinical data, some data suggests that other rations, such as 1:2, may be more optimal. Have you had the opportunity to vary the ratio of pRBCs to plasma in your model? Does one-to-one prove superior to other ratios in your model system?

Fourth, your data showed that changes in the resuscitation strategy did not influence the measured values of PT or fibrinogen and that FFP alone decreased PTT values. This is interesting and suggests that these traditional tests are inadequate under these conditions. Do you think that these findings suggest that TEG really should be the standard of care for determining coagulopathy in trauma patients?

Finally, in the midst of all of this great data, we should not overlook your exciting findings about lyophilized plasma. Lyophilized plasma performed very well in this study and appeared to be nearly as good or better than FFP. Do you think there is actually a clinically significant difference between lyophilized plasma and FFP? Should we start looking at lyophilized plasma more intensely in the clinical setting? Thanks for the opportunity to review and discuss this paper and I look forward to your comments.

Dr. Nicholas Spoerke (Portland, Oregon): Thank you for those insightful questions, Dr. Pritts. The first question was in regard to the platelets and their role and whether they were actually inactivated. As I briefly touched upon, the platelets were refrigerated and while they were not destroyed and are therefore counted by the platelet analyzer, they were irreversibly inactivated.

Additionally, had the platelets contributed significantly to the coagulation, we would have expected to see a difference in the TEG tracings, specifically the maximum amplitude. Though I did not show that data, there was no difference between groups with respect to the MA at any time point.

The second question was whether there was an optimal hematocrit or a threshold hematocrit for which the optimal coagulation milieu would be created. There have been some studies that have looked at that. We did not actually design the study to answer that question and unfortunately, in the polytrauma situation, the coagulation environment is so complicated that simple things like choosing one hematocrit is often not applicable.

The third question is whether we used any other ratios or considered using any other ratios. Though the one-to-one recommendations are based on retrospective data, we follow the current combat recommendations, because we believe them to be most useful immediately. While we're currently participating in the PROMMT trial, which prospectively is looking at one-to-one ratios in massive transfusion and focusing on the possible bias for early deaths, the PROMMT trial will be used to create a prospective randomized trial and we're eagerly awaiting those results.

The fourth question was whether the TEG is becoming the standard of care and touching on the clinical uses of TEG. While the TEG is not yet considered a standard of care, we found it most useful clinically to chose components for therapy along the whole spectrum of coagulation abnormalities.

In the massive transfusion setting, the TEG is a point-of-care test which rapidly tells which products are necessary. There is some limited data showing the TEG can reduce unnecessary product administration. In addition, we're also using it in the hypercoagulable setting, to help adjust low molecular weight dosing in these unique situations.

The last question was whether there's a difference between fresh frozen plasma and lyophilized plasma. We have found so far, through our in vitro and in vivo analysis of the hemodynamic data and mortality data, using a similar model, that FFP and LP are equally safe and effective.

Our in vitro studies have shown that it has superior clotting factor retention, retaining, on average, 86 percent of its clotting factor activity. Thank you very much.

Dr. Myung Park (Rochester, Minnesota): Thank you for the wonderful presentation. I have several questions. I noted that the R time is significantly shorter as you go from the pure FFP group to the LP packed red blood cell group. However, these numbers, albeit statistically significant, may not necessarily translate to any clinically significant difference.

Have you noticed in your animal group, of these four groups, any clinical difference in terms of blood pressure or pulse, something to correlate with the significantly different R times?

Question number two, did you perform a necropsy on these animals and if so, have you noted any evidence of acute thrombosis within the large vasculature of these animals and, lastly, have you looked at other TEG variables besides R

time, such as alpha angle, which shows you the rate of thrombin generation, and MA, which is reflective of the total clot strength?

Dr. Nicholas Spoerke (Portland, Oregon): The first question was in regard to pointing out that the R time was different among groups and was questioning whether the hemodynamic characteristics of the animals were different between various groups. We have analyzed the mortality data, blood loss data, mean arterial pressure, and heart rate data and there were no significant differences in the hemodynamic characteristics between groups depending on which resuscitation fluid was used.

We did not specifically look for evidence of acute thrombosis in any of the large arteries. However, there was no evidence of this occurring. In addition, each animal, at the conclusion of study, had a hepatectomy to evaluate the liver injury and there was, again, no evidence of acute thrombosis there.

The other TEG variables, alpha angle, the maximum amplitude, LY30, we have looked at those. We've analyzed all of them and I did not present that data. I only presented the R data because the other variables, there were no statistically significant differences with regard to those characteristics.

Unidentified: Nice work and nice presentation. I just want to caution you about R time. R time, we think it's more a reflection of the endogenous inhibitor in blood rather than the tendency of the coagulation. I'll give you an example.

If you hemodilute the animal by 50 percent, you actually see R times shorter than become longer, but really, the most important aspect of the TEG that is producing hemostatic clot is alpha and MA. If you see change in those, then you can attribute that one resuscitation was better than the other. If you don't see it, R time alone does not give you any indication of what product restores coagulation better. The other question is was there any survival benefit among these three groups?

Dr. Nicholas Spoerke (Portland, Oregon): There was not. There were no premature deaths prior to the end of study on any animals.

Dr. Joseph L. Pfeifer (Pittsfield, Massachusetts): It was a very good study and a very interesting outcome and I have a question. Did you look at arterial lactate or arterial ph in the red cell versus non-red cell group and was there any difference if you looked at it, suggesting that maybe improved oxygen delivery to tissues might have had some difference in clot formation?

Dr. Nicholas Spoerke (Portland, Oregon): That's a very good question and we did actually look at that and that's presented with our data, with the hemodynamic data, but specifically, there was no difference. We carefully looked at the lactate values and there was no difference in lactate values between groups at any time point after resuscitation with the various fluids.

Dr. Jeffry L. Kashuk (Hershey, Pennsylvania): I rise to congratulate the Oregon group and their continuing TEG work. Just a couple of comments. While I think it's useful, as some of the other discussants have commented, of looking at the R value, the issue of the platelet's contribution is still not really clear on the basis of this presentation.

I think there's some nice data from electron microscopy looking at various clot specimens from the Japanese showing that very early, even within the R portion of the TEG, there is platelet contributions and it appears that there's a significant platelet-RBC interaction for the establishment of the clot. While we may think that the platelets aren't functional, they may have an important role.

In addition, we have to keep in mind that a significant contributing aspect of the red blood cell is through ADP stimulating platelet action and initial clot formation. Also, I think we all recognize that in the patient, the red blood cells are an integral part of the clot, . . .as well as plasma- therefore, isolating the red blood cell from the plasma as you have done, isn't really truly reflective of the clinical milieu.

Lastly, perhaps you should looking at the TEG G value, in addition to the R, which will reflect the contribution of platelets as well as the enzymatic to the clot.

Dr. Nicholas Spoerke (Portland, Oregon): I would agree that a situation is more complicated than any of the analyzing equipment that we have can actually anticipate. Our bias with the TEG is that in the big spectrum between spun plasma and adding rapid phospholipids to the solution to recreate the in vivo coagulation cascade is really not ideal.

The TEG gives a number of different values, which unfortunately are a little bit open to interpretation. However, the value is immense in terms of managing patients in massive transfusion and a hypercoagulable state. Thank you very much.