Published in final edited form as:

J Surg Res. 2012 March ; 173(1): 26–30. doi:10.1016/j.jss.2011.04.047.

Hemodilution is Not Critical in the Pathogenesis of the Acute Coagulopathy of Trauma

M. Wohlaue¹, E.E. Moore^{1,2}, N. Droz⁵, J. Harr¹, E. Gonzalez¹, M. Frago¹, and C.C. Silliman^{3,4}

¹ Department of Surgery, University of Colorado Denver, Denver, CO

² Department of Surgery, Denver Health Medical Center, Denver, CO

³ Department of Pediatrics, University of Colorado Denver, Denver, CO

⁴ Research Department, Bonfils Blood Center, Denver, CO

⁵ Creighton University Medical Center, Omaha, NE

Abstract

Introduction—The acute coagulopathy of trauma is multifactorial; but generally believed to be aggravated by co-existing acidosis, hypothermia, and hemodilution. While acidosis and hypothermia have been extensively evaluated, there is a paucity of data on the independent role of hemodilution in this scenario. We therefore hypothesized that hemodilution will impair coagulation following experimental trauma and hemorrhagic shock.

Methods—Adult male Sprague-Dawley rats underwent trauma and hemorrhagic shock, followed by resuscitation with 2× SBV using normal saline (NS). Thrombelastography (TEG) was performed before and after shock.

Results—In this trauma model, resuscitated resulted in a hemodilution of 50% ($43 \pm 4.05\%$ vs. $19.8 \pm 3.96\%$ Hct preshock vs. postshock, $p < 0.0001$). Despite the substantial hemodilution, there was no significant change in clot strength (12.96 ± 2.84 vs. 11.79 ± 1.28 dynes/cm² G preshock vs. postshock, $p = 0.13$). Similarly, the onset of coagulation (R time) was not impaired (1.68 ± 1.74 sec vs. 1.75 ± 0.63 sec R time preshock vs. postshock, $p = 0.45$).

Conclusion—In the absence of hypothermia and acidosis, hemodilution ($\leq 50\%$) has a trivial effect on coagulation following trauma and hemorrhagic shock. These data call to question the commonly held belief that hemodilution *per se* is critical in the development of postinjury coagulopathy.

Keywords

blood coagulation disorders; coagulation; coagulopathy; acute coagulopathy of trauma; hemorrhagic shock; transfusion; massive transfusion; hemodilution; dilutional coagulopathy; resuscitation; critical care; crystalloid

© 2011 Elsevier Inc. All rights reserved.

Corresponding Author: Ernest E. Moore, MD, Phone: 303-436-6561 Fax: 303-436-6572 ernest.moore@dhha.org.

Presented at Academic Surgical Congress, Huntington Beach, February 3, 2011.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Beginning with the discovery that postinjury shock was caused by acute blood loss,¹ research has continued in its endeavor to optimize resuscitation strategies. In World War II, whole blood transfusion was finally accepted as beneficial for resuscitation of traumatic shock.^{2,3} With the discovery of ABO blood groups,⁴ blood component separation⁵ and storage,⁶ initial investigation focused on replacement of these components following massive transfusion.⁷ As a result, much of the emphasis on the coagulopathy following life threatening injury was attributed to the dilution of clotting factors as a result of component therapy.⁸ In fact, avoiding hemodilution formed the basis for hypotensive resuscitation, i.e. the dual benefit of avoiding blood pressure dislodgement of a hemostatic plug and hemodilution that would provoke a coagulopathy.⁹ For the past 30 years, contributions of physiologic derangements, beyond plasma clotting factors, added a further dimension to the investigative process, notably the bloody vicious cycle emphasizing the role of coexisting hypothermia and acidosis.^{10,11} It is believed that the acute coagulopathy of trauma is multifactorial; aggravated by co-existing acidosis, hypothermia, and hemodilution. While the independent contributions of acidosis and hypothermia have been extensively evaluated, there is a paucity of data on the independent role of hemodilution in this scenario. Traditionally, evaluation and treatment of postinjury coagulopathy has been based largely on the platelet-poor plasma assessment of the extrinsic pathway (prothrombin time, PT) and the intrinsic contact pathway (activated partial thromboplastin time, aPTT). These tests, however, reflect <5% of the thrombin generated during clot formation.¹² Previous clinical studies have shown that up to 35% of the variability in the INR and 20% of the variability of the PTT are unrelated to underlying clotting factor levels.^{7,24} For the past decade, coagulation research has been redirected based on the cell-model of hemostasis, recognizing the central role of platelets, the endothelium, and their interaction with the plasma elements.¹³ Thrombelastography (TEG), employed in liver transplant¹⁴ and cardiac surgery¹⁵ for half a century, has recently been applied to the trauma setting, facilitated by the modification of provoking coagulation with tissue factor, i.e., the rapid TEG. Additionally, TEG has been shown to be superior to PTT and INR in detecting clinically relevant clotting abnormalities following experimental trauma and hemorrhagic shock.¹⁶ We therefore hypothesized that hemodilution has an independent effect on the acute coagulopathy of trauma, using TEG as a comprehensive assessment of clot integrity.

Materials and Methods

All reagents were purchased from Sigma-Aldrich Corp. (St. Louis, Mo) unless otherwise specified. Thrombelastography equipment and supplies were obtained from Haemonetics Corporation (Niles, Ill); 0.9% injection grade normal saline (NS) was purchased from Baxter Healthcare (Deerfield, Ill); iSTAT equipment and supplies were purchased from Abbott Laboratories (Abbott Point of Care, Princeton, NJ). Polyethylene tubing was acquired from Fisher Scientific (Pittsburgh, PA). No heparin was given in this study.

Trauma Model

Adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 350–450 g were housed under barrier-sustained conditions and allowed free access to food and water before use. All animals were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, and this study was approved by the University of Colorado Health Sciences Center Animal Care and Use Committee. Animals (n=10) were anesthetized with inhaled isoflurane, and a cardiac puncture was performed to collect a blood sample for a preshock TEG. The femoral artery and vein were then cannulated with polyethylene (PE-50) tubing for continuous invasive pressure monitoring and to establish venous access for resuscitation. A tracheotomy was performed, at which

point the animal was placed on 40% O₂ using an air-oxygen mixer (Sechrist, Anaheim, CA) at a flow rate of 2 liters per minute. After a 45-minute observation period, a laparotomy was performed to simulate tissue injury, and hemorrhagic shock was induced over a period of 10-minutes through the arterial catheter to maintain a mean arterial pressure (MAP) of 35 mmHg for 30 minutes. NS was infused at a rate of 0.4 ml/hr to maintain patency of the femoral arterial line during shock. At the end of shock, animals were resuscitated with 2× shed blood volume in NS over a 30-minute period. Animals were then observed for 1.5 hours following resuscitation. TEG was performed at baseline, and at the completion of the experiment, 120 minutes postshock. Euthermia was maintained throughout the experiment with the use of a heat lamp. Preshock and postshock arterial blood gas measurements were performed to confirm the absence of metabolic acidosis, as well as preshock and postshock hematocrit levels to confirm that 50% hemodilution was achieved (Abbott Point of Care, Princeton, NJ).^{17,18}

Thrombelastography

Thrombelastography (TEG) was performed with blood collected from cardiac puncture at preshock and at the end of the experiment. 1 ml blood was anticoagulated with 100 µl 3.2% sodium citrate and vortexed briefly to avoid platelet clumping.¹⁹ The citrated whole blood was allowed to sit for 30 minutes according to the manufacturer's instructions. Kaolin was used as an activator, and 340 µl of the blood was added to 20 µl of .2M calcium chloride in a disposable plastic cup, with the assay performed on a TEG 5000 Thrombelastograph haemostasis analyzer (Haemonetics, Niles, IL) at 37° C within 2 hours of blood collection.

All TEG parameters were recorded from standard tracings: reaction time (R, minutes), coagulation time (K, minutes), angle (α , degrees), maximum amplitude (MA, mm), clot strength (G, dynes/cm²), and estimated percentage lysis (LY30, Lysis at 30 min, %). The various components of the TEG tracing are depicted in Figure 1.¹² The SP is a measure of the time to initial clot formation, interpreted from the earliest resistance detected by the TEG analyzer causing the tracing to split; this is the terminus of all other platelet-poor plasma clotting assays (e.g., PT and aPTT). The R value, the time elapsed from start of the test until the developing clot provides enough resistance to produce a 2 mm amplitude reading on the TEG tracing, represents the initiation phase of enzymatic clotting factors. K measures the time from clotting factor initiation (R) until clot formation reaches amplitude of 20 mm. The angle (α) is formed by the slope of a tangent line traced from the R to the K time measured in degrees. K time and angle (α) denote the rate at which the clot strengthens and is most representative of thrombin cleavage of the fibrinogen into fibrin. The MA indicates the point at which clot strength reaches its maximum amplitude in millimeters on the TEG tracing, and reflects the end result of platelet-fibrin interaction via the GPIIb-IIIa receptors. G is a calculated measure of total clot strength derived from amplitude (A, mm) $G = \frac{A}{5000 - A}$. The process of fibrinolysis leads to clot dissolution, leading to a decrease in clot strength; estimated percent lysis (LY30) measures the degree of fibrinolysis 30 minutes after MA is reached.

Statistical analysis

Data are reported as the mean + standard deviation, with significance determined using Student's t-tests.

Results

In the absence of hypothermia and acidosis, hemodilution does not have a significant impact on coagulation

In this trauma model, rats were resuscitated from hemorrhagic shock, resulting in a hemodilution of 50% ($43 \pm 4.05\%$ vs. $19.8 \pm 3.96\%$ Hct preshock vs. postshock, $p < 0.0001$). Temperature was monitored throughout the experiment and euthermia maintained with the use of a heat lamp (97.4 ± 0.39 vs. 97.2 ± 0.39 degrees F preshock vs postshock, $p = 0.37$). Additionally blood pH was measured and unchanged (7.41 ± 0.02 vs. 7.40 ± 0.02 preshock vs postshock, $p = 0.33$). (Results are summarized in Table 1).

Clot integrity is maintained following hemodilution

Despite the substantial hemodilution, there was no statistically significant change in clot strength following fluid resuscitation (12.96 ± 2.84 vs. 11.79 ± 1.28 dynes/cm² G preshock vs. postshock, $p = 0.13$). The maximum amplitude (MA) of the clot was not impaired by hemodilution (71.37 ± 5.65 mm vs. 70.07 ± 2.39 mm MA preshock vs. postshock, $p = 0.26$).

Enzymatic activity is preserved following hemodilution

Similarly, the onset of coagulation (R time) was not impaired (1.68 ± 1.74 min vs. 1.75 ± 0.63 min R time preshock vs. postshock, $p = 0.45$). Neither the K time (0.91 ± 0.24 min vs. 0.8 ± 0.00 min K time preshock vs. postshock, $p = 0.09$) nor the Angle (α) (81.55 ± 4.99 degrees vs. 82.04 ± 1.21 degrees angle (α) preshock vs. postshock, $p = 0.40$) were altered by hemodilution.

Hemodilution does not induce fibrinolysis

In addition to the preservation of clot strength and clotting factor activity, hemodilution did not influence fibrinolysis ($0.28 \pm 0.43\%$ vs. $1.44 \pm 3.33\%$ LY30, preshock vs. postshock, $p = 0.16$).

Discussion

This *in vivo* model simulating trauma and hemorrhagic shock does not indicate an independent adverse effect of hemodilution. Our model used crystalloid, not stored blood as the resuscitation fluid in order to directly investigate the role of dilution of clotting factors in the development of microvascular bleeding, and found that neither the clot integrity (G) nor the onset of coagulation (R time) was impaired by hemodilution. In contrast to the role of hemodilution, the adverse effects of acidosis and hypothermia on postinjury coagulopathy have been evaluated *in vitro*,²⁰ in animal experiments²¹ and in clinical studies.^{10,11}

Even though dilution of clotting factors contributing to postinjury coagulopathy has been widely accepted in clinical practice, the literature is divided on the subject. Simmons et. al. concluded in their landmark Vietnam War study that simple dilution effects did not explain the major clinical bleeding they were observing in the wounded soldiers.⁷ Miller et al., however, attributed microvascular bleeding to dilutional thrombocytopenia in their cohort of massively transfused battle casualties.²² In a civilian study, Counts et al. in 1979 concluded that microvascular bleeding was due to dilutional thrombocytopenia in massively transfused patients, which became the rationale for a randomized prospective trial evaluating preemptive platelet transfusion.²³ After their study showed that prophylactic platelet or FFP transfusion did not improve hemostasis or mortality,²⁴ and noting that clotting factors declined suddenly and out of proportion to the expected decline based on dilution alone, the authors ultimately concluded that hemodilution played an inconsequential role in the development of microvascular bleeding.²⁵ A similar trial failed to show benefit for

prophylactic platelet transfusion during cardiac surgery.²⁶ The results imply that platelets are released into the circulation to counteract the effects of hemodilution.

Various clinical studies have shown that INR and PTT fail to correlate with clinical bleeding in trauma patients.^{7,24} Martini et al. have shown that the INR and PTT were not sensitive in detecting these coagulation defects, whereas TEG differentiated the mechanism related to clotting abnormalities in experimental trauma and hemorrhagic shock.¹⁶ Due to the limitations of INR and PTT in predicting microvascular bleeding unless greatly prolonged, thrombelastography was used as a global assessment of coagulation integrity in this study.

A potential limitation of our study is that fibrinogen and platelet levels were not measured directly. The TEG parameters that reflect these parameters: fibrinogen activity (K time, angle), fibrin clot stability (LY30), and platelet integrity (MA), however, were unaltered. Also, the study is limited by variations in coagulation status between animals in this study and other studies. Problems with variability in coagulation-based assays have been demonstrated clinically as well,²⁷ leading the authors to search for reasons to account for these variations. A previous study by Kim et. al, for example, evaluating the effect of hemodilution on coagulation in Sprague Dawley rats, employed twice the recommended concentration of citrate as an anticoagulant, which could explain the relative hypocoagulability in their results. Furthermore, the Kim study corroborated our findings, that coagulopathy was not observed at mild (25%) or moderate (50%) hemodilution, highlighting the important functional reserve of coagulation factors in the setting of trauma and hemorrhagic shock.²⁸

While our focus was specifically on the role of clotting factor dilution on coagulation integrity, our results showed that resuscitating with a crystalloid equivalent to the rat's blood volume did not contribute to the development of microvascular bleeding; however, the direct contribution of stored blood components, specifically pRBCs on coagulation integrity was not investigated in this study. Hemodilution of 50% was chosen in order to avoid critical anemia (< 7 g/dl hemoglobin). A previous study of experimental hemorrhagic shock implicated hemodilution in the uniform lethality that ensued; however, this study was confounded by the fact that rats were resuscitated with crystalloid to a hematocrit of 5%. Alternatively, a model of hemorrhagic shock in which critical anemia was avoided demonstrated that hemodilution had no effect on mortality.²⁹ Therefore, in the presence of critical anemia, it is conceivable that irreversible hemorrhagic shock leads to lethality due to profoundly impaired oxygen carrying capacity rather than dilution of clotting factors.

Ruttman et. al investigated the role of dilution of clotting factors on clot formation using TEG.³⁰ Unexpectedly, administration of a 1 L NS bolus to healthy subjects induced a transient hypercoagulable state associated with a reduction in antithrombin III (ATIII) levels, leading to a decrease in the onset of coagulation (R) without difference in K, Angle, or MA.³¹ ATIII exhibits second-order kinetics, making it exceptionally sensitive to a reduction in concentration compared to other clotting factors.³²

In summary, there was no change in mean postshock coagulation values reported in our study and, in spite of the variation between the rodents, none of the animals in the study developed clinically detectable microvascular bleeding.

Hemodilution ($\leq 50\%$) had an inconsequential effect on coagulation following trauma and hemorrhagic shock as an independent factor. These data call to question the commonly held belief that hemodilution is critical in the development of postinjury coagulopathy. The highly variable clinical response to hemorrhagic shock, compounded by the effects of massive transfusion of stored blood components, disseminated intravascular coagulation,

and qualitative platelet defects are more likely to contribute to the complex milieu in which the development of postinjury coagulopathy occurs, rather than hemodilution by itself.

Acknowledgments

This work was supported by NIH/NIGMS Grants T32-GM008315 and P50-GM49222.

References

1. Blalock A. Experimental shock: The cause of the low blood pressure produced by muscle injury. *Arch Surg.* 1930; 20:959–996.
2. Robertson LB. The transfusion of whole blood: A suggestion for its more frequent employment in war surgery. *Br Med J.* 1916; 2:38–40. [PubMed: 20768200]
3. Hoyt DB. Blood and war—lest we forget. *J Am Coll Surg.* 2009; 209:681–6. [PubMed: 19959034]
4. Landsteiner K. Individual differences in human blood. *Science.* 1931; 73:403–9. [PubMed: 17755773]
5. Cohn EJ, Strong LE. Preparation and properties of serum and plasma proteins; a system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. *J Am Chem Soc.* 1946; 68:459–75. [PubMed: 21015743]
6. Lewisohn R. The importance of the proper dosage of sodium citrate in blood transfusion. *Ann Surg.* 1916; 64:618–23. [PubMed: 17863633]
7. Simmons RL, Collins JA, Heisterkamp CA, et al. Coagulation disorders in combat casualties. I. Acute changes after wounding. II. Effects of massive transfusion. 3. Post-resuscitative changes. *Ann Surg.* 1969; 169:455–82. [PubMed: 5774736]
8. Hirshberg A, Dugas M, Banez EI, et al. Minimizing dilutional coagulopathy in exsanguinating hemorrhage: a computer simulation. *J Trauma.* 2003; 54:454–63. [PubMed: 12634523]
9. Bickell WH, Wall MJ Jr, Pepe PE, et al. Immediate versus delayed fluid resuscitation for hypotensive patients with penetrating torso injuries. *N Engl J Med.* 1994; 331:1105–9. [PubMed: 7935634]
10. Kashuk JL, Moore EE, Millikan JS, et al. Major abdominal vascular trauma—a unified approach. *J Trauma.* 1982; 22:672–9. [PubMed: 6980992]
11. Cosgriff N, Moore EE, Sauaia A, et al. Predicting life-threatening coagulopathy in the massively transfused trauma patient: Hypothermia and acidosis revisited. *J Trauma.* 1997; 42:857–61. [PubMed: 9191667]
12. Gonzalez E, Kashuk JL, Moore EE, et al. Differentiation of enzymatic from platelet hypercoagulability using the novel thrombelastography parameter delta (delta). *J Surg Res.* 2010; 163:96–101. [PubMed: 20605586]
13. Hoffman M, Monroe DM 3rd. A cell-based model of hemostasis. *Thromb Haemost.* 2001; 85:958–65. [PubMed: 11434702]
14. Starzl TE, Marchioro TL, Von Kaulla KN, Hermann, et al. Homotransplantation of the liver in humans. *Surg Gynecol Obstet.* 1963; 117:659–76. [PubMed: 14100514]
15. Von Kaulla KN, Swan H. Clotting deviations in man during cardiac bypass: Fibrinolysis and circulating anticoagulant. *J Thorac Surg.* 1958; 36:519–30. [PubMed: 13588709]
16. Martini WZ, Cortez DS, Dubick MA, et al. Thrombelastography is better than PT, aPTT, and activated clotting time in detecting clinically relevant clotting abnormalities after hypothermia, hemorrhagic shock and resuscitation in pigs. *J Trauma.* 2008; 65:535–43. [PubMed: 18784565]
17. Mock T, Morrison D, Yatscoff R. Evaluation of the i-STAT system: a portable chemistry analyzer for the measurement of sodium, potassium, chloride, urea, glucose, and hematocrit. *Clin Biochem.* 1995; 28:187–92. [PubMed: 7628079]
18. Thomas FO, Hoffman TL, Handrahan DL, et al. The measure of treatment agreement between portable and laboratory blood gas measurements in guiding protocol-driven ventilator management. *J Trauma.* 2009; 67:303–13. [PubMed: 19667883]
19. Tvedten H, Korcal D. Vortex mixing of feline blood to disaggregate platelet clumps. *Vet Clin Pathol.* 2001; 30:104–106. [PubMed: 12024307]

20. Meng ZH, Wolberg AS, Monroe DM III, et al. The effect of temperature and pH on the activity of factor VIIa: Implications for the efficacy of high-dose factor VIIa in hypothermic and acidotic patients. *J Trauma*. 2003; 55:886–891. [PubMed: 14608161]
21. Martini WZ, Pusateri AE, Uscilowicz JM, et al. Independent contributions of hypothermia and acidosis to coagulopathy in swine. *J Trauma*. 2005; 58:1002–1009. [PubMed: 15920416]
22. Miller RD, Robbins TO, Tong MJ, et al. Coagulation defects associated with massive blood transfusions. *Ann Surg*. 1971; 174:794–801. [PubMed: 5113461]
23. Counts RB, Haisch C, Simon TL, et al. Hemostasis in massively transfused trauma patients. *Ann Surg*. 1979; 190:91–9. [PubMed: 464685]
24. Reed RL 2nd, Ciavarella D, Heimbach DM, et al. Prophylactic platelet administration during massive transfusion. A prospective, randomized, double-blind clinical study. *Ann Surg*. 1986; 203:40–8. [PubMed: 3510591]
25. Ciavarella D, Reed RL, Counts RB, et al. Clotting factor levels and the risk of diffuse microvascular bleeding in the massively transfused patient. *Br J Haematol*. 1987; 67:365–8. [PubMed: 3689699]
26. Simon TL, Akl BF, Murphy W. Controlled trial of routine administration of platelet concentrates in cardiopulmonary bypass surgery. *Ann Thorac Surg*. 1984; 37:359–64. [PubMed: 6370157]
27. Chitlur M, Lusher J. Standardization of thromboelastography: values and challenges. *Semin Thromb Hemost*. 2010; 36:707–11. [PubMed: 20978991]
28. Kim HW, Stubdal H, Greenburg AG. Coagulation dynamics after hemodilution with polyhemoglobin. *Surg Gynecol Obstet*. 1992; 175:219–26. [PubMed: 1381112]
29. Singh G, Chaudry KI, Chaudry IH. Crystalloid is as effective as blood in the resuscitation of hemorrhagic shock. *Ann Surg*. 1992; 215:377–82. [PubMed: 1558419]
30. Ruttman TG. Haemodilution enhances coagulation. *Br J Anaesth*. 2002; 88:470–472. [PubMed: 12066719]
31. Ruttman TG, Jamest MF, Lombard EH. Haemodilution induced enhancement of coagulation is attenuated in vitro by restoring antithrombin III to pre-dilution concentrations. *Anaesth Intensive Care*. 2001; 29:489–493. [PubMed: 11669429]
32. Jesty J. The kinetics of inhibition of thrombin by antithrombin in the presence of components of the hemostatic system. *Blood*. 1985; 66:1189–95. [PubMed: 4052633]

Figure 1. TEG diagram

The following TEG parameters were recorded from standard tracings: reaction time (R, minutes), coagulation time (K, minutes), angle (α , degrees), maximum amplitude (MA, mm), clot strength (G, dynes/cm²), and estimated percentage lysis (LY30, Lysis at 30 min, %).

Table 1

	Pre Shock	Post Shock	t-test pre vs. post shock
Hematocrit	43 ± 4.05	19.8 ± 3.96	< 0.0001
Clot Strength (G)	12.96 ± 2.84	11.79 ± 1.28	0.13
R	1.68 ± 1.74	1.75 ± 0.63	0.45
K	0.91 ± 0.24	0.8 ± 0.00	0.09
Angle	81.55 ± 4.99	82.04 ± 1.21	0.40
MA	71.37 ± 5.65	70.07 ± 2.39	0.26
Lysis at 30 min	0.28 ± 0.43	1.44 ± 3.33	0.16
Temperature (o F)	97.4 ± 0.39	97.2 ± 0.39	0.37
pH of blood	7.41 ± 0.02	7.40 ± 0.02	0.33