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14. ABSTRACT

The purpose of this grant was to support a national coordinating center for trauma research funding. The infrastructure/call for proposals and review process established was streamlined and efficient leading to the selection of research projects based on a solid scientific, peer review of submitted research proposals. Over the last six years, four trauma research studies have been completed across nine academic trauma centers enrolling a total of 3,817 trauma patients as study participants. In total, this award resulted in fifteen scholarly articles and twenty-five presentations at local, state and national trauma association/professional meetings and advanced scientific knowledge and best practice regarding the care of trauma patients in military and civilian settings.

15. SUBJECT TERMS

Trauma, ICU, education, research, training, analysis, practice, coagulation, transfusion

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INTRODUCTION

The University of Texas Health Science Center at San Antonio (UTHSCSA) proposed to utilize $2,101,000 in congressional funding to work collaboratively with the National Trauma Institute (NTI) to build on the establishment of NTI as a national coordinating center for trauma research funding. In addition, a forum for dissemination of trauma research information was provided for the trauma community through the NTI Annual Trauma Conference. No-cost extensions were approved in September 2011 and 2012. A 24-month no-cost extension was approved September 2013. A final one-year no-cost extension was requested in June of 2015 and approved by the COR. This final year allowed for the last research study to be completed.

BODY

Statement of Work

A. NTI supported a national coordinating center for trauma research funding.

1. NTI prepared and issued requests for proposals (RFP) based on areas of scientific merit in trauma and emergency or critical care.
2. NTI's Science Committee scored proposals according to scientific merit, clinical impact and ability to perform.
3. NTI's Board updated trauma research subject areas based upon their impact on survival or care of patients, existing funding, and funding availability annually.
4. NTI performed award management and compliance activities, including all appropriate USAMRMC HRPO requirements.
5. NTI provided research funding for the following proposals that sought to address areas of urgent need in the treatment of trauma.
    a) Timing and Mechanism of Traumatic Coagulopathy, PI - Mitchell Cohen, MD, University of California, San Francisco.
    b) Comparative Effectiveness of Clinical Care Processes in Resuscitation and Management of Moderate to Severe Traumatic Injuries. PI - Shahid Shafi, MPH, MD, FACS, Baylor Research Institute
    c) Characterization of the Effects of Early Sex-Hormone Environment Following Injury, PI - Jason L. Sperry, MD, MPH, University of Pittsburgh
    d) Vasopressin Supplementation during the Resuscitation of Hemorrhagic Shock, PI - Carrie Sims, MD, MS, University of Pennsylvania

B. NTI provided a forum for dissemination of trauma research information to the trauma community.

A. National Coordinating Center for Trauma Research Funding:

Tasks 1 and 2: Issuing requests for proposals and performing scientific peer review

NTI's national request for proposals attracted 85 pre-proposals. The NTI Science Committee invited 15 to submit full proposals, giving priority to clinical and translational research studies. Final proposals were evaluated on the following criteria: relevance to NTI research objectives, scientific excellence, clinical relevance and impact, multicenter collaboration, military relevance, innovation, potential for follow-on studies, and feasibility of completing the objectives in the funding period. The NTI Science Committee conducted scientific peer-review of all research proposals submitted, then held a face-to-face meeting in order to evaluate, review, and make final award recommendations. The NTI Science Committee ultimately recommended four proposals, and these were approved by the NTI Board of Directors for funding.

NTI used an online software product to facilitate the submission, management, and review of proposals. This web-based system supported reporting, financial tracking, and ongoing communication with awardee researchers. Throughout the grant lifecycle, NTI conducted award management and compliance tasks related to the studies, including maintenance of the appropriate USAMRMC HRPO requirements.

Tasks 3-5: Updating priorities, providing research funding for proposals that address areas of urgent need in the treatment of trauma, and performing compliance activities

Project 1:
Project Title: Timing and Mechanism of Traumatic Coagulopathy
PI Name: Mitchell Cohen, MD
PI Institution: University of California, San Francisco
HRPO: Log#: A-16375.3a
Participating Centers: a) UTHSC-Houston, Center for Translational Injury Research (CeTIR), Dr. Bryan Cotton. HRPO Log# A-16375.b; b) University of California, Berkeley, Adam Hubbard, PhD. HRPO Log # A-16375.c

Project Abstract:
Trauma remains the leading cause of death and disability in patients less than 40 years old. Coagulopathy is common following severe trauma and is associated with poor outcome. Unfortunately, the mechanisms for clotting problems are poorly characterized and, as a result, diagnosis is difficult and treatment options are limited. In addition, there is a newly-described link between early coagulopathy and later multiple organ failure (MOF), infection, and mortality. Despite this link, the mechanisms for this clotting abnormality, and later inflammation after trauma, are unknown. This is clinically important because it is impossible to optimize therapeutic efficacy to control bleeding and at the same time minimize the risks of late thrombotic, infectious, and inflammatory complications without completely understanding the spectrum of coagulation abnormalities seen after injury.

This clinically significant problem was addressed by this study with two inter-related aims. Aim 1 was to completely characterize coagulation parameters in severely injured patients by means of a prospective, multi-center observational study at two major trauma centers. Aim 2 was to use systems biology methods to identify the central mediators involved in the coagulopathic phenotypes after trauma and to produce a predictive model to provide decision support to diagnose coagulopathy and guide treatment after severe trauma.

Uncontrolled hemorrhage, to which clotting abnormalities contribute, is responsible for the majority of trauma related deaths in the first 24 hours. Vast experience gleaned in civilian and military trauma centers has confirmed that the initial treatment of the injured must center on a resuscitation protocol that prioritizes control of hemorrhage and treatment of coagulopathy and seeks to prevent the late inflammatory dysfunction. This study sought to improve understanding of traumatic coagulopathy and guide resuscitation toward better patient outcomes.

Results:
This study, with a total of 317 enrolled participants over 32 months, demonstrated that clinically significant platelet dysfunction after trauma exists in the presence of an otherwise reassuring platelet count and clotting studies, with profound implications for mortality. Impedance aggregometry reliably identifies this dysfunction in injured patients, and admission arachidonic acid and collagen responsiveness are significant predictors of both early and late mortality. This study prospectively quantified platelet function using multiple electrode aggregometry to identify previously undetected platelet dysfunction in trauma patients. The study found that a low Glasgow Coma Score (GCS) was

an independent predictor of platelet hypofunction and highlighted the importance of further investigation into the link between traumatic brain injury and platelet dysfunction. The clinical availability of rapid, point-of-care platelet function testing will lead to improved triage, more appropriately targeted therapy, and better outcomes after trauma (Kutcher ME, Redick BJ, McCreery RC, et al. 2012; Appendix C).

Acute coagulopathy occurs with massive tissue injury and hypoperfusion and results in both impairment of new clot formation and enhanced fibrinolysis in trauma patients. Prior to this study, clinical guidelines for treatment of hyper-fibrinolysis (HF) were sparse. When this study was initiated, HF was diagnosed with thromboelastography but these devices were not widely available. This study addressed a critical gap in scientific knowledge by identifying hypothermia, acidosis, and coagulopathy as well as relative thrombocytopenia as predictors of HF. It also identified significant depletion of factor V and factor IX as well as elevation of activated protein C, in patients with HF. Overall, the study confirmed and extended the literature on the recognition and treatment of HF after trauma through specific thromboelastography-based diagnostic criteria, multivariate risk factor assessment, and receiver operator characteristics-based clinical guidelines for empiric treatment. (Kutcher ME, Cripps MW, McCreery RC, et al. 2012; Appendix D)

Tissue injury leads to the release of damage-associated molecular patterns (DAMP's) that may drive a sterile inflammatory response; however, prior to this study, the role of extracellular histone levels after trauma was unexplored. Data from this study demonstrated that extracellular histone levels are elevated in response to traumatic injury and correlate with fibrinolysis and systemic anticoagulant activation. This study was the first to detail characterization of the patient and injury characteristics associated with extracellular histone levels after trauma and the first to demonstrate a correlation with activated protein C (aPC). These findings highlighted the parallels between mechanisms of pathogen-induced sepsis and those underlying the sterile inflammatory response to trauma, suggesting that insights and therapies from the critical care and sepsis literature may be applicable earlier in the hospital course to the care of the acutely injured trauma patient. (Kutcher ME, Xu J, Vilardi RF, et al. 2012; Appendix A)

The importance of specific clotting factor abnormalities to the complex phenomenon of acute traumatic coagulopathy is poorly understood. As part of this study, the research team investigated the use of principal component analysis (PCA) as a mathematical tool to more clearly identify and examine the clotting factor relationships underlying clinical patterns and correlate these with outcomes among critically injured patients. Results suggested that PCA accurately identifies patterns embedded in the complex milieu of the coagulation cascade. The independent consumptive and fibrinolytic components identified showed robust correlation with patient-level outcomes. (Kutcher ME, Ferguson AR, Cohen MJ. 2013; Appendix F)

At the time this study was initiated, identification and characterization of which massively transfused trauma patients die of anatomically nonsurvivable injury versus which patients exsanguinate from anatomically survivable injury versus which patients survive initially only to perish from late physiological collapse hadn't been performed. This study expanded previous research on the potential survivability of deaths on the battlefield by separating the potentially survivable cause of death into hemorrhage, early physiologic collapse and late physiologic collapse among severely injured civilian patients. (Cripps MW, Kutcher ME, Daley A, et al. 2013; Appendix G)

Mounting evidence on the benefits of hemostatic resuscitation has led to a renewed interest in whole blood (WB) and reconstituted WB (RWB), however, there were few data characterizing the clotting profiles of these variants. As part of this study, Kornblith et al. characterized extensive factor-level and functional profiles of banked nonmodified and modified variants of RWB. (Kornblith LZ, Howard BM, Cheung CK, et al. 2014; Appendix H)

The researchers also investigated differences in longitudinal clotting factor levels in a cohort of trauma patients who were not transfused to establish an early natural history of coagulation and longitudinal changes in coagulation profiles in transfused trauma patients. This was the first "natural history" study of longitudinal clotting factors after severe injury. The data showed that targeting ratios of red blood cells (RBC) and fresh frozen plasma (FFP) to RBC:FFP ≤ 1.5:1 leads to earlier correction of prothrombin time and partial thromboplastin time and earlier and prolonged repletion of specific clotting factor deficits compared with higher ratio transfusion strategies. (Kutcher ME, Kornblith LZ, Vilardi RF, et al. 2014; Appendix K)

Dr. Cohen and his research team presented and published the results of this study in multiple venues—see the Reportable Outcomes section.

Project 2:
Project Title: Comparative Effectiveness of Clinical Care Processes in Resuscitation and Management of Moderate to Severe Traumatic Injuries
PI Name: Shahid Shafi, MPH, MD, FACS
PI Institution: Baylor Research Institute
HRPO: Log#A-16375.2a
Participating Centers: University of Texas Health Science Center, Houston, HRPO Log#16375.2c; University of California at Los Angeles HRPO Log# A-16375.2d; Massachusetts General Hospital HRPO Log# A-16375.2e

Project Abstract:
Injured patients treated at designated trauma centers are more likely to survive than patients treated at non-designated hospitals. However, the PI of this project had earlier demonstrated that risk-adjusted mortality rates are highly variable across trauma centers despite availability of similar resources as ensured by their designation. Donabedian principles of quality management suggest that outcomes depend upon institutional structures and processes. If trauma centers have similar structures, then variable outcomes are likely related to variations in clinical care processes.

The hypothesis for this study was that clinical care processes for management of trauma patients are variable across centers, and specific processes that improve patient outcomes can be identified. Also, it was hypothesized that processes that increase costs without improving outcomes can be determined. This was a multicenter retrospective study of four Level I trauma centers over three years that serve a diverse population exceeding 13 million. Adult patients with moderate to severe injuries were included (n=3,107, 2006-2010). Clinical care processes related to initial assessment, resuscitation, hemorrhage control, operative care, critical care, and rehabilitation, as well as patient outcomes (in-hospital mortality, complications, length of stay, hospitalization costs) were measured. Compliance with 22 trauma-specific Processes of Care (T-POC) was measured. The association between compliance with T-POC and patient outcomes was measured after adjusting for patient demographic and clinical characteristics.

The specific aims of the study were as follows: Specific Aim 1: To identify processes of care that are independent predictors of risk-adjusted outcomes (mortality, complications, costs, length of stay). Specific Aim 2: To measure variations in practices, and their associated costs, between and within institutions. Specific Aim 3: To calculate the potential impact of improved adoption of processes of care identified in Specific Aim 1 on patient outcomes. This study was conducted in two phases. The first phase was a retrospective review at one trauma center of a random sample of 1,000 trauma patients that hypothesized that improved compliance with trauma-specific clinical processes of care is

associated with reduced in-hospital mortality. Additional analyses of this cohort sought to identify patients least likely to receive optimal care.

Results: The first phase of this study focused on 1,000 trauma patients at one trauma center. Study findings demonstrated large variations in clinical practice resulting in inadequate compliance with several commonly recommended clinical T-POC that are necessary for optimal management of trauma patients. Study patients were eligible for a total of 2,603 trauma processes of care (T-POC), of which only 1,515 (58%) were provided to the patients. Compliance was highest for T-POC involving resuscitation (83%) and was lowest for neurosurgical interventions (17%). (Shafi S, Rayan N, Barnes SA, et al. 2012; Appendix O)

In the second phase of the study, a retrospective observational study was conducted at 4 Level 1 trauma centers. Median compliance score with T-POC was 80 (interquartile range, 50-100). Compliance with each T-POC ranged from as low as 13% to as high as 95%. There were wide variations in practices between centers and within each center. After adjusting for patient demographics and injury severity, each 10% increase in compliance with T-POC was associated with a 12.5% reduction in the risk of death, 4.3% increase in hospital length of stay, 4.6% increase in direct costs, and 9% increase in risk of complications. Compliance with recommended T-POC remains suboptimal at trauma centers with significant variations in clinical practices. Improved adoption of recommended care may reduce mortality but will likely increase costs and complications. (Rayan N, Barnes SA, Fleming N, et al. 2012; Appendix P) (Shafi S, Barnes SA, Rayan N, et al. 2014; Appendix Q)

Dr. Shafi presented and published the results of this study in multiple venues—see the Reportable Outcomes section.

Project 3:
Project Title: Characterization of the Effects of Early Sex-hormone Environment Following Injury
Principal Investigator: Jason L. Sperry, MD, MPH
Site: University of Pittsburgh
HRPO: *Log#: A-16375.1*

Project Abstract:
Although significant advances in the care of the injured patient have occurred over the last decade, those who survive their initial injury continue to be plagued with the development of multiple organ failure and sepsis and their attributable morbid effects. One important and persistent finding has been that males and females respond differently following traumatic injury and hemorrhagic shock, with a relative protection afforded to females. A large body of literature has evolved attempting to elucidate the mechanisms responsible for these differences; however, a significant divide continues to exist between what experimental animal investigations have revealed and what has been shown clinically in humans. The ultimate elucidation of the mechanisms responsible for these outcome differences will allow future risk factors and therapeutic targets to be discovered and characterized, having significant potential to improve outcomes in both males and females following injury. The early sex-hormone environment may help shape or determine the intensity of the early inflammatory response that follows injury and may provide a predisposition toward maintenance of any excessive or inadequate response once initiated. Similarly, the early sex-hormone environment may affect the need for resuscitation or blood component transfusion, and knowledge of this early hormonal milieu may allow those patients at highest risk of poor outcome post-injury to be identified.

The overarching goal of this study was to further characterize and investigate the early sex-hormone environment following injury and the associations of early estrogen and testosterone levels with the strength of the innate immune response, the coagulation response, resuscitation requirements, and clinical outcomes following injury. As few effective interventions exist to alter the morbidity and mortality that inherently follow traumatic injury, investigation into novel mechanisms that may result in a protective effect can provide a route to reduce these sequelae post-injury.

Results:
This study characterized an X chromosome-linked IL-1 receptor-associated kinase (IRAK-1) polymorphism as an alternative mechanism responsible for sex differences post-injury. IRAK-1 is a key intermediate in the Toll-like receptor (TLR) pathway thought to drive inflammation post-injury. In a cohort of 288 subjects enrolled in 2011-2012, the prevalence of the IRAK-1 variant was 12.5%. Subjects with and without the variant were similar in age, injury severity, and 24-hour blood transfusion. After controlling for important confounders, the IRAK1 variant was independently associated with over a six-fold (OR 6.4; 95% CI1.8-23) and five-fold (OR 5.8; 95% CI1.4-24) greater risk of multiple organ failure (MOF) and mortality, respectively. These differences were most prominent in males, while females heterozygous for the variant demonstrated worse outcome in a dose-dependent fashion. Thus, the IRAK1 polymorphism is a strong independent predictor of MOF and mortality post-injury and represents a common variant with prognostic potential. These data demonstrate the importance of TLR signaling post-injury and support that a genetic mechanism may drive gender outcome differences post-injury. (Zolin SJ, Vodovotz Y, Forsythe RM, et al. 2015: Appendix V)

Additional funding was provided by NTI though W81XH-11-1-0841; and a larger cohort of 321 subjects allowed the use of Multiple Organ Failure (MOF) and nosocomial infections (NI) as the outcomes of interest and provided appropriate power for the analysis. The results demonstrated that despite a paucity of sex-specific differences in a moderate-sized blunt injury cohort of patients, the evolving sex hormone environment after injury is associated with both clinical outcome and innate immune response differences. Rising total testosterone levels at six hours post-injury were significantly associated with more than a five-fold and two-fold higher independent risk of MOF and NI, respectively. Concurrently, estrogen levels were found to be strongly associated with detrimental clinical outcome at the delayed 24-hour period alone. (Sperry JL, Zolin S, Zuckerbraum BS, et al. 2014: Appendix S)

Dr. Sperry presented and published the results of this study in multiple venues—see the Reportable Outcomes section.

Project 4:
Project Title: Vasopressin Supplementation during the Resuscitation of Hemorrhagic Shock
PI Name: Carrie Sims, MD, MS
PI Institution: University of Pennsylvania
HRPO: Log # A-16375.4

Project Abstract:
Trauma remains the leading cause of death for those under the age of forty in the United States, with a large percentage of patients dying from hemorrhagic shock within the initial hours after a severe injury. Although aggressive treatment with intravenous fluids and blood products has been the gold standard, massive resuscitation profoundly alters the neuroendocrine milieu needed to maintain vasomotor tone and is associated with the development of a vasopressin deficiency. Vasopressin is a critical hormone needed for blood pressure support during shock, and low levels are associated with recalcitrant hypotension, increased transfusion requirements, and additional morbidity. Although high

dose vasopressin supplementation has been shown to improve blood pressure, decrease blood loss and improve survival in animal models of lethal hemorrhage, clinical studies investigating vasopressin are limited to case reports, with no prospective clinical studies to date. The goal of this research was to develop targeted interventions to address hemorrhagic shock. The pilot study (Vasopressin Supplementation during the Resuscitation of Hemorrhagic Shock – the AVERT Shock Trial) evaluated the clinical applicability of using the biomarker copeptin for predicting the need for resuscitation resources and as a means of monitoring the development of vasopressin deficiency. Identifying and targeting neuroendocrine deficiencies during the resuscitation of trauma patients is a novel approach to ameliorate the profound hypotension seen in late stage shock; limiting the need for aggressive volume and blood product resuscitation and decreasing the incidence of resuscitation associated complications.

Results:
Because this study required an Exception from Informed Consent (EFIC), an extensive community consultation process was undertaken, with interviews conducted with 309 trauma patients, their family members and geographic community members. In addition, seven focus groups were held at community-based organizations in the neighborhood surrounding the Level 1 trauma center in order to assess and incorporate the community's perspective into the research design. Trauma patients, their family members and community members expressed a high degree of support for the AVERT Shock Trail, EFIC and emergency research in general. By allowing participants the opportunity to ask questions and provide direct feedback to the research team via focus groups and semi-structured interviews, the trauma community became "partners" in the research process. This method of community consultation may have contributed to the overwhelming support of the AVERT Shock Trial. (Sims CA, Isserman JA, Holena D, et al. 2013: Appendix Y)

In a subset of these participants (n=179), the research team calculated proximity to violence (distance from home address zip code to violence hot spot zones in the surrounding community) and examined whether race, socioeconomic status and closer proximity to violence were correlated with attitudes toward the AVERT Shock Study and skepticism of EFIC research. There was no correlation between AVERT attitude/willingness scores and race, sex, age, income or status as a trauma patient. Proximity to violence did not correlate with perception of EFIC, willingness to participate in emergency research or violent mechanism of injury. (Maher, Z, Grill, EK, Smith, BP, Sims, CA. 2015: Appendix AA)

The AVERT Shock Trial concluded trauma patient participant enrollment and data collection in September 2016 (n=100). Currently, Dr. Sims is analyzing the clinical study data.

Dr. Sims and her colleagues presented and published the initial results of this study pertaining to study design, the EFIC process and the influence of proximity to violence on community attitudes toward EFIC in multiple venues—see the Reportable Outcomes section.

B. Provide a Forum for Dissemination of Research Outcomes to the Trauma Community.

The 16th National Trauma Institute Annual Symposium was held August 30-September 1, 2010. There were more than 400 attendees: 45% military and 55% civilian. Attendees included physicians, nurses, and pre-hospital care providers from 28 states. Up to 15.5 hours of AMA PRA Category 1 Credits or Continuing Nursing Education credits were offered, and more than 100 speakers made pro/con style presentations in both general and breakout sessions.

The general sessions included PTSD and Provider Resiliency, Traumatic Brain Injury, and Resuscitation. The concurrent breakout sessions included Trauma Critical Care/Emergency

Medicine/Anesthesia, Craniofacial Trauma, Neurosurgery, Orthopedic Trauma, Trauma, Nursing/Allied Health, and Burn Surgery.

A three-hour Advanced Airway Workshop (AAW) was also held with 100 attendees--38% military and 62% civilian—following the symposium. This AAW included live demonstrations on manikins and adjunct materials that mimicked the difficult airway. The attendees rotated through 13 learning stations and utilized various fiber optic intubating devices, practiced surgical airway approaches, and used video laryngoscopes and supraglottic airway devices.

KEY RESEARCH ACCOMPLISHMENTS

Project 1:
Project Title: Timing and Mechanism of Traumatic Coagulopathy

This study advanced the science of traumatic coagulopathy by characterizing coagulation parameters in severely injured patients and using systems biology methods to identify the central mediators involved in the coagulopathic phenotypes after trauma and to produce a predictive model to provide decision support to diagnose coagulopathy and guide treatment after severe trauma. Key research accomplishments were:

- First characterization of Acute Traumatic Coagulopathy (ATC)
- First principle component analysis of drivers of traumatic coagulopathy after injury
- First advanced dynamic statistical models of coagulopathy after injury
- First measurement of the effect of plasma based resuscitation on coagulation and outcome after injury
- First “natural history” study of longitudinal clotting factor levels after severe injury
- The study findings supported the empiric treatment of fibrinolysis in patients with trauma who present with acidosis, hypothermia, coagulopathy, and relative thrombocytopenia as a strategy for appropriately sensitive empiric treatment in clinical settings in which thromboelastography and other advanced diagnostic equipment is unavailable

This study also produced a presentation (Kornblith LZ, et al. 2014) that was the winner of the Earl Young Competition at the 2014 Western Trauma Association annual meeting.

Project 2:
Project Title: Comparative Effectiveness of Clinical Care Processes in Resuscitation and Management of Moderate to Severe Traumatic Injuries

This was the first multicenter study to measure compliance with commonly recommended care at four trauma centers and to assess the association between trauma-specific clinical process of care (T-POC) compliance and patient outcomes. Key research accomplishments were:

- Median compliance score was 80 (inter-quartile range, 50 to 100). This indicates that half of the patients in the study received less than 80% of the recommended care.
- There were differences in compliance between the four centers, with median compliance score ranging from 67 to 100.
- Compliance with each process of care ranged from as low as 13% for thoracotomy in ED to as high as 95% for endotracheal intubation. There were wide variations in compliance with specific processes between centers and within each center.

- Each 10% increase in compliance with recommended care was associated with 12.5% reduction in mortality after accounting for patient demographics and injuries.
- Each 10% increase in compliance score was associated with a 4.3% increase in hospital length of stay, 4.6% increase in direct costs, and 9% increase in the risk of complications.
- Compliance with 11 specific processes was associated with reduced risk of mortality, while none was associated with increased risk of death.
- Full compliance with the 22 processes of care included in this study could potentially save 128 lives, an overall 56% reduction in mortality in the entire study population from 11.5% to 6.6%.

Project 3:
Project Title: Characterization of the Effects of Early Sex-hormone Environment Following Injury

Key research findings of this study that further characterize the early sex-hormone environment following injury and the associations of early estrogen and testosterone levels with the strength of the innate immune and coagulation responses, resuscitation requirements, and clinical outcomes were:

- The IRAK1 polymorphism is a strong independent predictor of MOF and mortality post-injury.
- The evolving sex hormone environment after injury is associated with both clinical outcome and innate immune response differences.
- Rising total testosterone levels at six hours post-injury are significantly associated with more than a fivefold and twofold higher independent risk of MOF and NI, respectively.
- Estrogen levels are strongly associated with detrimental clinical outcome at the delayed 24-hour period.

Project 4:
Project Title: Vasopressin Supplementation during the Resuscitation of Hemorrhagic Shock

Key research accomplishments regarding use of Exception from Informed Consent (EFIC) were:

- Roughly 29% of participants in semi-structured interviews or focus groups regarding EFIC had previously participated in a clinical study and only 4-6% had been asked to consent on behalf of another person.
- When asked who would be the best person to consent on their behalf for an emergency research trial, the majority (93-96%) listed a family member.
- The overwhelming majority supported the need for more research in trauma (95-99%) and indicated that the AVERT Shock Trial was an important study to perform (95-99%).

Dr. Sims received the American College of Surgeons Jacobson Promising Investigator Award in 2014 for her AVERT presentation titled “Novel Resuscitative Strategies”.

REPORTABLE OUTCOMES

Project 1:
Project Title: Timing and Mechanism of Traumatic Coagulopathy

Cohen MJ. Mechanisms of Acute Traumatic Coagulopathy. Advanced technology applications to combat casualty care (ATACCC), St. Petersburg FL, August 18, 2011.

Kutcher ME, Xu J, Vilardi RF, Ho C, Esmon CT, Cohen MJ. DAMPened Inflammation: Extracellular histone release in response to traumatic injury: Implications for a compensatory role of activated Protein C. Presented at Western Trauma Association Annual Meeting, Vail CO, February 2012. (Appendix A)

Narayan R, Curd, V, Singh T, Dailey A, McCreery RC, Xu J, Crane IM, Greenberg MD, Nelson MR, Knudson MM, Fiebig E, Kutcher ME, Cohen MJ. Timing and clinical accuracy of massive transfusion protocol activation. Presented at Pacific Coast Surgical Association Annual Meeting, Napa CA, February 2012. (Appendix B)

Kutcher ME, Redick BJ, McCreery RC, Crane IM, Greenberg MD, Cachola LM, Nelson MR, Cohen MJ. Characterization of platelet dysfunction after trauma. *J Trauma Acute Care Surg*. 2012 Jul;73(1):13-9. (Appendix C)

Kutcher ME, Cripps MW, McCreery RC, Crane IM, Greenberg MD, Cachola LM, Redick BJ, Nelson MF, Cohen MJ. Criteria for empiric treatment of hyperfibrinolysis after trauma. *J Trauma Acute Care Surg*. 2012 Jul;73(1):87-93. (Appendix D)

Kutcher ME, Ferguson AR, Cohen MJ. The principal components of acute traumatic coagulopathy. Oral presentation at the American Association for the Surgery of Trauma Meeting (AAST) 2012 Annual Conference, Kauai HI, September 12, 2012. (Appendix E)

Kutcher ME, Xu J, Vilardi RF, Ho C, Esmon CT, Cohen MJ. Extracellular histone release in response to traumatic injury: Implications for a compensatory role of activated protein C. *J Trauma Acute Care Surg*. 2012 Dec;73(6):1387-92. (Appendix A)

Kutcher ME, Ferguson AR, Cohen MJ. A principal component analysis of coagulation after trauma. *J Trauma Acute Care Surg*. 2013; 74(5): 1223-1230. (Appendix F)

Cripps MW, Kutcher ME, Daley A, McCreery RC, Greenberg MD, Cachola LM, Redick BJ, Nelson MF, Cohen MJ. Cause and timing of death in massively transfused trauma patients. *J Trauma Acute Care Surg*. 2013; 75(2), Supp 2: S255-S262. (Appendix G)

Kornblith LZ, Howard BM, Cheung CK, Dayter Y, Pandey S, Busch MP, Pati S, Callcut RA, Vilardi RF, Redick BJ, Nelson MF, Cohen MJ. The whole is greater than the sum of its parts: Hemostatic profiles of whole blood variants. Oral presentation at the Western Trauma Association (WTA) 2014 Annual Conference, Steamboat Springs, CO, March 5, 2014. (Appendix H)

Cohen MJ, Howard BM, Kornblith LZ, Redick BJ, Mann K, Orfeo T, Vilardi RF, Nelson MF, Brummel-Ziedins K. Computational modeling of thrombin generation in trauma. Poster presentation at 2014 Military Health System Research Symposium (MHSRS), Ft. Lauderdale, Florida, August 19, 2014. (Appendix I)

Kornblith LZ, Kutcher M, Howard BM, Vilardi RF, Redick BJ, Nelson MF, Cohen MJ. The natural history and effect of resuscitation ratio on coagulation after trauma. Poster presentation at 2014 Military Health System Research Symposium (MHSRS), Ft. Lauderdale, Florida, August 19, 2014. (Appendix J)

Kutcher ME, Kornblith LZ, Vilardi RF, Redick BJ, Nelson MF, Cohen MJ. The natural history and effect of resuscitation ratio on coagulation after trauma: A prospective cohort study. *Ann Surg* 2014; 260(6): 1103-1111. (Appendix K)

Kornblith LZ, Howard BM, Cheung CK, Dayter Y, Pandey S, Busch MP, Pati S, Callcut RA, Vilardi RF, Redick BJ, Nelson MF, Cohen MJ. The whole is greater than the sum of its parts: Hemostatic profiles of whole blood variants. *J Trauma Acute Care Surg*. 2014; 77(6): 818-827. (Appendix H)

Kornblith LZ, Cohen MJ. Letter to the Editor Response: The whole is greater than the sum of its parts: Hemostatic profiles of whole-blood variants. *J Trauma Acute Care Surg*. 2014; 77(6): 1003-1004. (Appendix L)

Project 2:
Project Title: Comparative Effectiveness of Clinical Care Processes in Resuscitation and Management of Moderate to Severe Traumatic Injuries

Shafi S, Rayan N, Barnes SA, Fleming N, Gentilello LM, Ballard D. Trauma core measures: Clinical processes that improve patient outcomes. Oral presentation at the American Association for the Surgery of Trauma (AAST) 2010 annual meeting, Boston, MA, September 2010. (Appendix M)

Rayan N, Barnes SA, Fleming N, Kudyakov R, Ballard D, Gentilello L, Shafi S. Barriers to evidence based care in trauma. Oral presentation at the American Association for the Surgery of Trauma (AAST) 2011 annual meeting, Chicago, IL, September 2011. (Appendix N)

Shafi S, Rayan N, Barnes SA, Fleming N, Gentilello LM, Ballard D. Moving from “optimal resources” to “optimal care” at trauma centers. *J Trauma Acute Care Surg*, 2012. Vol.72(4): 870-877. (Appendix O)

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Shafi S, Barnes SA, Rayan N, Kudyakov R, Foreman M, Cryer HG, Alam HB, Hoff W, Holcomb J. Compliance with recommended care at trauma centers: association with patient outcomes. *Journal of the American College of Surgeons*, 2014, August Vol. 219(2): 189-198. (Appendix Q)

Project 3:
Project Title: Characterization of the Effects of Early Sex-hormone Environment Following Injury

Sperry JL, Zuckerbraun BS, Zolin S, Vodovotz Y, Namas R, Peitzman AB Ferrell RE, Billiar TR. X-Chromosome linked IRAK1 polymorphism is strong predictor of multiple organ failure and mortality post-injury. Presented at the 134th Annual Meeting of the American Surgical Association, Boston MA, April 12, 2014. (Appendix R)

Sperry JL, Zolin S, Zuckerbraum BS, Vodovotz Y, Namas R, Neal MD, Ferrell RE, Rosengart MR, Peitzman AB, Billiar TR. X-Chromosome linked IRAK1 polymorphism is strong predictor of multiple organ failure and mortality post-injury. *Ann Surg.* 2014. Vol. 260(4) 698-705. (Appendix S)

Zolin SJ, Vodovotz Y, Forsythe RM, Rosengart M, Namas R, Peitzman AB. Billiart TR, Sperry JL. The early evolving sex hormone environment is associated with significant clinical outcome and inflammatory response differences post-injury. Oral presentation at the American Association for the Surgery of Trauma (AAST) 2014 annual meeting, Philadelphia, PA, September 2014. (Appendix T)

Sperry JL, Zhou T, Zolin S, Peitzman AB, Billiar TR. Sex based thromboelastography disparities post-injury: Independently different early on but why? Presented at the 28th Eastern Association for the Surgery of Trauma (EAST) Annual Scientific Assembly, Lake Buena Vista, FL, January 13-17, 2015. (Appendix U)

Zolin SJ, Vodovotz Y, Forsythe RM, Rosengart MR, Namas R, Brown JB, Peitzman AB, Billiar TR, Sperry JL. The early evolving sex hormone environment is associated with significant outcome and inflammatory response differences after injury. *J Trauma Acute Care Surg*, 2015, Vol. 78(3): 451-458. (Appendix V)

Project 4:
Project Title: Vasopressin Supplementation during the Resuscitation of Hemorrhagic Shock

Sims CA, Isserman JA, Holena D, Sundaram LM, Tolstoy N, Greer S, Sonnad S, Pascual J, Reilly P. Exception from informed consent for emergency research: Consulting the trauma community. Presented at the American Association for the Surgery of Trauma Meeting (AAST) 2012 Annual Conference, Kauai HI, September 12, 2012. (Appendix W)

Greer SE, Speck RM, Sundaram LM, Isserman J, Nathanson PG, Sonnad S, Sims CA. The community speaks: Analyzing attitudes about the Avert Shock trial and exception from informed consent (EFIC) in emergency research. Presented at Association for Academic Surgery and Society of University of Surgeons (ASC) 8th Annual Academic Surgical Congress, New Orleans LA, February, 2013. (Appendix X)

Greer SE, Speck RM, Sundaram LM, Isserman J, Nathanson PG, Sonnad S, Sims CA. The community speaks: Analyzing attitudes about the Avert Shock trial and exception from informed consent (EFIC) in emergency research. *J of Surg Res.* 2013:179(2): 337. (Appendix X)

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Maher, Z. AVERT shock trial presented at the University of Pennsylvania Injury Research and Prevention Symposium, poster, April 9, 2014.

Maher, Z. AVERT shock study presented at the University of Pennsylvania Emergency Medicine Research Symposium, poster, April 9, 2014.

Smallwood, A. AVERT Trial presented at the 2014 Pennsylvania Committee on Trauma Paper Competition, Harrisburg, PA, October 22-24, 2014.

Sims, CA. Novel Resuscitative Strategies presented at the American College of Surgeons Clinical Congress, San Francisco, CA, October, 2014.

Sims, CA. AVERT Trial presented at the American Heart Association Resuscitation Science Symposium (AHA ReSS) meeting, Chicago, IL, November, 2014 (pro con debate about vasopressors during the resuscitation of hemorrhagic shock).

Maher, Z, Grill EK, Smith BP, Sims CA. Does proximity to violence negatively influence attitudes toward exception from informed consent in emergency research? Presented at the Eastern Association for the Surgery of Trauma (EAST) Scientific Assembly, Orlando, FL, January 16, 2015. (Appendix Z)

Maher, Z. AVERT Shock Trial presented at Shock Trauma Surgical Grand Rounds, Rutgers Surgical Grand Rounds, February, 2015.

Maher, Z. AVERT Shock Trial presented at Pennsylvania Acute Research Collaboration as a work in progress, February 2015.

Maher, Z, Grill, EK, Smith, BP, Sims, CA. Does proximity to violence negatively influence attitudes toward exception from informed consent in emergency research? *J Trauma Acute Care Surg*. 2015:79(3): 364-371. (Appendix AA)

Overall Research Effort

Jenkins, DH. Impact of Department of Defense Funded Research to the National Trauma Institute. Presented at the Military Health System Research Symposium, Orlando FL, August 17, 2016. (Appendix AB)

National Trauma Institute Research Group, Price MA, Beilman GJ, Fabian TC, Hoyt DB, Jurkovich GJ, Knudson MM, MacKenzie EJ, Marshall VS, Overton KE, Peitzman AB, Phillips MJ, Pruitt BA, Smith SL, Stewart RM, Jenkins DH. The National Trauma Institute: Lessons learned. Presented at the University of Texas Health Science Center at San Antonio, Department of Surgery, Research Resident Conference, San Antonio TX, March 24, 2016. (Appendix AC)

CONCLUSION

The University of Texas Health Science Center at San Antonio (UTHSCSA) proposed to utilize $2,101,000 in congressional funding to work collaboratively with the National Trauma Institute (NTI) to build on the establishment of NTI as a national coordinating center for trauma research funding. In addition, a forum for dissemination of trauma research information was provided for the trauma community through the NTI Annual Trauma Conference. Over the last six years, four trauma research studies have been completed across nine academic trauma centers enrolling a total of 3,817 trauma patients as study participants. In total, this award resulted in 15 scholarly articles and 25 presentations at local, state and national trauma association/professional meetings.

Findings from these studies advanced scientific knowledge and best practices in the areas of timing and mechanism of traumatic coagulopathy, compliance with and comparative effectiveness of trauma specific processes of care in resuscitation and management of moderate to severe traumatic injuries, characterization of the effects of the early sex-hormone environment following injury, and community attitudes pertaining to exception form informed consent for emergency research.

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Greer SE, Speck RM, Sundaram LM, Isserman J, Nathanson PG, Sonnad S, Sims CA. The community speaks: Analyzing attitudes about the Avert Shock trial and exception from informed consent (EFIC) in emergency research. *J of Surg Res.* 2013:179(2): 337. (Appendix X)

Jenkins, DH. Impact of Department of Defense Funded Research to the National Trauma Institute. Presented at the Military Health System Research Symposium, Orlando FL, August 17, 2016. (Appendix AB)

Kornblith LZ, Cohen MJ. Letter to the Editor Response: The whole is greater than the sum of its parts: Hemostatic profiles of whole-blood variants. *J Trauma Acute Care Surg*. 2014; 77(6): 1003-1004. (Appendix L)

Kornblith LZ, Howard BM, Cheung CK, Dayter Y, Pandey S, Busch MP, Pati S, Callcut RA, Vilardi RF, Redick BJ, Nelson MF, Cohen MJ. The whole is greater than the sum of its parts: Hemostatic profiles of whole blood variants. Oral presentation at the Western Trauma Association (WTA) 2014 Annual Conference, Steamboat Springs, CO, March 5, 2014. (Appendix H)

Kornblith LZ, Howard BM, Cheung CK, Dayter Y, Pandey S, Busch MP, Pati S, Callcut RA, Vilardi RF, Redick BJ, Nelson MF, Cohen MJ. The whole is greater than the sum of its parts: Hemostatic profiles of whole blood variants. *J Trauma Acute Care Surg*. 2014; 77(6): 818-827. (Appendix H)

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Maher, Z. AVERT shock trial presented at the University of Pennsylvania Injury Research and Prevention Symposium, poster, April 9, 2014.

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Maher, Z. AVERT Shock Trial presented at Shock Trauma Surgical Grand Rounds, Rutgers Surgical Grand Rounds, February, 2015.

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Maher, Z, Grill EK, Smith BP, Sims CA. Does proximity to violence negatively influence attitudes toward exception from informed consent in emergency research? Presented at the Eastern Association for the Surgery of Trauma (EAST) Scientific Assembly, Orlando, FL, January 16, 2015. (Appendix Z)

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Shafi S, Rayan N, Barnes SA, Fleming N, Gentilello LM, Ballard D. Trauma core measures: Clinical processes that improve patient outcomes. Oral presentation at the American Association for the Surgery of Trauma (AAST) 2010 annual meeting, Boston, MA, September 2010. (Appendix M)

Shafi S, Rayan N, Barnes SA, Fleming N, Gentilello LM, Ballard D. Moving from “optimal resources” to “optimal care” at trauma centers. *J Trauma Acute Care Surg*, 2012. Vol.72(4): 870-877. (Appendix O)

Shafi S, Barnes SA, Rayan N, Kudyakov R, Foreman M, Cryer HG, Alam HB, Hoff W, Holcomb J. Compliance with recommended care at trauma centers: association with patient outcomes. *Journal of the American College of Surgeons*, 2014, August Vol. 219(2): 189-198. (Appendix Q)

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Sperry JL, Zolin S, Zuckerbraum BS, Vodovotz Y, Namas R, Neal MD, Ferrell RE, Rosengart MR, Peitzman AB, Billiar TR. X-Chromosome linked IRAK1 polymorphism is strong predictor of multiple organ failure and mortality post-injury. *Ann Surg.* 2014. Vol. 260(4) 698-705. (Appendix S)

Sperry JL, Zuckerbraun BS, Zolin S, Vodovotz Y, Namas R, Peitzman AB Ferrell RE, Billiart TR. X-Chromosome linked IRAK1 polymorphism is strong predictor of multiple organ failure and mortality post-injury. Presented at the 134th Annual Meeting of the American Surgical Association, Boston MA, April 12, 2014. (Appendix R)

Zolin SJ, Vodovotz Y, Forsythe RM, Rosengart M, Namas R, Peitzman AB. Billiart TR, Sperry JL. The early evolving sex hormone environment is associated with significant clinical outcome and inflammatory response differences post-injury. Oral presentation at the American Association for the Surgery of Trauma (AAST) 2014 annual meeting, Philadelphia, PA, September 2014. (Appendix T)

Zolin SJ, Vodovotz Y, Forsythe RM, Rosengart MR, Namas R, Brown JB, Peitzman AB, Billiar TR, Sperry JL. The early evolving sex hormone environment is associated with significant outcome and inflammatory response differences after injury. *J Trauma Acute Care Surg*, 2015, Vol. 78(3): 451-458. (Appendix V)

ABBREVIATIONS

AAST	American Association for the Surgery of Trauma
CeTIR	Center for Translational Injury Research
HRPO	Human Research Protection Office
HQDA	Headquarters Department of the Army
ICU	Intensive Care Unit
IRB	Institutional Review Board
LAR	Legal Authorized Representative
MOF	Multi Organ Failure
NCE	No Cost Extension
NI	Nosocomial Infection
NTI	National Trauma Institute
ORP	Office of Research Protections
OTSG	Office of the Surgeon General
PI	Principal Investigator
POC	Processes of Care
RFP	Request for Proposal
TEG	Thromboelastography
UTHSCSA	University of Texas Health Science Center San Antonio
UCLA	University of California Los Angeles
UCSF	University of California San Francisco

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APPENDICES

Appendices A – AC follow in the order they are listed in the Reportable Outcomes section.

Extracellular histone release in response to traumatic injury: Implications for a compensatory role of activated protein C

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BACKGROUND: Tissue injury leads to the release of DAMPs (damage-associated molecular patterns) that may drive a sterile inflammatory response; however, the role of extracellular histone levels after traumatic injury remains unexplored. We hypothesized that extracellular histone levels would be increased and associated with poor outcomes after traumatic injury.

METHODS: In this prognostic study, plasma was prospectively collected from 132 critically injured trauma patients on arrival and 6 hours after admission to an urban Level I trauma intensive care unit. Circulating extracellular histone levels and plasma clotting factors were assayed and linked to resuscitation and outcome data.

RESULTS: Of 132 patients, histone levels were elevated to a median of 14.0 absorbance units (AU) on arrival, declining to 6.4 AU by 6 hours. Patients with elevated admission histone levels had higher ISS (Injury Severity Score), lower admission GCS (Glasgow Coma Scale) score, more days of mechanical ventilation, and higher incidences of multiorgan failure, acute lung injury, and mortality (all $p \leq 0.05$). Histone levels correlated with prolonged international normalized ratio and partial thromboplastin time, fibrinolytic markers D-dimer and tissue-type plasminogen activator, and anticoagulants tissue factor pathway inhibitor and activated protein C (aPC; all $p < 0.03$). Increasing histone level from admission to 6 hours was a multivariate predictor of mortality (hazard ratio, 1.005; $p = 0.013$). When aPC level trends were included, the impact of histone level increase on mortality was abrogated ($p = 0.206$) by a protective effect of increasing aPC levels (hazard ratio, 0.900; $p = 0.020$).

CONCLUSION: Extracellular histone levels are elevated in response to traumatic injury and correlate with fibrinolysis and activation of anticoagulants. An increase in histone levels from admission to 6 hours is predictive of mortality, representing evidence of ongoing release of intracellular antigens similar to that seen in sepsis. Concomitant elevation of aPC abrogates this effect, suggesting a possible role for aPC in mitigating the sterile inflammatory response after trauma through the proteolysis of circulating histones. (*J Trauma Acute Care Surg*. 2012;73: 1389–1394. Copyright © 2012 by Lippincott Williams & Wilkins)

LEVEL OF EVIDENCE: Prognostic study, level III.

KEY WORDS: Trauma; DAMPs; histones; protein C.

Intuitive parallels exist between the systemic inflammatory responses seen in sepsis, ischemia-reperfusion, and trauma, and there is growing recognition of an underlying cross talk between systemic inflammation and the coagulation system that unites all of these phenomena.[1] As a canonical example, the tissue injury and end-organ damage seen in sepsis are initiated by a pathogenic stimulus but largely mediated by cytokine and leukocyte signaling. Recent work in models of sepsis has identified circulating extracellular histones, either derived from apoptotic cells[2] or secreted and incorporated into neutrophil extracellular traps (NETs),[3] as major mediators of endothelial apoptosis, organ failure, and death during sepsis.[4] Furthermore, direct intravascular injection of histones leads to macrovascular and microvascular thrombosis,[4] and histone-containing NETs have been shown to promote platelet aggregation and thrombus formation.[5,6] Both the proinflammatory and procoagulant effects of extracellular histones have recently been linked to signaling via Toll-like receptor 2 (TLR2) and TLR4.[6,7]

A countervailing role in the cross talk between inflammation and coagulation is held by the protein C system.[8] Activated protein C (aPC) mediates direct endothelial cytoprotection during ischemia[9] and reduces cytokine elaboration in sepsis.[10] Furthermore, aPC leads to anticoagulation via cleavage of activated factors Va and VIIIa and to derepression of fibrinolysis via consumption of plasminogen activator inhibitor 1.[11] Interestingly, aPC has also been shown to proteolytically cleave extracellular histones, mitigating the lethality of histone-induced systemic inflammatory response in animal models.[4]

Early theories that bacterial translocation from ischemic gut caused a sepsis-like state after traumatic injury have been challenged by the discovery that circulating mitochondrial DAMPs (damage-associated molecular patterns) mediate a sterile inflammatory response to trauma as a direct result of tissue injury by a pathogen.[12] Extracellular histone release

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may work through a similar mechanism in the inflammatory and prothrombotic milieu after trauma, as has been previously described in sepsis. Our group and others have previously characterized the anti-inflammatory and anticoagulant effects of activation of the protein C system in response to tissue injury and shock.[13,14] To investigate the inflammatory and coagulation cross talk mediated by extracellular histones and the protein C system in traumatic injury, we investigated levels of circulating extracellular histones in critically injured trauma patients, hypothesizing that elevated histone levels would be associated with end-organ damage and mortality and that these effects would be mitigated by activation of the protein C system.

MATERIALS AND METHODS

From June 2006 to January 2010, all patients who were activated at the highest tier of a three-tiered trauma activation system in use at San Francisco General Hospital and Trauma Center were eligible for study enrollment under a waiver of consent, with subsequent sample and data collection only completed in those patients who directly or via surrogate provided informed consent. Patients who were younger than 18 years, pregnant, transferred from another hospital, or incarcerated were excluded. The study was approved by the University of California, San Francisco Committee on Human Research. Patient enrollment for this study is described in detail elsewhere.[15]

Blood samples were prospectively collected via initial placement of a 16-gauge or larger peripheral intravenous catheter into citrated tubes containing 10 mmol/L of the protease inhibitor benzamidine within 10 minutes of emergency department arrival and again 6 hours after admission to the intensive care unit. Samples were centrifuged, and extracted plasma was stored at −80°C. Extracellular histone levels were measured using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) assay (Cell Death Detection ELISA^{plus} Kit; Roche Diagnostics, Indianapolis, IN). Briefly, 20 μL of citrated patient plasma was diluted 1:4 in 1% bovine serum albumin, 0.5% Tween, and 1 mmol/L ethylenediamine tetra-acetic acid in phosphate buffered saline and added to streptavidin-coated microtiter plates containing biotinylated mouse anti-histone antibody and peroxidase-conjugated anti-DNA antibodies. After standard washing steps, peroxidase activity of the retained immunocomplexes was developed by incubation with ABTS (2,2′-azino-di[3-ethylbenzthiazoline-sulfonate]) and read in a spectrophotometer at 405 nm; results are reported as absorbance units (AU). The mouse anti-histone antibody (clone H11-4) reacts with histones H1, H2A, H2B, H3, and H4. Coagulation factors were analyzed on an STA Compact coagulation analyzer (Diagnostica Stago, Inc., Parsippany, NJ). Activated protein C was assayed using an established ELISA method reported elsewhere.[14]

All data are presented as mean ± SD, median (interquartile range [IQR]), or percentage. Unpaired univariate comparisons were made using Student's *t*-test assuming unequal variance for normally distributed data, Wilcoxon rank-sum test for skewed data, and Fisher's exact test for proportions; paired data were compared using signed rank tests. Injury was assessed by ISS (Injury Severity Score).[16] Acute lung injury was identified based on the American-European consensus conference definition.[17] The diagnosis of multiple organ failure was defined as a multiple organ dysfunction score of 3 or higher using established Denver criteria.[18] Standard logistic regression was performed to identify predictors of histone level elevation. The Cox proportional hazards regression model was used to identify adjusted predictors of mortality. An $\alpha = 0.05$ was considered significant. All data analysis was performed by the authors using Stata version 12 (StataCorp, College Station, TX).

RESULTS

Overall characteristics for the 132 patients are presented in Table 1. Using an ELISA assay, extracellular histones were detectable at a median of 14.0 AU on arrival and declined to 6.4 AU by 6 hours. Because no agreed-on reference range for circulating histone levels exists clinically, to identify the characteristics of patients with elevated histone levels at admission, we dichotomized the study population into those in the highest quartile of histone levels at admission (≥50 AU, n = 24) compared with the lower three quartiles (<50 AU, n = 108). Patients with elevated (median, 164 AU; IQR, 102–364 AU) histone levels had significantly higher ISS and significantly lower GCS (Glasgow Coma Scale) score than those with lower histone levels (median, 10; IQR, 4–19) (Table 2). Histone level elevation was also significantly associated with higher mechanical ventilation requirements, a 1.8-fold higher incidence of acute lung injury, 3.2-fold higher incidence of multiorgan failure, and 2.1-fold greater mortality (all $p < 0.05$; Table 2). Although admission histone levels were higher in patients with severe injury (ISS > 20; $p = 0.040$), median histone levels fell by 6 hours in both severely and moderately injured (ISS ≤20) patients ($p < 0.05$) (Fig. 1). We further investigated trends in histone levels from admission to 6 hours in 85 patients with histone data at both time points, identifying 31 (36.5%) patients with an increased or unchanged histone level

TABLE 1. Overall Patient Characteristics

	(N = 132)
Age, y	40.9 ± 18.7
BMI, kg/m^2	27.7 ± 5.2
Penetrating, %	40.0
ISS	24.2 ± 13.4
GCS score	9 (4–15)
Base deficit	−6.7 ± 5.8
Temperature, °C	35.5 ± 0.9
Prehospital IVF, mL	50 (0–500)
Hospital days	10 (5–23)
ICU days	4.5 (2–10.5)
Vent-free days/28 days	23 (1–26)
Multiorgan failure, %	20.2
Acute lung injury, %	36.9
Mortality, %	21.2

Data are presented as mean ± SD or median (IQR).
BMI, body mass index; ICU, intensive care unit; IVF, intravenous fluids.

TABLE 2. Population Characteristics by Admission Histone Level

	Extracellular Histone Level		
	≥50 AU (n = 24)	<50 AU (n = 108)	*p*
Histone level	164 (102–364)	10 (4–19)	—
Age, y	39.9 ± 16.9	41.1 ± 19.2	0.760
BMI, kg/m^2	28.6 ± 4.7	27.5 ± 5.4	0.342
Penetrating injury, %	25.0	43.5	0.111
ISS*	30.5 ± 13.0	22.8 ± 13.1	0.013
GCS score*	5 (3–12.5)	10 (5–15)	0.034
Base deficit	−7.2 ± 4.6	−6.6 ± 6.1	0.620
Temperature, °C	35.5 ± 1.2	35.5 ± 0.8	0.890
Prehospital IVF , mL	0 (0–500)	75 (0–500)	0.949
24-h RBC	4 (0–9)	2 (0–6)	0.124
24-h FFP	2 (0–4)	0 (0–4)	0.660
24-h Plts	0 (0–1)	0 (0–0)	0.396
Hospital days	11.5 (3.5–28.5)	8.5 (5–22.5)	0.843
ICU days	6.5 (2.5–11.5)	4 (2–9.5)	0.303
Vent-free days/28 days*	7 (0–23)	25 (7.5–26)	0.007
Acute lung injury, %*	58.3	32.1	0.020
Multiorgan failure, %*	45.8	14.3	0.001
Mortality, %*	37.5	17.6	0.050

*$p < 0.05$ by Student's *t*-test, Mann-Whitney U-test, or Fisher's exact test.
Data are presented at mean ± SD or median (IQR range).
BMI, body mass index; ICU, intensive care unit; IVF, intravenous fluids; RBC, red blood cell units; FFP, fresh-frozen plasma units; Plts, platelet units.

at 6 hours, compared with 54 (63.5%) patients with decreasing histone levels. Despite no identifiable statistical differences in patient demographics or injury characteristics between these populations (data not shown), those with increasing histone levels had 2.5-fold higher mortality (32.3 vs. 13.0%, $p = 0.048$).

We then investigated correlations between coagulation-relevant measures by admission histone level. Patients with elevated histone levels had significantly higher median international normalized ratio and partial thromboplastin time at admission ($p = 0.006$ and $p = 0.047$, respectively; Fig. 2). Among clotting cascade proteins, no significant differences existed in procoagulant factors V, VII, VIII, IX, or X (all $p > 0.1$), in antithrombin III ($p = 0.860$), or in unactivated protein C ($p = 0.621$). However, patients in the highest quartile of admission histone level had significantly higher levels of the fibrinolytic markers D-dimer and tissue-type plasminogen activator, as well as the systemic anticoagulants tissue factor pathway inhibitor and aPC (all $p < 0.05$; Fig. 3).

To interrogate the temporal relationship between extracellular histone and aPC levels in terms of outcome, we used Cox proportional hazards regression to examine the multivariate association of changes in histone level with mortality. In unadjusted analysis, each 1 AU rise in histone level from admission to 6 hours was significantly associated with mortality (hazard ratio, 1.006; $p = 0.009$); when adjusted for age, injury severity, base deficit, and admission GCS, increasing histone level remained a robust predictor of later mortality (hazard ratio, 1.005; $p = 0.013$). The mean increase in histone level from 0 to 6 hours in the 31 patients with increased or unchanged levels at 6 hours was 53.5 AU; this increase is associated with a hazard ratio for mortality of 1.347 ($p = 0.013$) in adjusted analysis. Conversely, the mean decrease in the 54 patients with decreasing histone levels was −71.6 AU, with a protective hazard ratio of 0.676 ($p = 0.013$). However, when adjusted for the simultaneous change in aPC levels, the association between increasing histone level and mortality was abrogated ($p = 0.206$, not significant) by a protective association of increasing aPC levels with reduced mortality (hazard ratio, 0.900; $p = 0.020$; final model given in Table 3).

DISCUSSION

Here we report a prospective analysis of circulating extracellular histone levels in 132 critically injured trauma patients. Patients within the highest quartile of extracellular histone levels at admission had significantly higher ISS and lower GCS, as well as a 1.8-fold higher incidence of acute lung injury, 3.2-fold higher incidence of multiorgan failure, and 2.1-fold greater mortality. Elevated admission histone levels are significantly associated with coagulopathy, fibrinolysis, and activation of systemic anticoagulants. Using Cox proportional hazards regression, we identify an ongoing rise in histone levels as an independent predictor of mortality when adjusted for age, injury severity, shock, and impaired GCS. We further identify a statistical association between concomitant aPC activation and mitigation of the apparent effect of increasing histone levels. The finding that an ongoing rise in histone levels correlates with poor outcomes after trauma is clinically intuitive, as a reflection of ongoing cellular damage or continued inflammatory cytokine milieu. Previous studies have noted the presence of extracellular DNA possibly in complex with circulating histones,[19,20] and two studies have measured histone-complexed DNA directly as part of a biomarker screen in injured patients.[21,22] But to our knowledge, this is the first detailed characterization of the patient and injury characteristics associated with extracellular histone levels after trauma and the first to demonstrate a correlation with aPC. Taken together, this work serves as an initial exploration of the role of extracellular histones and aPC in inflammatory and immune cross talk.

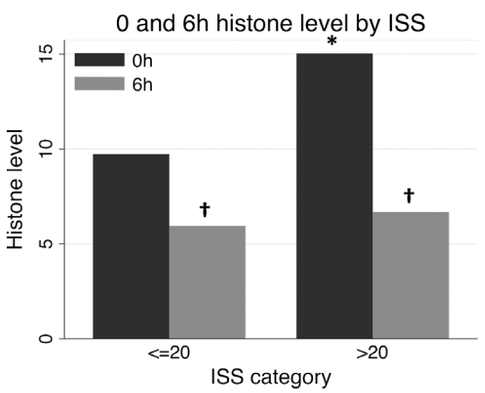

Figure 1. Median 0- and 6-hour histone levels by injury severity. *$p < 0.05$ between categories by Mann-Whitney U-test; †$p < 0.05$ between time points by paired signed rank test.

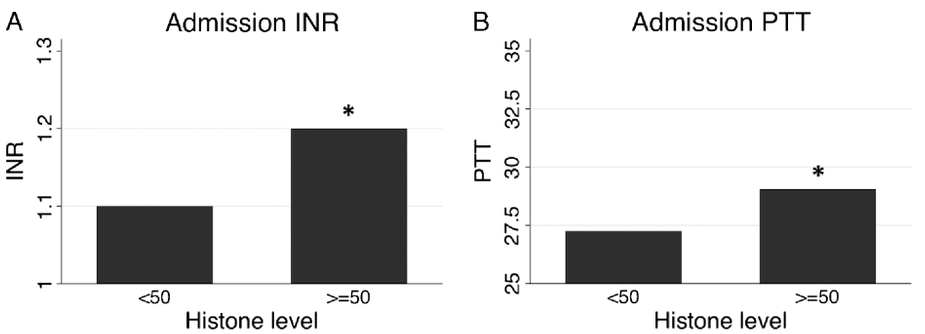

Figure 2. Admission international normalized ratio ([INR] *A*) and partial thromboplastin times ([PTT] *B*) by admission histone level. *$p < 0.05$ by Wilcoxon rank-sum testing.

This work points to three avenues for potential novel anti-inflammatory therapies in the care of trauma patients. First, TLR2 and TLR4 were recently identified as principal signaling mediators downstream of extracellular histones.[6,7] In addition to histones, other endogenous TLR2 and TLR4 ligands exist that are also released in response to inflammatory stimuli, including heat shock proteins,[23] tenascin C,[24] and high-mobility group box 1;[25,26] several of these have been specifically evaluated as prognostic biomarkers in trauma patients.[27,28] Thus, the TLR2 and TLR4 receptors and their signaling apparatus are attractive therapeutic targets for pharmacologic suppression of the sterile inflammatory response to trauma, although initial trials of novel anti-TLR4–based therapies are preliminary and have not yet demonstrated a clinical benefit.[29] Second, proteomic analysis of circulating biomarkers in lipopolysaccharide (LPS)-induced septic shock has revealed citrullination of extracellular histones as an element of the early response of neutrophils to inflammatory stimuli[30] and the subsequent incorporation of citrullinated histones into NETs in response to infection.[31,32] Ongoing work by Li et al. has demonstrated that treatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid significantly reduces the circulating levels of both native and citrullinated histone H3[33] and improves survival[34,35] in mouse models of LPS-induced septic shock. Third, recombinant aPC was, until its recent removal from the market, the only drug shown to reduce mortality in adult patients with severe sepsis.[36] Importantly, the anti-inflammatory effects of aPC are mediated via interaction with the endothelial protein C receptor, leading to secondary activation of the protease-activated receptor 1,[37,38] whereas its anticoagulant effects are mediated by receptor-independent proteolysis of activated factors Va and VIIIa. Kerschen et al.[39] have recently shown that a recombinant form of aPC with preserved receptor binding sites but targeted

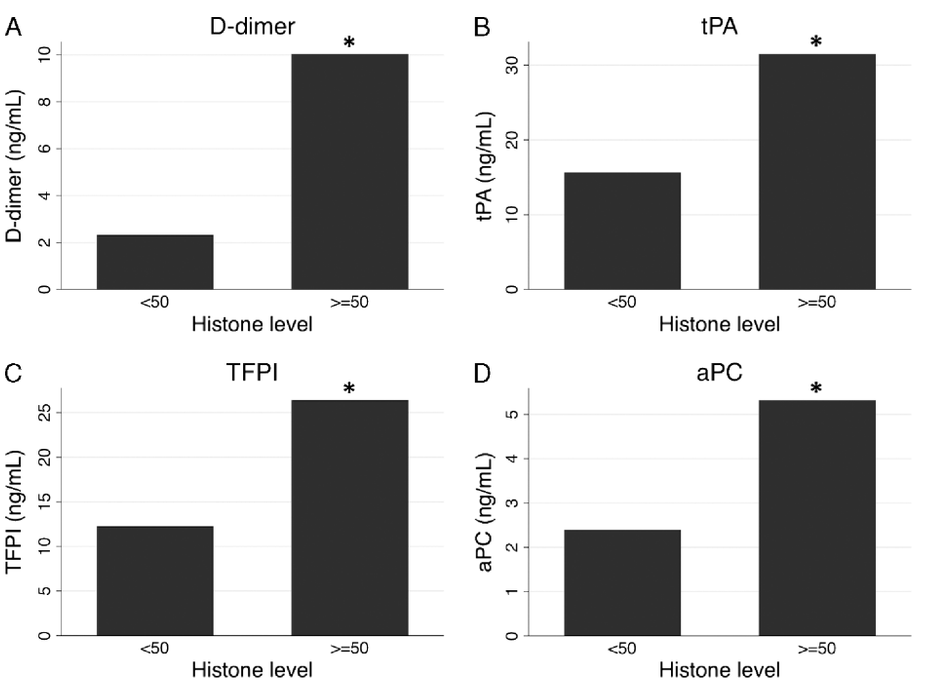

Figure 3. Differences by admission histone level in D-dimer (*A*), tissue-type plasminogen activator ([tPA] *B*), tissue factor pathway inhibitor ([TFPI] *C*), and aPC (*D*). *$p < 0.05$ by Wilcoxon rank-sum testing.

TABLE 3. Cox Proportional Hazards Regression for Predictors of Mortality

	OR	*p*	CI
Age*	1.104	0.007	(1.027–1.186)
ISS	1.047	0.467	(0.926–1.183)
Base deficit	0.884	0.213	(0.792–1.073)
Arrival GCS	0.818	0.211	(0.597–1.121)
Histone increase, 0–6 h	0.993	0.206	(0.982–1.004)
aPC increase, 0–6 h*	0.900	0.020	(0.823–0.983)

*$p < 0.05$ by Wald test; Harrell's C = 0.743 for the model.
CI, confidence interval; OR, odds ratio.

mutation leading to less than 10% anticoagulant activity is equivalent to the native recombinant aPC in reducing mortality associated in both LPS-induced and polymicrobial sepsis models in mice. However, Xu et al.[7] showed that proteolytic cleavage of histones by aPC was receptor independent and was augmented by the presence of membrane phospholipid similarly to the aPC-mediated cleavage of activated factor Va. Our group has also previously shown that selective inhibition of the anticoagulant function of aPC prevents coagulopathy with no impact on survival, whereas antibody blockade of both anticoagulant and cytoprotective functioning of aPC caused pulmonary thrombosis, perivascular hemorrhage, and increased mortality in a mouse model of trauma and hemorrhagic shock.[13] Further in vitro and animal model work will be required to specifically delineate whether the mechanism of aPC-mediated cleavage of histones is independent of its anticoagulant function. In any case, histone burden as a potential biomarker in trauma and sepsis may revive interest in aPC-based therapies by providing better patient selection and more goal-directed therapy.

Several limitations exist that are important for interpretation of this study. Ours remains an initial single-center experience and is subject to all attendant biases; further work is needed to confirm and extend these findings in a larger series, other centers, and with extended temporal resolution. In particular, the physiologic stress and additional tissue injury encountered during operative interventions may produce a "second hit" phenomenon that is not accounted for here; sequential data collection at later time points may identify additional late sequelae of histone level elevation. Furthermore, the difficulty in identifying an appropriate control group for trauma patient comparison impairs our ability to identify residual confounding. Taken together, these limitations highlight the fact that these data identify associations between histone release and concomitant activation of protein C after traumatic injury but should not be taken to prove causality. Further in vitro, in vivo, and clinical studies are required to identify mechanisms of histone level elevation and clearance after trauma and to clearly delineate whether histone release is causative of, as opposed to merely associated with, poor outcomes after traumatic injury.

Here we identify histone release and clearance as promising areas of investigation in trauma resuscitation and suggest a biologically plausible interaction with the protein C system as a potential mechanism for further study. This highlights an intuitive potential explanation for the activation of the protein C system in the service of the innate immune response to tissue injury in which uncontrolled systemic activation may be an unintended side effect leading to the systemic coagulopathy seen after trauma.[14] This suggests a place for the interaction between histones and aPC at a critical junction in the cross talk between inflammation and coagulation. This work further highlights the parallels between mechanisms of pathogen-induced sepsis and those underlying the sterile inflammatory response to trauma, suggesting that insights and therapies from the critical care and sepsis literature may be applicable earlier in the hospital course to the care of the acutely injured trauma patient.

AUTHORSHIP

M.E.K. and M.J.C. prepared the article and performed all data analysis and take full responsibility for the data as presented. J.X. and C.T.E. performed histone level measurements and provided critical article review. R.F.V. and C.H. performed clotting factor and protein C level measurements.

DISCLOSURE

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8D

Timing and Clinical Accuracy of Massive Transfusion Protocol Activation

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Presenter: Raja Narayan

Objectives: Massive transfusion (MT) is a poorly characterized practice with multiple predictive scores, activation criteria, and institution-specific protocols. Detailed characterization will identify optimal practices to refine our resuscitation strategy, improving outcome after trauma.

Design: Retrospective review.

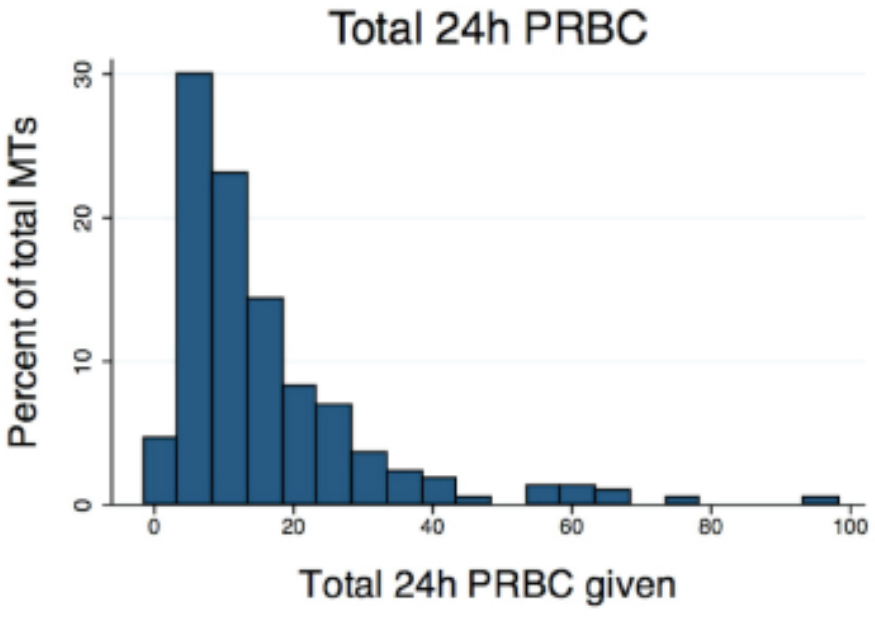
Setting: Level I urban trauma center.

Patients: A total of 226 patients who either received an MT (>10 U red blood cells [RBCs] over 24 hours) or had activation of the institutional massive transfusion protocol (MTP).

Main Outcome Measures: Patient demographics, MTP activation criteria, blood product use, activation timing, and outcomes were evaluated.

Results: Of 226 patients evaluated, a median of 11 U RBCs was given; 48% of the patients received true MTs (median: 18 U RBCs), compared with 52% false alarms requiring fewer RBCs (median: 5). The mean ratio of RBC to fresh frozen plasma units during MTP was 1:2, and the ratio of RBCs to platelets was 11.6:1. Median time to blood bank sending of MTP RBCs after protocol activation was 9 minutes. Trauma accounted for 85.3% of MTP activations and required larger amounts of RBCs (median: 12 vs. 7 U, P = .005) than medical/obstetric activations. Of MTP activations, 36.1% were delayed until after more than 4 U RBCs had been given, and 42.9% were activated more than 1 hour after admission. Of four validated MT prediction scores, only the McLaughlin score differed statistically between true and false protocol activations (2.0 ± 1.2 vs. 1.6 ± 1.1, P = .034), and none satisfactorily distinguished patients receiving true MTs.

Conclusions: Despite data showing mortality benefits from early blood product resuscitation, the design and implementation of MT protocols are problematic because of uncertainty regarding activation criteria and timing . More accurate clinical predictive scoring and criteria are required.



		Median (IQR)
Pre-MT	Crystalloid (L)	0.6 (0–2)
	PRBC	2 (1–4)
	FFP	0 (0–0)
	Plt	0 (0–0)
	RBC:FFP ratio	1.3 (1–2)
MTP	Crystalloid (L)	2 (1–3)
	PRBC	6 (3–12)
	FFP	6 (2–12)
	Plt	0 (0–1)
	RBC:FFP ratio	1 (0.8–1.5)
24-hour total	Crystalloid (L)	4 (2–8)
	PRBC	11 (6–19)
	FFP	8 (4–15)
	Plt	1 (0–2)
	RBC:FFP ratio	1.3 (1.1–1.8)

Characterization of platelet dysfunction after trauma

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BACKGROUND: The increased morbidity and mortality associated with coagulopathy and thrombocytopenia after trauma are well described. However, few studies have assessed platelet function after injury.

METHODS: Blood samples were prospectively collected from 101 patients with critical injury and trauma on arrival to the emergency department and serially after admission to a Level I urban trauma intensive care unit from November 2010 to October 2011 and functionally assayed for responsiveness to adenosine diphosphate, thrombin receptor-activating peptide, arachidonic acid (AA), and collagen using multiple electrode impedance aggregometry.

RESULTS: Of the 101 enrolled patients, 46 (45.5%) had below-normal platelet response to at least one agonist ("platelet hypofunction") at admission, and 92 patients (91.1%) had platelet hypofunction some time during their intensive care unit stay. Admission platelet hypofunction was associated with low Glasgow Coma Scale score and a nearly 10-fold higher early mortality. Logistic regression identified admission Glasgow Coma Scale (odds ratio, 0.819; $p = 0.008$) and base deficit (odds ratio, 0.872; $p = 0.033$) as independent predictors of platelet hypofunction. Admission AA and collagen responsiveness were significantly lower for patients who died ($p < 0.01$), whereas admission platelet counts were similar ($p = 0.278$); Cox regression confirmed thrombin receptor-activating peptide, AA, and collagen responsiveness as independent predictors of in-hospital mortality ($p < 0.05$). Receiver operating characteristic analysis identified admission AA and collagen responsiveness as negative predictors of both 24-hour (AA area under the curve [AUC], 0.874; collagen AUC, 0.904) and in-hospital mortality (AA AUC, 0.769; collagen AUC, 0.717).

CONCLUSION: In this prognostic study, we identify clinically significant platelet dysfunction after trauma in the presence of an otherwise reassuring platelet count and standard clotting studies, with profound implications for mortality. Multiple electrode impedance aggregometry reliably identifies this dysfunction in injured patients, and admission AA and collagen responsiveness are sensitive and specific independent predictors of both early and late mortality. (*J Trauma Acute Care Surg*. 2012;73: 13–19.

LEVEL OF EVIDENCE: Prognostic study, level II.
KEY WORDS: Platelets; impedance aggregometry; multiple electrode aggregometry.

Platelets play a pivotal role in hemostasis after injury.[1] Recent evidence identifies that admission platelet counts are inversely correlated with early mortality and transfusion for patients with critical injury and trauma, even for platelet counts well into the normal reference range.[2] Quantitative platelet deficits also predict progression of intracranial hemorrhage and mortality after traumatic brain injury.[3] Although the increased morbidity and mortality associated with enzymatic coagulopathy after trauma is well described, near-total impairment of clot formation can also occur as a result of platelet dysfunction despite the presence of reference range coagulation studies and platelet count.[4] Thorough study of platelet dysfunction has been hindered by the technical complexity of existing platelet function assays; however, recent advances in impedance-based platelet aggregometry allow for rapid, point-of-care assessment of platelet function.[5]

Impedance aggregometry assays platelet aggregation via electrical resistance across sets of silver-coated copper electrodes immersed in whole blood; nonthrombogenic resting platelets are activated using specific platelet agonists, causing platelets to aggregate on the charged surface and increasing impedance in proportion to the degree of platelet activation.[6] This principle underlies the recently developed Multiplate multiple electrode aggregometer, which uses disposable test cells containing duplicate pairs of sensor wires to measure platelet aggregation in response to agonists of interest in citrated, heparinized, or hirudin-anticoagulated whole blood.[5] Impedance aggregometry has been cross-validated with single platelet counting, turbidimetric platelet aggregation, vasodilator-stimulated phosphoprotein phosphorylation, and light aggregometry[5,7,8] in normal controls and in monitoring clopidogrel and aspirin effects; however, only preliminary investigations exist using impedance aggregometry to characterize platelet dysfunction related to trauma.[9]

Therefore, the purpose of this study was to prospectively quantify platelet function using multiple electrode aggregometry to identify previously undetected platelet dysfunction in patients with trauma. We further sought to relate any observed dysfunction to outcomes after severe injury.

PATIENTS AND METHODS

Blood samples were prospectively collected from 101 patients with critical injury and trauma on arrival and at 6, 12, 24, 48, 72, 96, and 120 hours after admission to a Level I urban trauma intensive care unit (ICU) from November 2010 to October 2011. Admission samples were collected via initial placement of a 16G or larger peripheral intravenous line; subsequent samples were collected via indwelling arterial catheters. Standard laboratory vacuum-sealed tubes containing 3.2% (0.109 mol/L) sodium citrate were used for all draws. A total of 376 samples were analyzed, with a median of three samples per patient (interquartile range, 2–4). Demographics, resuscitation data, clinical laboratory results, and outcomes were collected in parallel. Informed consent was obtained from all patients, as approved by the University of California Committee on Human Research.

Platelet function was assessed at point of care using the Multiplate multiple electrode aggregometer (Verum Diagnostica GmbH, Munich, Germany) immediately after sample collection. Briefly, 0.3 mL of whole blood was diluted in warmed isotonic sodium chloride solution containing 3-mmol/L $CaCl_2$ and incubated for 3 minutes at 37°C with continuous stirring in a Multiplate test cell. Each test cell contains two sets of 3-mm silver-coated copper wires, across which electrical resistance is measured at 0.57-second intervals. Platelet activation was induced by adenosine diphosphate (ADP; final concentration, 6.5 μmol/L; via P2 receptors), thrombin receptor-activating

peptide-6 (TRAP; final concentration, 32 μmol/L; via PAR receptors), arachidonic acid (AA; final concentration, 0.5 mmol/L; via the cyclo-oxygenase pathway), or collagen (final concentration, 3.2 μg/mL; via GpIa/IIa and GpVI receptors). Platelet adhesion to the electrodes was detected as increasing electrical impedance, measured by duplicate sets of sensor wires in each test cell. Agonist responses are reported as area under the aggregation curve in units (U) during a 6-minute measurement period. Reference ranges for citrated whole blood were provided by the manufacturer based on studies of healthy controls.

Data are presented as mean (SD), median (interquartile range), or percentage; univariate comparisons were made using Student's *t* test for normally distributed data, Wilcoxon rank sum testing for skewed data, and Fisher's exact test for proportions. Logistic regression was performed to identify predictors of platelet hypofunction. Kaplan-Meier time-to-event analysis was used to assess differences in mortality; Cox proportional hazards regression was used to identify adjusted predictors of mortality. Nonparametric receiver operating characteristic (ROC) analysis was performed to characterize the ability of continuous agonist responses to classify binary outcomes. An $\alpha = 0.05$ was considered significant. All analysis was performed by the authors using Stata version 12 (StataCorp, College Station, TX).

RESULTS

Our study population composed of 101 patients had a mean (SD) age of 41.3 (19.3) years and a mean (SD) Injury Severity Score (ISS) 23.2 (5.4); there was 31.0% penetrating injury and 61.2% brain injury. Mean platelet responsiveness to ADP, TRAP, AA, and collagen at admission were in the low reference range according to manufacturer-provided reference values (Table 1). Notably, the mean (SD) admission platelet count was 274.4 (85.4) $\times$ 10^3/μL, with no admission platelet count below 140 $\times$ 10^3/μL (Table 1). Significant correlations between agonist response and platelet count were observed for all agonists, with linear correlation between platelet response extending well into the clinically "normal" platelet range (Fig. 1). Platelet responsiveness was then longitudinally evaluated from ICU admission to ICU discharge or 120 hours. For all agonists, mean platelet responsiveness fell sharply to below the reference range by 6 hours (Fig. 2). TRAP and collagen responsiveness returned to the low reference range by 24 hours, whereas ADP and AA responsiveness (Fig. 2*A* and *B*) remained significantly impaired until 96 hours (ADP; Fig. 2*C*) and 120 hours (AA; Fig. 2*D*), respectively. Mean platelet count remained more than 100 $\times$ 10^3/μL for the entirety of the ICU stay (Fig. 2*E*).

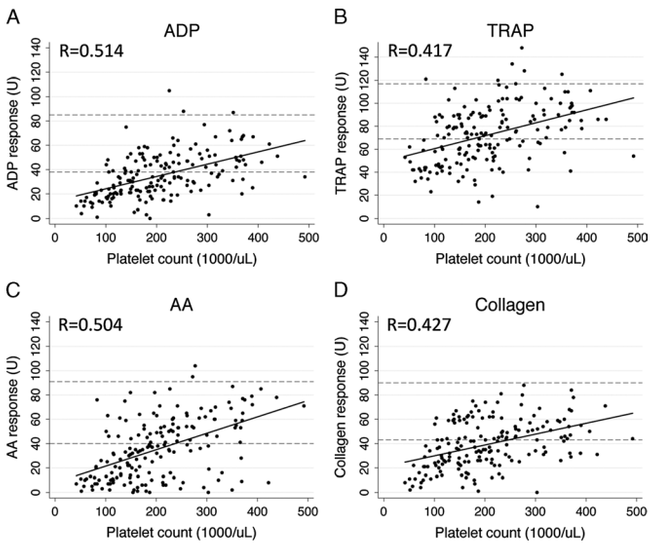

Figure 1. Scatter plots showing correlation between platelet count and ADP (*A*), TRAP (*B*), AA (*C*), and collagen (*D*) responsiveness using matched data from all time points collected. Manufacturer-provided reference ranges indicated by reference lines at the lower (5th percentile) and upper (95th percentile) boundaries. ρ values for each pairwise correlation given at upper left of each graph, with all associated $p < 0.001$.

Using the lower bound (fifth percentile) of manufacturer-provided reference ranges, 46 patients (45.5%) had below-normal platelet response to at least one agonist at admission; 92 patients (91.1%) had a below-normal response some time during their ICU stay. Of the 42 patients with confirmed prehospital medication data, 4 patients were taking aspirin and 1 patient was taking clopidogrel (Plavix) at the time of injury. Patients taking aspirin had significantly lower admission AA responsiveness (mean [SD], 5.8 [3.3] U vs. 48.0 [26.1] U; $p < 0.001$) and a trend toward lower collagen responsiveness (mean [SD], 24.5 [18.6] U vs. 46.7 [18.1] U; $p = 0.092$) but did not differ significantly in responsiveness to other agonists or by admission platelet count (all $p > 0.400$). Similarly, a single patient known to be taking Plavix had an admission ADP responsiveness of 27 U (below the 25th percentile in the study population) and a below-normal AA responsiveness of 38 U (reference range, 40–91 U). For all subsequent analysis, patients known to be taking aspirin or Plavix were excluded unless otherwise noted.

We then dichotomized the study population into 39 patients (42.9%) with a below-normal response to any agonist at admission ("platelet hypofunction") compared with 52 patients (57.1%) with all reference range responses ("normal function"). Platelet hypofunction was associated with low admission Glasgow Coma Scale (GCS) score ($p = 0.007$), higher mechanical ventilation requirements ($p = 0.040$), and a nearly 10-fold higher early mortality ($p = 0.009$; Table 2). Logistic regression identified base deficit (odds ratio [OR], 0.872;

TABLE 1. Admission Platelet Agonist Responses and Platelet Counts

	Admission Values (n = 78), Mean (SD)	Observed Range	Reference Range
ADP, U	44.6 (20.4)	0–105	38–85
TRAP, U	86.6 (27.0)	10–170	69–117
AA, U	44.3 (28.3)	0–104	40–91
Collagen, U	44.7 (19.7)	0–88	43–90
Platelets, $\times 10^3$/μL	274.4 (85.4)	140–605	150–400

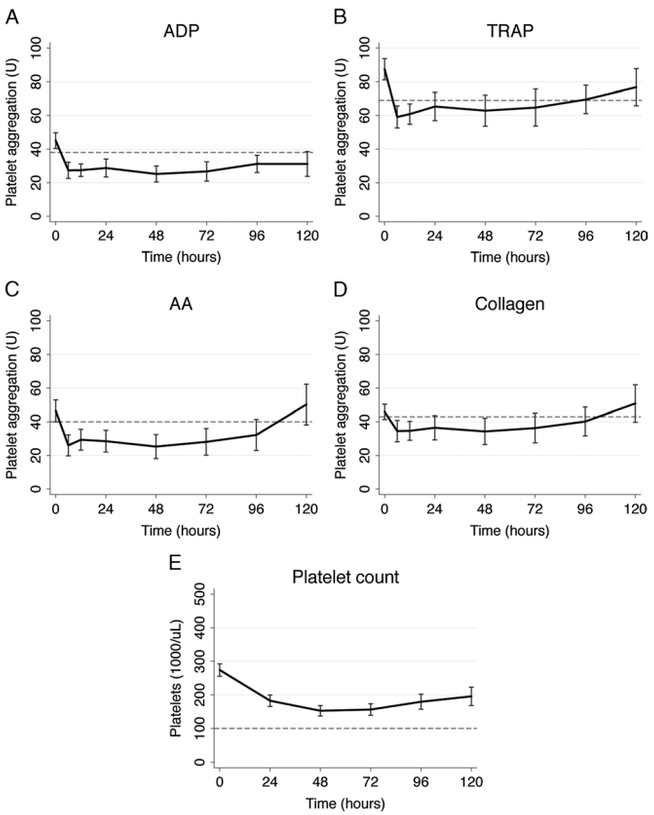

Figure 2. Platelet ADP (*A*), TRAP (*B*), AA (*C*), and collagen (*D*) responsiveness as area under the aggregation curve in units (U) over time. Platelet count measurements (*E*) are shown for comparison. Data points are mean values, with *capped bars* indicating 95% confidence intervals; *dotted lines* indicate the lower bound (fifth percentile) of normal values for each measurement.

$p = 0.033$) and GCS score (OR, 0.819, $p = 0.008$) as independent predictors of admission platelet hypofunction; platelet count was not a significant predictor of hypofunction ($p = 0.150$). Analysis was repeated in an intention-to-treat fashion including patients taking aspirin and Plavix: ORs and significance were similar for base deficit and GCS score, with older age identified as an additional significant predictor (OR, 1.041; $p = 0.032$).

To identify patient factors related to differential agonist responses, the study population was dichotomized by age (≥55 vs. <55 years), admission base deficit (≤−6 vs. >−6), traumatic brain injury (head Abbreviated Injury Scale [AIS] score, ≥3 vs. <3), and admission GCS score (≥8 vs. <8). Older patients had significantly lower responsiveness to TRAP (mean [SD], 74.5 [27.2] U vs. 91.9 [27.2] U; $p = 0.030$) and AA (mean [SD], 26.8 [23.5] U vs. 51.8 [26.2] U; $p = 0.001$); ADP ($p = 0.074$) and collagen ($p = 0.375$) responsiveness did not differ by age. Patients in shock as defined by admission base deficit had significantly lower responsiveness to collagen (mean [SD], 34.7 [20.5] U vs. 50.8 [16.7] U; $p = 0.011$), with no differences in ADP, TRAP, or AA responsiveness ($p > 0.200$). No significant differences were observed for patients with traumatic brain injury as identified by AIS score (all $p > 0.500$); however, patients with lower admission GCS score had lower responsiveness to ADP (mean [SD], 38.6 [20.7] U vs. 48.6 [20.3] U; $p = 0.016$) and collagen (mean [SD], 35.5 [20.1] U vs. 47.2 [18.3] U; $p = 0.008$), with no differences in TRAP or AA ($p > 0.300$). Admission platelet count did not statistically differ by age, base deficit, head AIS score, or GCS score (all $p > 0.100$).

Agonist responses were then examined for differences by mortality. For patients who died in-hospital at any time, admission AA and collagen responsiveness were significantly lower than those of survivors (AA: mean [SD], 22.4 [24.3] U vs. 48.8 [25.6] U; $p = 0.001$ (collagen: mean [SD], 29.6 [21.4] vs. 47.0 [17.6] U; $p = 0.008$), whereas admission platelet count did not differ significantly ($p = 0.278$). To account for the contribution of other patient and injury characteristics, Cox proportional hazards regression was used to adjust for age, GCS score, base deficit, and platelet count. In multivariate analysis, low TRAP (hazards ratio, 0.980; $p = 0.047$), AA (hazards ratio 0.968, $p = 0.003$), and collagen responsiveness (hazards ratio, 0.955; $p = 0.031$) were independent predictors of in-hospital mortality (Fig. 3). These results were unchanged when including patients taking aspirin or Plavix.

TABLE 2. Patient Characteristics by Platelet Hypofunction on Admission

	Platelet Hypofunction (n = 39)	Normal Function (n = 52)	*p*
Age, mean (SD), y	44.4 (20.7)	36.7 (16.5)	0.060
BMI, mean (SD), kg/m^2	26.3 (5.7)	25.6 (4.9)	0.543
Blunt injury, %	65.7	70.2	0.811
ISS, mean (SD)	22.8 (14.3)	25.4 (15.3)	0.513
GCS score,* median (IQR)	7 (3–10)	13 (6–15)	0.007
Temperature, mean (SD), °C	35.7 (0.7)	35.8 (0.8)	0.539
Prehospital IVF, median (IQR), mL	250 (50–1,000)	250 (50–750)	0.711
pH, mean (SD)	7.23 (0.20)	7.31 (0.14)	0.178
Base deficit, mean (SD)	−6.9 (6.4)	−3.9 (5.6)	0.072
INR, median (IQR),	1.2 (1.1–1.3)	1.2 (1.1–1.3)	0.875
PTT, median (IQR), s	28.6 (26.3–31.5)	26.4 (25.3–31.1)	0.322
Hematocrit, mean (SD), %	40.4 (5.5)	39.7 (4.8)	0.553
Platelet count, mean (SD), × 10^3/μL	257.7 (75.3)	285.7 (88.5)	0.125
RBC/24 h, median (IQR)	0 (0–1)	0 (0–4)	0.688
FFP/24 h, median (IQR)	0 (0–2)	0 (0–2)	0.795
Plts/24 h, median (IQR)	0 (0–0)	0 (0–0)	0.405
Hospital days, median (IQR),	6 (2–27)	10 (6.5–20)	0.090
ICU days, median (IQR)	3.5 (1–14)	3 (2–14)	0.436
Vent-free days/28 d,* median (IQR)	12 (0–26)	26 (7.5–27)	0.040
Multiorgan failure, %	31.7	27.3	0.656
24 h mortality,* %	20.0	2.1	0.009
In-hospital mortality, %	34.3	14.6	0.062

*$p < 0.05$ by Student's *t* test, Mann-Whitney *U* test, or Fisher's exact test.

BMI, body mass index; FFP, fresh frozen plasma units; INR, international normalized ratio; IQR, interquartile range; IVF, intravenous fluid; Plts\, platelet units; PTT, partial thromboplastin time; RBC, red blood cell units.

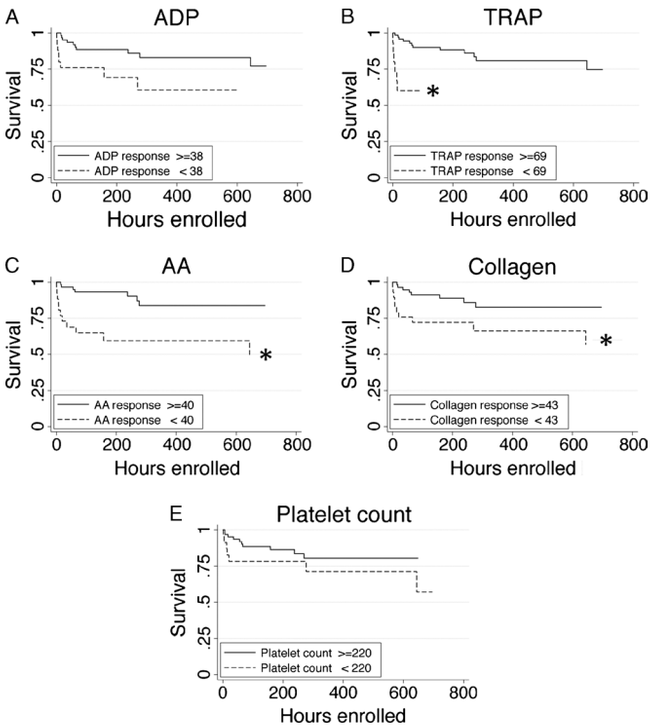

Figure 3. Kaplan-Meier 30-day survival curves showing survival differences between patients with below-normal admission platelet responsiveness to ADP (*A*), TRAP (*B*), AA (*C*), and collagen (*D*). Survival curves for patient admission platelet counts below the 25th percentile (*E*) are shown for comparison. *$p < 0.05$ by log-rank test.

To establish the utility of admission platelet function testing, nonparametric ROC analysis was used to identify clinically relevant cutoff values for admission agonist responsiveness as predictors of in-hospital mortality. Based on ROC curves (Fig. 4), AA (area under the curve, 0.769) and collagen (area under the curve, 0.717) responsiveness were robust predictors of mortality; in contrast, admission ADP responsiveness, TRAP responsiveness, and platelet count did not statistically differ from chance. Specifically, an admission AA responsiveness of 35 U or higher had 80.0% sensitivity and 68.8% specificity for in-hospital mortality (negative likelihood ratio, 0.291), correctly classifying mortality in 77.3% of patients; admission collagen responsiveness of 20 U or higher had 96.0% sensitivity and 37.5% specificity (negative likelihood ratio, 0.107), correctly classifying 81.8% of the patients.

DISCUSSION

Here, we report a prospective, impedance aggregometry-based analysis of platelet dysfunction after trauma. Using the Multiplate multiple electrode aggregometer, we serially assayed platelet activation to ADP, TRAP, AA, and collagen in 101 patients with critical injury on arrival and then serially for the remainder of their ICU stay. Despite uniformly normal admission platelet counts, platelet hypofunction was strikingly common, occurring in 45.5% of patients at admission and 91.1% some time during their ICU stay; mean responsiveness to some agonists remained abnormal for up to 120 hours after admission. We identified severe base deficit and low GCS score as multivariate predictors of admission platelet hypofunction. Using Cox proportional hazards regression, we demonstrated that admission platelet hyporesponsiveness to TRAP, AA, and collagen were independent predictors of mortality when adjusted for other patient and injury characteristics. Using ROC analysis to identify the most informative agonist responses, admission AA and collagen were found to be significant predictors of in-hospital mortality.

Platelet dysfunction after trauma has been systematically described in only three other studies. Jacoby et al.[10] used the platelet function analyzer-100 (PFA-100; which measures shear-induced occlusion of an aperture in an agonist-impregnated cartridge) and flow cytometric markers of platelet activation (platelet microparticles, P-selection, and activated glycoprotein IIb/IIIa) to prospectively assess platelet function in 100 patients with trauma. In this study, significantly impaired collagen/epinephrine closure times were observed in six nonsurvivors at later time points, although admission values were statistically similar to 94 survivors; similarly, closure times were impaired in 22 patients with significant head injury (head AIS score, ≥4) compared with 78 patients without head injury at 24 hours, with no difference at admission. This parallels our finding that

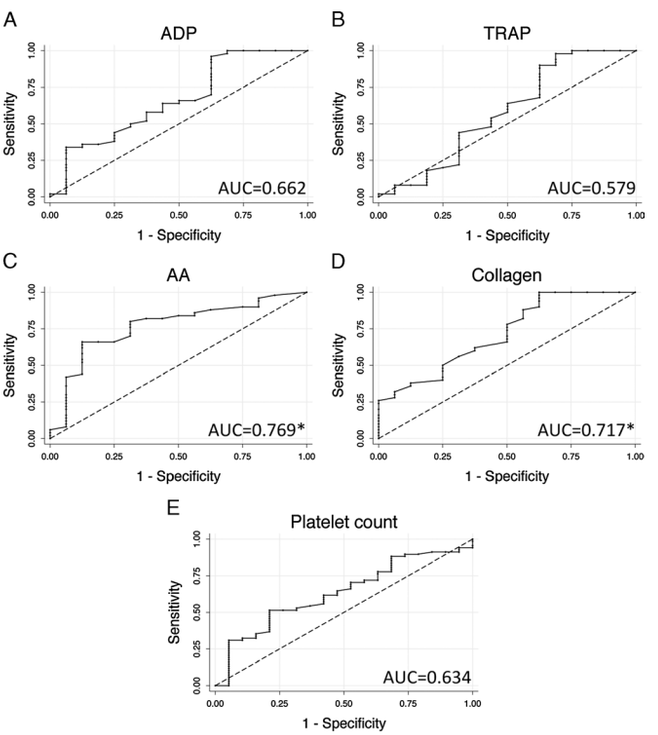

Figure 4. Receiver operating characteristic curves using admission platelet responsiveness to ADP (*A*), TRAP (*B*), AA (*C*), and collagen (*D*) as predictors of in-hospital mortality. Curve for admission platelet count (*E*) is shown for comparison. Area under the receiver operating characteristic curve (AUC) values given at the lower right in each graph. *Areas under the curve for which 95% confidence intervals differ significantly from chance.

platelet dysfunction is associated with mortality and that brain injury is a significant predictor of platelet dysfunction. This study found no differences in nonsurvivors or patients with brain injury at admission based on aperture closure time, although platelet microparticle levels were significantly higher at admission in these populations; this indicates that alterations in platelet function present on arrival are not reliably detected by PFA-100 aggregation. We here identify that impedance aggregometry is sensitive to these early differences and that poor admission AA-induced and collagen-induced responsiveness are associated with later mortality. Impedance aggregometry appears superior to PFA-100 aggregation in identifying platelet dysfunction on arrival, potentially allowing better triage and earlier targeted therapy.

Solomon et al.[9] recently reported a retrospective study of impedance aggregometry responses to ADP, TRAP, and collagen at admission in 163 patients with trauma. The incidence of platelet hypofunction in their study was notably lower than that reported here. They found platelet hyporesponsiveness to ADP in 13.9% of patients, TRAP in 13.7%, and collagen in 5.6%: comparatively, we identified hyporesponsiveness to ADP in 30.7%, TRAP in 18.7%, and collagen in 34.7%. Similarly, they identified only a weak correlation between platelet count and agonist responsiveness but did not report statistical significance. Many of these differences are likely attributable to differences in study population: their population had median ISS of 18 and overall mortality of 12.3%, compared with our median ISS of 25 and mortality rate of 22.7%. Because no adjusted analysis was performed, the absence of additional findings may be caused by the predominance of milder injury in their population. Their finding that ADP and TRAP responsiveness were significantly lower in seven patients with ISS of 50 or higher compared with 113 patients with ISS of 25 or lower supports this explanation. Despite these differences, the association of platelet dysfunction with mortality in their study parallels the unadjusted and multivariate analysis presented here.

Nekludov et al.[11] reported a smaller experience using thromboelastography (TEG)-based platelet mapping to evaluate platelet response to ADP and AA in 30 patients with trauma compared with controls. TEG-based platelet mapping measures the maximal amplitude of clot formation in heparin-treated and reptilase-treated whole blood in response to ADP and AA, comparing it to kaolin-activated maximal amplitude to generate an agonist-specific percentage of platelet inhibition.[12] The authors found significantly impaired AA responsiveness in patients with brain injury compared with patients with trauma without brain injury as well as controls but were unable to detect platelet dysfunction in 10 patients with trauma without brain injury. Although platelet mapping has been correlated with light aggregometry results,[13] to our knowledge, no direct comparison of TEG-based platelet mapping and impedance aggregometry exists to facilitate comparison of results. However, although we describe broader impaired AA responsiveness for patients with and without brain injury, our finding that low admission GCS score is a multivariate predictor of platelet hypofunction parallels the findings of Nekludov et al., pointing to important associations between platelet dysfunction and brain injury. These data have clear clinical implications for identifying patients at risk for intracranial hemorrhage progression.

The mechanisms underlying trauma-associated platelet dysfunction are poorly understood. One potential mechanism is suggested by Jacoby et al.,[10] who identified that flow cytometric markers of platelet activation were elevated in patients with trauma, despite impaired functional aperture closure times. In nontrauma studies, prolonged circulation of activated but hypofunctional platelets has been observed for up to 96 hours after activation.[14] These data mirror our finding that platelet function falls within 6 hours of admission and remains suppressed for up to 120 hours after injury. Taken together, this suggests that immediate platelet activation in response to tissue injury may induce a prolonged refractory state, in which a fraction of activated platelets remain in circulation but are dysfunctional. In light of the critical role played by platelets in the cell-based model of coagulation, this platelet hypofunction may correlate with functionally impaired thrombin generation even in the absence of classical explanations for coagulopathy (such as clotting factor depletion or hyperfibrinolysis) or may partially mediate the effects of hypothermia, hemodilution, and acidosis on clot formation. The ability to identify this state and assess the impact of targeted therapies would allow better guidance for the conduct of resuscitation and operative intervention.

Several limitations that are important for interpretation of this study exist. Similar to other platelet function studies, ours remains an initial, single-center experience; further work is needed to confirm and extend these findings. Although previous studies have cross-validated impedance aggregometry with several other assays of platelet function,[5,7,8] these studies were performed in healthy controls or were designed to detect antiplatelet medication effects. The reference ranges derived from these studies may not be ideal measures of platelet hypofunction in the setting of trauma. Point-of-care instrument use in a busy trauma center poses additional challenges in sample handling and evaluation of results that need to be addressed before these results can be clinically applied. Finally, although physiologic relevance is suggested by the prospective correlation of platelet function with later stent thrombosis in the cardiovascular literature,[15] further study is required to confirm that platelet aggregation in a laboratory test cell is a meaningful surrogate for hemostatic function in the bleeding trauma patient.

These results highlight two important clinical issues. First, although impairment of platelet response to ADP and AA have been characterized in response to clopidogrel and aspirin, respectively,[16] there is no a priori sense of which agonists are relevant in the setting of trauma. Given the wide availability of over-the-counter medications with antiplatelet effects and the known variability of platelet function in the population at large,[17] one could posit that the dysfunction of AA and collagen pathways seen here may be the result of an occult medication–related effect, as opposed to a trauma-related phenomenon. However, the observed hyporesponsiveness to TRAP argues that this platelet dysfunction is related to injury because neither thrombin generation nor platelet activation downstream of thrombin receptors is affected by cyclo-oxygenase-pathway blockade.[18] The data presented here

provide evidence for a specific platelet dysfunction induced by traumatic injury, but careful additional in vitro and clinical characterizations are required to elucidate the mechanism and to validate trauma-relevant agonists, reference ranges, and indications for clinical action.

Second, although we clearly identify the grave prognosis associated with platelet hypofunction, few therapeutic options exist to address it, calling into question the clinical utility of identifying an untreatable pathologic finding. Studies of platelet function-targeted therapy have been impeded by a lack of well-validated assays with which to demonstrate efficacy. However, the recent development of new functional analyzers has fostered a growing literature on several potential proplatelet therapies, including studies of desmopressin[19] and tranexamic acid[20] in reversing platelet dysfunction in cardiac surgical patients. Ongoing in vitro aggregometry studies and further prospective clinical studies will provide a platform for the evaluation of novel proplatelet agents, adding to the clinical armamentarium for treatment of trauma-associated platelet dysfunction.

Here, we demonstrate that clinically significant platelet dysfunction after trauma exists in the presence of an otherwise reassuring platelet count and clotting studies, with profound implications for mortality. Impedance aggregometry reliably identifies this dysfunction in injured patients, and admission AA and collagen responsiveness are significant predictors of both early and late mortality. The significance of low GCS score as an independent predictor of platelet hypofunction highlights the importance of further investigation into the link between traumatic brain injury and platelet dysfunction. The clinical availability of rapid, point-of-care platelet function testing will lead to improved triage, more appropriately targeted therapy, and better outcomes after trauma.

AUTHORSHIP

M.E.K. and M.J.C. prepared the article, performed all data analysis, and take full responsibility for the data as presented. B.J.R. and M.F.N. made significant contributions to the study design and implementation. R.C.M., I.M.C., M.D.G., and L.M.C. performed all clinical data collection.

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DISCLOSURE

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Criteria for empiric treatment of hyperfibrinolysis after trauma

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BACKGROUND: Recent studies identify a survival benefit from the administration of antifibrinolytic agents in patients with severe injury and trauma. However, identification of hyperfibrinolysis requires thromboelastography, which is not widely available. We hypothesized that analysis of patients with thromboelastography-diagnosed hyperfibrinolysis would identify clinical criteria for empiric antifibrinolytic treatment in the absence of thromboelastography.

METHODS: From November 2010 to March 2012, serial blood samples were collected from 115 patients with critical injury on arrival to the emergency department of an urban Level I trauma center. Rotational thromboelastography was performed to assess viscoelastic properties of clot formation in the presence and absence of aprotinin to identify treatable hyperfibrinolysis. For 20 patients identified with treatable hyperfibrinolysis, clinical predictors were investigated using receiver operating characteristic analysis.

RESULTS: Of the 115 patients evaluated, 20% had hyperfibrinolysis, defined as an admission maximal clot lysis of 10% or higher, reversible by aprotinin treatment. Patients with hyperfibrinolysis had significantly lower temperature, pH, and platelet counts and higher international normalized ratio, activated partial thromboplastin time, and D-dimer. Hyperfibrinolysis was associated with multiorgan failure (63.2% vs. 24.6%, $p = 0.004$) and mortality (52.2% vs. 12.9%, $p < 0.001$). We then evaluated all non–rotational thromboelastography clinical and laboratory parameters predictive of hyperfibrinolysis using receiver operating characteristic analysis to evaluate potential empiric treatment guidelines. The presence of hypothermia (temperature ≤36.0°C), acidosis (pH ≤7.2), relative coagulopathy (international normalized ratio ≥1.3 or activated partial thromboplastin time ≥30), or relative thrombocytopenia (platelet count ≤200) identified hyperfibrinolysis with 100% sensitivity and 55.4% specificity (area under the curve, 0.777).

CONCLUSION: Consideration of empiric antifibrinolytic therapy is warranted for patients with critical injury and trauma who present with acidosis, hypothermia, coagulopathy, or relative thrombocytopenia. These clinical predictors identified hyperfibrinolysis with 100% sensitivity while simultaneously eliminating 46.6% of inappropriate therapy compared with the empiric treatment of all injured patients. These criteria will facilitate empiric treatment of hyperfibrinolysis for clinicians without access to thromboelastography. (*J Trauma Acute Care Surg*. 2012;73: 87–93. Copyright © 2012 by Lippincott Williams & Wilkins)

LEVEL OF EVIDENCE: Prognostic study, level III.

KEY WORDS: Fibrinolysis; thromboelastography; rotational thromboelastometry; ROTEM.

Hemorrhage remains the leading cause of potentially preventable death after trauma, exacerbated in up to a third of injured patients by abnormal coagulation that is present on arrival to the emergency department.[1] This phenomenon, referred to as acute traumatic coagulopathy (ATC), exists independently of the classically identified coagulopathy risk factors of hypothermia, hemodilution, and acidosis.[2] ATC occurs in the setting of massive tissue injury and hypoperfusion and results in both impairment of new clot formation and enhanced fibrinolysis of existing clot.[3,4] Although the early empiric administration of plasma[5,6] and damage control strategies[7] have been shown to mitigate the effects of impaired clot formation, guidelines addressing treatment of clinically significant fibrinolysis after injury are sparse. This enhanced fibrinolysis, termed *hyperfibrinolysis* (HF), has been identified as an integral component of ATC. Recent intriguing data suggest that the use of plasminogen-targeted inhibitors of enzymatic fibrinolysis such as tranexamic acid (TXA) may provide the missing pharmacologic treatment of the hyperfibrinolytic component of ATC.[8,9]

Although several groups have reported on varying degrees of HF after injury, the precise incidence of HF remains unclear, owing in part to heterogeneity in diagnostic technique and lack of consensus definitions.[10–13] Currently, the criterion standard for diagnosing HF is thromboelastography; however, these devices are not yet widely available, and protocols for their use in guiding trauma resuscitation have to date been limited to single-center experiences.[14–17] The clinical relevance of treating HF is clear based on the CRASH-2 large randomized controlled trial of empiric administration of TXA in patients with critical injury and trauma, which identified a significant mortality benefit to empiric TXA therapy in patients with critical injury.[9] However, no diagnostic criteria were used to specifically identify HF before enrollment, suggesting that a large cohort of these patients were overtreated.

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Clearly, specific identification of the patients that are at highest risk for developing HF would facilitate appropriate therapy for the hyperfibrinolytic component of ATC while avoiding the cost and potential adverse effects of overtreatment. Therefore, the aims of this study were as follows: (1) to identify patients with treatable HF using thromboelastography, (2) to characterize the relationship of HF with injury characteristics and outcomes, and (3) to identify clinical and basic laboratory value–based guidelines for appropriate empiric treatment of HF in the absence of thromboelastography.

PATIENTS AND METHODS

Blood samples were prospectively collected from 115 patients with critical injury on arrival to the emergency department of an urban Level I trauma center. Patients who were younger than 18 years, had more than 5% body surface area burns, received more than 2 L of intravenously administered fluid before arrival, or were transferred from another institution were excluded. Admission blood samples were collected via initial placement of a 16 gauge or larger peripheral intravenous line into 3.2% (0.109 mol) sodium citrate and processed within 3 hours of being drawn; sample collection methodology is described in detail elsewhere.[3] After a waiver of consent was applied for initial blood draws, informed consent was obtained for all study patients as approved by our institutional committee on human research.

Point-of-care rotational thromboelastometry (ROTEM) was performed to assess viscoelastic properties of clot formation. Briefly, 300-μL samples of citrated whole blood were warmed to 37°C, recalcified, and activated with tissue factor-containing rabbit brain thromboplastin in the presence (APTEM) and absence (EXTEM) of aprotinin. An enzymatic fibrinolysis index (EFI) was defined as the difference in maximal clot lysis percentage between EXTEM and APTEM testing, identifying HF amenable to treatment and eliminating variability not related to enzymatic fibrinolysis. Massive transfusion was defined as transfusion of 10 U of red blood cell counts (RBCs) or more within the first 24 hours of admission for patients surviving to 24 hours; to include patients who received high-volume transfusion but did not survive to 24 hours, scaled transfusion of 5 U or more for patients dying by 12 hours or 2.5 U or more for patients dying by 6 hours were also included. Activity levels of factors II, V, VII, VIII, IX, and X; antithrombin III; and protein C as well as antigen levels of fibrinogen and D-dimer were assayed using a Stago Compact Functional Coagulation Analyzer (Diagnostica Stago, Parsippany, NJ). Activated protein C was assayed using an established enzyme-linked immunosorbent assay method reported elsewhere.[4] Standard laboratory, resuscitation, and outcome data were prospectively collected in parallel.

All data are presented as mean (SD), median (interquartile range [IQR]), or percentage; univariate comparisons were made using Student's t test for normally distributed data, Wilcoxon rank sum test for skewed data, and Fisher's exact test for proportions. Standard logistic regression was performed to identify predictors of HF. Kaplan-Meier time-to-event analysis and log-rank tests were used to assess differences in 24-hour and in-hospital mortality between groups. Nonparametric receiver operating characteristic (ROC) analysis was performed to characterize the ability of clinical predictors to identify HF. An $\alpha = 0.05$ was considered significant. All data analysis was performed by the authors using Stata version 12 (StataCorp; College Station, TX).

RESULTS

The overall study population had a mean (SD) age of 40.8 (19.2) years, 26.1% penetrating injury, 54.9% neurologic injury, mean (SD) Injury Severity Score (ISS) of 22.0 (14.5), mean (SD) base deficit of −5.4 (6.0), and Glasgow Coma Scale (GCS) score of 10 (5–14). Of the 115 patients with complete ROTEM data at admission, 23 patients (20%) had HF as defined by an admission EFI of 10% or higher (Table 1). Sample tracings for representative patients with HF are provided in Figure 1. For clinical characteristics, patients with HF had significantly lower admission temperature, higher incidence of both early (<6 hours) red blood cell transfusion and massive transfusion, higher mechanical ventilation requirements, and higher incidence of multiorgan failure and mortality (Table 2). As confirmed by Kaplan-Meier survival analysis, patients with HF die significantly earlier and more often than those without (log rank, $p < 0.001$; Fig. 2). For admission clinical laboratory values, patients with HF had significantly higher international normalized ratio (INR), activated partial thromboplastin time (PTT), and D-dimer levels, as well as lower pH and platelet count (all $p < 0.05$; Table 3). The clotting factor and anticoagulant profile demonstrates that HF is further associated with significant depletion of factors V and factor IX, as well as elevation of activated protein C (all $p < 0.03$; Table 3). We then evaluated all population differences with $p < 0.200$ using both univariate and multivariate logistic regression to identify predictors of HF (Table 4). This identified lower temperature, pH, and platelet count and higher INR, PTT, and D-dimer levels as significant univariate predictors of HF; however, none of these parameters alone were independent predictors of HF when adjusted for ISS and admission base deficit.

Because no individual non-ROTEM parameters independently predicted HF, we then evaluated combinations of univariate predictors of HF using ROC analysis to identify potential empiric treatment guidelines. To identify broad guidelines, we included only univariate predictors that are widely available as part of a standard trauma clinical and laboratory workup as candidates, excluding specialized clotting factor measurements, fibrinogen, and D-dimer levels. For continuous values, we selected cutoffs at the value yielding the highest percentage of patients correctly classified as having HF or not. Using these pooled cutoffs, the presence of hypothermia (temperature ≤36.0°C), acidosis (pH ≤7.2), relative coagulopathy (INR ≥1.3 or PTT ≥30), or relative thrombocytopenia (platelet count ≤200) identified HF with 100% sensitivity, 55.4% specificity, and positive likelihood ratio 2.24 (area under the curve, 0.777; 95% confidence interval, 0.726–0.828).

To avoid the inclusion of moribund patients in whom empiric treatment of HF would be futile, we repeated all previous analysis excluding patients in whom care was withdrawn as a surrogate for nonsurvivable injury. Seventeen

TABLE 1. Demographic and Injury Characteristics of Patients With HF

ID	Age, y	Sex	Mechanism	Injuries	EX ML, %	AP ML, %	EFI, %	24-h RBC	MT	Outcome
1	32	M	Gunshot to axilla, thigh	Rib fracture, lung laceration, soft tissue injury	13	2	11	1	N	Discharged, hospital day 6
2	62	M	Assault	Tracheal fracture, subgaleal hematoma	19	6	13	0	N	Discharged, hospital day 5
3	40	M	10-foot fall down stairs	Mandibular fracture, T12/L1 compression fractures, subgaleal hematoma	28	15	13	0	N	Discharged, hospital day 6
4	68	F	Pedestrian vs. bicycle	Skull base fractures, SDH, SAH, carotid dissection	20	6	14	2	N	Died of hypoxia, hospital day 27
5	80	F	Pedestrian vs. automobile	SDH, SAH, IPH	27	11	16	4	N	Discharged, hospital day 59
6	19	M	Multiple gunshots to shoulder, axilla, abdomen, back, perineum	Rib fracture, lung laceration, retroperitoneal hematoma, rectal wall hematoma	34	17	17	0	N	Discharged, hospital day 7
7	26	M	4-story fall	Facial and rib fractures; liver, splenic, renal, and pancreatic lacerations; duodenal hematoma	37	16	21	14	Y	Discharged, hospital day 28
8	22	M	Pedestrian vs. automobile	Skull fractures, SAH, SDH, T5 vertebral body fracture, pulmonary contusion, clavicle and scapular fractures	36	12	24	2	N	Discharged, hospital day 24
9	32	M	Gunshots to chest, upper extremity	Rib fracture, hemothorax, kidney laceration, T10-11 spinal cord injury, soft tissue injury	58	16	42	0	N	Discharged, hospital day 26
10	23	M	Motor vehicle collision with ejection	Skull base fracture, SDH, SAH, facial fractures, upper-extremity fracture	54	9	45	14	Y	Discharged, hospital day 32
11	71	F	Blunt head injury	SDH, SAH	95	12	83	8	Y	Care withdrawn, ICU day 1
12	24	M	Helmeted motorcycle crash with ejection and 40-foot fall	Carotid disruption, SAH, C1/C2 fracture, pulmonary contusion, renal and splenic lacerations	100	10	90	6	Y	Care withdrawn, ICU day 1
13	52	M	Helmeted motorcycle crash; preexisting cirrhosis	SDH, pulmonary contusion; rib, vertebral body, scapular, and clavicular fractures	100	8	92	40	Y	Care withdrawn, ICU day 10
14	48	M	Fall from standing; intoxicated	SAH	100	7	93	0	N	Discharged, hospital day 3
15	69	M	Hanging	Diffuse anoxic brain injury	100	6	94	0	N	Care withdrawn, ICU day 2
16	84	F	20-foot fall	Pelvic fractures, lower extremity fractures	100	5	95	9	Y	Died of cardiac arrest, day 1
17	37	M	Gunshot to head	SAH, cerebral edema, uncal herniation	100	4	96	2	N	Care withdrawn, ICU day 1
18	22	M	Gunshot to head	SAH, SDH	99	3	96	0	N	Care withdrawn, ICU day 1

(Continued on next page)

TABLE 1. *(continued)*

ID	Age, y	Sex	Mechanism	Injuries	EX ML, %	AP ML, %	EFI, %	24-h RBC	MT	Outcome
19	35	M	Stab wound to neck	Tracheal disruption, traumatic arrest requiring emergency thoracotomy	99	2	97	2	Y	Died of cardiac arrest, day 1
20	56	M	20-step fall	EDH, SDH, SAH	99	2	97	5	N	Discharged, hospital day 6
21	25	F	Bicycle vs. automobile	SAH, carotid artery dissection, sternal fracture, lung laceration, liver laceration, pelvic fractures	99	2	97	13	Y	Care withdrawn, ICU day 1
22	74	F	Fall from standing; preexisting cirrhosis	Humerus fracture, femoral arterial injury	100	0	100	56	Y	Died of hemorrhage, day 1
23	85	M	Fall from standing	SAH, SDH	100	0	100	0	N	Died of multiorgan failure, ICU day 7

AP ML, APTEM maximum lysis; EDH, epidural hematoma; EX ML, EXTEM maximum lysis; ICU, intensive care unit; IPH, intraparenchymal hemorrhage; MT, massive transfusion; RBC, red blood cell units transfused during the first 24 hours; SAH, subarachnoid hemorrhage; SDH, subdural hemorrhage.

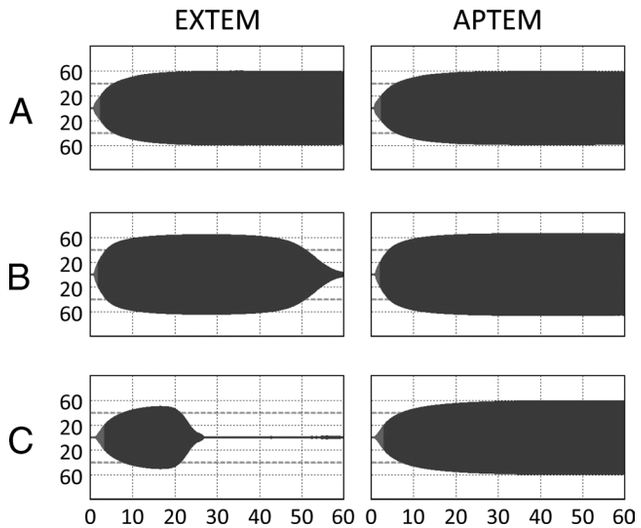

Figure 1. Representative admission ROTEM EXTEM and APTEM tracings for patients with no HF (A), secondary HF with normal maximal clot firmness (B), and primary HF with impaired maximal clot firmness (C).

patients met these criteria (Table 1). When excluding these patients, mortality remained significantly worse for patients with HF ($p = 0.006$). In univariate analysis for predictors of HF, INR ($p = 0.062$), pH ($p = 0.093$), platelet count ($p = 0.136$), and D-dimer ($p = 0.055$) were no longer significant, whereas temperature and PTT remained predictive ($p < 0.05$). Excluding patients with nonsurvivable injury, the previouly identified criteria for empiric treatment remained 100% sensitive for HF, with slightly improved specificity (59.0% specificity; positive likelihood ratio, 2.44) and a similar and still significant area

TABLE 2. Patient Characteristics by HF

	EFI ≥ 10 (n = 23)	EFI 0–10 (n = 92)	*p*
Age, mean (SD), y	47.2 (22.6)	39.2 (18.1)	0.125
Penetrating injury, %	26.1	26.1	1.000
Neurologic injury, %	60.9	53.3	0.640
ISS, mean (SD)	23.0 (13.2)	21.8 (14.8)	0.767
GCS score, median (IQR)	9 (3–14)	10 (6–14)	0.304
Temperature, mean (SD)	35.4 (0.8)	36.1 (0.6)	0.012*
Prehospital IVF, median (IQR)	150 (50–250)	225 (0–1,000)	0.988
Transfused RBC in 6 h, %	60.9	33.8	0.029*
Massively transfused, %	39.1	5.5	<0.001*
Hospital days, median (IQR)	6 (1–25)	10 (4–18)	0.073
ICU days, median (IQR)	2 (1–11)	4 (2–12.5)	0.119
Vent-free days/28 d, median (IQR)	2 (0–25)	25 (10.5–27)	0.004*
Multiorgan failure, %	63.2	24.6	0.004*
24-h mortality, %	34.8	3.5	<0.001*
In-hospital mortality, %	52.2	12.9	<0.001*

*$p < 0.05$ by Student's *t*, Mann-Whitney *U*, or Fisher's exact tests.
EFI, enzymatic HF index; ICU, intensive care unit; IVF, intravenously administered fluid.

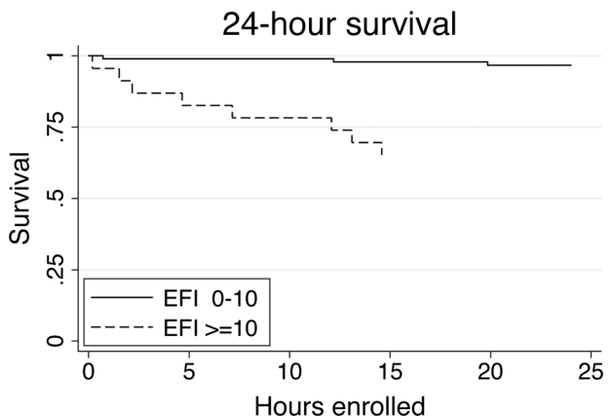

Figure 2. Kaplan-Meier 24-hour survival curves showing early survival differences between patients with (*dashed line*) and without (*solid line*) HF. *$p < 0.001$ by log-rank test.

under the ROC curve of 0.795 (95% confidence interval, 0.742–0.848).

DISCUSSION

Although intriguing data exist regarding a survival benefit to the pharmacologic treatment of HF after trauma, broad clinical application of this knowledge has been impaired by a lack of specific diagnostic criteria and the absence of guidelines for empiric therapy. Several recent single-center studies have investigated HF after trauma using thromboelastography; however, heterogeneity in study populations, enrollment criteria, thromboelastography assays, and viscoelastic parameters assessed has led to variable estimates of the incidence of HF, ranging from 3% to 20% of all patients with trauma.[13] Despite significant uncertainty regarding the incidence, there is no clinical disagreement that HF portends a markedly worse prognosis, with the same studies identifying mortality rates of 38.5% to 100% for patients with HF. The data presented here add significantly to this growing body of knowledge regarding diagnostic utility, patient-level characterization, and clinically applicable guidelines for empiric treatment.

Two commercially available systems exist for thromboelastography: ROTEM and the TEG Thromboelastograph hemostasis analyzer (TEG; Haemonetics, Braintree, MA). Two studies use TEG-based diagnostic criteria to identify HF, using a 15% decrement in clot firmness either 30[10] or 60[18] minutes after maximal clot amplitude. Similar results have been published by several groups using comparable ROTEM-based maximal clot lysis parameters.[12,19–21] Using ROTEM APTEM test–based criteria provides the additional theoretical advantage of identifying only fibrinolysis that is reversible by treatment with aprotinin, thereby eliminating spurious clot amplitude reduction owing to sample drying and clot retraction while simultaneously providing a gauge of potential treatment efficacy. To our knowledge, only one other group has published data using an APTEM-based definition; this study by Levrat et al.[11] identified HF in 87 patients with trauma admitted to a large academic trauma center in France, identifying six patients (5%) with a more than 7% increase in maximal clot firmness between EXTEM- and APTEM-activated samples. Here, we use a similar definition, identifying 15 patients (20%) with a more than 10% EFI; both studies identify significant associations between HF and increased injury severity, coagulopathy, and mortality.

We further extend this analysis by identifying hypothermia, acidosis, and coagulopathy as well as relative thrombocytopenia as predictors of HF in univariate analysis. Of the other studies addressing HF after trauma, only that of Kashuk et al.[10] systematically investigated adjusted predictors of HF in their cohort of 11 patients with TEG-based HF of 61 patients receiving transfusions. Both our study as well as that of Kashuk et al. identified emergency department coagulopathy as a critical predictor of HF. We further identify significant depletion

TABLE 3. Admission Laboratory Values by HF

	EFI ≥ 10 (n = 23)	EFI 0–10 (n = 85)	*p*
INR, median (IQR)	1.3 (1.1–1.4)	1.1 (1.0–1.2)	<0.001*
PTT, median (IQR)	32.0 (28.0–41.9)	27.0 (24.6–30.0)	<0.001*
pH, mean (SD)	7.17 (0.17)	7.29 (0.15)	0.035*
Base deficit, mean (SD)	−7.3 (5.4)	−4.7 (6.2)	0.109
Hematocrit, mean (SD)	39.1 (6.2)	40.6 (5.8)	0.302
Platelet count, mean (SD)	238 (82)	278 (76)	0.043*
Fibrinogen, mean (SD)	164 (89)	292 (164)	0.116
D-dimer, median (IQR)	7.8 (5.7–9.2)	1.8 (0.6–8.1)	0.019*
Factor II, mean (SD)	65.2 (22.2)	70.4 (18.0)	0.415
Factor V, mean (SD)	27.1 (16.8)	45.7 (25.3)	0.005*
Factor VII, mean (SD)	88.1 (33.9)	89.1 (47.7)	0.927
Factor VIII, mean (SD)	173.3 (93.1)	188.6 (109.3)	0.626
Factor IX, mean (SD)	92.7 (46.0)	132.2 (54.2)	0.011*
Factor X, mean (SD)	72.2 (26.7)	75.3 (21.8)	0.690
AT3, mean (SD)	75.2 (31.2)	80.5 (23.9)	0.574
PC, mean (SD)	105.7 (46.6)	89.9 (34.8)	0.266
aPC, median (IQR)	25.5 (10.7–51.5)	3.2 (2.1–15.1)	0.028*

*$p < 0.05$ by Student's *t*, Mann-Whitney *U*, or Fisher's exact tests.
aPC, activated protein.

TABLE 4. Univariate and Multivariate Predictors of HF

	Univariate		Multivariate	
	Odds Ratio	*p*	Odds Ratio	*p*
Age	1.021	0.077	1.027	0.201
Temperature	0.229	0.006*	0.233	0.078
INR	1.332	0.007*	1.177	0.135
PTT	1.125	0.002*	1.099	0.055*
pH	0.633	0.036*	0.781	0.722
Base deficit	0.931	0.131	0.913	0.126
Platelet count	0.993	0.034*	0.990	0.075
Fibrinogen	0.987	0.109	0.969	0.207
D-dimer	1.238	0.018*	1.345	0.057

*$p < 0.05$ by Wald test.
Logistic regression was used to generate both unadjusted univariate odds ratios as well as multivariate, ISS and base deficit–adjusted odds ratios for HF.
Odds ratios for INR and pH scaled for differences of 0.1.

of factor V and factor IX, as well as elevation of activated protein C, in patients with HF. Taken together, the clear clinical relationship between impairment of clot formation and activation of fibrinolysis lends credence to the recently described dual role of the activated protein C system in coagulopathy after trauma via cleavage of activated factors Va and VIIIa as well as derepression of fibrinolysis.[3,4]

The recent prospective, randomized, placebo-controlled CRASH-2 trial evaluated more than 20,000 patients with trauma, identifying a significant decrease in both all-cause (14.5% vs. 16.0%) and hemorrhage-related (4.9% vs. 5.7%) mortality in trauma patients treated empirically with TXA.[9] However, inclusion criteria for the study were broad, with only half of enrolled patients receiving a blood transfusion or requiring an emergency operation. Based on the 1.5% absolute mortality risk reduction identified, the number needed to treat empirically with TXA to save one life is 67; this modest risk reduction observed likely reflects significant overtreatment. These generalizability concerns are partially addressed by the recent Military Application of Tranexamic acid in Trauma Emergency Resuscitation (MATTERs) study, which retrospectively evaluated the use of TXA in 293 of 896 patients with combat injury with respect to total blood product use, thromboembolic complications, and mortality.[8] The authors found that the TXA-treated group had lower unadjusted mortality than the non-TXA–treated group (17.4% vs. 23.9%, respectively, with an absolute mortality risk reduction of 6.5%) despite more severe injury. Notably, the rate of thromboembolic complications was significantly greater in the TXA-treated compared with non-TXA–treated group in this study. Although TXA treatment was not an independent predictor of thromboembolism in adjusted analysis performed in the MATTERs study and the incidence of thromboembolic complications did not significantly differ between TXA- and placebo-treated patients in the CRASH-2 trial, this highlights the important concern of potentially increased thromboembolic risk with TXA treatment. In the more selective TXA treatment assessed in the MATTERs study compared with that of CRASH-2, the overall number needed to treat to save 1 life is 15; this is further reduced to as low as 7 for patients requiring massive transfusion (absolute risk reduction, 13.7%). Overall, available evidence indicates that a subset of patients with trauma exist for whom empiric TXA treatment would provide a significant survival benefit while minimizing treatment cost and the risk of adverse effects.

The data presented here provide a first attempt to specifically address this critical gap in the growing literature on the management of HF after trauma by outlining criteria for empiric antifibrinolytic treatment. Specifically, we provide data that treatment of any patient with critical injury and hypothermia (temperature $\leq$36.0°C), acidosis (pH $\leq$7.2), relative coagulopathy (INR $\geq$1.3 or PTT $\geq$30), or relative thrombocytopenia (platelet count $\leq$200) would identify HF with 100% sensitivity and 55.4% specificity. Furthermore, data from both the MATTERs study and those from Kashuk et al. highlight the increased incidence of HF for patients receiving massive transfusions. Specifically, the MATTERs study identified an enhanced absolute mortality risk reduction associated with TXA treatment of 13.7% for patients requiring massive transfusion compared with 6.5% in the overall study population.[8] Similarly, Kashuk et al. show that 34% of patients requiring more than 6 U of red blood cells within 6 hours of admission had TEG-based HF compared with 18% of patients overall, suggesting that patients requiring massive transfusion are at higher risk of HF. In our patient population, transfusion of RBC within 6 hours of admission was significantly more common, and massive transfusion within the first 24 hours was more than sevenfold more common, for patients with HF. Taking these data together, we would advocate that any patient with clinically significant injury presenting with acidosis, hypothermia, coagulopathy, or relative thrombocytopenia should be considered for empiric treatment with an antifibrinolytic agent within 1 hour of arrival and that empiric early antifibrinolytic therapy should be considered as an addition to institutional massive transfusion protocols.

As with the other currently available studies of HF after trauma, several limitations are important in the interpretation of the results presented here. With 115 patients available for analysis, sample size limits both the generalizability as well as the statistical power of this study, highlighting the need for further prospective, multicenter evaluation of both diagnostic criteria and treatment protocols for HF before their widespread adoption. Although thromboelastography is the current de facto criterion standard for the diagnosis of HF, the significant heterogeneity of findings using several biochemical markers of fibrinolysis has made specific validation of functional testing such as ROTEM- and TEG-based thromboelastography elusive. Addressing this technical concern, Levrat et al. have previously validated the ROTEM EXTEM and APTEM tests in a cohort of 23 patients using a euglobulin clot lysis time of less than 90 minutes as a criterion standard, showing 100% specificity of ROTEM in the diagnosis of HF.[11] Furthermore, the enzymatic HF index defined herein relies on the ROTEM APTEM maximal clotting firmness, using the requirement of reversibility by aprotinin to eliminate spurious results due to mechanical clot retraction and confounding by other factors unrelated to true enzymatic fibrinolysis. The release of tissue thromboplastins leading to local hypercoagulability and systemic disseminated intravascular coagulation-like consumptive coagulopathy has been posited to underlie coagulopathy in severe traumatic brain injury, although this mechanism remains poorly understood. Although reversal of hyperfibrinolytic coagulopathy may mitigate the threat of progressive hematoma enlargement and herniation in the setting of active intracranial hemorrhage, this must be balanced with the theoretical risk of worsening ischemic damage in the penumbra of injury. Furthermore, the CRASH-2 trial did not demonstrate a mortality reduction in head injury–related death in the TXA-treated group compared with placebo,[9] and the MATTERs study found that admission GCS score 8 or less was an independent predictor of mortality, even when adjusted for TXA treatment.[8] Taken together, these results imply that antifibrinolytic therapy may be less efficacious in this group. As with all empiric guidelines, clinical judgment must balance the potential risks and benefits of treatment given the clinical uncertainty that remains in select settings such as traumatic brain injury. Finally, although both aprotinin and TXA bind and inhibit plasmin, differences in the specificity of the bovine-derived polypeptide aprotinin and

the synthetic lysine analogue TXA exist, and their efficacy in reversal of fibrinolysis may not be equivalent;[22] therefore, inferences about the potential efficacy of TXA presented here should be interpreted with caution.

Overall, this study confirms and extends the growing body of literature on the recognition and treatment of HF after trauma through specific thromboelastography-based diagnostic criteria, multivariate risk factor assessment, and receiver operator characteristic–based clinical guidelines for empiric treatment. These data highlight the clinical utility of thromboelastography and confirm a mechanistic link between ATC and HF. Most importantly, data presented here support the empiric treatment of fibrinolysis in patients with trauma who present with acidosis, hypothermia, coagulopathy, and relative thrombocytopenia as a strategy for appropriately sensitive empiric treatment in clinical settings in which thromboelastography and other advanced diagnostic equipment is unavailable.

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AUTHORSHIP

M.E.K., M.W.C., and M.J.C. prepared the article, performed all data analysis, and take full responsibility for the data as presented. R.C.M., I.M.C., M.D.G., and L.M.C performed clinical data collection. B.J.R. and M.F.N. made significant contributions to the study design and implementation.

DISCLOSURE

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THE PRINCIPAL COMPONENTS OF ACUTE TRAUMATIC COAGULOPATHY

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Introduction: Clotting factor abnormalities in acute traumatic coagulopathy are poorly understood, with application of traditional regression techniques confounded by collinearity. We hypothesized that principal components analysis (PCA), a pattern-finding technique, would identify clinically predictive patterns in the complex clotting factor milieu after trauma.

Methods: Plasma was prospectively collected from 163 critically-injured trauma patients. Prothrombin, Factors V, VII, VIII, IX, X, D-dimer, activated and native Protein C, and antithrombin III levels were assayed, and subjected to PCA to identify principal components (PCs).

Results: Of 163 patients, 19.0% had coagulopathy (INR≥1.3). PCA identified 3 PCs, accounting for 67.5% of variance (see Figure). PC1 identified global clotting factor depletion; PC2 the activation of Protein C and fibrinolysis; and PC3 Factor VII elevation and VIII depletion. PC1 score correlated with penetrating injury and injury severity, predicting coagulopathy (OR 4.67, p<0.001) and mortality (OR 1.47, p=0.032). PC2 score correlated with injury severity, acidosis, and shock, and significantly predicted ventilator-associated pneumonia (OR 1.59, p=0.008), acute lung injury (OR 2.24, p<0.001), multiorgan failure (OR 1.83, p=0.002), and mortality (OR 1.62, p=0.006). PC3 did not significantly predict outcomes.

	PC1	PC2	PC3
Prothrombin	-0.86	-0.04	0.11
Factor V	-0.78	0.01	-0.11
Factor VII	-0.62	0.01	0.47
Factor VIII	-0.35	0.34	-0.73
Factori IX	-0.69	0.07	0.03
Factor X	-0.88	-0.01	0.20
D-dimer	0.25	0.80	0.00
aPC	0.20	0.74	0.39
Protein C	-0.80	0.11	-0.05
AT III	-0.74	0.16	-0.17

Conclusion: PCA identifies distinct patterns of coagulopathy: depletion coagulopathy predicts mortality and INR elevation, while fibrinolytic coagulopathy predicts infection, end-organ failure, and mortality, without detectable differences in INR or PTT. These disparate patterns identify specific perturbations to target directed resuscitation and treatment.

A principal component analysis of coagulation after trauma

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BACKGROUND: Clotting factor abnormalities underlying acute traumatic coagulopathy are poorly understood, with application of traditional regression techniques confounded by colinearity. We hypothesized that principal components analysis (PCA), a pattern-finding and data reduction technique, would identify clinically predictive patterns in the complex clotting factor milieu after trauma.

METHODS: Plasma was prospectively collected from 163 critically injured trauma patients. Prothrombin; factors V, VII, VIII, IX, X; D-dimer; activated and native protein C; and antithrombin III levels were assayed and subjected to nonlinear PCA to identify principal components (PCs).

RESULTS: Of 163 patients, 19.0% were coagulopathic on admission. PCA identified 3 significant PCs, accounting for 67.5% of overall variance. PC1 identified global clotting factor depletion; PC2 the activation of protein C and fibrinolysis; and PC3 factor VII elevation and VIII depletion. PC1 score correlated with penetrating injury and injury severity, predicting coagulopathy (odds ratio [OR], 4.67; $p < 0.001$) and mortality (OR, 1.47; $p = 0.032$). PC2 score correlated with injury severity, acidosis, and shock, and significantly predicted ventilator-associated pneumonia (OR, 1.59; $p = 0.008$), acute lung injury (OR, 2.24; $p < 0.001$), multiorgan failure (OR, 1.83; $p = 0.002$), and mortality (OR, 1.62; $p = 0.006$) but was not associated with international normalized ratio (INR)–based or partial thromboplastin time (PTT)–based coagulopathy ($p > 0.200$). PC3 did not significantly predict outcomes.

CONCLUSION: PCA identifies distinct patterns of coagulopathy: depletion coagulopathy predicts mortality and INR/PTT elevation, while fibrinolytic coagulopathy predicts infection, end-organ failure, and mortality, without detectable differences in INR or PTT. While depletion coagulopathy is intuitive, fibrinolytic coagulopathy may be a distinct but often overlapping entity with differential effects on outcomes. (*J Trauma Acute Care Surg*. 2013;74: 1223–1230. Copyright © 2013 by Lippincott Williams & Wilkins)

LEVEL OF EVIDENCE: Prognostic study, level III.

KEY WORDS: Coagulopathy; principal components analysis; fibrinolysis.

Hemorrhage remains the leading cause of potentially preventable death after trauma, complicated in up to a third of injured patients by coagulation abnormalities present on arrival to the emergency department.[1] Although well-studied, the importance of specific clotting factor abnormalities to the complex phenomenon of acute traumatic coagulopathy is poorly understood. Strong correlations between clotting factor levels pose a significant challenge to identifying the isolated importance of any individual factor. This colinearity makes standard regression techniques prone to unstable results, difficult to generalize, and at risk of identifying spurious statistical significance. Several mathematical techniques exist to more clearly describe the patterns that exist in such complex, correlated data sets and to associate these patterns with binary outcomes; principal component analysis (PCA) is one such method.

PCA is a statistical pattern detection tool that distills a complex set of intercorrelations down to essential clusters of variables that move together as groups. To begin, a data set of correlated variables is decomposed into a smaller set of uncorrelated synthetic multivariables. A best-fit plane is described in this multivariate space, the axes of which are termed *principal components* (PCs); the location of each individual data point in multivariate space can then be specifically described relevant to these PCs. In this sense, PCA can be thought of as a multivariate form of the Pearson correlation, in which the best-fit line is replaced by a best-fit multivariate plane. The data set described here contains arrival clotting factor measurements in a panel of critically injured patients, in which many of the individual factor levels are highly correlated with each other. The application of PCA transforms each patient's individual clotting factor measurements into a smaller set of PC "scores," which can be interpreted as individual patient locations within multivariate outcome space. Furthermore, each PC can be broken down into "factor loadings" that describe both the contribution of each individual clotting factor into the calculation of that PC score as well as each factor's relationships with other factors. A detailed explanation of the rationale and methodology of PCA is available in the Supplemental Digital Content (see Document, Supplemental Digital Content 1, http://links.lww.com/TA/A243).

As an example, a PC controlled entirely by a single predictor would have a loading coefficient of +1.0 for the predictor in question, with all other predictor coefficients equal to zero; in contrast, a PC determined by mixed contributions from multiple factors would have loading coefficients spanning values from −1.0 to +1.0 for each contributing factor, corresponding to positive and inverse Pearson correlations. Intuitively, PCs

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describe the internal structure of a set of correlated measurements allowing patients to be grouped by global patterns of clotting factor perturbations as defined by high or low PC scores, and the clotting factor interrelationships that define each group can be described. For a set of highly interrelated predictors such as clotting factor measurements, PCA accommodates degrees of colinearity that would make standard regression approaches unstable.

Therefore, we hypothesized that PCA would identify clinically predictive patterns in the complex clotting factor milieu after trauma. In this study, we apply PCA to identify and examine the clotting factor relationships underlying these clinical patterns and correlate these with outcomes in a panel of critically injured patients.

PATIENTS AND METHODS

Plasma was collected from 163 critically injured trauma patients on arrival to the emergency department of an urban Level I trauma center from February 2005 to October 2010 as part of an ongoing clinical study.[2] Consecutive patients triggering activation of the highest level of a two-tiered triage system during the study period were prospectively enrolled under a waiver of consent; patients younger than 18 years, those with more than 5% body surface area burns, those who received more than 2 L of intravenous fluid before arrival, and those transferred from another institution were excluded. Patients were retrospectively excluded if informed consent was unobtainable or declined. Admission blood samples were collected via initial placement of a 16-gauge or larger peripheral intravenous line into 3.2% (0.109 M) sodium citrate and processed within 3 hours of being drawn; sample collection methodology is described in detail elsewhere.[2] Activity levels of factors II, V, VII, VIII, IX, and X; antithrombin III and protein C; as well as antigen D-dimer levels were assayed using a Stago Compact Functional Coagulation Analyzer (Diagnostica Stago, Parsippany, NJ). Activated protein C was assayed using an established enzyme-linked immunosorbent assay method reported elsewhere.[3] Demographics, laboratory and resuscitation data, and outcomes were collected in parallel. Injury was assessed by Injury Severity Score (ISS).[4] Acute lung injury was based on the American-European consensus definition.[5] Multiorgan failure was defined as a multiple organ dysfunction score of greater than 3 using established Denver criteria.[6] The study was approved by the University of California Committee on Human Research.

Nonlinear PCA was performed using SPSS categories CAT-PCA (IBM, Chicago, IL); specific details are available in the Supplemental Digital Content Methods (see Document, Supplemental Digital Content 1, http://links.lww.com/TA/A243). Possible nonlinear correlations among coagulation factors were accounted for in the input stage, resulting in an output of continuous, linear PC scores and PC loadings;[7] (see Figure, Supplemental Digital Content 2, http://links.lww.com/TA/A241). All measured factor levels were included as predictors: prothrombin; factors V, VII, VIII, IX, and X; D-dimer; activated protein C; protein C; and antithrombin III. PCs were considered significant for eigenvalues greater than or equal to 1.0 (see Figure, Supplemental Digital Content 3, http://links.lww.com/TA/A242); factor loadings were considered significant for coefficients greater than or equal to 0.3. Continuous PC scores were calculated for each patient along each PC axis, and differences between the highest and lowest quartile of patients for each significant PC were examined using standard univariate statistics. The predictive capacities of independent PC scores were evaluated using unadjusted logistic regression for the binary outcomes of mortality, multiorgan failure, acute lung injury, and ventilator-associated pneumonia (VAP) and for standard definitions of coagulopathy determined by admission international normalized ratio (INR) and partial thromboplastin time (PTT).

Data are presented as mean ± SD, median (interquartile range [IQR]), or percentage; univariate comparisons were made using Student's *t* test for normally distributed data, Wilcoxon rank-sum testing for skewed data, and Fisher's exact test for proportions. Missing predictor data were imputed using multiple imputation; results were similar to those obtained using only complete data as well as using a data set completed with population means (data not shown). An α of 0.05 was considered significant. All analysis was performed by the authors using SPSS categories (IBM) and Stata version 12 (Stata Corp; College Station, TX).

RESULTS

Our 163-patient study population had a mean age of 41.3 ± 19.3 years and a mean ISS of 23.2 ± 5.4; there was 31.0% penetrating and 61.2% brain injuries. Of 163 patients, 22 (19.0%) were coagulopathic on arrival as defined by INR of 1.3 or greater, and 56 (34.4%) were coagulopathic as defined by PTT of 30 or greater. Nonlinear PCA identified seven independent PCs, together accounting for 92.0% of the variance present in the data. PCs 1, 2, and 3 were considered significant (eigenvalues > 1.0); these together accounted for 67.5% of total variance. Eigenvalues, percentage of variance explained, and the factor loading matrix for all individual clotting factors for the three significant PCs are shown in Table 1. Factor loadings were considered significant for loading coefficients greater than or equal to 0.3.

PC1 accounted for 43.9% of overall variance, including significant negative factor loading on (analogous to inverse Pearson correlation with) prothrombin; factors V, VII, VIII, IX, and X; protein C; and antithrombin III (Table 1). To identify patient-level characteristics associated with high PC1 scores, patients with the highest quartile of PC1 score were compared with those in the lowest quartile (Table 2). Patients in the highest quartile of PC1 score had significantly more common penetrating injury (34.2% vs.12.5%, $p = 0.035$) and more severe injury (mean ISS, 32.2 vs. 23.8; $p = 0.011$) compared with those in the lowest quartile. High-PC1 patients also had significantly elevated admission INR (median, 1.2 vs. 1.0; $p < 0.001$) and PTT (median, 32.6 seconds vs. 27.0 seconds, $p < 0.001$), as well as lower admission platelet count (mean, $245 \times 10^3/\mu L$ vs. $311 \times 10^3/\mu L$; $p < 0.001$). High-PC1 patients also had significantly higher transfusion requirements for red blood cells (median, 5 U vs. 0 U, $p < 0.001$), plasma (median, 2 U vs. 0 U; $p < 0.001$), and platelets (median [IQR], 0 U [0–2 U] vs. 0 U [0 U]; $p = 0.003$) and significantly higher mechanical ventilation requirements (median, 6.5 ventilator-free days vs. 17.5 ventilator-free days; $p = 0.016$). Expressed in odds ratios, each unit increase in PC1 score was associated with a 4.68-fold higher incidence of INR-based coagulopathy ($p < 0.001$), a

TABLE 1. Composition of PCs

	PC1	PC2	PC3
Eigenvalue	4.39	1.35	1.01
Percentage of variance	43.91	13.45	10.12
Prothrombin	−0.86	−0.04	0.11
Factor V	−0.78	0.01	−0.11
Factor VII	−0.62	0.01	0.47
Factor VIII	−0.35	0.34	−0.73
Factor IX	−0.69	0.07	0.03
Factor X	−0.88	−0.01	0.20
D-dimer	0.25	0.80	0.00
aPC	0.20	0.74	0.39
Protein C	−0.80	0.11	−0.05
AT III	−0.74	0.16	−0.17

Heatmap: −1.00 0.00 1.00

PC eigenvalues, percentage of variance accounted for, and factor loading magnitudes. Positive loading (analogous to a positive Pearson correlation) is indicated in shades of red, with negative correlation (analogous to inverse Pearson correlation) indicated in shades of blue. The magnitude of the loading for each factors is shown in text, and considered significant for values of greater than 0.30.

TABLE 2. Patient Characteristics by First PC (PC1)

	Low PC1 (n = 40)	High PC1 (n = 41)	*p*
PC score	−1.16 ± 0.52	1.29 ± 0.78	—
Age	39.8 ± 14.2	42.1 ± 19.9	0.564
BMI	28.5 ± 5.5	27.5 ± 5.9	0.466
Penetrating injury	12.5%	34.2%	0.035
ISS	23.8 ± 12.8	32.2 ± 16.2	0.011
GCS score	7 (4.5–12)	7 (3–14)	0.782
Temperature	35.4 ± 0.9	35.3 ± 1.1	0.690
Prehospital IVF	200 (0–600)	0 (0–150)	0.118
pH	7.29 ± 0.11	7.26 ± 0.12	0.143
Base deficit	−5.8 ± 4.5	−8.1 ± 6.7	0.089
INR	1.0 (1.0–1.1)	1.2 (1.1–1.4)	<0.001
PTT	27.0 (24.9–28.1)	32.6 (27.5–38.5)	<0.001
Platelet count	311 ± 85	245 ± 84	<0.001
RBC per 24 h	0 (0–0)	5 (1–14)	<0.001
FFP per 24 h	0 (0–0)	2 (0–9)	<0.001
Platelets per 24 h	0 (0–0)	0 (0–2)	0.003
Hospital days	14 (6.5–29)	14 (6–30)	0.769
ICU days	9 (3–16)	9 (3–21)	0.861
Ventilator-free days	17.5 (1.5–26)	6.5 (0–22.5)	0.016
VAP	42.5%	39.0%	0.823
Acute lung injury	38.2%	51.4%	0.341
Multiorgan failure	17.5%	17.1%	1.000
Mortality	17.5%	31.7%	0.198

*$p < 0.05$ by Student's *t*, Mann-Whitney, or Fisher's exact testing.

Data from the lowest ("low PC1") and highest ("high PC1") patient quartiles are presented as mean ± SD or median (IQR).

BMI, body mass index; FFP, fresh frozen plasma units; IVF, intravenously administered fluid; RBC, red blood cell units.

TABLE 3. Patient Characteristics by Second PC (PC2)

	Low PC2 (n = 48)	High PC2 (n = 40)	*p*
PC score	−0.79 ± 0.21	1.38 ± 1.09	—
Age	43.7 ± 16.1	44.5 ± 20.7	0.831
BMI	26.1 ± 5.0	27.8 ± 4.7	0.114
Penetrating injury	14.6%	17.5%	0.775
ISS	23.0 ± 15.3	37.3 ± 16.6	<0.001
GCS score	7 (3–9)	8 (4–14)	0.122
Temperature	35.6 ± 0.8	35.5 ± 0.9	0.526
Prehospital IVF	500 (125–775)	0 (0–0)	0.001
pH	7.32 ± 0.10	7.27 ± 0.12	0.032
Base deficit	−5.5 ± 5.3	−8.4 ± 5.6	0.029
INR	1.1 (1.0–1.2)	1.1 (1.0–1.2)	0.440
PTT	29.4 (26.2–32.4)	28.8 (25.6–37.5)	0.756
Platelet count	238 ± 71	288 ± 107	0.015
RBC per 24 h	0 (0–3)	6 (1–14)	<0.001
FFP per 24 h	0 (0–3)	4 (0–12)	<0.001
Platelets per 24 h	0 (0–0)	0 (0–2)	0.002
Hospital days	8.5 (3–23)	22.5 (9.5–40.5)	0.004
ICU days	4.5 (2–12.5)	12 (6–22)	0.003
Ventilator-free days	22 (0–26)	2 (0–19)	0.002
VAP	29.8%	55.0%	0.028
Acute lung injury	26.7%	73.5%	<0.001
Multiorgan failure	4.2%	32.5%	<0.001
Mortality	16.7%	37.5%	0.031

*$p < 0.05$ by Student's *t*, Mann-Whitney, or Fisher's exact testing.

Data from the lowest ("low PC2") and highest ("high PC2") patient quartiles are presented as mean ± SD or median (IQR).

BMI, body mass index; FFP, fresh frozen plasma units; IVF, intravenously administered fluid; RBC, red blood cell units.

3.35-fold higher incidence of PTT-based coagulopathy ($p < 0.001$), and 1.48-fold higher mortality ($p = 0.032$).

PC2 accounted for 13.4% of overall variance, including significant positive factor loading on (analogous to positive Pearson correlation with) factor VIII, D-dimer, and activated protein C levels (Table 1). Similarly to those previously mentioned, patients in the highest PC2 quartile were again compared with those in the lowest (Table 3). High PC2 patients had more severe injury (mean ISS, 37.3 vs. 23.0; $p < 0.001$), acidosis (mean pH, 7.27 vs. 7.23; $p = 0.032$), and base deficit (mean, −8.4 vs. −5.5, $p = 0.029$). High-PC2 patients received less prehospital intravenous fluid (median, 0 mL vs. 500 mL), consistent with expedited transport. Despite these differences in injury and shock severity, admission INR and PTT did not differ significantly by PC2 quartile ($p = 0.440$ and $p = 0.756$, respectively), although admission platelet count was higher in the high PC2 population (mean 288 × 10^3/μL vs. 238, $p = 0.015$). High-PC2 patients had significantly higher transfusion requirements for red blood cells (median, 6 U vs. 0 U; $p < 0.001$), plasma (median, 4 U vs. 0 U; $p < 0.001$), and platelets (median [IQR], 0 U [0–2] vs. 0 U [0 U]; $p = 0.002$). In outcomes, high-PC2 patients had prolonged intensive care unit (ICU) (median, 12 days vs. 4.5 days; $p = 0.003$) and total hospital stays (median 22.5 days vs. 8.5 days; $p = 0.004$), and significantly higher mechanical ventilation requirements (median, 2 ventilator-free days vs. 22 ventilator-free days; $p = 0.002$). The incidences of VAP

(55.0% vs. 29.8%; $p = 0.028$), acute lung injury (73.5% vs. 26.7%; $p < 0.001$), multiorgan failure (32.5% vs. 4.2%; $p < 0.001$), and mortality (37.5% vs. 16.7%; $p = 0.031$) were all markedly higher in the high-PC2 population. Expressed in odds ratios, PC2 score was not associated with significant increases in the incidence of either INR-based or PTT-based coagulopathy ($p = 0.220$ and $p = 0.340$, respectively); however, each unit increase in PC2 score was associated with a 1.59-fold higher incidence of VAP, a 2.24-fold higher incidence of acute lung injury, a 1.83-fold higher incidence of multiorgan failure ($p = 0.002$), and 1.62-fold higher mortality ($p = 0.006$).

PC3 accounted for 10.1% of overall variance, including significant positive factor loading with factor VII and activated protein C and significant negative loading with factor VIII (Table 1). Patients in the highest PC3 quartile had significantly better Glasgow Coma Scale (GCS) score (median 9 vs. 6; $p = 0.034$) and lower admission PTT (median 26.6 seconds vs. 28.8 seconds; $p < 0.001$); high-PC3 patients also had a lower incidence of acute lung injury (40.0% vs. 67.6%; $p = 0.033$; Table 4). Unit increases in PC3 were associated with a 1.44-fold increase in the incidence of PTT-based coagulopathy ($p = 0.041$); however, PC3 scores were not significantly associated with other measured outcomes (all other $p > 0.05$).

TABLE 4. Patient Characteristics by Third PC (PC3)

	Low PC3 (n = 41)	High PC3 (n = 40)	p
PC score	−1.19 ± 0.63	1.22 ± 0.74	—
Age	43.4 ± 18.7	41.0 ± 18.7	0.567
BMI	27.3 ± 5.8	28.2 ± 5.8	0.550
Penetrating injury	17.1%	32.5%	0.128
ISS	27.9 ± 15.2	26.4 ± 11.9	0.644
GCS score	6 (3–9)	9 (4–15)	0.034
Temperature	35.7 ± 0.7	35.5 ± 1.0	0.395
Prehospital IVF	0 (0–450)	0 (0–300)	0.953
pH	7.31 ± 0.10	7.28 ± 0.17	0.429
Base deficit	−6.1 ± 4.9	−7.0 ± 7.1	0.560
INR	1.1 (1.0–1.2)	1.1 (1.0–1.2)	0.654
PTT	28.8 (27.0–34.3)	26.6 (23.8–29.8)	<0.001
Platelet count	276 ± 99	292 ± 81	0.435
RBC per 24 h	0 (0–6.5)	1.5 (0–5.5)	0.829
FFP per 24 h	0 (0–4.5)	0 (0–3.5)	0.730
Platelets per 24 h	0 (0–1)	0 (0–0)	0.325
Hospital days	11 (5–25)	14.5 (6.5–28)	0.192
ICU days	8 (3–18)	6 (3–15.5)	0.940
Ventilator-free days	8 (0–24)	20.5 (1–26)	0.084
VAP	48.8%	32.5%	0.176
Acute lung injury	67.6%	40.0%	0.033
Multiorgan failure	19.5%	10.0%	0.349
Mortality	34.1%	17.5%	0.128

*$p < 0.05$ by Student's *t*, Mann-Whitney, or Fisher's exact testing.

Data from the lowest ("low PC3") and highest ("high PC3") patient quartiles are presented as mean ± SD or median (IQR).

BMI, body mass index; FFP, fresh frozen plasma units; IVF, intravenously administered fluid; RBC, red blood cell units.

To graphically assess interrelationships between PCs and their relationship to outcomes, scatter plots were generated representing each patient in multivariate space by PC1 and PC2 score (Fig. 1). Visually, the majority of patients with a prolonged INR and PTT are seen to have an elevated PC1 score; these are equally balanced between high and low PC2 scores (Fig. 1*A* and *B*). In comparison, most patients with multiorgan failure and mortality are seen to have an elevated PC2 score; these are equally balanced between high and low PC1 scores (Fig. 1*C* and *D*). Odds ratio data for all binary outcomes assessed are summarized in Table 5.

DISCUSSION

Here, we describe the use of PCA to interrogate the data structure of clotting factor levels in a panel of 163 critically injured trauma patients. Three significant uncorrelated multivariate components were identified, together explaining 67.5% of the total variance in observed clotting factor measurements. PC1 accounted for 43.2% of overall variance and consisted of significant negative loading coefficients for all procoagulant clotting factors as well as the anticoagulants protein C and antithrombin III. Intuitively, PC1 identifies global clotting factor depletion, and its patient-level values are associated with increased incidences of admission coagulopathy and mortality. PC2 accounted for 13.4% of variance, consisting principally of significant positive factor loading on D-dimer and activated protein C levels. Intuitively, PC2 identifies a fibrinolytic component to the clotting factor milieu; interestingly, this component is not significantly associated with admission coagulopathy by INR-based or PTT-based definitions but is significantly associated with VAP, acute lung injury, multiorgan failure, and mortality. PC3 accounted for 10.1% of variance, consisting principally of significant negative loading on factor VIII, with smaller contributions from factor VII and activated protein C. Intuitively, PC3 may account for an element of coagulopathy associated with consumption-driven depletion of factor VIII; PC3 was associated only with admission PTT-based coagulopathy.

Depletion Coagulopathy

The global procoagulant depletion phenotype described by PC1 matches the clinical intuition that patients with overwhelming tissue injury and acute hemorrhage frequently present with coagulopathy resulting from clotting factor consumption. The early recognition of this "vicious cycle" of self-perpetuating consumptive coagulopathy is a mainstay of trauma resuscitation,[8] with recent studies extending these clinical observations to confirm the association of specific clotting factor deficits to poor outcomes after injury.[9] The global procoagulant depletion phenotype reflected by PC1 and its association with both coagulopathy and mortality reflect the clinical intuition that clotting factor depletion must be anticipated and treated early to minimize its deleterious effects; precisely this insight has driven recent trends in plasma-based hemostatic resuscitation therapy for critically injured patients.[10,11]

Fibrinolytic Coagulopathy

The fibrinolytic phenotype described by PC2 is significantly associated with multiple functional outcomes but is interestingly not associated with standard admission laboratory

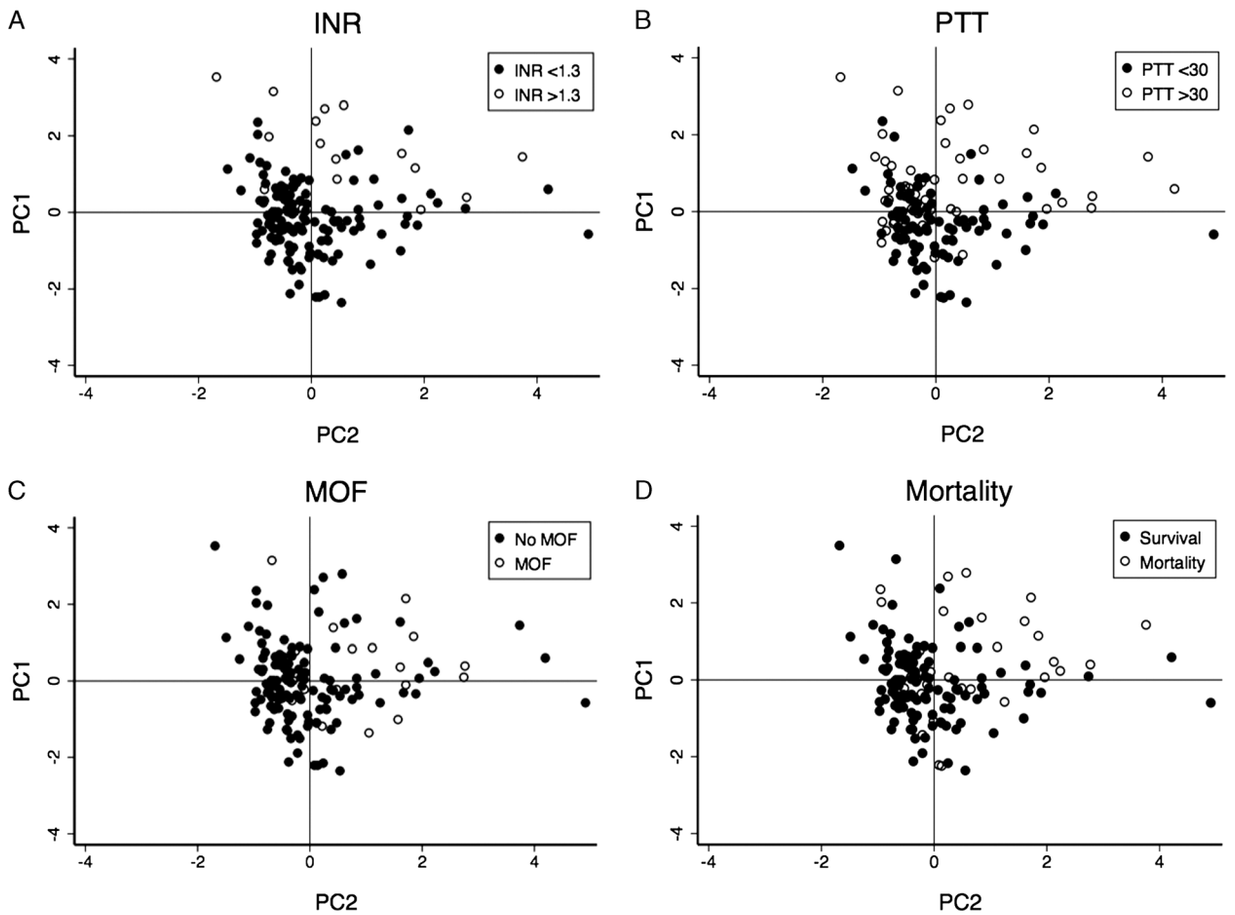

Figure 1. Plots of patient scores within multivariate PC space, by binary outcomes. Representative two-dimensional (PC1-PC2 space) plots provided. *A*, INR of 1.3 or greater; *B*, PTT of 30 or greater; *C*, multiorgan failure; and *D*, mortality. Markers indicate outcome status (*filled circles*, absence; *hollow circles,* presence).

values such as INR or PTT. This subtlety supports the hypothesis that injury-induced activation of endogenous anticoagulants and other systemic effectors, as opposed to consumptive depletion of procoagulant factors alone, plays a critical role in the pathophysiology of acute traumatic coagulopathy. Recent work suggests several candidate biochemical pathways that may mediate dysfunctional coagulation after trauma independently of clotting factor depletion, including catecholamine-mediated degradation of the endothelial glycocalyx[12,13] and activation of the protein C system.[2,3] While activated protein C mediates receptor-independent proteolysis of activated factors Va and VIIIa as well as derepression of fibrinolysis,[2] it may also play an additional receptor-dependent role in potentiating the systemic inflammatory response.[3] Kerschen et al.[14] recently showed that a recombinant form of activated protein C with targeted mutation leading to less than 10% anticoagulant activity is equivalent to

TABLE 5. PC Scores as Predictors of Outcomes

		PC1	PC2	PC3
Odds ratio	Mortality	1.48 (1.03–2.12) AUC, 0.616	1.62 (1.15–2.29) AUC, 0.669	—
	Multiorgan failure	—	1.83 (1.25–2.68) AUC, 0.750	—
	Acute lung injury	—	2.24 95% CI: AUC:	—
	VAP	—	1.59 (1.13–2.24) AUC, 0.628	—
	INR ≥1.3	4.68 (2.37–7.66) AUC, 0.844	—	—
	PTT ≥ 30	3.35 (2.06–5.45) AUC, 0.769	—	1.44 (1.02–2.04) AUC, 0.593

Significant odds ratios derived from continuous PC scores in univariate, unadjusted logistic regression models. 95% confidence intervals and area under the receiver operator characteristic curve ("AUC") are given for all significant predictors. Nonsignificant (Wald test $p > 0.05$) odds ratios marked as em dashes.

native protein in reducing mortality in sepsis models in mice. A prospective study of clotting factor levels in 71 injured patients identified a significant negative correlation between severity of systemic hypoperfusion after injury and the activity of several procoagulant factors but found that decreased factor V activity may occur via a consumption-independent mechanism such as protein C–mediated cleavage.[15]

This identification of PC2 as a fibrinolytic component is also consistent with a growing recognition of the importance of hyperfibrinolysis to acute traumatic coagulopathy. Hyperfibrinolysis is estimate to occur from 3% to 20% of significantly injured patients, with mortality spanning 38.5% to 100%.[16] At the biochemical level, the presence of hyperfibrinolysis is associated with significantly elevated levels of D-dimer and activated protein C.[17] Recent intriguing data suggest that the use of plasminogen-targeted antifibrinolytics such as tranexamic acid may provide the missing pharmacologic treatment for the hyperfibrinolytic component of acute traumatic coagulopathy.[18,19] Taken together, these results suggest that aggressive clotting factor repletion by empiric plasma-based therapies may inadequately treat the fibrinolytic component of acute traumatic coagulopathy and that targeted therapies are a promising area of active investigation.

Study Limitations

As with other single-center prospective studies examining the relationship between admission clotting factor studies and outcomes, several limitations are important to interpretation of these data. Although our sample size is modest, highly cited work in PCA suggests that 5 to 10 samples for each predictor included is adequate for robust results,[20,21] which our 163-patient panel well exceeds for our 10-predictor model. Importantly, this analysis does not provide for strict causal interpretation; neither PCs themselves nor the individual patient PC scores provided by PCA are directly clinically interpretable. Furthermore, the PCA model itself is not generalizable or portable but is only applicable to the data set from which it is derived; PC scores cannot be derived for novel patients outside of the data set presented here. For this exploratory analysis, we included all classical clotting factor measurements available; however, other relevant physiologic measures (such as pH or temperature) and clotting cascade elements (such as calcium and fibrinogen) were not uniformly available. Thus, the final PCA model may be sensitive to predictor selection. Cognates to common predictor selection strategies for regression analysis (such as forward/backward and information criteria-based selection) are not well-established for the construction of PCA models and will require detailed sensitivity analyses to validate. Overall, however, the purpose of the current study was not to construct a comprehensive predictive model but instead to interrogate the clotting cascade for clinically compelling patterns. We suggest that these patterns identify unique groups of patients that would otherwise go undetected based on standard clinical characteristics alone and that the further analysis of these groups may identify novel molecular markers and potential therapeutic targets.

Summary

Taken together, these results suggest that PCA accurately identifies patterns embedded in the complex milieu of the coagulation cascade in injured patients. The independent consumptive and fibrinolytic components identified here show robust correlation with patient-level outcomes and match prevailing clinical intuition regarding drivers of acute traumatic coagulopathy. In particular, the fibrinolytic phenotype is associated with markedly poor outcomes but not with abnormalities in INR or PTT, highlighting the inadequacy of these measures in describing the complexity of traumatic coagulopathy and the need for validated markers of abnormal fibrinolysis after trauma. Whereas standard regression techniques are problematic for investigating the role of clotting factors in isolation, PCA is well suited to address just such highly collinear systems of predictors as patterns. The pattern finding capability demonstrated here holds promise for elucidating critical mechanisms underlying the pathophysiology of acute traumatic coagulopathy. Further development of data-driven analytical methods such as PCA may ultimately provide critical insights to drive advances in clinical care.

AUTHORSHIP

M.E.K., A.R.F., and M.J.C. prepared the article, performed all data analyses, and take full responsibility for the data as presented.

DISCLOSURE

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DISCUSSION

Dr. Sandro Rizoli (Toronto, Canada): This study, as I understand it, has two components. It explores the mechanisms underlying acute coagulopathy of trauma and, second, it explores the use of a mathematical statistical tool to identify patterns in a complex system such as coagulation.

The mathematical tool is called Principal Component Analysis and even after the brilliant presentation I still am not sure that I could explain to anyone what it really is.

Very little is known about coagulation and trauma. We know it is complex, multifactorial, it changes over time and we know it changes in response to surgical interventions.

We also know it very difficult to determine the importance of each clotting element in this complex process. So a mathematical tool that can make it easier to understand is welcome and worthwhile testing.

So over a five-year period, Dr. Cohen and his group drew blood from 163 severely-injured patients on arrival to hospital and performed many coagulation assays. Of the 163 patients included, only 22 were coagulopathic, defined by an INR of 1.3, which could be a soft definition considering that 1,5 is more commonly used.

So my first question is considering that the goal of this study was to understand coagulopathy as a disease, shouldn't the statistical analysis be done only on the 22 patients that were in fact coagulopathic?

Otherwise this study was done on non-coagulopathic patients and might simply demonstrate the variations in clotting factors after trauma, which do not necessarily lead to coagulopathy.

The second question is, many of the differences found between patients within the same PC were statistically significant but not clinically relevant. A platelet count of 250 versus 300 or a PTT of 32 versus 27 doesn't seem clinically relevant to me. So how can this statistically but not clinically relevant difference be taken to explain coagulopathy?

And, finally, concerning your interpretation of the role of hyperfibrinolysis in trauma, could we extrapolate your conclusions and say that every trauma has a component of hyperfibrinolysis and thus should receive Tranexamic acid?

So, once again, I want to congratulate the authors for their brilliant work and I thank the ASAT for the privilege.

Dr. Ronald V. Maier (Seattle, Washington): Very nice study, attempting to better elucidate the complex coagulopathic patterns in the injured patient.

One of the issues is to follow the process serially over time in these patients with different treatments. But using the early data you have demonstrating differential treatments prehospital, can you assess how much crystalloid is required to increase bleeding significantly?

You should have had enough variability in these patients as far as prehospital volume of resuscitation that you can interrogate the impact of a large volume of crystalloid on coagulopathy or type of coagulopathy at the time of hospital presentation. Thank you.

Dr. Susan Rowell (Portland, Oregon): Given the worse outcomes associated with PC2 that are not detectible by INR and PTT, I am curious as to whether you have done any platelet function analysis or have correlated this with TEG data which could be more easily performed at the bedside? Thank you.

Dr. Scott G. Thomas (South Bend, Indiana): I'm interested in your thoughts on recent discussions with regard to TEG replacing INR. And, at your institution, are you looking at that as a primary modality of looking at coagulopathy and trauma? As we know, Dr. Holcomb just recently published a paper saying that this may be the new trend. And is this your plan in the future for looking at coagulopathic patients in trauma? Thank you.

Dr. Andre Cap (Houston, Texas): I'm curious about why we didn't see fibrinogen results in your table of coagulation factors. I was wondering if you had measured fibrinogen and how those values align with the various patterns you're observing.

I agree with the previous comments about TEG analysis and how it correlates with other measures of coagulation function. Thanks very much.

Dr. Matthew Kutcher (San Francisco, California): Thank you for your insightful questions. In terms of Dr. Rizoli's question—whether these analyses should be performed on coagulopathic versus normal patients—interestingly, it turns out that the PC scores in coagulopathic versus normal patients were remarkably different.

In receiver-operator characteristic analyses, we found that a PC1 score is a very strong predictor of having an elevated admission INR with an area under the curve of about 800—so these scores really are clinically relevant.

I think the focus of this paper, however, was really as an exploratory analysis to understand whether these principal components would independently recapitulate these clinical patterns.

And so I think the question is: how do you identify patients who are at risk of bleeding to death, versus those who don't hemorrhage but who are at risk of having an uncontrolled inflammatory response to trauma.

That's where we're really looking to go with this work, and that's our interest in a closer exploration of the real meaning of PC2. This is biological work that remains to be done.

In terms of the question of clinical relevance, we simply use mean values to try to isolate and understand a subset of patients with high PC scores to see what the differences in these populations are. The difference in the mean platelet counts, for example, is not particularly large—but we already know what a low or high platelet count by itself means.

So: are these patterns differentiating patients who were or were not coagulopathic early in their clinical course, or, better yet, are not going to bleed to death but who will have later end-organ damage from their early inflammatory response to trauma? I think that most of our standard laboratory measurements are poor measures of that. That's how our interest in this work began in the first place.

In terms of Dr. Rizoli's comments on hyperfibrinolysis, we're very interested in the use of agents like aminocaproic and tranexamic acid for the treatment of hyperfibrinolysis. And I think the TEG is a great way of looking that.

TEG, in a way, is a little bit like Principal Components Analysis in that it distills the clotting cascade into a visual set of patterns from which more useful numbers like an R or an MA can be extracted. So a hyperfibrinolytic TEG has a collection of different numbers that you can use to describe it, but it has a particular shape. And in a sense that shape is that pattern that we look for.

So if you had a patient who came in with a very high PC1 score and a low to normal PC2 score—basically someone with depletion coagulopathy—you could treat that patient very well with fresh frozen plasma.

But if you had a similar patient with a high PC1 score but also a high PC2 score, plasma might fix their depletion coagulopathy but really wouldn't address the problem of their hyperfibrinolysis. This is why we've been interested in identifying guidelines for empiric and data-based anti-fibrinolytic therapies.

In terms of Dr. Maier's question about prehospital crystalloid, in San Francisco the average transport time is less than fifteen minutes and we're lucky if they have an IV by the time they get in the door, so our prehospital crystalloid volumes tend to be in the 0 to 500 range.

And so we don't really have the variance to determine many differences based on prehospital data but, as you point out, I think looking longitudinally during the course of in-hospital resuscitation we will have the ability to find out what perturbs this system over time, based on responses to crystalloid, red cells, FFP, anti-fibrinolytics and other resuscitative measures.

And we look forward to doing that work—it's just statistically more complex and is going to take more data and time.

I think platelet function and TEG would be excellent datasets to use the PCA technique on. We actually do have longitudinal platelet function as well as TEG data on some of these patients in addition to their clotting factor profiles. We look forward to doing the same sort of exploratory analyses in those arenas as well.

In response to Dr. Thomas' question about looking at TEG-based resuscitation, I think that this is exactly where this sense that we need a better understanding of patterns comes from.

And, as I alluded to, I think hyperfibrinolysis is one of the critically important patterns to identify early. The difference between PC1 and PC2 is exactly like one might see in a TEG, where you can identify hyperfibrinolysis, or you can identify a separate platelet-based deficit, or a clotting factor-based deficit, or any number of these things occurring at the same time. And all of these are treated differently. Both the PCA data here and TEG used as a resuscitation guide clinically make sense, since it parallels the way we think. Patterns, not flow-charts.

And finally, in terms of fibrinogen as well as some other clotting cascade elements and adjuncts, we don't have as complete a set of measurements for these as we do from our standard factor level profiles, but we are in the process of expanding our standard battery of measurements as well as going back to re-measure some of these in our stored samples as well.

Thank you very much for the privilege of presenting our work today.

Cause and timing of death in massively transfused trauma patients

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BACKGROUND: The purpose of this study was to characterize the cause of death in severely injured trauma patients to define potential responses to resuscitation.

METHODS: Prospective analysis of 190 critically injured patients who underwent massive transfusion protocol (MTP) activation or received massive transfusion (>10 U of packed red blood cells [RBC] per 24 hours). Cause of death was adjudicated into one of four categories as follows: (1) exsanguination, (2) early physiologic collapse, (3) late physiologic collapse, and (4) nonsurvivable injury.

RESULTS: A total 190 patients underwent massive transfusion or MTP with 76 deaths (40% mortality), of whom 72 deaths were adjudicated to one of four categories: 33.3% died of exsanguination, 16.6% died of early physiologic collapse, 11.1% died of late physiologic collapse, while 38.8% died of nonsurvivable injuries. Patients who died of exsanguination were younger and had the highest RBC/fresh frozen plasma ratio (2.97 [2.24]), although the early physiologic collapse group survived long enough to use the most blood products ($p < 0.001$). The late physiologic collapse group had significantly fewer penetrating injuries, was older, and had significantly more crystalloid use but received a lower RBC/fresh frozen plasma ratio (1.50 [0.42]). Those who were determined to have a nonsurvivable injury had a lower presenting Glasgow Coma Scale (GCS) score, fewer penetrating injuries, and higher initial blood pressure reflecting a preponderance of nonsurvivable traumatic brain injury. The average survival time for patients with potentially survivable injuries was 2.4 hours versus 18.4 hours for nonsurvivable injuries ($p < 0.001$).

CONCLUSION: Severely injured patients requiring MTP have a high mortality rate. However, no studies to date have addressed the cause of death after MTP. Characterization of cause of death will allow targeting of surgical and resuscitative conduct to allow extension of the physiologic reserve time, therefore rendering previously nonsurvivable injury potentially survivable. (*J Trauma Acute Care Surg*. 2013;75: S255–S262. Copyright © 2013 by Lippincott Williams & Wilkins)

LEVEL OF EVIDENCE: Prognostic study, level III.

KEY WORDS: Massive transfusion; cause of death; epidemiology of trauma.

Considerable interest and research exist regarding optimal resuscitation of the severely injured trauma patients. Multiple recent studies have shown that early use of a balanced resuscitation via a massive transfusion protocol (MTP) decreases mortality in these patients.[1–6] Despite data that overwhelmingly support these plasma-based advances in resuscitation, there is considerable disparity in effectiveness from center to center and from study to study. Even in the most optimistic studies, the severely injured continue to have considerable mortality.[7,8] This high mortality rate lends to a large population of potentially preventable deaths, and the lack of data on the cause of early death after injury makes it difficult to determine the true effectiveness of balanced resuscitation protocols on patients with severe survivable injuries.

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Indeed, the majority of epidemiologic studies have examined the mechanism of injury rather than the cause of or physiology preceding death. It has been well described that trauma patients die at three distinct time points and that truncal hemorrhage is a leading cause of potentially preventable death.[9,10] Despite these characterizations, identification of which patients die of anatomically nonsurvivable (and hence not able to resuscitate) injury versus which patients exsanguinate from anatomically survivable injury versus which patients survive initially only to perish from late physiologic collapse has not been performed. Hence, we hypothesized that the characterization of the precise cause of death would not only provide significant insight into epidemiologic reasons for mortality but also elucidate reasons for the effectiveness of resuscitation protocols. Ultimately, this would help to more efficiently target resources and resuscitation of our injured war fighters and civilians that could allow extension of the physiologic reserve time, thereby rendering previously nonsurvivable injury potentially survivable.

PATIENTS AND METHODS

Data were prospectively collected from 190 critically injured trauma patients who met the highest level of trauma activation criteria on arrival to the emergency department of San Francisco General Hospital from 2005 to 2011. Inclusion

criteria consisted of all patients in whom the MTP was activated (MTP is defined later). Patients who were younger than 18 years, had greater than 5% body surface area burns, received more than 2 L of intravenously administered fluid before arrival, or were transferred from another institution were excluded. Admission blood samples were collected immediately upon arrival to the emergency department and processed as previously described elsewhere.[11] Standard laboratory, resuscitation, and outcome data were prospectively collected in parallel. Consent was obtained as approved by the University of California Institutional Committee on Human Research.

Activation of the MTP was based on clinician judgment, immediately on arrival, or if there were ongoing transfusion requirements. Massive transfusion(MT) was defined as transfusion of 10 or more units of red blood cells within the first 24 hours of admission, in patients surviving to 24 hours; to account for survivor bias and to include patients who received high-volume transfusion but did not survive to 24 hours, scaled transfusion of 5 or more units in patients dying by 12 hours or 2.5 or more units in patients dying by 6 hours were also defined as MT. MTP activation releases 4 U of fresh frozen plasma (FFP) and 6 U of packed red blood cell pRBC from the blood bank with apheresis platelets and cryoprecipitate being released on orders. At the time of the study, neither tranexamic acid nor factor VII was part of the MTP.

The cause of death was codified and adjudicated by two independent trauma surgeons. The cause of death categories were defined as follows: (1) exsanguination—the patient died because of uncontrolled surgical hemorrhage; (2) early collapse—the patient died within 24 hours of arrival to the hospital from physiologic collapse, after adequate control of surgical bleeding; (3) late cardiopulmonary collapse—the patient received surgical and/or intensive care therapy, had adequate hemorrhage control, and died of cardiopulmonary failure later than 24 hours after admission; (4) nonsurvivable injury—for the purposes of this analysis, survivability was based on chart documentation of the attending trauma surgeon or neurosurgeon, in whose judgment the patient was deemed to have a nonsurvivable injury by at least one of the following: physical examination, diagnostic imaging (i.e., computed tomographic scan), cerebral blood flow imaging, or operative findings. We then compared nonsurvivors in the three broad categories of exsanguination, early collapse, and late

TABLE 1. Demographics and Resuscitation of Those Who Survived Compared With Those Who Had a Potentially Survivable Injury but Still Died and Those With Anatomically Nonsurvivable Injuries

	Survivors (n = 114)	Potential Survivors (n = 44)	Anatomically Nonsurvivable (n = 28)	*p*
Age, y	35.8 (16.4)	38.3 (17.8)	45.7 (23.0)	0.033
Sex, male, %	74.6%	84.1%	64.3%	0.167
Penetrating injury, %	53.5	61.4	28.6	0.021
Head Abbreviated Injury Scale (AIS) score	2.2 (2.0)	2.4 (2.0)	2.9 (2.3)	0.511
ISS	26.0 (14.9)	31.5 (16.5)	35.9 (19.1)	0.009
GCS score	15 (11–15)	6 (3–12)	3 (3–5.5)	<0.001
Temperature, °C	35.8 (0.8)	35.1 (1.6)	35.3 (1.2)	0.016
Heart rate, beats/min	106.7 (28.5)	101.8 (45.9)	99.4 (36.6)	0.508
SBP, mm Hg	104.0 (32.6)	88.2 (43.8)	124.2 (52.3)	<0.001
pH	7.25 (0.15)	7.04 (0.19)	7.23 (0.13)	<0.001
Base deficit	−8.6 (5.3)	−15.9 (7.2)	−8.8 (6.0)	<0.001
Hemoglobin, g/dL	12.0 (2.3)	10.8 (2.6)	11.3 (2.0)	0.018
International normalized ratio (INR)	1.3 (1.1–1.5)	1.7 (1.4–2.3)	1.3 (1.2–1.6)	<0.001
Prothrombin time (PT)	15.4 (14.3–17.5)	19.7 (17.3–24.5)	15.9 (14.9–19.7)	<0.001
Partial thromboplastin time (PTT)	28.4 (24.9–32.9)	40.0 (33.3–54.8)	38.7 (31.5–55.0)	<0.001
Platelets × 10^3	276 (93)	180 (90)	243 (111)	<0.001
White blood cell × 10^3/μL	11.0 (8.0–14.2)	8.3 (5.3–11.6)	9.7 (6.4–13.6)	0.006
Creatinine, mg/dL	1.0 (0.8–1.3)	1.3 (1.1–1.4)	1.0 (0.9–1.3)	0.002
Prehospital Intravenously administered fluid (IVF), mL	250 (50–500)	100 (25–750)	150 (25–1,400)	0.892
24-h crystalloid, mL	6.3 (4.4–10.1)	4.0 (2.0–9.0)	8.3 (4.1–11.4)	0.017
24-h colloid, mL	0 (0–0)	0 (0–0)	0 (0–0)	0.362
24-h pRBC, U	10 (6–17)	23.5 (13–38.5)	13 (8.5–19)	<0.001
24-h FFP, U	8 (4–12)	13 (4.5–24)	10 (4.5–14)	0.035
24-h platelets, apheresis unit	1 (0–2)	1 (0–4)	1 (0–2)	0.572
%MT	56.1%	86.4%	67.9%	0.001
24-h RBC/FFP ratio	1.47 (0.86)	2.32 (1.76)	1.56 (0.73)	<0.001
Minutes to MTP	46.5 (26–103.5)	19.5 (15–25)	67 (35–93)	<0.001

Potential survivors had more penetrating injuries, were more hypotensive, were more acidotic, had a greater base deficit, were more coagulopathic compared with both the survivors and those with nonsurvivable injuries.

Normally distributed data are reported in mean (SD); skewed data are reported as median (interquartile range).

collapse to each other using analysis of variance, Kruskal-Wallis, and χ^2 tests as appropriate. The corresponding data for survivors are provided for reference.

All data are presented as mean (SD), median (interquartile range), or percentage; univariate comparisons were made using Student's *t* test for normally distributed data, Wilcoxon rank-sum test for skewed data, and Fisher's exact test for proportions. Kaplan-Meier time-to-event analysis and log-rank testing were used to assess differences in 24-hour and in-hospital mortality between groups. An α of 0.05 was considered significant. All data analysis was performed by the authors using Stata version 12 (StataCorp, College Station, TX).

RESULTS

One hundred ninety patients underwent MT or MTP, with 76 deaths (40% mortality), of which 72 deaths were adjudicated to the following: exsanguination, early cardiovascular collapse, late cardiovascular collapse, or nonsurvivable injury. Four deaths were excluded because of lack of available documentation that could clearly identify the cause of death. In the entire cohort of those patients who died, the mean (SD) age was 38.4 (18.5) years, with men constituting 75.3% of the population. Penetrating injuries were found in 50.5%, and the mean (SD) Injury Severity Score (ISS) was 28.3 (16.3).

Table 1 compares characteristics of survivors versus potentially survivable mortality versus anatomically nonsurvivable injury, of which 61% (n = 44) died of a potentially survivable injury, while 38.8% (n = 28) were deemed to have died of anatomically nonsurvivable injuries. Potential survivors had more penetrating injuries and a greater base deficit and were more hypotensive, acidotic, and coagulopathic compared with both the survivors and those with nonsurvivable injuries. The percentage of patients who received the full definition of an MT is listed as percent massive transfusion (%MT). The physiologic parameters in the anatomically nonsurvivable group were consistent with those seen in patients with traumatic brain injury, which was also identified by computed tomographic scan or physical examination in 96.4% of the patients (Table 5).

We next compared the survivors (n = 114) with those who had died despite having potentially survivable injury (n = 44) (Table 2) and found that these two groups were similar in age, mechanism, ISS, temperature, and heart rate. However, those who died presented with lower Glasgow Coma Scale (GCS) score and systolic blood pressure (SBP) and were significantly more acidotic and coagulopathic than those who survived. Moreover, the potentially survivable cohort used less crystalloid but significantly more blood products. The cause of death in patients with potentially survivable injuries was divided into (1) exsanguination, (2) early physiologic collapse, or (3) late physiologic collapse, and compared with the those with nonsurvivable injuries; survivor data are provided for reference (Table 3). Of patients in this cohort, 38.8% (n = 28) died of anatomically nonsurvivable injury and 33.3% died of exsanguination (n = 24). The percentage of deaths attributed to early physiologic collapse in this group was 16.6% (n = 12), while late physiologic collapse constituted 11.1% of deaths (n = 8).

Evaluation of the demographics of each group shows that the late physiologic collapse group was older and had significantly fewer penetrating injuries ($p < 0.02$). Those who were determined to have a nonsurvivable injury had a lower presenting GCS score, fewer penetrating injuries, and higher initial blood pressure (Table 3).

Analysis of the resuscitation of each group indicated that the late physiologic collapse group had significantly more 24-hour crystalloid use, but the RBC/FFP ratio was 1.50 (0.42). Patients who died of exsanguination were younger and had the highest RBC/FFP ratio, although the early physiologic collapse group survived long enough to use the most blood products ($p < 0.001$).

We then performed a Kaplan-Meier time-to-event analysis using log-rank testing to assess differences in 24-hour and in-hospital mortality between groups. The median survival time for patients who died of exsanguination was 1.6 hours versus 5.7 hours for those who died of early physiologic collapse. Those with nonsurvivable injuries died at a median of 18.4 hours after arrival, while those who died of late physiologic collapse had a median survival time to 338.6 hours ($p < 0.001$, Fig. 1).

TABLE 2. Demographics and Resuscitation of Those Who Survived Compared With Those Who Had Potentially Survivable Injuries but Died

	Survivors (n = 114)	Potential Survivors (n = 44)	*p*
Age, y	35.8 (16.4)	38.3 (17.8)	0.426
Sex, male, %	74.6	84.1	0.290
Penetrating injury, %	53.5	61.4	0.475
Head AIS score	2.2 (2.0)	2.4 (2.0)	0.739
ISS	26.0 (14.9)	31.5 (16.5)	0.115
GCS score	15 (11–15)	6 (3–12)	<0.001
Temperature, °C	35.8 (0.8)	35.1 (1.6)	0.082
Heart rate, beats/min	106.7 (28.5)	101.8 (45.9)	0.522
SBP, mm Hg	104.0 (32.6)	88.2 (43.8)	0.036
pH	7.25 (0.15)	7.04 (0.19)	<0.001
Base deficit	−8.6 (5.3)	−15.9 (7.2)	<0.001
Hemoglobin, g/dL	12.0 (2.3)	10.8 (2.6)	0.013
INR	1.3 (1.1–1.5)	1.7 (1.4–2.3)	<0.001
PT	15.4 (14.3–17.5)	19.7 (17.3–24.5)	<0.001
PTT	28.4 (24.9–32.9)	40.0 (33.3–54.8)	<0.001
Platelets × 10^3	276 (93)	180 (90)	<0.001
White blood cell × $10^3/\mu L$	11.0 (8.0–14.2)	8.3 (5.3–11.6)	0.002
Creatinine, mg/dL	1.0 (0.8–1.3)	1.3 (1.1–1.4)	<0.001
Prehospital IVF, mL	250 (50–500)	100 (25–750)	0.663
24-h crystalloid, mL	6.3 (4.4–10.1)	4.0 (2.0–9.0)	0.008
24-h colloid, mL	0 (0–0)	0 (0–0)	0.591
24-h pRBC, U	10 (6–17)	23.5 (13–38.5)	<0.001
24-h FFP, U	8 (4–12)	13 (4.5–24)	0.013
24-h platelets, apheresis unit	1 (0–2)	1 (0–4)	0.540
%MT	56.10%	86.4%	<0.001
24-h RBC/FFP ratio	1.47 (0.86)	2.32 (1.76)	0.005
Minutes to MTP	46.5 (26–103.5)	19.5 (15–25)	<0.001

These groups were similar in age, percentage of penetrating mechanism, ISS, temperature, and heart rate, but those who died presented with lower GCS score and SBP and were significantly more acidotic and coagulopathic compared with those who survived.

Normally distributed data are reported in mean (SD); skewed data are reported as median (interquartile range).

TABLE 3. Demographics and Resuscitation by Cause of Death With Survivors Shown for Reference

		Cause of Death				
		Potential Survivors			Anatomically Nonsurvivable	
	Survivors	Exsanguination	Early Collapse	Late Collapse		
	(n = 114)	(n = 24)	(n = 12)	(n = 8)	(n = 28)	*p*
Age, y	35.8 (16.4)	32.8 (16.4)	39.8 (16.3)	52.6 (17.5)	45.7 (23.0)	0.039
Sex, male, %	74.6	95.8	66.7	75.0	64.3	0.023
Penetrating injury, %	53.5	83.3	50.0	12.5	28.6	<0.001
Head AIS score	2.2 (2.0)	1.7 (2.1)	2.4 (2.3)	3.3 (1.7)	2.9 (2.3)	0.440
ISS	26.0 (14.9)	37.8 (19.5)	31.4 (12.2)	22.1 (11.4)	35.9 (19.1)	0.208
GCS score	15 (11–15)	4 (3–12)	6 (3–14)	11 (6–14)	3 (3–5.5)	0.035
Temperature, °C	35.8 (0.8)	35.3 (1.6)	35.6 (0.9)	34.6 (2.1)	35.3 (1.2)	0.673
Heart rate, beats/min	106.7 (28.5)	93.8 (58.1)	113.5 (30.4)	105.4 (22.7)	99.4 (36.6)	0.623
SBP, mm Hg	104.0 (32.6)	77.7 (44.7)	94.9 (42.7)	108.1 (38.1)	124.2 (52.3)	0.008
pH	7.25 (0.15)	6.99 (0.20)	7.05 (0.20)	7.16 (0.15)	7.23 (0.13)	<0.001
Base deficit	−8.6 (5.3)	−18.1 (8.3)	−13.2 (6.2)	−14.4 (5.0)	−8.8 (6.0)	<0.001
Hemoglobin, g/dL	12.0 (2.3)	10.9 (2.8)	10.7 (1.6)	10.5 (3.5)	11.3 (2.0)	0.817
INR	1.3 (1.1–1.5)	1.9 (1.7–2.3)	1.5 (1.2–3.0)	1.8 (1.1–2.6)	1.3 (1.2–1.6)	0.018
PT	15.4 (14.3–17.5)	20.7 (19.3–23.8)	17.6 (16.2–30.4)	20.8 (14.6–26.8)	15.9 (14.9–19.7)	0.121
PTT	28.4 (24.9–32.9)	43.2 (39.0–60.9)	41.2 (32.6–52.8)	33.6 (27.4–78.5)	38.7 (31.5–55.0)	0.475
Platelets × 10^3	276 (93)	171 (87)	205 (100)	158 (85)	243 (111)	0.072
White blood cell × $10^3/\mu L$	11.0 (8.0–14.2)	5.6 (5.0–8.4)	8.6 (6.6–13.6)	11.3 (9.2–14.7)	9.7 (6.4–13.6)	0.004
Creatinine, mg/dL	1.0 (0.8–1.3)	1.3 (1.2–1.4)	1.4 (1.2–1.5)	1.1 (0.8–1.3)	1.0 (0.9–1.3)	0.017
Prehospital IVF, mL	250 (50–500)	100 (0–750)	—	100 (100–100)	150 (25–1,400)	0.892
24-h crystalloid, mL	6.3 (4.4–10.1)	3.0 (2–4.9)	4.0 (2–7.5)	16.0 (9–19.5)	8.3 (4.1–11.4)	<0.001
24-h colloid, mL	0 (0–0)	0 (0–0)	0 (0–0.5)	0 (0–0.6)	0 (0–0)	0.141
24-h pRBC, U	10 (6–17)	18.5 (10–28.5)	36.5 (19.5–65)	23.5 (20–39)	13 (8.5–19)	<0.001
24-h FFP, U	8 (4–12)	6.5 (3–13)	25.0 (15–35.5)	18.0 (14–23.5)	10 (4.5–14)	<0.001
24-h platelets, apheresis unit	1 (0–2)	0 (0–0)	3.5 (1–5)	3 (2.5–4.5)	1 (0–2)	<0.001
%MT	56.10%	75.0%	100.0%	100.0%	67.9%	0.049
24-h RBC/FFP ratio	1.47 (0.86)	2.97 (2.24)	1.74 (0.63)	1.50 (0.42)	1.56 (0.73)	0.004
Minutes to MTP	46.5 (26–103.5)	19 (15–24)	24 (14–47)	21 (21–21)	67 (35–93)	0.002

There is a higher RBC/FFP ratio in the exsanguination group as well as significantly higher crystalloid use in the late collapse cohort. In addition, all patients in the early and late collapse cohorts received full MTP, while only 75% and 67.9% of exsanguination and nonsurvivable, respectively, received full MTP.

Normally distributed data are reported in mean (SD); skewed data are reported as median (interquartile range).

We next evaluated those patients with anatomically nonsurvivable injury (n = 28) and compared them with the rest of the study population at large (n = 158), to identify predictive factors for anatomically nonsurvivable injuries (Table 4). Again, the group with nonsurvivable injuries had fewer penetrating injuries ($p < 0.013$), significantly lower presenting GCS score ($p < 0.001$), and higher initial SBP ($p < 0.023$). There were no identifiable differences in the resuscitation of the two groups.

Finally, we compared only the patients who died, divided into those who had a potentially survivable injury but still succumbed to their injuries, versus those who were deemed to have an anatomically nonsurvivable injury (Table 5). This continued to show traumatic brain injury as the driving force in patients with nonsurvivable injury. The patients with nonsurvivable injuries had statistically significantly lower GCS score, higher SBP, fewer penetrating wounds, less acid-base derangement, and less coagulopathy. The patients with nonsurvivable injuries did use more crystalloid, used fewer pRBCs, and had a lower RBC/FFP ratio than those with potentially survivable injuries. There was also a significantly longer time to activation of the MTP in patients with nonsurvivable injuries of 67 minutes versus 19.5 minutes ($p < 0.001$).

DISCUSSION

The cause of death confounds any discussion regarding the optimal resuscitation of severely injured patients. Whether these deaths result from the failure of surgical care and resuscitation or are the sequelae of overwhelming injury, which is refractory to any resuscitative conduct, remains an open question. To address this requires investigation into the causes of death and a classification of anatomically nonsurvivable versus potentially survivable injury. Hence, the aim of this article was to codify the cause of death of the severely injured trauma patients to learn more about the reasons for death and to better characterize which of our patients have a chance of survival and would potentially benefit from aggressive and progressive resuscitation.

Often, patients are said to bleed to death; however, this description can be highly variable. Some patients bleed to death from giant holes in major vessels. In these cases, resuscitation

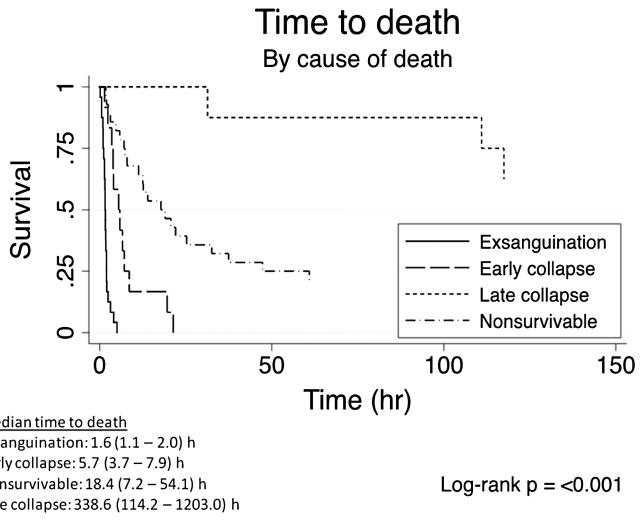

Figure 1. Time-to-death analysis. Those who died of exsanguination died the earliest at a median time of 1.6 hours, while those who died of late physiologic collapse died at a median time of approximately 2 weeks (338.6 hours).

and the biology of coagulation are irrelevant; overwhelmed by the severity of injury, these patients are in need of rapid transport to an operating theater for definitive surgical control of their injuries. Other patients are described as having succumbed to their injuries despite surgical control, with some dying of physiologic degradation in the operating room, while others slowly decompensate in the intensive care unit. While these codifications seem intuitive, they have yet to be specifically described in the literature.

Since the landmark trauma epidemiology article by Baker et al.[12] in 1980, there have been multiple studies[10,13–18] evaluating the epidemiology of trauma. These publications are roughly divided into examinations of mechanism of injury and physiologic cause of death. Although the vast majority of these epidemiology articles deal with the mechanism of injury for the use of regional trauma management and injury prevention programs, there are few which have focused on the reasons for death. To date, however, no studies have examined the potential survivability after injury and effects of resuscitation.

Previous studies that have evaluated the cause of death demonstrate that central nervous system injury and hemorrhage remain the two most significant causes of death, with each contributing 30% to 40%[10,16,19,20] of the mortality in trauma patients. In our study, we saw an injury percentage rates similar to these previous reports, with exsanguination causing death in 33.3% of the patients and 38.8% having injuries that were deemed to be nonsurvivable (96.4% had brain injury).

Analysis of the cause of death in the recent military conflicts provides insight into potentially survivable versus nonsurvivable injuries.[21–23] Similar to our study, these studies show that traumatic brain injury is the leading cause of nonsurvivable injury,[22] while hemorrhage and, more specifically, truncal hemorrhage remains as the leading cause of potentially survivable death in the military population.[21–23] While these studies highlight the improvements in survival made by tactical combat casualty care, our study has increased the granularity of these military analyses by separating the potentially survivable cause of death into hemorrhage, early physiologic collapse, and late physiologic collapse.

The use of hemostatic resuscitation has been shown to improve survival in severely injured patients. However, the ability to identify early which patients need hemostatic resuscitation and the mathematical phenomenon of "decedent dropout" can hinder these studies. Early activation of an MTP has been demonstrated to improve survival[1–6] but can result in unnecessary MTP activation. We have attempted to address this by including the %MT to provide more accurate description of how many patients in each category achieved a "full MT" as defined in the Patients and Methods section. Decedent dropout is the result of early death in trauma patients, which results in an elevated RBC/FFP ratio. This elevated ratio is not necessarily reflective of the efficacy of the resuscitation but, instead, can indicate death before FFP being available for transfusion.

TABLE 4. Demographics and Resuscitation for All Patients With Survivable Injuries Versus Those With Anatomically Nonsurvivable Injuries to Identify Predictive Factors for Anatomically Nonsurvivable Injuries

	Survivable Injured (n = 158)	Anatomically Nonsurvivable (n = 28)	*p*
Age, y	36.5 (16.8)	45.7 (23.0)	0.052
Sex, male, %	77.2	64.3	0.158
Penetrating injury, %	55.7	28.6	0.013
Head AIS score	2.2 (2.0)	2.9 (2.3)	0.296
ISS	27.1 (15.4)	35.9 (19.1)	0.031
GCS score	14 (6–15)	3 (3–5.5)	<0.001
Temperature, °C	35.7 (1.1)	35.3 (1.2)	0.197
Heart rate, beats/min	105.4 (33.9)	99.4 (36.6)	0.420
SBP, mm Hg	99.6 (36.6)	124.2 (52.3)	0.023
pH	7.20 (0.19)	7.23 (0.13)	0.268
Base deficit	−10.3 (6.6)	−8.8 (6.0)	0.236
Hemoglobin, g/dL	11.7 (2.4)	11.3 (2.0)	0.369
INR	1.3 (1.2–1.6)	1.3 (1.2–1.6)	0.895
PT	15.8 (14.5–18.7)	15.9 (14.9–19.7)	0.255
PTT	30.2 (26–38.1)	38.7 (31.5–55.0)	0.001
Platelets × 10^3	252 (101)	243 (111)	0.696
White blood cell × $10^3/\mu L$	9.8 (7.5–13.4)	9.7 (6.4–13.6)	0.938
Creatinine, mg/dL	1.1 (0.9–1.4)	1.0 (0.9–1.3)	0.416
Prehospital IVF, mL	250 (50–500)	150 (25–1,400)	0.910
24-h crystalloid, mL	5.9 (3.1–9.8)	8.3 (4.1–11.4)	0.215
24-h colloid, mL	0 (0–0)	0 (0–0)	0.187
24-h pRBC, U	13 (7–21)	13 (8.5–19)	0.976
24-h FFP, U	8 (4–17)	10 (4.5–14)	0.864
24-h platelets, apheresis unit	1 (0–2)	1 (0–2)	0.396
%MT	64.6%	67.9%	0.832
24-h RBC/FFP ratio	1.71 (1.24)	1.56 (0.73)	0.381
Minutes to MTP	37 (19–77)	67 (35–93)	0.103

There is a preponderance of blunt injury, depressed GCS score, and elevated blood pressure in the group with nonsurvivable injuries.

Normally distributed data are reported in mean (SD); skewed data are reported as median (interquartile range).

TABLE 5. Demographics and Resuscitation of Those Who Had Survivable Injuries but Still Died Versus Those With Anatomically Nonsurvivable Injuries Identify Traumatic Brain Injury as the Driving Force of the Cohort With Anatomically Nonsurvivable Injuries

	Potential Survivors (n = 44)	Anatomically Nonsurvivable (n = 28)	*p*
Age, y	38.3 (17.8)	45.7 (23.0)	0.154
Sex, male, %	84.1	64.3	0.086
Penetrating injury, %	61.4	28.6	0.008
Head AIS score	2.4 (2.0)	2.9 (2.3)	0.462
ISS	31.5 (16.5)	35.9 (19.1)	0.367
GCS score	6 (3–12)	3 (3–5.5)	0.018
Traumatic brain injury	20.5%	96.4%	<0.001
Temperature, °C	35.1 (1.6)	35.3 (1.2)	0.695
Heart rate, beats/min	101.8 (45.9)	99.4 (36.6)	0.807
SBP, mm Hg	88.2 (43.8)	124.2 (52.3)	0.004
pH	7.04 (0.19)	7.23 (0.13)	<0.001
Base deficit	−15.9 (7.2)	−8.8 (6.0)	<0.001
Hemoglobin, g/dL	10.8 (2.6)	11.3 (2.0)	0.360
INR	1.7 (1.4–2.3)	1.3 (1.2–1.6)	0.005
PT	19.7 (17.3–24.5)	15.9 (14.9–19.7)	0.036
PTT	40 (33.3–54.8)	38.7 (31.5–55.0)	0.801
Platelets × 10^3	180 (90)	243 (111)	0.019
White blood cell × $10^3/\mu L$	8.3 (5.3–11.6)	9.7 (6.4–13.6)	0.073
Creatinine, mg/dL	1.3 (1.1–1.4)	1.0 (0.9–1.3)	0.012
Prehospital IVF, mL	100 (25–750)	150 (25–1,400)	0.632
24-h crystalloid, mL	4.0 (2–9)	8.3 (4.1–11.4)	0.033
24-h colloid, mL	0 (0–0)	0 (0–0)	0.355
24-h pRBC, U	23.5 (13–38.5)	13 (8.5–19)	0.001
24-h FFP, U	13 (4.5–24)	10 (4.5–14)	0.133
24-h platelets, apheresis unit	1 (0–4)	1 (0–2)	0.267
%MT	86.40%	67.9%	0.077
24-h RBC/FFP ratio	2.32 (1.76)	1.56 (0.73)	0.016
Minutes to MTP	19.5 (15–25)	67 (35–93)	<0.001

Normally distributed data are reported in mean (SD); skewed data are reported as median (interquartile range).

Ho et al.[24] addresses decedent dropout by using a mathematical model to show the significant risk of survivorship bias in some observational studies comparing low and high FFP administration. Our comparison of cause of death identified the exsanguination group as having a statistically significant higher RBC/FFP ratio. We believe that this is not a failure of transfusion ratios but more likely a lack of surgical control of hemorrhage from massive injury rendering resuscitative conduct moot. Those who died of early or late physiologic collapse had similar ratios to the survivors, raising interesting questions. Did the early physiologic collapse need ratios closer to 1:1? Were patients in the late physiologic collapse group overresuscitated early, leading toward later collapse? The differences in transfusion ratios in our study call for the codification of cause of death in clinical studies so that physiologic responses to resuscitation strategies can be accurately stratified.

The injured patient in whom surgical control has been obtained and a vigorous resuscitation has been used but who still succumbs to his or her injuries from early physiologic collapse proves to be a very frustrating cohort. Anecdotally, these are the patients with significant injury who despite surgical control of the injury and an initial trajectory toward survival becomes refractory to resuscitation and enter a spiral of decompensation and die. These frustrating patients present with no predicting factors as compared with those who exsanguinate, despite having achieved surgical hemorrhage control. What effect our resuscitative efforts have on this cohort remains an open question. One hypothesis is that our better resuscitative conduct allows for correction of coagulopathy and restoration of appropriate physiology, providing more time for surgical bleeding control in a patient that would have previously exsanguinated. In a subset of these patients, large-volume blood product–based resuscitation may have merely prolonged survival past initial hemorrhage control in patients who essentially had nonsurvivable injuries, shifting their cause of death to early physiologic collapse. Their significantly higher blood product use, despite not reaching optimal ratios, hints at this. Another theory is that these patients have a survivable injury which, with better resuscitation, would not cross the threshold of physiologic collapse. In this case, efforts at better resuscitation would prevent exhaustion of physiologic reserve and ultimately lead to survival. This group uses the most blood products and still has a poor outcome. This intuitively survivable cohort in whom resuscitation practices may make the critical difference between survival and death should receive increased trauma resuscitation research.

The prolonged median time to death from late physiologic collapse indicates that we are improving in our ability to keep these patients alive longer than demonstrated in previous

studies.[10,14,16,19,20] The time-to-death analysis are comparable with older studies that[16,20] reported 84.5% of their patients died within 48 hours and only 41 patients (6.5%) lived greater than 1 week. Our analysis showed that 89% of patients died within 48 hours and 11% had a median time to death of 2 weeks. This is a clear indication that our critical care techniques are improving and that specific analysis of the cause of death in future studies will aid in identifying targeted methods to improve outcomes.

Our data show that those patients who were deemed to have a nonsurvivable injury who still underwent a MTP did so with an average activation time of 67 minutes versus 19.5 minutes for those who were deemed to have a potentially survivable injury but died anyway. It is interesting that there is such a long delay in these patients. This was not affected by withdrawal of care, as only three patients who had care withdrawn (data not shown). We hypothesize that this increase in lag time to the activation of an MTP is secondary to the prominence of traumatic brain injury in these patients, which may delay MTP activation until there is progression of bleed, deterioration in clinical examination, and so on. The earlier identification of these patients may be prudent to assist families in decision making and to allocate resources in a more efficient manner.

The choice of the cause-of-death categories is based on physiologic principles and the clinical observation that patients who die after undergoing MT fall into distinct general physiologic conditions. While we recognize that there are additional subsets of potential interest, such as the presence or absence of coagulopathy, our study is limited by the total number of patients in our data set. The selection of these groups inherently affect some aspects of the study results (such as time to death), but the differences in physiologic parameters and resuscitation for each group identified here lends credence to these categories as clinically relevant.

As a result of our analysis, we believe that a staged approach to the goals of resuscitation should be considered in discussions of trauma epidemiology. Patients who die of uncontrolled hemorrhage are ultimately best served by injury prevention efforts and optimization of evacuation to definitive surgical treatment. Those who die of early physiologic collapse need better-targeted resuscitation. Those who die of late physiologic collapse need improvements in critical care; however, the early resuscitation of these patients may also play a critical role in their long-term trajectory.

CONCLUSION

This article supports the need for a multi-institutional study and the start of a national data bank that codifies the cause of death in trauma patients to specifically identify risk factors and better-targeted therapy for exsanguination, physiologic collapse, and nonsurvivable injuries. The inclusion of these data into quality improvement programs could help identify areas of improvement in trauma resuscitation.

AUTHORSHIP

M.W.C., M.E.K., M.J.C. contributed to the literature search, study design, data analysis and interpretation, writing, and critical revision. A.D., R.C.M., M.D.G., L.M.C., B.J.R., M.F.N. contributed to the study design, data collection, and critical revision of the manuscript.

DISCLOSURE

The authors declare no conflicts of interest.

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The whole is greater than the sum of its parts: Hemostatic profiles of whole blood variants

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BACKGROUND: Mounting evidence highlighting the benefits of hemostatic resuscitation has led to a renewed interest in whole blood (WB) and reconstituted WB (RWB). However, few data exist to characterize the clotting profiles of these variants. This study characterizes banked WB variants and RWB in standard 1:1:1 and 2:1:1 transfusion ratios of packed red blood cells, fresh frozen plasma, and platelets (PLTs). We hypothesized that the global hemostatic profile of 1:1:1 RWB is superior to 2:1:1 RWB and that PLT-modified WB (MWB) is superior to 1:1:1 RWB.

METHODS: Twenty-three units of packed red blood cells, fresh frozen plasma, and PLTs were obtained from the regional blood collection center and mixed to create 23 1:1:1 and 23 2:1:1 RWB units. Freshly donated WB units were obtained and used to create 11 of each nonmodified WB (NMWB) (room temperature and cooled) and MWB (room temperature and cooled) variants. International normalized ratio (INR)/partial thromboplastin time (PTT), complete blood cell count, functional studies, and an extensive panel of procoagulant and anticoagulant factor assays were performed on all products.

RESULTS: The 1:1:1 RWB had significantly lower INR and PTT (1.31 vs. 1.55, $p = 0.0029$; 42 seconds vs. 50 seconds, $p = 0.0008$) and higher activity of factors II, V, VII, VIII, IX, and X; antithrombin III, as well as protein C and higher fibrinogen levels than did 2:1:1 RWB (factor IX, 86% vs. 70%, $p = 0.0313$; fibrinogen, 242 mg/dL vs. 202 mg/dL, $p = 0.0385$). There were no differences in INR/PTT or factor activity between MWB and NMWB. However, MWB had greater maximum clot firmness (MCF) by rotational thromboelastometry tissue factor–activated extrinsic clotting cascade measures than did NMWB (MCF, 61 mm vs. 50 mm, $p = 0.0031$). MWB also had greater MCF by rotational thromboelastometry tissue factor–activated extrinsic clotting cascade measures than did 1:1:1 RWB (MCF, 61 mm vs. 45 mm, $p = 0.0005$).

CONCLUSION: Although 1:1:1 RWB had a superior clotting profile relative to 2:1:1 RWB, MWB exhibited even better global hemostasis than did 1:1:1 RWB. Characterization of factor-level and functional clotting differences between WB variants is imperative for understanding the clinical benefits of hemostatic resuscitation. (*J Trauma Acute Care Surg*. 2014;77: 818–827. Copyright © 2014 by Lippincott Williams & Wilkins)

KEY WORDS: Whole blood; reconstituted whole blood; characterization.

The assorted medicinal uses of blood date back to ancient times, yet it was not until World War I that the US Army Medical Department officially adopted transfusion with citrated whole blood (WB) as a lifesaving intervention on the battlefield.[1] This practice continued through World War II, the Korean War, and the Vietnam Conflict.[2–5] However, with blood banking advances in processing and storage in the 1960s and the 1970s, component therapy (packed red blood cells [RBCs], fresh frozen plasma [FFP], and platelets [PLTs]) replaced WB as the preferred treatment of hemorrhagic shock in both military and civilian populations.[6] Remarkably, during this transition, there were no studies evaluating the efficacy or superiority of component therapy in the treatment of hemorrhagic shock.[7] Despite this lack of data, the shift to component therapy accelerated in the 1980s, with attention toward optimum resource allocation. This was due in large part to concern over transfusion-transmitted diseases including human immunodeficiency virus and viral hepatitis. Ultimately, the use of fresh WB was precluded, except in special circumstances, because of the expansion of donor testing for serologic markers and nucleic acids in centralized laboratories coupled with the belief that PLTs stored at 2°C to 6°C were cleared faster from circulation than PLTs stored at 22°C.[8–11] However, the identification and the characterization of acute traumatic coagulopathy,[12–20] along with emerging evidence suggesting that balanced component resuscitation improves outcomes in military and civilian injured

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populations,[20–25] have renewed interest in the potential benefits of WB.[7,26–32]

Despite this renewed interest, only one single-center randomized trial of WB (PLT-modified WB [MWB]) compared with component therapy in injured patients has been completed.[7] In addition, limited in vitro data exist to begin to completely characterize the differences in clotting profiles of banked WB variants and reconstituted WB (RWB) made from components mixed in varying ratios.[33,34] These data are critical for understanding the clinical benefits seen with hemostatic resuscitation and designing a clinical trial with optimal product comparisons. We sought to characterize extensive factor-level and functional profiles of banked nonmodified WB (NMWB) and MWB variants as well as RWB in standard 1:1:1 and 2:1:1 transfusion ratios of RBC/FFP/PLTs. We hypothesized that the global hemostatic profile of 1:1:1 RWB is superior to 2:1:1 RWB and that the hemostatic profile of MWB is superior to 1:1:1 RWB.

MATERIALS AND METHODS

Donor informed consent and standard blood donation guidelines of the regional blood collection center (RBCC) were followed for all products obtained. All products were deidentified. Donor characteristics (age, sex, and blood type), volume, collection date, and banked anticoagulant were obtained.

Product Selection/Sample Generation of WB Variants

Our WB generation schema is detailed in Figure 1*A*. Briefly, the RBCC identified 11 freshly donated nontransfusable, nonleukoreduced WB units. All products were cooled for less than 24 hours at 2°C to 6°C during processing, typing, and weighing. Each WB unit meeting weight criteria (overweight or underweight per blood center protocol; overweight defined as >665 grams; underweight defined as < 550 grams) was then divided into four equal bags using a Triple Bag Aliquot System (300-mL satellite bags, Charter Medical Ltd., Winston-Salem, NC). Two of the bags were brought to room temperature after initial processing, and two of the bags remained cooled at 2°C to 6°C overnight. The next morning, leukoreduced recently expired (24–48 hours) PLTs (mean age of PLTs 6.64 ± 1.10 days) were added to one of the bags stored at 2°C to 6°C and one of the bags stored at room temperature to create MWB variants (modified room temperature and modified cooled), which were WB units enriched by the addition of PLTs. All PLTs used were collected by apheresis. A blood-type compatibility schema was used for optimal matching (see Supplement 1a, Supplemental Digital Content 1, http://links.lww.com/TA/A454). The volume of PLTs added to create the two types of MWB variants was equal to one twenty-fourth of the original PLTs unit (11.26 [2.70] mL) to mimic the published transfusion ratio of 6 U of WB:1 U of PLTs ([donated WB unit/4] × [1 U of PLTs/6 U of WB] = one twenty-fourth).[7] The following WB variants were produced from each donated WB unit: 11 room temperature WB (RTWB) (140.90 [37.72] mL), 11 cooled WB (CWB) (135.45 [33.01] mL), 11 modified CWB (MCWB) (136.09 [32.11] mL), and 7 modified RTWB (MRTWB) (122.14 [38.64] mL). The RTWB and CWB together composed the NMWB variants, and the MRTWB and MCWB together composed the MWB variants.

Product Selection/Sample Generation of RWB Variants

Our RWB unit generation schema is detailed in Figure 1*B*. Briefly, 23 of each RWB variant were mixed from components identified by the RBCC. The RWB units were composed of leukoreduced recently expired (24–48 hours) RBCs (mean age of RBCs 42.74 ± 1.64 days; see Supplement 1b, Supplemental Digital Content 1, http://links.lww.com/TA/A454) stored at 1°C to 6°C, mixed with leukoreduced recently expired (24–48 hours) PLTs (mean age of PLTs 6.64 ± 1.10 days; see Supplement 1b, Supplemental Digital Content 1, http://links.lww.com/TA/A454) stored at 20°C to 24°C on a horizontal shaker, and nontransfusable (due to potential for human leukocyte/neutrophil antibodies) FFP stored at −20°C mixed in 1:1:1 (1 U of RBC:1 U of FFP:1/6 U of PLTs) and 2:1:1 (2 U of RBCs:1 U of FFP:1/6 U of PLTs) ratios. A blood-type compatibility schema was used for optimal matching (see Supplement 1b, Supplemental Digital Content 1, http://links.lww.com/TA/A454). A Triple Bag Aliquot System (150-mL satellite bags, Charter Medical Ltd., Winston-Salem, NC) was attached to the RBC unit. Plasma products were thawed in a plasma water bath (Precision Scientific, Inc., Winchester, WV) at 37°C for 15 minutes to 30 minutes per transfusion protocol. RWB variants were mixed on a rotational rocker at 60 rpm for 1 minute.

Laboratory Analysis

The product was withdrawn from the bags after previously defined processing via a 16-gauge to 18-gauge needle into a 60-mL syringe and then injected into standard laboratory vacuum-sealed 4.5-mL tubes containing 3.2% (0.109 mol/L) sodium citrate (Monoject, Franklin Lakes, NJ), 3-mL tubes containing 56 United States Pharmacopeia (USP) units of lithium heparin (BD Vacutainer, Franklin Lakes, NJ), and 7-mL tubes containing EDTA K3EDTA 15% Solution, 0.081 mL, 12.15 mg (BD Vacutainer), for later-defined laboratory testing.

Our methodology for WB laboratory analysis has been previously described.[35,36] Rotational thromboelastometry (ROTEM) was performed to assess viscoelastic properties of clot formation with the ROTEM delta (Pentapharm GmbH, Munich, Germany). Four tests were run simultaneously for each sample: ROTEM tissue factor–activated extrinsic clotting cascade measures (EXTEM) (recalcifier: star-TEM, activator: ex-TEM tissue factor); ROTEM contact-activated intrinsic clotting cascade measures (INTEM) (mild intrinsic coagulation activation using recalcifier: star-TEM, activator: in-TEM [intrinsic coagulation activator]); ROTEM tissue factor–activated/aprotinin fibrinolytic-inhibited clotting cascade measures (APTEM) (fibrinolysis inhibitor aprotinin and recalcifier: ap-TEM, activator: ex-TEM); and ROTEM tissue factor–activated/cytochalasin D PLT-inhibited clotting cascade measures (FIBTEM) (recalcifier and thrombocyte inhibitor: fib-TEM, activator: ex-TEM). Each sample was warmed to 37.0°C in the TEM cup, prepared accordingly with the use of an automated pipette, and the test was initiated within 20 seconds of mixing 300 μL of blood with the recalcifier and the activator. The presence of fibrinolysis was defined as APTEM maximum clot firmness (MCF) greater than EXTEM MCF.

Samples were immediately centrifuged to platelet-poor plasma, and plasma was extracted and analyzed; all sample analyses were performed by researchers blinded to data. Levels

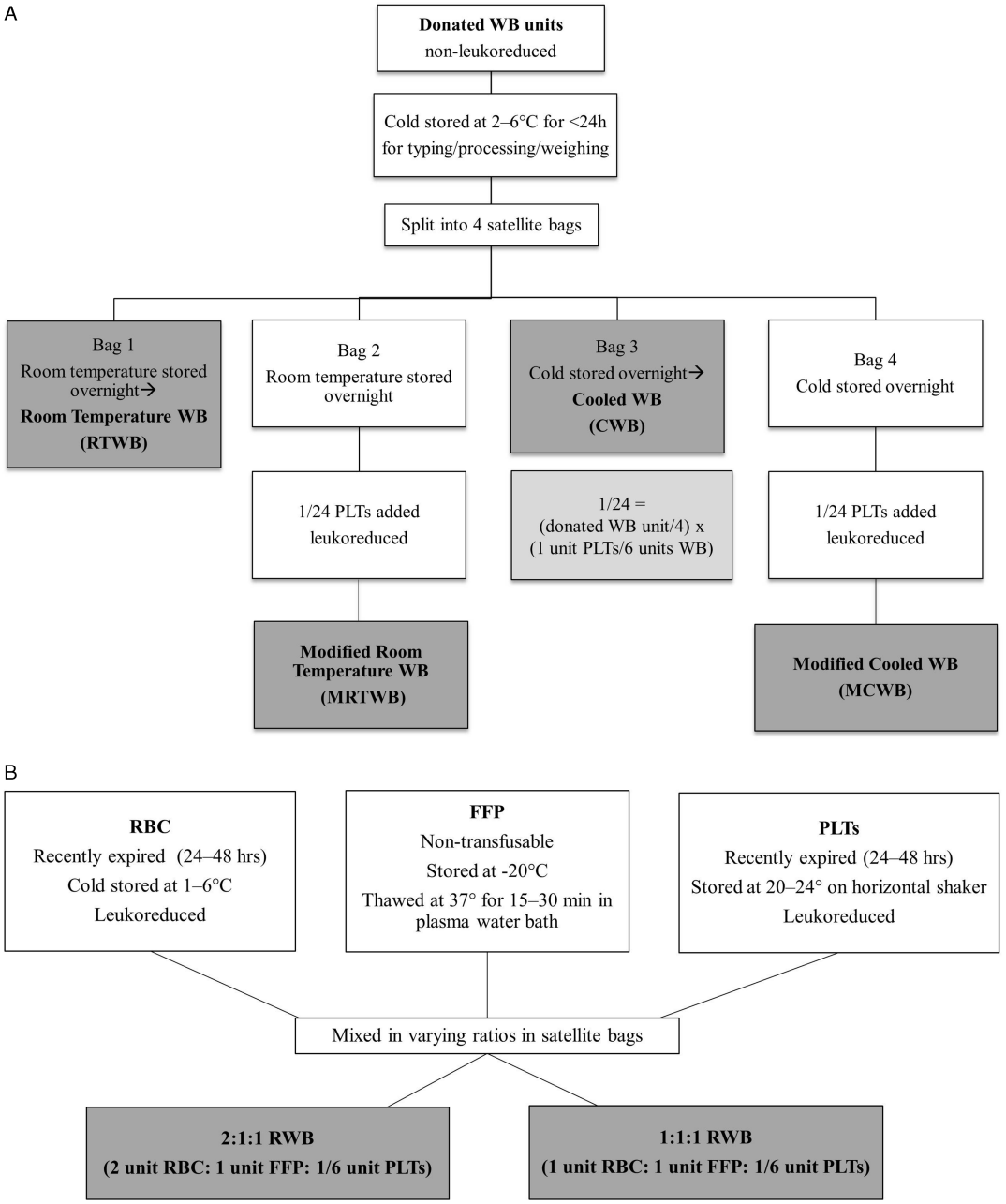

Figure 1. *A*, Product selection and sample generation of WB variants. Four WB variants (RTWB, MRTWB, CWB, and MCWB) boxed in dark gray. Calculation of amount of PLTs used for modification boxed in light gray. WB collected in 500-mL citrate phosphate double dextrose solution (CP2D) or citrate phosphate dextrose adenine solution 1 (CPDA-1) bags (Haemonetics Corporation, Braintree, MA). *B*, Product selection and sample generation of RWB variants. Two RWB variants (2:1:1 RWB and 1:1:1 RWB) boxed in dark gray. RBC from WB collected with citrate phosphate double dextrose solution (CP2D) or by apheresis with acid citrate dextrose A solution (ACD-A). PLTS, apheresis PLT unit.

of fibrinogen; the activity of the procoagulant factors II, V, VII, VIII, IX, and X; as well as the endogenous anticoagulants antithrombin III (ATIII) and protein C were assayed. Fibrinogen; factors II, V, VII, VIII, IX, and X; ATIII; and protein C were measured with a Stago Compact Coagulation Analyzer (Diagnostica Stago Inc., Parsippany, NJ) in accordance with manufacturer instructions. Complete blood cell count was performed in accordance with San Francisco General Hospital Clinical Laboratory standard procedures.

Statistical Analysis

All data are presented as mean (SD). Univariate comparisons were made using the Student's *t* test or analysis of variance

for normally distributed data, the Wilcoxon rank-sum or the Kruskal-Wallis test for skewed data, and the Fisher's exact test for proportions. Intergroup comparisons between multiple groups were judged significant only when corrected for multiple comparisons using a standard Bonferroni correction. An α of 0.05 was considered significant. All statistical analyses were performed by the authors using Stata version 12 (StataCorp, College Station, TX).

TABLE 1. Clotting Profiles by RWB Variants (1:1:1 Versus 2:1:1)

	1:1:1		2:1:1		
	n = 23	SD	n = 23	SD	*p*
INR	1.31	0.18	1.55	0.31	0.0029
PT, s	15.98	1.71	18.20	2.75	0.0024
PTT, s	41.76	4.68	49.92	8.12	0.0008
Factor II, %	65.00	19.81	59.96	17.47	0.1348
Factor V, %	50.91	21.56	41.55	18.80	0.0579
Factor VII, %	62.26	27.41	58.63	22.08	0.2671
Factor VIII, %	76.35	32.17	62.61	24.07	0.1111
Factor IX, %	86.22	25.39	69.78	21.85	0.0313
Factor X, %	66.52	16.12	58.09	14.93	0.0724
ATIII, %	72.65	41.19	62.09	35.00	0.1240
Protein C, %	75.52	24.53	67.65	16.88	0.0782
Fibrinogen, mg/dL	241.65	67.16	202.04	58.40	0.0385
D-dimer, μg/mL	0.25	0.06	0.23	0.05	0.3497
EXTEM CT, s	145.91	42.81	199.61	159.16	0.2025
EXTEM CFT, s	760.57	1560.60	952.43	1772.07	0.0060
EXTEM α, degrees	56.27	16.00	48.65	9.46	0.0032
EXTEM a10, mm	38.57	14.04	30.43	12.35	0.0430
EXTEM a20, mm	45.83	15.45	38.82	14.30	0.0019
EXTEM MCF, mm	50.35	15.48	43.22	15.74	0.0018
EXTEM ML, %	3.83	7.48	1.04	3.76	0.0054
INTEM CT, s	379.70	99.82	454.13	148.88	0.1088
INTEM CFT, s	698.83	1510.72	936.30	1775.14	0.0249
INTEM α, degrees	52.09	14.22	44.41	13.43	0.0243
INTEM a10, mm	39.87	12.56	31.26	10.92	0.0007
INTEM a20, mm	48.74	14.00	40.43	13.37	0.0001
INTEM MCF, mm	53.57	14.76	46.35	14.57	0.0005
INTEM ML, %	1.00	2.56	0.83	2.92	0.7996
APTEM CT, s	174.00	110.53	192.96	116.48	0.3392
APTEM CFT, s	790.35	1530.98	1034.91	1759.29	0.0517
APTEM α, degrees	53.77	12.06	43.71	13.07	0.0124
APTEM a10, mm	34.78	13.36	28.26	12.03	0.0890
APTEM a20, mm	43.22	14.95	36.00	14.19	0.0142
APTEM MCF, mm	48.43	16.52	40.96	15.62	0.0054
APTEM ML, %	1.00	2.07	2.13	4.85	0.9175
FIBTEM MCF, mm	12.04	2.96	7.91	2.13	0.0000
WBC, $\times 10^3$/μL	0.01	0.03	0.00	0.00	0.1522
Hgb, g/dL	9.05	1.07	12.15	1.22	0.0000
Hct, %	28.93	3.46	39.19	3.90	0.0000
Plts, $\times 10^9$/L	129.62	22.23	95.48	21.85	0.0000

Data are given as mean and SD. Significance assessed by the Student's *t* test for normally distributed data and Wilcoxon rank-sum test for nonnormally distributed data. $p < 0.05$, significant. CT, time to initial clot formation; CFT, time to 20 mm of clot firmness; α, angle of tangent at 2 mm of clot firmness.

D-dimer, dimerized plasmin fragment D; ML, maximum lysis; PT, prothrombin time.

RESULTS

Product Characteristics

The donor and product characteristics of the 23 RBC, FFP, and PLT units are detailed in Supplement 2a (Supplemental Digital Content 2, http://links.lww.com/TA/A455). The donor and product characteristics of the 11 WB units are detailed in Supplement 2b (Supplemental Digital Content 2, http://links.lww.com/TA/A455). All products were analyzed for differences in complete blood cell count measures, standard coagulation measures, factors, and functional clotting parameters.

1:1:1 Versus 2:1:1 RWB

Both 1:1:1 RWB and 2:1:1 RWB had a low white blood cell count (WBC) (0.01 [0.03] $\times 10^3$/μL vs. 0.00 [0.00] $\times 10^3$/μL, $p = 0.1522$; Table 1) consistent with the leukoreduction of the RBC. Appropriately, 1:1:1 RWB had a significantly lower hemoglobin (Hgb) and hematocrit (Hct) than did 2:1:1 RWB but a higher platelet count (Plts) (Hgb, 9.05 [1.07] g/dL vs. 12.15 [1.22] g/dL, $p < 0.001$; Hct, 28.93% [3.46%] vs. 39.19% [3.90%], $p < 0.001$; Plts, 129.62 [22.23] $\times 10^9$/L vs. 95.48 [21.85] $\times 10^9$/L, $p < 0.001$; Table 1). Notably, 1:1:1 RWB had a significantly lower international normalized ratio (INR) and partial thromboplastin time (PTT) than did 2:1:1 RWB (INR, 1.31 [0.18] vs. 1.55 [0.31], $p = 0.0029$; Table 1, Fig. 2*A*); higher factor activity for factors II, V, VII, VIII, IX, and X, ATIII, as well as protein C; and a higher fibrinogen level (factor IX, 86.22% vs. 69.78%, $p = 0.0313$; fibrinogen, 241.65 mg/dL vs. 202.04 mg/dL, $p = 0.0385$; remaining $p > 0.05$; Table 1, Fig. 2*B*).

Overall, 1:1:1 RWB had a superior clotting profile by ROTEM compared with 2:1:1 RWB. Tissue factor activated extrinsic clotting cascade measures (EXTEM) in 1:1:1 RWB compared with 2:1:1 RWB demonstrated a trend toward faster clot initiation with a shorter clotting time (CT), a significantly shorter clot formation time (CFT) ($p = 0.0060$), and a greater α angle ($p = 0.0032$; Table 1). In addition, 1:1:1 RWB compared with 2:1:1 RWB demonstrated stronger clot with a greater clot firmness at 10 minutes (a10) ($p = 0.0430$) and 20 minutes (a20) ($p = 0.0019$) as well as a greater MCF ($p = 0.0018$; Table 1, Fig. 2*C*). This pattern was also seen in the contact activated intrinsic clotting cascade measures (INTEM). The 1:1:1 RWB compared with 2:1:1 RWB showed a trend toward shorter CT; a significantly shorter CFT ($p = 0.0249$); greater α ($p = 0.0243$); and a stronger a10 ($p = 0.0007$), a20 ($p = 0.0001$), and MCF ($p = 0.0005$; Table 1). The 1:1:1 RWB had higher levels of functioning fibrinogen than did 2:1:1 RWB as demonstrated by tissue factor activation with cytochalasin D PLT inhibition (FIBTEM) with a stronger MCF ($p < 0.0001$; Table 1). Neither 1:1:1 RWB nor 2:1:1 RWB demonstrated fibrinolysis (Table 1).

RTWB Versus CWB Versus MCWB Versus MRTWB

There were no differences in WBC, Hgb, or Hct between the four WB variants. As expected, the MCWB had significantly higher Plts than did the CWB (299.40 [74.19] $\times 10^9$/L vs. 191.90 [66.97] $\times 10^9$/L, $p < 0.0083$ corrected for multiple

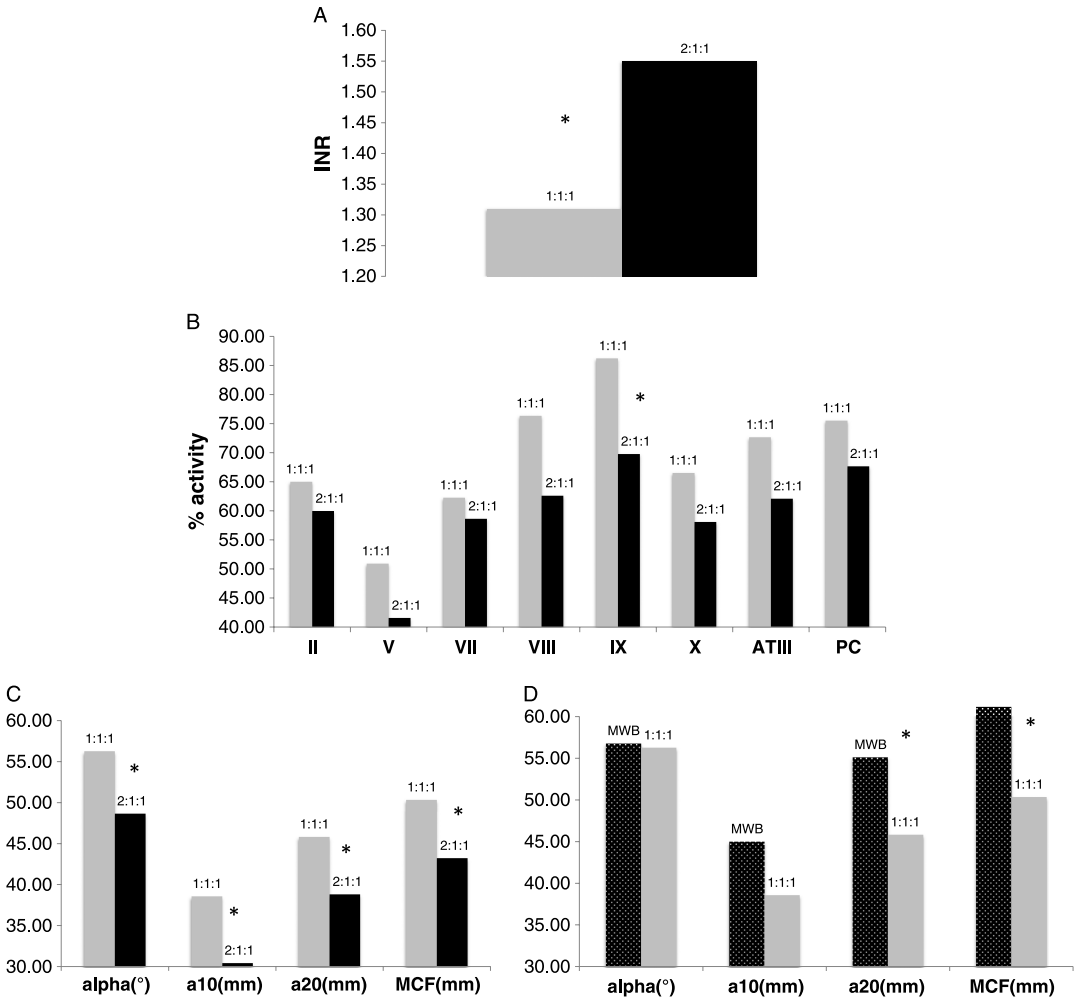

Figure 2. *A*, INR of 1:1:1 versus 2:1:1 RWB. *B*, Percentage of factor activity of 1:1:1 versus 2:1:1 RWB. *C*, Functional clotting measures of 1:1:1 versus 2:1:1 RWB by EXTEM ROTEM. α, angle of tangent at 2 mm of clot firmness. *D*, Functional clotting measures of MWB versus 1:1:1 RWB by EXTEM ROTEM. *$p < 0.05$. II, factor II; V, factor V; VII, factor VII; VIII, factor VIII; IX, factor IX; X, factor X; PC, protein C.

comparisons; Table 2), and the MRTWB trended toward higher Plts than did the RTWB ($p = 0.0128$; Table 2), demonstrating successful addition of PLTs to the WB. There were no differences in INR, PTT, or factor activity/levels between RTWB, CWB, MCWB, and MRTWB (all $p > 0.05$; Table 2). By EXTEM, however, MCWB had a significantly stronger MCF than did CWB ($p < 0.0083$ corrected for multiple comparisons; Table 2). Although not significant, there was a trend toward greater measures of clot strength by EXTEM and INTEM for a10, a20, and MCF for the MWB variants (MCWB and MRTWB) compared with their NMWB counterparts (CWB and RTWB) (Table 2). None of the WB variants demonstrated fibrinolysis (Table 2).

MWB Versus NMWB

Given that the four WB variants had no differences in standard coagulation measures or factor activity/levels, but the MWB variants had improved functional clotting profiles compared with the NMWB variants, we next combined the NMWB variants to compare with the combined MWB counterparts to assess the effects of PLT modification. There was no difference in WBC or Hgb between NMWB and MWB (all $p > 0.05$; Table 3). However, MWB compared with NMWB had a significantly lower Hct and appropriately higher Plts (Hct, 34.51% [3.43%] vs. 37.24% [3.39%], $p = 0.0207$; Plts, 302.59 [73.75] $\times 10^9$/L vs. 197.00 [65.76] $\times 10^9$/L, $p = 0.0002$; Table 3). We found no differences in INR, PTT, factor activity, or levels between MWB and NMWB (all $p > 0.05$; Table 3). Although there were no significant differences between MWB and NMWB in EXTEM or INTEM clot initiation measures (CT, CFT, or α), MWB had significantly greater EXTEM clot strength measures than did NMWB (a10, $p = 0.0203$; a20, $p = 0.0138$; MCF, $p = 0.0031$; Table 3). Similarly, by INTEM, MWB had a trend toward stronger a10 and significantly stronger a20 and MCF (a20, $p = 0.0347$; MCF, $p = 0.0137$; Table 3). Despite the difference in EXTEM and INTEM MCF, there was nosignificant difference between fibrinogen function as measured by FIBTEM MCF between MWB and NMWB (Table 3). Neither MWB nor NMWB demonstrated fibrinolysis (Table 3).

TABLE 2. Clotting Profiles by WB Variants (RTWB Versus CWB Versus MCWB Versus MRTWB)

	RTWB		CWB		MCWB		MRTWB		
	n = 11	SD	n = 11	SD	n = 11	SD	n = 7	SD	*p*
INR	1.29	0.20	1.27	0.20	1.27	0.15	1.32	0.14	0.8897
PT, s	15.77	1.92	15.61	1.94	15.70	1.46	16.09	1.35	0.9498
PTT, s	53.89	18.45	52.55	16.79	48.84	11.73	56.36	16.71	0.7353
Factor II, %	68.64	7.51	73.73	7.99	72.73	10.30	70.71	9.07	0.3171
Factor V, %	59.55	13.82	56.36	14.60	49.18	19.66	52.14	12.58	0.4571
Factor VII, %	70.45	21.30	71.27	19.87	72.27	18.02	71.57	8.54	0.9983
Factor VIII, %	55.18	35.60	56.73	27.76	55.91	26.19	59.29	24.86	0.8139
Factor IX, %	86.18	17.56	89.73	19.07	82.00	17.04	87.86	12.69	0.7644
Factor X, %	74.00	12.37	74.82	14.38	73.55	13.79	72.14	13.16	0.9812
ATIII, %	73.73	18.28	77.82	18.98	77.18	19.16	77.29	18.90	0.677
Protein C, %	81.00	14.39	82.18	15.64	82.82	15.17	84.71	10.72	0.9607
Fibrinogen, mg/dL	225.82	56.04	227.73	55.60	225.91	43.33	213.43	39.66	0.9392
D-dimer, μg/mL	4.66	6.69	4.51	5.87	4.00	6.16	4.02	5.84	0.9302
EXTEM CT, s	220.00	216.31	184.00	119.74	200.27	136.46	207.14	151.87	0.9983
EXTEM CFT, s	622.72	995.07	762.36	1557.49	209.27	122.30	203.86	141.08	0.4145
EXTEM α, degrees	49.90	20.07	48.50	18.81	56.27	13.30	57.57	16.26	0.5672
EXTEM a10, mm	35.09	18.05	34.09	14.44	44.64	9.92	45.57	13.59	0.1749
EXTEM a20, mm	42.82	18.14	43.18	15.87	55.27	8.42	54.86	11.44	0.1052
EXTEM MCF, mm	48.91	17.14	49.00	15.54	61.73	3.55	60.29	7.09	0.0318
EXTEM ML, %	2.45	5.26	1.64	2.34	11.64	12.55	7.57	10.85	0.0468
INTEM CT, s	465.27	256.24	442.73	227.86	396.91	175.89	436.57	158.62	0.9323
INTEM CFT, s	368.09	342.49	368.56	317.20	222.73	114.00	250.57	205.09	0.6404
INTEM α, degrees	48.55	20.59	48.60	14.87	55.27	12.08	59.00	13.55	0.5581
INTEM a10, mm	39.00	16.44	36.18	14.85	45.64	9.04	44.71	15.34	0.3781
INTEM a20, mm	49.09	15.95	47.82	13.43	57.27	5.68	56.29	9.39	0.1789
INTEM MCF, mm	55.64	12.89	54.27	11.55	62.91	4.13	63.71	5.12	0.0840
INTEM ML, %	0.64	0.81	0.82	0.87	0.45	0.52	0.57	0.79	0.8242
APTEM CT, s	206.27	131.50	212.82	104.95	213.36	134.78	190.86	149.99	0.8402
APTEM CFT, s	206.27	131.50	479.45	488.68	312.63	209.27	311.14	296.96	0.5491
APTEM α, degrees	45.73	20.47	44.55	19.32	51.45	15.14	55.00	15.97	0.5808
APTEM a10, mm	29.27	16.99	31.82	14.42	35.91	13.08	39.86	15.88	0.4808
APTEM a20, mm	38.36	16.59	40.82	14.85	46.00	11.45	49.43	14.22	0.3966
APTEM MCF, mm	45.64	17.00	47.91	13.30	55.82	6.63	60.00	6.51	0.0563
APTEM ML, %	9.64	29.99	0.73	0.65	0.36	0.67	0.29	0.49	0.4123
FIBTEM MCF, mm	8.64	4.20	10.82	4.40	10.64	4.84	9.71	1.98	0.6023
WBC, $\times 10^3/\mu L$	4.40	1.31	4.51	1.28	4.15	1.32	4.01	1.51	0.7594
Hgb, g/dL	11.24	1.47	11.52	1.26	10.39	1.22	10.90	1.27	0.2698
Hct, %	37.22	3.80	37.26	3.13	33.77	3.34	35.56	3.55	0.0973
PLTs, $\times 10^9$/L	202.10	67.72	191.90	66.97	299.40	74.19	307.14	78.80	0.0031

Data are given as mean and SD. Significance assessed by analysis of variance for normally distributed data and the Kruskal-Wallis test for nonnormally distributed data. $p < 0.05$, significant for overall comparison; $p < 0.0083$, for intergroup comparisons between multiple groups using standard Bonferroni correction. CT, time to initial clot formation; CFT, time to 20 mm of clot firmness; α, angle of tangent at 2 mm of clot firmness.

D-dimer, dimerized plasmin fragment D; ML, maximum lysis; PT, prothrombin time.

NMWB Versus 1:1:1 RWB

NMWB had significantly higher Hgb, Hct, and Plts than did 1:1:1 RWB (all $p < 0.05$; see Supplement 3, Supplemental Digital Content 3, http://links.lww.com/TA/A456). There was no difference in INR, but NMWB had a significantly prolonged PTT compared with 1:1:1 RWB (53.22 [17.22] seconds vs. 41.76 [4.68] seconds; $p = 0.0117$; see Supplement 3, Supplemental Digital Content 3, http://links.lww.com/TA/A456), which we attribute to the significantly higher factor VIII activity in 1:1:1 RWB compared with NMWB (76.35% [32.17%] vs. 55.95% [31.16%]; $p = 0.0193$). There were no other statistically significant factor differences or functional clotting differences by ROTEM (see Supplement 3, Supplemental Digital Content 3, http://links.lww.com/TA/A456).

MWB Versus 1:1:1 RWB

Finally, given the superior clotting profile of 1:1:1 RWB compared with 2:1:1 RWB and of MWB compared with NMWB, but the lack of notable differences between NMWB and 1:1:1, we lastly compared the clotting profiles of 1:1:1 RWB with MWB in search of the most hemostatic product.

TABLE 3. Clotting Profiles of NMWB Variants Versus MWB Variants

	NMWB		MWB		
	n = 22	SD	n = 18	SD	*p*
INR	1.28	0.20	1.32	0.14	0.5771
PT, s	53.22	17.22	56.36	1.35	1.0000
PTT, s	15.69	1.88	16.09	16.71	0.7605
Factor II, %	71.18	8.00	71.94	9.61	0.5859
Factor V, %	57.95	13.96	50.33	16.89	0.1453
Factor VII, %	70.86	20.11	72.00	14.73	0.9458
Factor VIII, %	55.95	31.16	57.22	24.99	0.4303
Factor IX, %	87.95	17.98	84.28	15.37	0.5766
Factor X, %	74.41	13.10	73.00	13.17	0.7377
ATIII, %	75.77	18.30	77.22	18.49	0.4218
Protein C, %	81.59	14.68	83.56	13.30	0.6599
Fibrinogen, mg/dL	226.77	54.48	221.06	41.22	0.7079
D-dimer, μg/mL	4.59	6.14	4.01	5.87	0.7115
EXTEM CT, s	202.00	171.60	202.94	138.22	0.8919
EXTEM CFT, s	692.54	1277.40	207.17	125.82	0.0972
EXTEM α, degrees	49.20	18.94	56.78	14.07	0.1692
EXTEM a10, mm	34.60	15.96	45.00	11.10	0.0203
EXTEM a20, mm	43.00	16.63	55.11	9.37	0.0138
EXTEM MCF, mm	48.95	15.96	61.17	5.07	0.0031
EXTEM ML, %	2.05	3.99	10.06	11.76	0.0084
INTEM CT, s	454.00	236.90	412.33	165.76	0.9783
INTEM CFT, s	368.32	322.13	233.56	150.62	0.2424
INTEM α, degrees	48.57	17.65	56.59	12.32	0.1911
INTEM a10, mm	37.59	15.36	45.28	11.46	0.1055
INTEM a20, mm	48.45	14.40	56.89	7.10	0.0347
INTEM MCF, mm	54.95	11.96	63.22	4.41	0.0137
INTEM ML, %	0.72	0.83	0.50	0.62	0.4434
APTEM CT, s	209.55	116.15	204.61	136.94	0.7874
APTEM CFT, s	585.18	713.02	312.06	238.51	0.2517
APTEM α, degrees	45.14	19.43	52.83	15.10	0.3327
APTEM a10, mm	30.55	15.44	37.44	13.91	0.1958
APTEM a20, mm	39.59	15.42	47.33	12.31	0.1499
APTEM MCF, mm	46.77	14.94	57.44	6.72	0.0273
APTEM ML, %	5.18	21.19	0.33	0.59	0.4092
FIBTEM MCF, mm	9.73	4.34	10.28	3.92	0.6764
WBC, $\times 10^3/\mu L$	4.46	1.26	4.09	1.36	0.3215
Hgb, g/dL	11.38	1.34	10.60	1.23	0.0732
Hct, %	37.24	3.39	34.51	3.43	0.0207
PLTs, $\times 10^9/L$	197.00	65.76	302.59	73.75	0.0002

Data are given as mean and SD. Significance assessed by the Student's *t* test for normally distributed data and the Wilcoxon rank-sum test for nonnormally distributed data. $p < 0.05$, significant. CT, time to initial clot formation; CFT, time to 20 mm of clot firmness; α, angle of tangent at 2 mm of clot firmness.

D-dimer, dimerized plasmin fragment D; ML, maximum lysis; PT, prothrombin time.

Similar to previous comparison, although there was no difference in INR between the 1:1:1 RWB and the MWB (1.31 [0.18] vs. 1.32 [0.14], $p = 0.8232$), MWB had a significantly prolonged PTT compared with 1:1:1 RWB (56.35 [16.71] seconds vs. 41.76 [4.68] seconds; $p = 0.0117$; Table 4). Again, we attributed this to the significantly higher factor VIII activity in 1:1:1 RWB compared with MWB (76.35% [32.17%] vs. 57.22% [24.99%]; $p = 0.0201$). There were no other notable factor differences to explain this PTT disparity (Table 4). However, overall MWB variants had superior functional clotting profiles by EXTEM and INTEM measures of clot strength than did 1:1:1 RWB. Although there were no significant differences between MWB and 1:1:1 RWB in EXTEM or INTEM CT, CFT, or α, MWB had significantly stronger EXTEM a20 and MCF than did 1:1:1 RWB (a20, $p = 0.0088$; MCF, $p = 0.0005$; Table 4, Fig. 2*D*). Similarly, MWB had

TABLE 4. Clotting Profiles of MWB Variants Versus 1:1:1 RWB

	MWB Composite		1:1:1		
	n = 18	SD	n = 23	SD	*p*
INR	1.32	0.14	1.31	0.18	0.8232
PT, s	56.35	16.71	41.76	4.68	0.0117
PTT, s	16.09	1.35	15.98	1.71	0.7858
Factor II, %	71.94	9.61	65.00	19.81	0.0848
Factor V, %	50.33	16.89	50.91	21.56	0.4942
Factor VII, %	72.00	14.73	62.26	27.41	0.1032
Factor VIII, %	57.22	24.99	76.35	32.17	0.0201
Factor IX, %	84.28	15.37	86.22	25.39	0.6453
Factor X, %	73.00	13.17	66.52	16.12	0.1648
ATIII, %	77.22	18.49	72.65	41.19	0.0697
Protein C, %	83.56	13.30	75.52	24.53	0.1891
Fibrinogen, mg/dL	221.06	41.22	241.65	67.16	0.2345
D-dimer, μg/mL	4.01	5.87	0.25	0.06	0.0026
EXTEM CT, s	202.94	138.22	145.91	42.81	0.2319
EXTEM CFT, s	207.17	125.82	760.57	1560.60	0.7426
EXTEM α, degrees	56.78	14.07	56.27	16.00	0.8489
EXTEM a10, mm	45.00	11.10	38.57	14.04	0.1094
EXTEM a20, mm	55.11	9.37	45.83	15.45	0.0088
EXTEM MCF, mm	61.17	5.07	50.35	15.48	0.0005
EXTEM ML, %	10.06	11.76	3.83	7.48	0.0386
INTEM CT, s	412.33	165.76	379.70	99.82	0.8233
INTEM CFT, s	233.56	150.62	698.83	1510.72	0.9476
INTEM α, degrees	56.59	12.32	52.09	14.22	0.6218
INTEM a10, mm	45.28	11.46	39.87	12.56	0.1661
INTEM a20, mm	56.89	7.10	48.74	14.00	0.0095
INTEM MCF, mm	63.22	4.41	53.57	14.76	0.0009
INTEM ML, %	0.50	0.62	1.00	2.56	0.1436
APTEM CT, s	204.61	136.94	174.00	110.53	0.6647
APTEM CFT, s	312.06	238.51	790.35	1530.98	0.8131
APTEM α, degrees	52.83	15.10	53.77	12.06	0.8320
APTEM a10, mm	37.44	13.91	34.78	13.36	0.5400
APTEM a20, mm	47.33	12.31	43.22	14.95	0.6739
APTEM MCF, mm	57.44	6.72	48.43	16.52	0.0734
APTEM ML, %	0.33	0.59	1.00	2.07	0.4339
FIBTEM MCF, mm	10.28	3.92	12.04	2.96	0.1225
WBC, $\times 10^3/\mu L$	4.09	1.36	0.01	0.03	0.0000
Hgb, g/dL	10.60	1.23	9.05	1.07	0.0003
Hct, %	34.51	3.43	28.93	3.46	0.0000
PLTs, $\times 10^9/L$	302.59	73.75	129.62	22.23	0.0000

Data are given as mean and SD. Significance assessed by the Student's *t* test for normally distributed data and the Wilcoxon rank-sum test for nonnormally distributed data. $p < 0.05$, significant. CT, time to initial clot formation; CFT, time to 20 mm of clot firmness; α, angle of tangent at 2 mm of clot firmness.

D-dimer , dimerized plasmin fragment D; ML, maximum lysis; PT, prothrombin time.

significantly stronger INTEM a20 and MCF than did 1:1:1 RWB (a20, $p = 0.0095$; MCF, $p = 0.0009$; Table 4). Despite the difference in EXTEM and INTEM MCF, there was no significant difference between fibrinogen function as measured by FIBTEM MCF between MWB and 1:1:1 RWB. Neither demonstrated fibrinolysis (Table 4).

DISCUSSION

Heightened understanding of traumatic coagulopathy[12–16,36] and the benefits of hemostatic resuscitation[17–25] has prompted numerous clinical studies examining various ratios of component and WB therapy in military and civilian populations for the treatment of hemorrhagic shock.[7,26–32] However, the scientific foundation of this growing body of literature is limited by the lack of complete characterization of factor-level and functional clotting between banked WB variants and RWB. Without these critical data, we cannot completely understand the clinical or mechanistic benefits of hemostatic resuscitation. This study characterizes and compares the global clotting profiles of banked WB variants and RWB.

We found that the clotting profile of 1:1:1 RWB was significantly more hemostatic relative to 2:1:1 RWB by all measures of standard coagulation, factor activity/levels, and functional clotting. This global hemostatic superiority of 1:1:1 RWB may contribute to the clinical benefits seen in patients receiving balanced hemostatic resuscitation. Significant differences were not found in INR, PTT, or factor activity/levels between the four WB variants (RTWB, CWB, MCWB, and MRTWB). This finding suggests that the factor-rich plasma in the PLTs does not lead to measurable differences in factor activity in the MWB variants compared with the NMWB variants. However, we did find that the addition of PLTs led to greater measures of clot strength by ROTEM in the MRTWB and MCWB compared with their NMWB counterparts. Because fibrin and platelets are the primary contributors to fundamental clot strength, this finding can be ascribed to the added PLTs, given the lack of significant difference in fibrinogen levels or fibrinogen function by FIBTEM MCF. Interestingly, despite the historic dogma that the cooling of platelets leads to dysfunction,[8–10] paradoxically, the MCWB had the highest measure of EXTEM MCF of the WB variants. This finding is consistent with accumulating data demonstrating that cold-stored PLTs have better functional and inflammatory profiles than room temperature–stored PLTs,[37–39] which, if confirmed, would allow for profound advances in the banking and distribution of WB because of the ability to cold store both PLTs and WB. This could make use of WB universally feasible and perhaps preferable for the treatment of hemorrhagic shock in civilian populations.

Despite the lack of differences in standard coagulation measures or factor activity/levels between the four WB variants, significantly superior functional clotting profiles were found in the MWB variants over the NMWB variants. Consistent with the lack of differences in factor activity, there was no difference in clot initiation. However, there was a nonsignificant trend toward a shorter CFT and a greater α in the MWB variants. These measures of the speed of clot formation are strongly influenced by functioning platelets. Most importantly, the MWB variants had significantly greater measures of clot strength. This finding, combined with the lack of difference in fibrinogen levels and fibrinogen function measured by FIBTEM MCF between the MWB and the NMWB, leads us to attribute the superior clot strength to the added PLTs.

Lastly, in search of the optimal functionally hemostatic resuscitation product, we compared 1:1:1 RWB with both NMWB and MWB. Importantly, there were no significant differences in clotting function between 1:1:1 RWB and NMWB. However, we found that MWB variants exhibited even better global hemostasis than did 1:1:1 RWB. Surprisingly, both NMWB and MWB had a prolonged PTT compared with 1:1:1 RWB, which, we hypothesize, may be reflective of the contribution of factor VIII–rich FFP in the 1:1:1 RWB. Alternatively, this may be explained by the difference in storage durations between the WB units and the RBC units used to create the RWB.[34] Despite this, the MWB ultimately had a superior functional clotting profile over 1:1:1 RWB, with significantly greater absolute clot strength. Interestingly, the 1:1:1 RWB trended toward a higher fibrinogen level compared with the MWB. Despite this, MWB still had greater clot strength compared with 1:1:1 RWB, which we accredit to the contribution of PLTs. Importantly, the EXTEM MCF in the MWB was within "normal" published ROTEM manufacturer range whereas it was not for the NMWB or the 1:1:1 RWB. This further supports that MWB may be a superior hemostatic product to NMWB and to 1:1:1 RWB.

Finally, despite our crucial finding that the MWB variants demonstrated unparalleled functional clotting profiles, several limitations need to be addressed. We acknowledge the limitation of studying aged components in comparison with freshly donated WB. We recognize the possibility that the superior hemostasis of the MWB may be partially attributed to its youth, and perhaps, using freshly donated components for creation of the RWB would approach the hemostatic potential of the MWB. It will be important in the future to alter the age of the products compared to completely characterize their differences; nevertheless, aged components are important to characterize and compare because they are frequently supplied in massive transfusion situations. In addition, we cannot account for differences due to leukoreduction or the variability in platelet counts and established storage lesions of the products.[40–45] In future study, leukoreduction of the WB with platelet-sparing filters could be performed to standardize leukoreduction yet retain platelet function. The WB used was nontransfusable due to weight and thus all units were either slightly over- or underweight. Overweight units therefore had a lower anticoagulant:WB ratio and underweight units had a higher anticoagulant: WB ratio. In the future, standardization of these ratios will be critical. Lastly, we are lacking important platelet aggregometry data; however, we have some insight into platelet function via proxy ROTEM measurements and the known dual contribution of fibrin and platelets to clot strength. As previously discussed, the incumbent impact of cooling on platelets must be completely elucidated given that even the room temperature variants underwent a brief period of cooling for initial typing and processing, which may have had some impact on platelet function. However, we believe that fresh warm WB is a futile product to investigate because it logistically may

never be available for massive transfusion in civilian populations because of requirements for infectious disease testing and its limited storage capacity.[46] Therefore, it is of greatest interest to direct future studies at investigating cold-stored WB. The finding that our cold-stored WB trended toward stronger clot by EXTEM MCF than did the RTWB is supported by studies of preservation of coagulation function of cold-stored WB out to 21 days (extending well beyond current banking practices)[2,47] combined with data demonstrating that cold-stored PLTs are functionally superior to room temperature–stored PLTs.[37,38] Lastly, we recognize that clotting potential is probably not solely responsible for the benefits of hemostatic resuscitation, and we appreciate the need to elucidate the role of inflammamodulatory elements between these products. Future investigations of age and leukocyte reduction variations with cold storage of WB and PLTs will be fundamental to ultimately defining the optimal resuscitation product and making paradigm shifts in blood banking and distribution.

AUTHORSHIP

L.Z.K., B.M.H., C.K.C., Y.D., S.P., and M.J.C. contributed to study design, data collection, data analysis, data interpretation, writing, and manuscript revision. M.P.B., S.P., R.A.C., R.F.V., B.J.R., and M.F.N. contributed to study design, data collection, and manuscript revision.

DISCLOSURE

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Computational Modeling of Thrombin Generation in Trauma

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Background

Thrombin plays a central role in the acute response to injury, and its global measurement provides dynamic information beyond conventional clot-based tests. Computational models of thrombin generation describe the coagulation network using ordinary differential equations, with individual coagulation factor input data. We applied an established computational coagulation model to trauma.

Aims

To assess the efficacy and predictive capacity of a computational coagulation model in a trauma population.

Methods

- Plasma samples collected from 41 critically-injured trauma patients at time of admission.
- Coagulation factor levels (II, V, VII, VIII, IX, X, tissue factor pathway inhibitor, antithrombin and protein C) for each individual used as inputs in a previously validated mathematical model; thrombin profiles generated, corresponding dynamic parameters extracted.
- Demographic, resuscitation, and outcomes data were collected in parallel.
- Analysis by dichotomization of model variables
- Acute traumatic coagulopathy (ATC) defined as two or more of the following:
  INR > 1.3, ISS > 15, BD < -6, aPC > 5

Results

Figure 1: Derivation of Computational Model

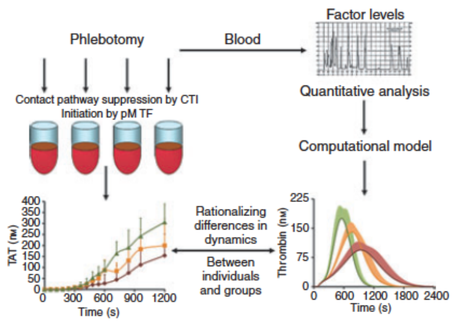

Figure 2: Thrombin Model Parameters

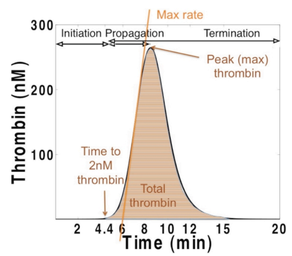

Table 1: Time to 10nm Thrombin Formation

	Long onset (n=19)	Short onset (n=19)	*p*-value
Age (years)	34 (22 – 45)	36 (24 – 70)	0.148
Male gender	95%	84%	0.604
Blunt mechanism	37%	53%	0.515
Injury Severity (ISS)	17(10 - 22)	25 (17 - 38)	**0.035**
AIS-Head	2 (0 - 3)	3 (0 - 5)	0.130
Admit base deficit	-5.5 +/- 5.1	-6.9 +/- 7.6	0.499
Admit INR	1.1 (1 - 1.2)	1.1 (1 - 1.2)	0.975
Admit PTT (s)	27 (25 - 29)	31 (28 - 36)	**0.001**
Massive Transfusion	0%	37%	**0.008**
Total ICU days	3 (2 – 8)	3 (2 - 12)	0.847
Multi-organ failure	5%	11%	1.000
Mortality at discharge	5%	32%	0.090

Dichotomized at median time to 10nm thrombin, 312.5 sec.

Percentages unless specified; median (interquartile range), mean +/- SD.

Table 2: Max Rate Thrombin Formation

	Slow rate (n=20)	Fast rate (n=21)	*p*-value
Age (years)	45 (30 - 68)	31 (21 - 43)	**0.027**
Male gender	85%	95%	0.343
Blunt mechanism	65%	29%	**0.029**
Injury Severity (ISS)	25 (15 - 41)	18 (16 - 24)	0.170
AIS-Head	4 (1 - 5)	0 (0 - 3)	**0.007**
Admit base deficit	-6.0 +/- 6.4	-7.2 +/- 6.5	0.569
Admit INR	1.1 (1 – 1.4)	1.1 (1 - 1.2)	0.500
Admit PTT (s)	31 (29 - 38)	27 (25 - 29)	**<0.001**
Massive Transfusion	35%	10%	0.067
Total ICU days	5 (2 - 17)	3 (2 - 6)	0.301
Multi-organ failure	10%	10%	1.000
Mortality at discharge	35%	10%	0.067

Dichotomized at median maximum rate, 0.9 nm/sec.

Table 3: Peak Thrombin Formation

	Low peak (n=21)	High peak (n=20)	*p*-value
Age (years)	44 (28 - 66)	32 (22 - 44)	0.078
Male gender	86%	95%	0.606
Blunt mechanism	62%	30%	0.062
Injury Severity (ISS)	25 (16 - 41)	18 (14 - 25)	0.196
AIS-Head	4 (0 - 5)	1 (0 - 3)	**0.019**
Admit base deficit	-6.3 +/- 6.4	-7.0 +/- 6.6	0.758
Admit INR	1.1 (1 - 1.4)	1.1 (1 - 1.2)	0.282
Admit PTT (s)	31 (28 - 36)	27 (26 - 29)	**<0.001**
Massive Transfusion	38%	5%	**0.020**
Total ICU days	4 (2 – 12)	3 (2 – 6.5)	0.483
Multi-organ failure	10%	10%	1.000
Mortality at discharge	33%	10%	0.130

Dichotomized at median peak thrombin level, 116.8 nm.

Table 4: Peak Thrombin Formation

	Low AUC (n=20)	High AUC (n=21)	*p*-value
Age (years)	42 (26 - 68)	33 (22 - 44)	0.103
Male gender	85%	95%	0.343
Blunt mechanism	60%	33%	0.121
Injury Severity (ISS)	25 (17 - 41)	18 (14 - 24)	0.114
AIS-Head	4 (1 - 5)	0 (0 - 3)	**0.007**
Admit base deficit	-6.4 +/- 6.5	-6.9 +/- 6.4	0.797
Admit INR	1.1 (1 – 1.5)	1.1 (1 - 1.2)	0.168
Admit PTT (s)	31 (28 - 38)	27 (25 – 29)	**0.001**
Massive Transfusion	40%	5%	**0.009**
Total ICU days	4 (2 - 17)	3 (2 - 7)	0.701
Multi-organ failure	10%	10%	1.000
Mortality at discharge	35%	10%	0.067

Dichotomized at median Area Under Curve (AUC), 33,590 nm*m.

Table 5: Thrombin Parameters by ATC

	ATC (n=22)	No ATC (n=19)	*p*
T to 10nM (s)	363 (313 - 604)	261 (249 - 381)	**0.017**
Max Rate (nm/s)	0.48 (0.09 - 1.13)	1.11 (0.56 - 1.51)	0.075
Peak Thrombin (nm)	71 (19 - 143)	150 (83 - 194)	0.056
Total thrombin (µm*m)	18.5 (5.1 - 42.9)	40.8 (21.6 – 55.2)	0.056

Conclusions

- In a trauma population at time of admission, computational thrombin modeling parameters correspond to profiles of injury severity and resuscitation requirements.
- Computational thrombin parameters differ in patients with acute traumatic coagulopathy.
- Modeling derived from baseline parameters at admission may describe and predict coagulation dynamics better than conventional measures.
- Ongoing work to delineate dynamic changes in computational model over time in critically injured, and resuscitated, patients

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The Natural History and Effect of Resuscitation Ratio On Coagulation After Trauma

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Background

- One third of trauma patients present with acute traumatic coagulopathy (ATC)
- ATC is associated with a 4x increased mortality
- Balanced transfusion ratios of red blood cell (RBC) to fresh frozen plasma (FFP) are associated with a survival advantage in ATC
- This survival benefit is present even in patients with normal-range coagulation studies on admission, suggesting mechanisms beyond simple coagulation factor repletion

Aims

- Examine longitudinal changes in coagulation profiles in transfused patients in relation to RBC:FFP transfusion ratios
- Correlate transfusion ratios to baseline demographics and clinical outcomes

Methods

- Citrated plasma samples prospectively collected from 336 trauma patients on arrival and serially during ICU stay up to 72h from 2005 – 2011
- Standard coagulation studies as well as extensive panel of pro- and anticoagulant clotting factors were measured
- RBC:FFP ratios calculated as whole RBC units divided by FFP units received within 6h, and dichotomized into 'low ratio' (RBC:FFP ≤ 1.5:1) and 'high ratio' (RBC:FFP > 1.5:1)

Results

Table 1: Summary statistics by transfusion

	Non-transfused (n = 193)	Transfused (n = 143)	*p*-value
Age (years)	43.4 ± 19.6	40.2 ± 19.5	0.135
Penetrating injury	18.8%	46.5%	<0.001*
ISS	21.8 ± 13.7	34.1 ± 16.0	<0.001*
AIS-head	4 (0 - 5)	4 (0 - 5)	0.976
GCS	7 (4 - 14)	8.5 (3 - 15)	0.615
pH	7.31 ± 0.14	7.22 ± 0.15	<0.001*
Base deficit	-4.9 ± 5.5	-9.1 ± 6.7	<0.001*
Prehospital IVF (mL)	200 (0 - 500)	100 (0 - 300)	0.141
Temperature (°C)	35.6 ± 0.9	35.5 ± 0.9	0.372
RBC units / 24h	0	9 (5 - 17)	-
FFP units / 24h	0	6 (4 - 12)	-
Platelet units / 24h	0	1 (0 – 2)	-
Factor VIIa given	0%	11.9%	<0.001*
PT (sec)	13.8 (13.1 - 15.4)	15.7 (14.2 - 19.6)	<0.050*
PTT (sec)	29.8 (26.7 - 33.8)	30.8 (26.9 - 38.3)	<0.050*
Factor V (%)	56.5 ± 27.9	41.5 ± 26.1	<0.050*
aPC (ng/mL)	3.1 (1.5 - 9.5)	12.1 (3.5 - 37.8)	<0.050*
tPA (ng/mL)	15.2 (8.4 - 27.3)	27.9 (12.1 - 33.4)	<0.050*
Multiorgan failure	12.5%	21.1%	<0.001*
Mortality	20.2%	47.6%	<0.001*

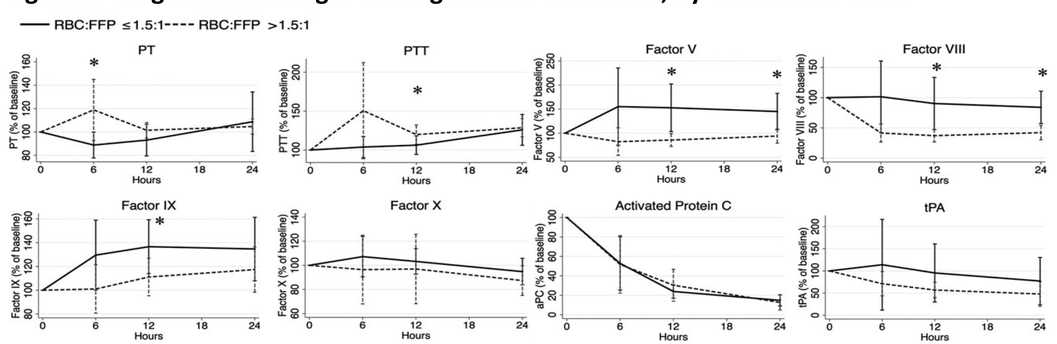
Dichotomized by whether patients received any transfusion within 6 hours. $p < 0.05$
Percentages unless otherwise specified; median (interquartile range), mean +/- SD.

Table 2: Summary statistics by RBC:FFP ratio

	Low Ratio RBC:FFP ≤ 1.5:1 (n = 91)	High Ratio RBC:FFP > 1.5:1 (n = 52)	*p*-value
RBC:FFP ratio	1 (0.8 - 1.2)	2.2 (2.0 - 3.0)	-
Age (years)	38.7 ± 18.3	42.8 ± 21.5	0.253
Penetrating injury	42.2%	53.8%	0.222
ISS	32.7 ± 16.2	36.6 ± 15.5	0.164
AIS-head	5 (0 - 5)	1 (0 - 5)	0.009*
GCS	6 (3 - 15)	12 (4 - 15)	0.029*
pH	7.23 ± 0.15	7.19 ± 0.14	0.145
Base deficit	-7.8 ± 6.4	-11.3 ± 6.7	0.004*
Prehospital IVF (mL)	150 (0 - 300)	0 (0 - 325)	0.196
Temperature (°C)	35.8 ± 0.8	35.0 ± 0.9	0.004*
RBC units / 24h	7 (4 - 14)	10 (7 - 22)	0.001*
FFP units / 24h	7 (4- 12)	4.5 (3 - 11)	0.160
Platelet units / 24h	0 (0 - 2)	1 (0 – 2)	0.525
Factor VIIa given	11.0%	13.5%	0.789
PT (sec)	16.7 (14.5 - 21.2)	16.2 (14.6 - 20.6)	>0.050
PTT (sec)	32.5 (27.5 - 42.2)	32.5 (27.6 - 41.8)	>0.050
Factor V (%)	38.7 ± 24.7	38 ± 26.8	>0.050
aPC (ng/mL)	15.3 (5.4 - 49.5)	13.7 (3.5 – 51.2)	>0.050
tPA (ng/mL)	31 (9 - 46.7)	25 (14.7 - 38.3)	>0.050
Multiorgan failure	25.6%	13.5%	0.134
Mortality	42.9%	55.8%	0.165

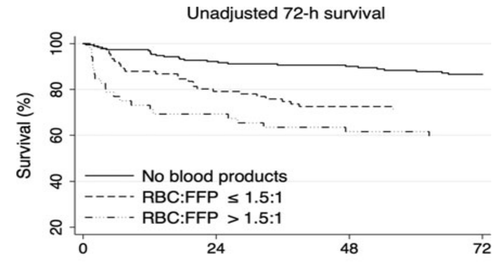
Percentages unless otherwise specified; median (interquartile range), mean +/- SD.

Figure 1: Longitudinal Changes in Coagulation Parameters, by Transfusion Ratio



N = 143. * $p < 0.05$ for comparison between "low" and "high" ratio patients at each time point.

Figure 2: Survival by RBC:FFP ratio



Unadjusted Kaplan-Meier survival by RBC:FFP ratio. N = 336.
Log rank p<0.001 between groups.

Table 3: Cox Proportional Hazards Model of In-Hospital Mortality

Variable	HR	95% CI	*p*-value
Age	1.038	(1.024 - 1.052)	<0.001
ISS	1.023	(1.008 - 1.038)	0.002
Arrival GCS	0.803	(0.749 - 0.861)	<0.001
Arrival base deficit	1.029	(0.989 - 1.069)	0.154
Arrival INR	0.958	(0.659 - 1.392)	0.820
Penetrating injury	2.362	(1.213 - 4.599)	0.011
Non-transfused	-	-	-
Low ratio	1.661	(0.943 - 2.925)	0.079
High ratio	3.402	(1.693 - 6.833)	0.001

N=336. In-hospital mortality outcome occurred in 107 cases. Harrell's C = 0.829.

Conclusions

- Balanced resuscitation leads to earlier correction of coagulopathy, and earlier and prolonged repletion of specific factors
- Mechanism of clinical benefit seen in balanced resuscitation remains unknown:
  - reversal of coagulopathy
  - inflammomodulatory changes
- Balanced resuscitation as interim standard of care for transfusion in the critically injured

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The Natural History and Effect of Resuscitation Ratio on Coagulation After Trauma

A Prospective Cohort Study

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Objective: To investigate the natural history of coagulation factor perturbation after injury and identify longitudinal differences in clotting factor repletion by red blood cell:fresh frozen plasma (RBC:FFP) transfusion ratio.

Background: Hemostatic transfusion ratios of RBC to FFP approaching 1:1 are associated with a survival advantage in traumatic hemorrhage, even in patients with normal coagulation studies.

Methods: Plasma was prospectively collected from 336 trauma patients during their intensive care unit stay for up to 72 hours from February, 2005, to October, 2011. Standard coagulation studies as well as pro- and anticoagulant clotting factors were measured. RBC:FFP transfusion ratios were calculated at 6 hours after arrival and dichotomized into "low ratio" (RBC:FFP $\leq$ 1.5:1) and "high ratio" (RBC:FFP $>$ 1.5:1) groups.

Results: Factor-level measurements from 193 nontransfused patients provide an early natural history of clotting factor-level changes after injury. In comparison, 143 transfused patients had more severe injury, prolonged prothrombin time and partial thromboplastin time (PTT), and lower levels of both pro- and anticoagulants up to 24 hours. PTT was prolonged up to 12 hours and only returned to admission baseline at 48 hours in "high ratio" patients versus correction by 6 hours in "low ratio" patients. Better repletion of factors V, VIII, and IX was seen longitudinally, and both unadjusted and injury-adjusted survival was significantly improved in "low ratio" versus "high ratio" groups.

Conclusions: Resuscitation with a "low ratio" of RBC:FFP leads to earlier correction of coagulopathy, and earlier and prolonged repletion of some but not all procoagulant factors. This prospective evidence suggests hemostatic resuscitation as an interim standard of care for transfusion in critically injured patients pending the results of ongoing randomized study.

Keywords: coagulation, injury, transfusion

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Trauma remains a major cause of morbidity and mortality in both civilian and military populations, and the majority of preventable traumatic deaths occur as a result of hemorrhage.[1–3] Although transfusion as a bridge to surgical hemostasis is an undisputed mainstay of therapy for hemorrhagic shock, the optimal practice of transfusion has changed in the last 10 years in response to the recognition of coagulopathy as a critical predictor of poor outcomes. Specifically, 25% to 38% of traumatically injured patients are found to have abnormal coagulation on arrival to the hospital, and patients with this acute traumatic coagulopathy (ATC) go on to be 4 times more likely to die.[2,4–6] Thus, a thorough understanding of optimal transfusion strategies to both treat the end-organ hypoperfusion of hemorrhagic shock as well as to correct (or prevent) coagulopathy are critical to the acute care of trauma patients. To this end, the practice of "hemostatic resuscitation"—the empiric transfusion of plasma at a fixed ratio with red blood cells—has evolved over many years as a potential therapeutic strategy to address both goals.[7–11]

Multiple large retrospective studies have demonstrated that empiric transfusion of red blood cell (RBC) and fresh frozen plasma (FFP) units in RBC:FFP ratios approaching 1:1 are associated with a survival advantage in hemorrhagic shock after traumatic injury in both civilian[12,13] and military settings.[14–16] Alongside clinical enthusiasm for this novel approach, however, exists significant concern regarding the survival bias inherent in such retrospective studies.[17,18] One of the major barriers to interpreting the observed survival advantage associated with hemostatic resuscitation is the lack of a well-understood biological mechanism, making dynamic monitoring of efficacy difficult and meaningful clinical endpoints unclear. Two competing hypotheses exist. One primary hypothesis suggests that plasma-based resuscitation leads to better repletion of coagulation factors by earlier- and higher-volume FFP transfusion. Although this is clinically intuitive, there are currently no data examining the relationship of plasma-based resuscitation and coagulation factor repletion. Alternatively, others have suggested that earlier and increased plasma transfusion may modulate the inflammomodulatory cascade triggered by severe injury,[19,20] thus treating an "endotheliopathy" of trauma. Evidence to support this hypothesis exists in the fact that the survival benefit to plasma-based resuscitation strategies is evident even in patients with normal-range coagulation studies on admission.[21]

To address these competing hypotheses, the first aim of this study was to investigate differences in longitudinal clotting factor levels in a critically injured cohort of trauma patients who were not transfused, to establish an early natural history of coagulation after injury. The second aim was to examine longitudinal changes in coagulation profiles in transfused patients in relation to RBC:FFP transfusion ratios, assessed by dichotomizing patients into "low ratio" (RBC:FFP $\leq$ 1.5:1) and "high ratio" (RBC:FFP $>$ 1.5:1) groups. Overall, these data provide a novel and comprehensive assessment of changes in the clotting factor milieu after critical injury by describing an early natural history to coagulation abnormalities, as well as identifying the differential effects of transfusion strategies on the correction of these abnormalities over time.

METHODS

Patient Sample, Study Design, and Clinical Data

We performed a prospective cohort study of patients requiring highest level trauma activation and subsequent intensive care unit

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(ICU) admission, beginning on arrival to the emergency department (ED) of San Francisco General Hospital from February 2005 to October 2011 as part of a longitudinal study examining perturbations in coagulation and inflammation after injury. Highest-level trauma activation was triggered by either prespecified physiologic (at least 1 prehospital or hospital systolic blood pressure <90, heart rate >110, or Glasgow Coma Score ≤ 8) or anatomic criteria (penetrating torso trauma or evidence of high-energy blunt trauma), or the clinical discretion of prehospital providers, ED triage nurses, or attending physicians. ICU admission was at the discretion of the attending trauma surgeon; patients who died in the operating room or ED before ICU admission were also included. During the study period, 3775 patients met criteria for highest level trauma activation, with 2066 subsequently admitted to the ICU and thus eligible for study enrollment. Of these, 289 patients were prospectively excluded for age less than 18 years, incarceration, pregnancy, transfer from another hospital, or administration of more than 2 L of crystalloid before initial blood draw. Patients were retrospectively excluded if they were found to be on warfarin or possessed a preexisting bleeding diathesis at the time of injury. Sample and data collection was performed for these 1777 patients under a waiver of consent approved by the University of California institutional review board. Consent was subsequently obtained from patients or appropriate surrogates in 535 patients; of these, 336 patients had serial clotting factor measurements performed and complete transfusion data available, constituting the final patient population for analysis.

As an observational study, the decision to transfuse and the specific array of blood products transfused were entirely at the discretion of the attending trauma surgeon. An institutional massive transfusion protocol exists, whose activation prompts delivery of "packs" of 4U RBC and/or 4U FFP and prompts consideration of a pooled donor platelet "6-pack" for platelets less than 100,000 per mL and 2U pooled cryoprecipitate for fibrinogen less than 100 mg/dL; protocol activation is at the discretion of the attending trauma surgeon or anesthesiologist. A minimum of 4U thawed AB plasma and 6U of type O blood are available in the blood bank at all times for immediate release. Adjunct hemostatic agents such as recombinant factor VIIa (NovoSeven RT; Novo Nordisk Inc, Plainsboro, NJ) and prothrombin complex concentrate (Bebulin VH; Baxter, Westlake Village, CA) were administered at the discretion of the attending trauma surgeon or anesthesiologist; no enrolled patient received tranexamic acid or other antifibrinolytic agent during the study period.

Our sample collection methodology has been described in detail previously.[5,22] Briefly, serial 10-mL samples of blood were drawn in citrated vacuum tubes upon arrival to the ED, and then serially at 6, 12, 24, 48, and 72 hours after admission to the ICU. Samples were immediately centrifuged, and plasma extracted and stored at -80°C; all analysis was performed by researchers blinded to all patient data. Levels of fibrinogen, the procoagulant factors II, V, VII, VIII, IX, and X; the endogenous anticoagulants antithrombin III (ATIII); protein C; activated protein C (aPC); and plasminogen activator inhibitor-1 (PAI-1) were assayed. Fibrinogen; factors II, V, VII, VIII, IX, and X; antithrombin III; and protein C were measured with a Stago Compact Coagulation Analyzer (Diagnostica Stago Inc, Parsippany, NJ) in accordance with manufacturer instructions. Activated protein C measurements were performed on samples collected in citrated tubes containing 10-mM benzamidine using an enzyme capture assay described in detail elsewhere.[5] PAI-1 measurements were performed with an enzyme-linked immunosorbent assay (eBiosciences, San Diego, CA).

Statistical Analysis

All data are presented as mean ± standard deviation, median [interquartile range (IQR)], or percentage. The study population was subdivided on the basis of 2 criteria: transfusion status and RBC:FFP ratio. First, we dichotomized the cohort into those who were transfused within 24 hours and those who received no blood products within 24 hours of admission. In the transfused patient group, RBC:FFP ratios were calculated as the number of whole RBC units divided by FFP units received in the first 6 hours. The 6 hours' time point was the earliest time point with available data after arrival; this was selected to capture patients requiring acute transfusion in response to hemorrhagic shock. Transfused patients were dichotomized into "low ratio" and "high ratio" groups using the median RBC:FFP ratio in the group rounded to the nearest $\frac{1}{2}$ unit (1.5 RBC:1 FFP) as a cut-point to ensure relative equality of group size. Univariate comparisons were made using the Student *t* test for normally distributed data, Wilcoxon rank sum testing for skewed data, and the Fisher exact test for proportions. Paired continuous factor-level measurements were compared using the paired *t* test for normally distributed data and the Wilcoxon signed rank test for skewed data. Kaplan-Meier time-to-event analysis and log-rank testing were used to assess differences in in-hospital mortality between groups. Cox proportional hazards regression was used to adjust for baseline demographic, injury, and physiology characteristics. An alpha of 0.05 was considered significant. All data analyses were performed by the authors using Stata version 12 (StataCorp LP, College Station, TX).

RESULTS

We prospectively enrolled, collected serial blood samples from, and assayed coagulation factors in 336 patients. This population represents a critically injured cohort of trauma patients: mean age was 42.1 ± 19.6 years, penetrating injury occurred in 30.6%, mean injury severity score (ISS) was 27.0 ± 15.9, mean base deficit was −6.9 ± 6.4, and in-hospital mortality was 31.8% (Table 1). Forty (11.9%) patients were coagulopathic [international normalized ratio (INR) ≥ 1.5] and 131 (40.0%) patients were in shock (base deficit ≤ -6) on arrival. Other cohort demographic information is detailed in Table 1.

To begin to investigate resuscitation practices in these patients, we dichotomized the cohort into those who were transfused within 24 hours (143 patients) and those who received no blood products within 24 hours of admission (193 patients); demographics of these cohorts are given in Table 2. As expected, the 143 transfused

TABLE 1. Summary Statistics for All Patients

Age (yr)	42.1 ± 19.6
Penetrating injury	30.6%
ISS	27.0 ± 15.9
AIS head	4 (0–5)
GCS	8 (3–14)
pH	7.27 ± 0.15
Base deficit	−6.9 ± 6.4
Prehospital intravenous fluid	100 (0–500)
Temperature (°C)	35.6 ± 0.9
RBC units/24 h	0 (0–7)
FFP units/24 h	0 (0–5)
Platelet units/24 h	0 (0–0)
Factor VIIa given	5.1%
Total hospital days	8 (3–24)
ICU days	4 (2–12)
Ventilator-free days / 28 d	15 (0–26)
Ventilator-associated pneumonia	27.4%
Acute lung injury	29.9%
Multiorgan failure	16.2%
Mortality	31.8%

N = 336.

TABLE 2. Summary Statistics for Patients by Transfusion Within 6 hours of Arrival

	Nontransfused (n = 193)	Transfused (n = 143)	*P*
Age (yr)	43.4 ± 19.6	40.2 ± 19.5	0.135
Penetrating injury	18.8%	46.5%	<0.001*
ISS	21.8 ± 13.7	34.1 ± 16.0	<0.001*
AIS head	4 (0–5)	4 (0–5)	0.976
GCS	7 (4–14)	8.5 (3–15)	0.615
pH	7.31 ± 0.14	7.22 ± 0.15	<0.001*
Base deficit	−4.9 ± 5.5	−9.1 ± 6.7	<0.001*
Prehospital intravenous fluid	200 (0–500)	100 (0–300)	0.141
Temperature (°C)	35.6 ± 0.9	35.5 ± 0.9	0.372
RBC units/24 h	0	9 (5–17)	—
FFP units/24 h	0	6 (4–12)	—
Platelet units/24 h	0	1 (0–2)	—
Factor VIIa given	0.0%	11.9%	<0.001*
Total hospital days	8 (3–20)	9 (2–30)	0.573
ICU days	4 (2–9)	4 (2–19)	0.487
Ventilator-free days/28 d	24 (0–26)	0 (0–22)	<0.001*
Ventilator-associated pneumonia	22.9%	33.3%	0.073
Acute lung injury	19.7%	44.4%	<0.001*
Multiorgan failure	12.5%	21.1%	<0.001*
Mortality	20.2%	47.6%	<0.001*

N = 336.
*$P < 0.05$.

TABLE 3. Summary Statistics for Patients by RBC:FFP Transfusion Ratio Within 6 Hours of Arrival

	Low Ratio RBC:FFP ≤ 1.5:1 (n = 91)	High Ratio RBC:FFP > 1.5:1 (n = 52)	*P*
RBC:FFP ratio	1 (0.8–1.2)	2.2 (2.0–3.0)	—
Age	38.7 ± 18.3	42.8 ± 21.5	0.253
Penetrating injury	42.2%	53.8%	0.222
ISS	32.7 ± 16.2	36.6 ± 15.5	0.164
AIS head	5 (0–5)	1 (0–5)	0.009*
GCS	6 (3–15)	12 (4–15)	0.029*
pH	7.23 ± 0.15	7.19 ± 0.14	0.145
Base deficit	−7.8 ± 6.4	−11.3 ± 6.7	0.004*
Prehospital intravenous fluid	150 (0–300)	0 (0–325)	0.196
Temperature (°C)	35.8 ± 0.8	35.0 ± 0.9	0.004*
RBC units/24 h	7 (4–14)	10 (7–22)	0.001*
FFP units/24 h	7 (4- 12)	4.5 (3–11)	0.160
Platelet units/24 h	0 (0–2)	1 (0–2)	0.525
Factor VIIa given	11.0%	13.5%	0.789
Total hospital days	11 (2–32)	6.5 (1.5–24)	0.135
ICU days	5 (2–22)	3 (1–12)	0.028*
Ventilator-free days/28 d	0 (0–24)	0 (0–20.5)	0.551
Ventilator-associated pneumonia	40.0%	23.4%	0.074
Acute lung injury	46.0%	41.7%	0.834
Multiorgan failure	25.6%	13.5%	0.134
Mortality	42.9%	55.8%	0.165

N = 143.
*$P < 0.05$.

patients had more severe injury, a higher percentage of penetrating injury, higher ISS, lower pH, more severe base deficit, fewer ventilator-free days, a higher incidence of acute lung injury, multiorgan failure, and higher overall mortality (all $P < 0.001$, Table 2). Sixty-four patients (44.8% of transfused patients, 19.0% of the overall cohort) received "massive" transfusions of 10 or more units RBC per 24 hours. Seventeen transfused patients (11.9%) received recombinant factor VIIa. The median RBC:FFP ratio in transfused patients at 6 hours was 1.25:1; therefore, we selected a clinically accessible ratio of 1.5:1 to subdivide the transfused cohort into "low ratio" (RBC:FFP ≤ 1.5:1) and "high ratio" (RBC:FFP > 1.5:1) based on the number of whole units of RBC and FFP transfused by 6 hours of admission. Of the 143 transfused patients, 91 patients received a "low ratio" with a median RBC:FFP ratio of 1:1 (IQR: 0.8-1.2:1). The other 52 transfused patients received a "high ratio" of RBC:FFP with a median of 2.2:1 (IQR: 2.0-3.0:1; Table 3). As expected, the 91 patients transfused with a "low ratio" received fewer RBC units per 24 hours, with a median of 7 (4–14) units versus 10 (7–22) units in the "high ratio" group ($P = 0.001$; Table 3). The "low ratio" group was comparable in age, mechanism of injury, and ISS to the "high ratio" group; however, they had higher AIS (abbreviated injury score) head score (median 5 vs 1, $P < 0.05$) and lower Glasgow coma score (GCS) (median 6 vs 12, $P < 0.05$). In addition, those transfused with a "high ratio" had a significantly more severe base deficit (mean = −11.3 vs −7.8, $P = 0.004$), lower temperature (mean = 35.0 vs 35.8°, $P = 0.004$), and a trend toward higher mortality rate (55.8% vs 42.9%, $P = 0.165$). Patients transfused with a "low ratio" had trends toward longer hospital stay (median = 11 vs 6.5 days, $P = 0.135$), and higher rates of ventilator-associated pneumonia (40.0% vs 23.4%, $P = 0.074$) and multiorgan failure (25.6% vs 13.5%, $P = 0.134$), as well as significantly longer ICU stay (median 5 vs 3 days, $P = 0.028$; Table 3).

After separately evaluating the demographics of the study population based on transfusion and resuscitation ratios, we then sought to describe specific differences in admission and longitudinal coagulation factor levels. The 193 nontransfused patients represent a cohort of injured patients in which serial coagulation factor measurements reflect an unperturbed course, thus describing the natural history of factor levels after injury. Differences between admission and later factor levels at all subsequent time points up to 48 hours were statistically significant for prothrombin time (PT), partial thromboplastin time (PTT), factor II, factor V, factor VIII, factor X, and activated protein C, as described in detail in Table 4. Overall, compared to admission baseline, PT and PTT became progressively prolonged, and factor levels of both endogenous procoagulants and anticoagulants became progressively depleted in the nontransfused cohort; factors V and IX were exceptions, in that they progressively increased over time (Table 4). In the cohort of 143 transfused patients, differences between admission and later factor levels at all subsequent time points up to 48 hours were statistically significant for PTT, factor VII, factor VIII, factor IX, protein C, and activated protein C, as described in Table 5. Overall, compared to admission baseline, PTT became progressively prolonged for 48 hours after admission and only returned to baseline at 72 hours. Similarly to the nontransfused cohort, factor VIII levels remained lower than admission baseline for 72 hours, factor IX levels rose from admission baseline to 72 hours, and activated protein C levels dropped significantly by 6 hours and remained low through 72 hours. In transfused patients, protein C levels remained depleted relative to admission baseline for up to 72 hours, compared to the repletion by 24 hours seen in the nontransfused cohort. Factor VII levels showed a varied pattern, with early repletion within 12 hours, followed by depletion relative to admission levels from 24 to 48 hours, and subsequent return to admission levels by 72 hours. Given the critical differences in the transfused versus nontransfused patient population, we also compared baseline factor levels between the transfused and nontransfused cohorts: PT and PTT were

TABLE 4. Longitudinal Factor Levels in Patients Receiving No Blood Products Within 72 Hours of Arrival

	0 h	6 h	12 h	24 h	48 h	72 h
PT (sec)	13.8 (13.1–15.4)*	14.7 (13.8–15.6)†	14.4 (13.7–15.7)†	15.6 (14.2–16.8)†	15.2 (14.0–16.2)†	14.6 (14.2–16.5)†
PTT (sec)	29.8 (26.7–33.8)*	30.9 (28.8–34.9)†	33.1 (29.3–36.5)†	38.6 (33.7–42.8)†	42.0 (37.1–49.5)†	44.6 (39.9–46.9)†
Fibrinogen (ng/mL)	234 (151–289)	260 (233–306)	251 (211–303)	233 (208–259)	506 (350–582)	720 (622–818)
Factor II (%)	81.4 ± 19.1*	74.7 ± 18.2†	75.9 ± 18.0†	73.1 ± 17.6†	69.5 ± 19.1†	83.2 ± 18.7
Factor V (%)	56.5 ± 27.9*	56.7 ± 24.0†	56.6 ± 21.2†	57.2 ± 22.1†	68.0 ± 22.3†	81.3 ± 29.3†
Factor VII (%)	95.4 ± 33.9*	95.9 ± 28.9	94.0 ± 30.6	67.6 ± 26.9†	61.3 ± 33.0†	81.1 ± 29.4
Factor VIII (%)	218.8 ± 171.8	136.6 ± 106.5†	128.5 ± 76.3†	137.6 ± 127.7†	139.4 ± 53.2†	177.0 ± 63.4†
Factor IX (%)	129.2 ± 40.9*	130.1 ± 45.9	138.1 ± 46.8†	140.8 ± 43.5†	172.2 ± 58.4†	226.2 ± 93.1†
Factor X (%)	86.3 ± 22.9*	78.7 ± 23.1†	79.8 ± 22.0†	74.5 ± 18.6†	66.3 ± 17.1†	76.2 ± 20.6
AT III (%)	89.8 ± 21.8*	87.3 ± 31.4	84.8 ± 22.2†	83.7 ± 21.0†	83.1 ± 23.4	84.1 ± 28.3
Protein C (%)	96.4 ± 28.8*	92.1 ± 23.3†	89.5 ± 27.0†	81.5 ± 24.7†	81.9 ± 23.0	87.0 ± 25.0
aPC (ng/mL)	3.1 (1.5–9.5)*	0.9 (0.2–2.1)†	1.0 (0.2–1.7)†	1.2 (0.2–1.7)†	1.0 (0.1–1.8)†	0.6 (0.3–1.4)†
D-dimer (μg/mL)	1.7 (0.5–4.0)*	1.6 (0.7–3.6)†	2.3 (0.9–4.1)†	2.4 (1.0–4.0)	1.7 (1.0–4.2)	1.9 (1.3–3.1)
tPA (ng/mL)	15.2 (8.4–27.3)*	12.1 (6.9–19.0)†	6.0 (4.6–14.2)†	6.7 (5.7–9.4)†	6	6.7
PAI-1 (ng/mL)	38.0 (9.6–60.7)	73.4 (44.1–91.4)	38.8 (19.3–55.2)	33.2 (17.8–71.0)	11.7 (8.1–15.0)	17.4

N = 193.
*$P < 0.05$ for comparison between transfused and nontransfused patients on arrival only.
†$P < 0.05$ for comparison between admission factor level and factor level at the indicated time.

TABLE 5. Longitudinal Factor Levels in Patients Receiving Blood Products Within 72 Hours

	0 h	6 h	12 h	24 h	48 h	72 h
PT (sec)	15.7 (14.2–19.6)*	15.6 (14.4–17.8)	15.7 (14.7–17.1)	16.7 (15.5–18.2)	16.6 (14.9–18.8)	15.5 (14.3–17.3)
PTT (sec)	30.8 (26.9–38.3)*	32.8 (28.9–38.0)†	34.3 (31.8–39.6)†	38.6 (34.5–43.6)†	40.8 (36.6–47.4)†	39.0 (33.8–43.8)†
Fibrinogen (ng/mL)	160 (125–221)	153 (124–226)	196 (152–237)	288 (189–340)†	398 (281–566)†	499 (294–716)†
Factor II (%)	66.5 ± 21.2*	62.2 ± 16.6†	63.8 ± 16.0†	64.0 ± 16.0†	65.6 ± 17.5	70.0 ± 15.4
Factor V (%)	41.5 ± 26.1*	41.4 ± 19.6†	43.3 ± 18.7	46.5 ± 21.0	54.6 ± 27.5†	74.2 ± 39.2†
Factor VII (%)	75.1 ± 34.9*	99.3 ± 61.8†	94.5 ± 60.2†	52.7 ± 28.8†	58.6 ± 29.4†	76.2 ± 30.3
Factor VIII (%)	219.6 ± 144.8	128.6 ± 94.5†	115.6 ± 73.7†	119.1 ± 71.8†	150.7 ± 124.2†	179.5 ± 88.9†
Factor IX (%)	103.6 ± 40.2*	114.9 ± 38.8†	115.6 ± 33.0†	119.6 ± 35.0†	155.7 ± 46.0†	185.2 ± 50.4†
Factor X (%)	66.7 ± 24.4*	68.3 ± 26.3	66.2 ± 21.3†	61.4 ± 15.7†	64.1 ± 15.6†	70.7 ± 17.6†
AT III (%)	78.1 ± 24.6*	72.1 ± 19.4†	76.3 ± 17.9	73.9 ± 18.3†	67.3 ± 19.6†	74.3 ± 23.0
Protein C (%)	80.6 ± 28.0*	69.9 ± 20.6†	71.7 ± 18.7†	66.8 ± 19.6†	67.0 ± 22.6†	73.7 ± 28.0
aPC (ng/mL)	12.1 (3.5–37.8)*	1.9 (0.7–3.5)†	1.2 (0.5–2.4)†	1.0 (0.2–1.7)†	1.3 (0.5–2.3)†	1.2 (0.6–2.4)†
D-dimer (μg/mL)	6.8 (2.9–9.8)*	6.5 (3.3–9.4)	6.6 (3.8–9.9)	5.2 (2.9–9.4)†	3.3 (1.9–6.1)†	3.6 (2.7–5.6)†
tPA (ng/mL)	27.9 (13.0–44.0)*	11.6 (9.9–18.9)†	11.8 (8.7–15.1)†	9.0 (5.9–12.9)†	4.6 (4.3–5.3)	4.5 (3.5–5.4)
PAI-1 (ng/mL)	25.3 (12.1–33.4)	130.2 (79.8–149.8)†	135.1 (84.8–237.4)†	70.4 (46.8–120.4)†	26.0 (14.0–33.2)	19.2 (13.8–24.6)

N = 143.
*$P < 0.05$ for comparison between transfused and nontransfused patients on arrival only.
†$P < 0.05$ for comparison between admission factor level and factor level at the indicated time.

prolonged; factors II, V, VII, IX, and X as well as ATIII and protein C were lower; and activated protein C and tPA were elevated in the transfused cohort on admission (all $P < 0.05$, Table 4 and 5).

Next, we analyzed comprehensive factor levels in those transfused with a "low ratio" versus "high ratio" of RBC:FFP units within the first 6 hours of admission, to identify differences in clotting factor levels over time associated with the 2 different transfusion strategies. On arrival and before transfusion, patients ultimately receiving "low ratio" transfusion had significantly higher levels of baseline factor II, and lower baseline levels of factor VIII compared with the "high ratio" cohort (Table 6 and 7). In the group of 91 patients transfused with a "low ratio," differences in admission and later factor levels at all subsequent time points up to 24 hours were statistically significant for factor VII, factor VIII, factor IX, activated protein C, and PAI-1 (Table 6). Within the group of 52 patients transfused with a "high ratio," differences in admission and later factor levels at all subsequent time points up to 24 hours were statistically significant for PTT, factor VIII, ATIII, protein C, activated protein C, tPA, and PAI-1 (Table 7). Evaluating point-by-point differences in factor levels between the 2 transfusion strategy cohorts, significant differences were seen at several time points. Although admission PT and PTT for both ratio groups were similar, the PTT was comparatively prolonged in the group transfused with a "high ratio" compared with those transfused with a "low ratio" at 6 hours and 12 hours after admission; furthermore, the prolonged PTT took 72 hours to return to admission baseline in the "high ratio" group, compared with correction within 24 hours in the "low ratio" group. Similarly, protein C levels remained depleted relative to admission baseline at all time points in the "high ratio" group, whereas protein C levels were repleted to baseline by 72 hours in the "low ratio" group. Early absolute clotting factor deficits in levels of factor V (at 6 hours), factor IX (at 6 and 12 hours), and factor X (at 6 hours) were better-corrected by "low" versus "high" ratio transfusion (Tables 6 and 7).

TABLE 6. Longitudinal Factor Levels in Patients Transfused With "Low Ratio" (RBC:FFP ≤ 1.5:1) at 6 Hours

	0 h	6 h	12 h	24 h	48 h	72 h
PT (sec)	16.7 (14.5–21.2)	15.3 (14.2–17.6)	15.6 (14.6–16.9)†	16.9 (15.5–19.1)	16.2 (14.9–18.8)	15.5 (14.1–17.8)†
PTT (sec)	32.5 (27.5–42.2)	33.2 (28.6–36.1)*	33.7 (30.8–38.7)*	38.2 (34.8–43.1)†	39.2 (36.4–47.4)	38.5 (32.9–43.8)
Fibrinogen (ng/mL)	157 (106–200)	142 (111–205)	180 (133–234)	288 (162–362)†	325 (264–514)†	494 (395–559)†
Factor II (%)	66.2 ± 21.3*	61.6 ± 17.1	64.4 ± 17.5	64.0 ± 18.5	66.8 ± 20.2	72.2 ± 14.4†
Factor V (%)	38.7 ± 24.7	43.2 ± 20.7*	43.7 ± 19.3	45.3 ± 23.4	58.8 ± 29.0†	79.6 ± 44.2†
Factor VII (%)	72.3 ± 31.7	107.0 ± 77.6†	100.3 ± 69.7†	48.2 ± 22.9†	61.8 ± 34.6	79.6 ± 29.8
Factor VIII (%)	188.1 ± 128.5*	115.3 ± 80.3†	105.2 ± 62.2†	110.6 ± 51.0†	179.3 ± 147.7	192.9 ± 94.6
Factor IX (%)	101.2 ± 40.6	116.3 ± 39.4*†	114.0 ± 35.4*†	114.6 ± 36.7†	151.6 ± 45.6†	184.4 ± 46.2†
Factor X (%)	64.1 ± 23.9	72.6 ± 33.4*	65.0 ± 19.9	60.7 ± 17.9	66.2 ± 16.8	72.6 ± 15.7
AT III (%)	75.5 ± 24.9	67.6 ± 18.9	73.8 ± 17.6	69.8 ± 16.7	66.8 ± 21.0	72.4 ± 22.6
Protein C (%)	77.4 ± 29.0	66.8 ± 17.0	72.0 ± 18.5	64.5 ± 22.4†	65.9 ± 21.6†	73.4 ± 30.4
aPC (ng/mL)	15.3 (5.4–49.5)	2.8 (0.7–4.7)†	1.2 (0.6–2.5)†	1.0 (0.5–1.9)†	0.5 (0.3–1.7)†	0.8 (0.6–2.4)†
D-dimer (μg/mL)	6.7 (2.4–9.8)	6.4 (3.2–9.4)	6.8 (3.8–10.6)	7.6 (3.9–11.4)	3.3 (2.1–5.0)	3.7 (3.1–6.1)
tPA (ng/mL)	31.0 (9.0–46.7)	15.3 (11.2–22.6)	13.5 (11.4–18.0)†	9.8 (6.1–15.3)†	4.3	—
PAI-1 (ng/mL)	25.3 (11.8–30.2)	146.9 (138.7–372.4)†	142.4 (134.2–278.9)†	86.1 (57.7–124.6)†	25.5 (8.3–33.5)	—

N = 91.
*$P < 0.05$ for comparison between "low" and "high ratio" patients at each time point.
†$P < 0.05$ for comparison between admission factor level and factor level at the indicated time.

TABLE 7. Longitudinal Factor Levels in Patients With 6 Hours RBC:FFP "High Ratio" (RBC:FFP > 1.5:1) at 6 Hours

	0 h	6 h	12 h	24 h	48 h	72 h
PT (sec)	16.2 (14.6–20.6)	16.2 (15.1–18.3)	15.9 (15.0–17.0)	17.0 (15.8–17.7)	17.6 (15.6–18.8)	15.6 (15.2–17.3)
PTT (sec)	32.5 (27.6–41.8)	36.6 (32.5–41.7)*†	35.9 (32.8–40.4)*†	39.8 (36.0–46.4)†	47.0 (38.6–49.5)†	41.5 (39.4–45.8)
Fibrinogen (ng/mL)	139 (125–205)	124 (101–153)	183 (157–230)	258 (213–291)†	547 (530–566)†	668 (566–780)†
Factor II (%)	57.7 ± 18.3*	54.1 ± 14.9	59.4 ± 12.1	60.6 ± 12.4	61.8 ± 14.2	70.2 ± 15.4
Factor V (%)	38.0 ± 26.8	33.3 ± 16.2*†	39.7 ± 16.7†	43.2 ± 17.6	47.7 ± 25.3	61.0 ± 23.0
Factor VII (%)	73.9 ± 46.3	86.3 ± 51.8	101.1 ± 59.4	61.1 ± 36.3†	52.9 ± 19.3†	78.7 ± 32.7
Factor VIII (%)	266.6 ± 172.0*	106.4 ± 77.4†	106.2 ± 78.5†	112.5 ± 43.7†	114.1 ± 53.4†	152.6 ± 78.1†
Factor IX (%)	96.5 ± 44.2	86.5 ± 29.7*	100.7 ± 18.5*	107.1 ± 25.1	142.9 ± 36.1†	179.0 ± 53.1†
Factor X (%)	61.0 ± 23.1	54.7 ± 16.9*	62.3 ± 20.9	58.2 ± 10.4†	57.9 ± 12.9†	72.7 ± 13.0
AT III (%)	73.8 ± 23.4	67.2 ± 19.7†	74.7 ± 16.7†	71.9 ± 20.0†	61.2 ± 18.9†	66.8 ± 24.1†
Protein C (%)	79.5 ± 27.1	63.9 ± 17.8†	69.7 ± 12.9†	64.4 ± 15.1†	57.7 ± 11.9†	61.3 ± 18.6†
aPC (ng/mL)	13.7 (3.5–51.2)	1.4 (0.6–2.7)†	1.0 (0.4–2.3)†	0.5 (0.0–1.4)†	1.2 (1.1–1.4)†	1.2 (0.5–13.4)
D-dimer (μg/mL)	7.5 (3.8–12.0)	6.5 (2.6–23.0)	7.4 (4.2–14.6)	7.4 (3.8–10.6)†	4.7 (2.8–6.7)	4.4 (3.2–6.7)
tPA (ng/mL)	25.0 (14.7–38.3)	12.5 (8.0–15.6)†	10.2 (8.4–14.3)†	6.8 (5.8–11.1)†	5.0 (4.6–5.3)	4.5 (3.5–5.4)
PAI-1 (ng/mL)	25.7 (12.4–38.4)	125.1 (49.5–132.6)†	141.6 (84.1–251.4)†	64.6 (43.8–143.4)†	27.1 (19.1–33.2)	19.2 (13.8–24.6)

N = 52.
*$P < 0.05$ for comparison between "low" and "high" ratio patients at each time point.
†$P < 0.05$ for comparison between admission factor level and factor level at the indicated time.

To better understand differences between transfusion practices given the differences in baseline population characteristics (Table 3) and clotting factor levels on admission (Tables 6 and 7), we then assessed longitudinal clotting factor levels as a percentage of their arrival baseline (Figure 1). Overall, patients transfused with a "low ratio" had significantly less prolonged PT (at 6 hours) and PTT (at 12 hours) than those transfused at a "high ratio." In terms of specific factor repletion, factors V and VIII were significantly better repleted at both 12 hours and 24 hours, as well as factor IX at 12 hours. No significant differences were seen compared to admission baseline for fibrinogen, procoagulant factors II, VII, X, anticoagulants factors ATII, protein C, activated protein C, or fibrinolysis-associated molecules D-dimer, tPA, or PAI-1.

To determine the overall association with survival, we performed Kaplan-Meier survival analysis comparing nontransfused patients with those transfused with a "low" versus "high" ratio of RBC:FFP, identifying an association between lower RBC:FFP transfusion ratios and improved survival to discharge (Figure 2, log-rank $P < 0.001$). Given the significant baseline differences in injury severity in these populations detailed in Table 2 and 3, we created a Cox proportional hazards model for in-hospital mortality to adjust for age, injury severity score, arrival GCS, arrival base deficit, arrival INR, penetrating versus blunt mechanism, and categorical transfusion status (nontransfused, low ratio, or high ratio); the study population had 107 in-hospital deaths, and Harrell's C for the model was 0.829 (Table 8). Compared to the referent category of nontransfused patients, those transfused with a "low" ratio had a nonsignificant hazard ratio for mortality of 1.661 [$P = 0.079$, 95% confidence interval (CI): 0.943–2.925], whereas those transfused with a "high" ratio had 3.402-fold significantly higher mortality ($P = 0.001$, 95% CI: 1.693-6.833). In terms of differences between transfusion strategies, those transfused with a "high" ratio had a significant

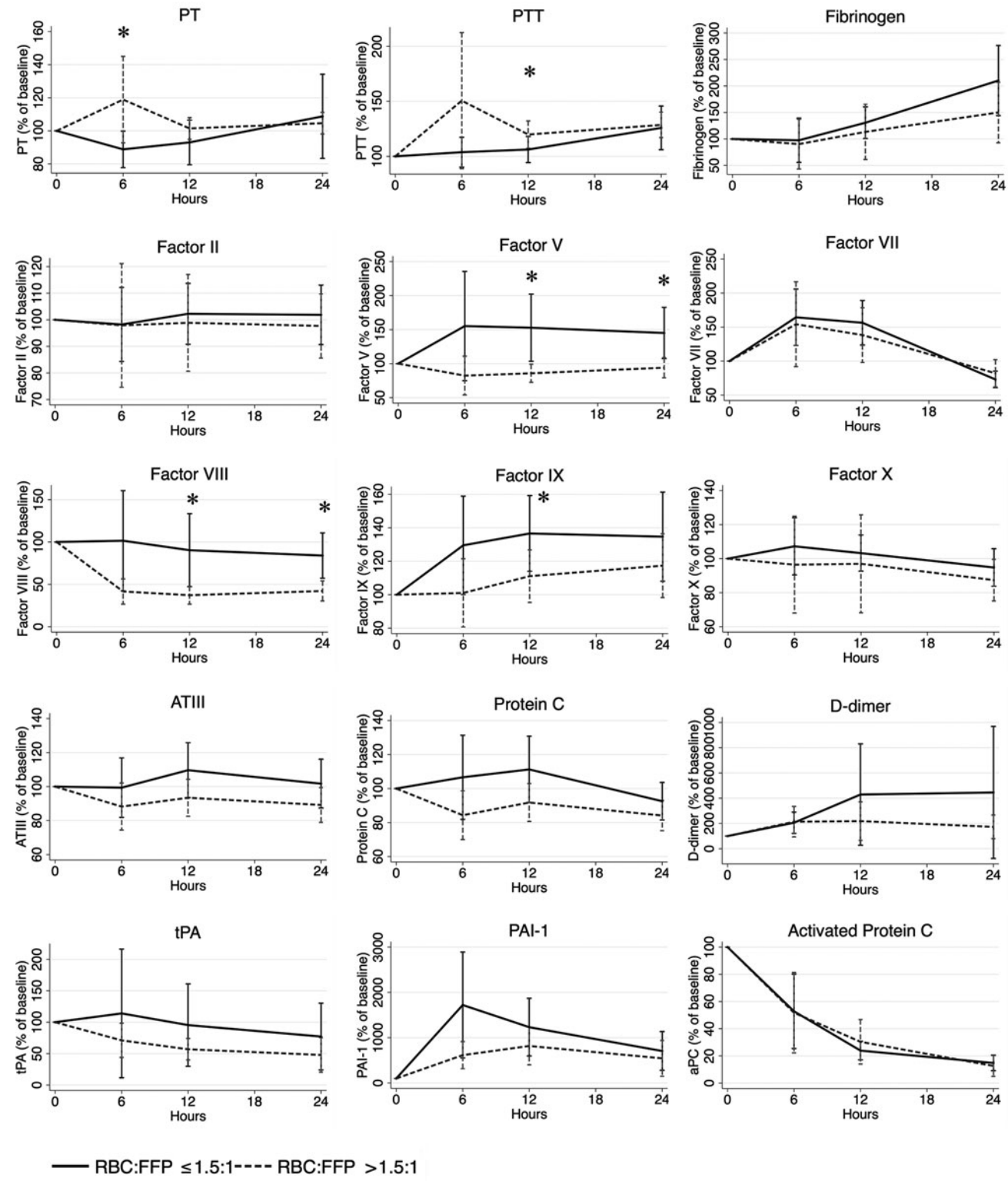

FIGURE 1. Longitudinal percentage change in coagulation studies by "high" versus "low" RBC:FFP resuscitation ratio compared to admission baseline. N = 143. $^{*}P < 0.05$ for comparison between "low" and "high" ratio patients at each time point.

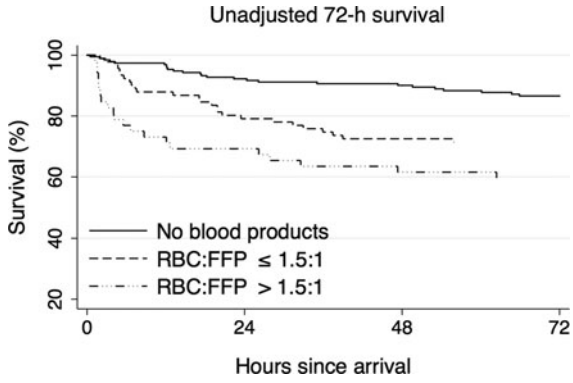

FIGURE 2. Unadjusted Kaplan-Meier survival curves by RBC:FFP ratio. N = 336. Log rank $P < 0.001$ between groups.

TABLE 8. Cox Proportional Hazards Model for In-Hospital Mortality

Variable	Hazard Ratio	*P*	95% CI
Age (yr)	1.038	<0.001	(1.024–1.052)
ISS	1.023	0.002	(1.008–1.038)
Arrival GCS	0.803	<0.001	(0.749–0.861)
Arrival base deficit	1.029	0.154	(0.989–1.069)
Arrival INR	0.958	0.820	(0.659–1.392)
Penetrating injury	2.362	0.011	(1.213–4.599)
Nontransfused	—	—	—
Low ratio	1.661	0.079	(0.943–2.925)
High ratio	3.402	0.001	(1.693–6.833)

N = 336. In-hospital mortality outcome occurred in 107 patients. Harrell's C = 0.829.

2.048-fold higher mortality than those transfused with a "low" ratio, even when adjusted for differences in age, injury characteristics, and admission physiology ($P = 0.027$, 95% CI: 1.087–3.858; Table 8).

DISCUSSION

Here, we present the first "natural history" study of longitudinal clotting factor levels after severe trauma. By prospective collection of both longitudinal biochemical and outcomes data beginning early after injury, we are here able to provide data on the course and serial effects of treatment regimes on coagulation after injury by presenting longitudinal profiles as affected by "low ratio" and "high ratio" hemostatic resuscitation, identifying significant differences in repletion of specific clotting factors based on transfusion strategy. In particular, hemostatic resuscitation ratio-based transfusion is associated with earlier correction of both PT and PTT, as well as earlier repletion of factors V, VIII, IX, and X after severe injury, both in terms of absolute levels and when compared with admission baseline. We further identify a survival advantage to the "low ratio" transfusion strategies, both in unadjusted analysis and when adjusted for patient demographics, injury characteristics, and admission physiology. Taken together, we believe this survival benefit appears most likely due to a mixed picture of both more optimal coagulopathy reversal and modulation of the inflammatory response to trauma.

The last 10 years have seen a paradigm change in the care of the severely injured and bleeding patient. This change is a consequence of the identification of ATC as a clinical entity, and of hemostatic resuscitation as its effective (and empiric) treatment. Before the clinical recognition of ATC, classic trauma resuscitation literature identified iatrogenic and resuscitation-associated causes as responsible for bleeding after trauma: hypothermia, metabolic acidosis, and dilutional coagulopathy were identified as primary drivers and were generally thought to be secondary to resuscitative efforts.[9] In response to this pioneering literature, trauma surgeons began to incorporate rewarming efforts, early correction of acidosis, and limitation of crystalloid as prime tenants of resuscitation strategy. Once these major causes of coagulopathy were avoided or appropriately treated, however, a distinct coagulopathy present in patients immediately after injury and before any resuscitative interventions still remained. Brohi et al[4] and Macleod et al[2] concurrently elucidated this in 2003 and defined ATC separately from the iatrogenic causes previously identified in coagulopathic bleeding after trauma. Our group and others subsequently codified the clinical and biochemical nature of this entity, reporting that ATC occurs only in the presence of both severe injury and shock, and is mediated by activation of the protein C system.[5,22,23] Concurrently, military and civilian studies on transfusion strategy suggested a survival benefits to earlier and more frequent use of plasma during massive transfusion. Beginning with Borgman's data from military casualties, multiple studies clearly demonstrated that approaching balanced ratio transfusion of RBC:FFP led to improved survival.[12–16,21] As these considerable retrospective data are compelling and prospective randomized studies in this arena are in early stages of progress, plasma-based resuscitation conduct has been overwhelmingly accepted by the trauma community despite the fact that the biochemical mechanisms underlying the survival benefit of plasma remain unclear.[24]

Whether this apparent survival benefit stems from earlier and more durable reversal of coagulopathy or is the result of as-yet undescribed inflammomodulatory effects remains unclear. Early suggestions that there is more to balanced resuscitation than the repletion of depleted clotting factors comes from clinical and basic science data. Clinically, Brown et al[21] reported that trauma patients derive benefit from plasma-based resuscitation independent of the presence of coagulopathy. Groundbreaking preclinical basic science data also support the inflammomodulatory hypothesis. Pati et al[20] described a cell culture model showing that plasma decreased endothelial cell permeability compared to crystalloid. Kozar et al[19] showed that in vivo endothelial glycocalyx degradation after hemorrhagic shock was partially restored by plasma, but not lactated Ringer's in a rat model.[19] These results suggest that an "endotheliopathy" induced by hemorrhagic shock may be treated by plasma administration via an inflammomodulatory mechanism, above and beyond the simple correction of clotting factor depletion. Given the known mortality benefit of balanced resuscitation confirmed here, further work identifying the nature of this endotheliopathy and the specific inflammomodulatory effects of plasma is required for a complete understanding of the biochemical mechanisms involved.

As an attempt to elucidate the biochemical framework of this clinical dilemma, in this study, we describe the natural history of coagulation factor perturbation after injury, both with and without transfusion. Those patients requiring transfusion had prolonged PT and PTT, lower levels of procoagulants, and higher levels of endogenous anticoagulants at each time point up to 24 hours. Notably, transfusion with a "low ratio" of RBC:FFP was associated with earlier correction of PT and PTT as well as repletion of factor V, factor VIII, and factor IX deficits compared with those transfused with higher ratios, suggesting that "low ratio" transfusion corrects specific critical clotting factor deficits more efficiently than higher RBC:FFP ratios. Interestingly, not all factors are significantly corrected in the "low ratio" group compared with the "high ratio' group. This suggests that reversal of ATC may be critically contingent on repletion of deficits in factor V, VIII, IX, and X, identifying specific factors of interest to target therapeutically and follow clinically. Confirming the clinical

significance of the differences in clotting factor repletion observed in "low" versus "high" ratio transfusion, we also identified a statistically significant survival benefit to "low ratio" transfusion in Kaplan-Meier survival analysis. Of critical importance, this was robust even when adjusted for elements of patient demographics, injury characteristics, and admission physiology that potentially confound this finding.

As with other single-center prospective studies examining the relationship between clotting factor studies and outcomes, several limitations are important to interpretation of this data. The clinical utility of targeting and/or monitoring individual factors suggested here is contingent upon specific definitions of critical levels of depletion for each of these factors, which remain uncharacterized. Rizoli et al[25] have suggested that depletion to a 30% level represents a critical threshold that should guide the appropriateness of plasma therapy; however, the data presented here suggest that not all coagulation factors are critical mediators of ATC, and that each factor may have its own independent level of critical depletion. Further study from a coagulopathy reversal point of view will require viscoelastic assays such as thromboelastography, as opposed to static PT and PTT measures as used here, to specifically map out these critical definitions using a functionally meaningful (and clinically applicable) endpoint; in parallel, identification of the specific importance of improved factor V, VIII, IX, and X repletion by "low ratio" resuscitation also suggests specific biochemical targets of interest in unraveling the basic science behind potential inflammomodulatory mechanisms of plasma-based therapy. Importantly, this study is also not powered to identify potential unintended consequences of empiric plasma administration risks; as is well-documented elsewhere, the risks of inappropriate plasma administration are ominous, particularly in patients who undergo early plasma-based resuscitation but ultimately do not require massive transfusion.[26] Most importantly, the recognition of potential survival bias is critical in the interpretation of the data presented here.[18] Specifically, a conventional crystalloid and RBC-based approach to trauma resuscitation (placing many patients who die early after admission in a "high" RBC:FFP ratio category, and reducing the apparent mortality rate in patients who simply survive long enough to receive substantial plasma) biases toward the finding a survival benefit in the "low ratio" group, whereas exclusion of patients who die before the 6-hour time point used to calculate ratios here (thereby excluding patients who may stand the greatest chance to benefit from early plasma therapy) subtly biases against a "low ratio" benefit; the ultimate balance of these potential biases cannot be derived from these data, mandating cautious interpretation of the survival benefit to "low ratio" resuscitation seen here.[27] Only "gold standard" randomized clinical trials will eliminate this bias; however, given the logistic, clinical, and ethical challenges that face these ongoing studies, we suggest that the data presented here provide an evidence base to justify hemostatic resuscitation as a defensible interim standard of care for critically injured trauma patients requiring transfusion until the results of randomized controlled trials are available to more definitively guide therapy.

CONCLUSIONS

We here detail the natural history of coagulation factors in both nontransfused and transfused patients after severe traumatic injury. We further demonstrate that targeting ratios of RBC:FFP $\leq$ 1.5:1 leads to earlier correction of PT and PTT, and earlier and prolonged repletion of specific clotting factor deficits compared with higher ratio transfusion strategies. Given both the unadjusted and injury-adjusted survival benefit seen in plasma-based resuscitation despite the correction of only a subset of factors, we hypothesize that hemostatic resuscitation may additionally prevent and/or treat the endotheliopathy of trauma via direct inflammomodulatory effects. Further studies must be done to continue to prospectively and definitively evaluate specific clotting factor deficits to trigger both plasma-based and clotting factor-targeted therapy, and to identify the specific biochemical mechanisms of both endotheliopathy and the inflammomodulatory cascade after trauma, and its specific responses to plasma-based therapy.

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Letter: The whole is greater than the sum of its parts: Hemostatic profiles of whole-blood variants

To the Editor:

We read with great interest the recent study on hemostatic profiles of whole-blood (WB) variants by Kornblith et al.[1] in this journal, where they aimed to study several laboratory parameters of donated WB and reconstituted whole blood (RWB) under several mixed ratios and conditions.

Interestingly, standard coagulation tests (SCTs) such as prothrombin time and partial thromboplastin time were substantially prolonged for both room temperature WB and cooled WB. The activity of coagulation factors were approximately 30% lower than previously reported in healthy volunteers.[2] This finding is hard to explain. Usually, 450 mL of donated blood is collected in a package prefilled with 63 mL of citrate phosphate dextrose anticoagulant to conserve the WB, but this results in an approximate 12% dilution of the coagulation factors.[3] Therefore, measurement of coagulation activity in donated WB should result in normal SCT result and ROTEM parameters. Weiss et al.[2] used a stepwise method to dilute WB and found that EXTEM clotting time (CT) exceeded the normal upper limit of 80 seconds after a 60% dilution of the coagulation factors. In contrast, EXTEM CT in the current study was 220 seconds, with an extremely high SD value of 216.3 seconds.

Importantly, RWB from red blood cells, fresh frozen plasma (FFP), and platelet concentrates mixed at a ratio of 1:1:1 also failed to normalize SCT results and concentration of coagulation factors. Moreover, EXTEM and INTEM CT were 1.5 times higher than the normal upper limit.[4] Interestingly, fibrinogen concentration was higher in RWB than in donated WB.

One explanation could be that donated and reconstituted WB variants were already anticoagulated with various forms of citrated additives in the initial collection bags, thereby inactivating all available calcium needed for coagulation. According to the authors, the samples for SCTs and ROTEM analyses were drawn from this already citrated WB bag into tubes containing 3.2% (0.109 mol/L) sodium citrate (usually in a 1:9 ratio of citrate to blood sample). First, this unnecessarily further dilutes the sample (usually by approximately 10%). Second and more importantly, a much higher dose for recalcification (e.g., star-TEM reagent) is needed for appropriate ROTEM analysis and an intact coagulation cascade. However, according to the authors, this was unfortunately not accounted for by the automated pipette program of the ROTEM device used. This presumably resulted in major flaws in all of the ROTEM results.

It seems that a major systematic error in the preparation and/or measurement of samples occurred. Therefore, these results must be interpreted with extreme caution. Furthermore, according to the presented results, it might be impossible to normalize SCT results or ROTEM CT by transfusion of red blood cells, FFP, and platelet concentrates at a ratio of 1:1:1. If the presented results mirror the true coagulation capacity of the transfused blood products, an alternative approach to high ratio FFP administration (e.g., coagulation factor concentrates) is necessary to normalize both SCT results and ROTEM CT.[5]

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Response: The whole is greater than the sum of its parts: Hemostatic profiles of whole-blood variants

In Reply:

We respectfully appreciate the AUVA's interest in our work and their specific concerns over the prolonged standard coagulation measures and out-of-range ROTEM measurements in our study. We strongly disagree, however, that there are any compelling methodological issues and additionally disagree that it is difficult to explain these findings in the banked products compared with healthy volunteer blood. Unfortunately, the editorial claims seem to be based on incorrect assumptions regarding methodology and results, which we seek to remedy here. To clarify, our regional blood collection center colleagues collected the donated whole blood in a collection bag prefilled with 70 mL of anticoagulant, primarily citrate phosphate dextrose as per standard protocol. The clinical standard acceptable volume of whole blood for collection is 450 mL to 550 mL with added 70 mL of anticoagulant (which results in variable 13–16% anticoagulant). The whole-blood units provided to us were either slightly overweight or underweight and therefore had a mean anticoagulant of 17%, only 1% greater than the clinical standard. Each whole-blood unit was split into four satellite bags without anticoagulant (designed specifically to avoid excess dilution and to standardize surface contact) and therefore retained their anticoagulant concentration after the split. For the reconstituted whole blood components, the red blood cells, platelets, and plasma (whole blood derived) all had an anticoagulant concentration of 15%. Therefore, the mean concentration of anticoagulant across all the products we analyzed was 15% to 17%, making it appropriate to compare coagulation measurements between these banked products.

Next, all samples were run in standard citrated tubes. The total citrate concentration in our samples most certainly inactivated some of the calcium, but we believe that the suggestion that all available calcium needed for coagulation was inactivated is incorrect. There is more than sufficient calcium to recalcify these blood samples, ensuring appropriate assay. In fact, the suggestion that inadequate recalcification occurred is contradicted by our ROTEM data demonstrating intact coagulation with overall clot strength measured by ROTEM within normal range for modified whole-blood variants (all of which had approximately 17% anticoagulant). Further evidence is provided by Mann et al.,[1] who demonstrated that alterations of coagulation with citrate anticoagulants found on thrombogram and thromboelastograms are not reversible by calcium replacement and that the effects of citrate may alter the natural dynamics of tissue factor–induced blood coagulation separate from chelation of calcium. Lastly and most importantly, we feel the discussion of dilution and recalcification is misguided, as the absolute coagulation measurements of the banked products were not the critical finding of our study but rather the comparison between the products, which demonstrated the superiority of 1:1:1 reconstituted whole blood over 2:1:1 and of modified whole blood over 1:1:1 reconstituted whole blood. These comparisons are the pivotal results, which provide important insight into the characterization of blood products and their use in resuscitation.

*The authors declare no conflicts of interest.

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Letter: The need for a definitive clinical trial of cryopreserved red blood cells

To the Editor:

The implications of a shorter shelf life for liquid-stored packed red blood cells (PRBCs) would be difficult to manage. This may be the situation faced by blood services across the world if the three large age-of-blood trials currently underway (ISRCTN44878718, NCT00991341, and NCT01638416) find convincing evidence of poor clinical outcomes as the result of transfusion with aged liquid-stored PRBC. In addition to the logistical advantages, cryopreserving PRBCs may be a viable alternative to liquid-storing PRBC for more than 1 week to 2 weeks. However, the superiority of cryopreserved PRBCs compared with aged liquid-stored PRBC on relevant clinical outcomes would first need to be demonstrated. Data to support this are limited and confined to surrogate outcomes. Hampton et al.[1] raise the possibility that cryopreserved PRBCs may even be superior to standard storage duration (median, 14 days; interquartile range, 9.8–27.3 days) liquid-stored PRBC. While in vitro investigations suggest mechanisms of such an effect, such as the removal of immunogenic material during the deglycerolization process[2] and the selective hemolysis of perisenescent donor red blood cells,[3] cryopreserved PRBCs, compared with fresh liquid-stored PRBC, have an increased mean corpuscular volume (which may return to normal once transfused),[3] increased osmotic fragility,[4] a lower intracellular pH,[3] and a decreased aggregability, which may impede movement through the microcirculation.[4] How the sum of these changes affects clinically relevant outcomes is unknown.

Cryopreserved PRBCs are already in limited use in a number of countries as a means of supplying rare blood groups. Before becoming a solution to the hypothesized aged liquid-stored PRBC problem, a large clinical trial will be warranted to demonstrate clinical and cost-effective (rather than biologic surrogate) outcomes. If there is clear superiority in clinical outcomes, the utility of cryopreservation will come down to the comparison between the magnitude of the benefit and the cost. After surveying stakeholders, the ABLE (ISRCTN44878718) investigators chose an absolute risk reduction of 5% as sufficient to change practice with regard to the age of liquid-stored PRBCs. With the use of the same estimate for the magnitude of superiority required to change practice, a cryopreserved PRBC trial would require more than 2,500 patients. If pilot effectiveness trials suggest that there is no clinical benefit (or detriment) to be found, the logistic advantages of cryopreserved PRBC may still warrant a trial. The question will then be whether a noninferiority trial (likely with many more thousands of patients required to exclude a clinically meaningful difference) will be required. How much worse a clinical outcome would we be prepared to accept to accrue the logistic advantages of cryopreservation? This would be a novel question for regulatory authorities. Hampton et al. are currently undertaking the first trial assessing clinical outcomes associated with transfusion of cryopreserved PRBCs, with encouraging clinical results reported after the first 57 patients enrolled in the pilot study.[5] However, with a planned enrolment of only 350 patients (NCT01038557), this first trial is unlikely to be definitive. It is perhaps timely then that consideration is given to the conduct of a definitive multicenter cryopreserved PRBC trial.

*The authors declare no conflicts of interest.

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Response: The need for a definitive clinical trial of cryopreserved red blood cells

In Reply:

Thank you for the opportunity to reply to the comments of Dr. Milford's group. In

Session I

Paper 1 12:50 PM

TRAUMA CORE MEASURES: CLINICAL PROCESSES THAT IMPROVE PATIENT OUTCOMES

Shahid Shafi*, MD MPH, Nadine Rayan, MHA, Sunni Barnes, PhD, Larry Gentilello*, MD, Neil S Fleming, PhD, David J Ballard, MD PhD. Baylor Institute for Health Care Research and Improvement.

Invited Discussant: Mark Hermila

Introduction: The Trauma Quality Improvement Program (TQIP) has shown that risk-adjusted mortality rates at some centers are nearly 50% higher than at others. The reasons for this significant "quality gap" are unknown, but may be due to different clinical practices or processes of care. We have previously shown that adoption of processes promoted as Core Measures by the Joint Commission (JC) and Center for Medicare and Medicaid Services (CMS) in hospitalized patients do not improve trauma patient outcomes. We hypothesized that improved compliance with trauma specific clinical processes of care (T-POC) is associated with reduced mortality.

Methods: Records of a random sample of 1000 patients admitted to a Level 1 trauma center who met TQIP criteria (AIS > 3) were retrospectively reviewed for compliance with twenty-five T-POC's endorsed by ATLS, EAST, the Brain Trauma Foundation, or the Glue Grant Consortium. All were evidence-based or expert consensus panel recommendations. Multivariate regression was used to determine the relationship between T-POC compliance and mortality, adjusted for age, gender, injury type, and severity.

Results: Median age was 41 years, 65% were males, 88% sustained a blunt injury, and mortality was 12%. Of these, 81% were eligible for at least one T-POC, and over 60% were eligible for 2 or more. There was wide variation in compliance with T-POC's, ranging from 99% for transfusions in hypotensive patients, to 8% for ICP monitoring in brain injured patients. Every 10% increase in compliance with T-POC's was associated with a 14% reduction in risk-adjusted mortality.

Conclusion: Unlike adoption of JC or CMS core measures, adoption of T-POC's is associated with reduced mortality in trauma patients. Trauma centers with excess mortality may improve patient outcomes by consistent application of T-POC. These processes should be explored for potential use as Core Trauma Center Performance Measures.

Session 8B

Paper 27 2:00 PM

BARRIERS TO EVIDENCE BASED CARE IN TRAUMA

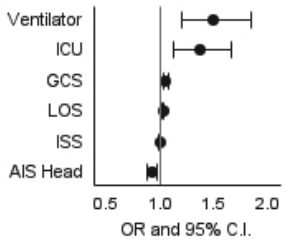
Nadine Rayan, MHA, Sunni Barnes, PhD, Neil Fleming, PhD, Rustam Kudyakov, MD, MPH, David Ballard, MD, PhD, Larry Gentilello*, MD, Shahid Shafi*, MD, MPH. Baylor Institute for Health Care Research and Improvement.

Invited Discussant: L.D. Britt

Objective: We previously demonstrated that evidence-based medicine (EBM) is often not practiced in trauma care, as patients receive less than two-thirds of the care recommended by EBM. We hypothesized that patients least likely to receive EBM-based care can be prospectively identified, which may be used to increase adoption of EBM in trauma care.

Methods: Records of a random sample of 1000 patients admitted to a Level 1 trauma center (2006-08) with moderate to severe injuries (AIS $\geq$ 3) were reviewed for compliance with 25 EBM-based processes of care (POC) endorsed by ATLS, EAST, the Brain Trauma Foundation, SCIP, and the Glue Grant Consortium. These encompassed all aspects of care, including initial evaluation, resuscitation, operative care, critical care, rehabilitation, and injury prevention. Multivariate logistic regression was used to identify patients likely to receive EBM-based POC's. Results are reported as odds of compliance with 95% C.I.

Results: 774 patients were eligible for 2603 POC's. However, only 1515 of these POC's (58%) were provided to patients. Compliance was highest for POC's involving resuscitation (83%), and were lowest for neurosurgical interventions (17%). Increasing injury severity (higher ISS, lower GCS, higher head AIS) was associated with lower compliance, while ICU stay and ventilator use were associated with higher compliance (Figure). There was no relationship between compliance and the following: age, gender, race, insurance status, household income, or initial care during a night or weekend shift.



Conclusion: Patients with increased injury severity who are in greatest need of optimal care are least likely to receive it. Hence, patients with the most severe injuries should be targeted to ensure compliance with EBM-based processes of care. Studies to investigate barriers to closing this quality chasm should focus on patients with the most severe injuries, especially those with significant traumatic brain injuries.

Moving from "optimal resources" to "optimal care" at trauma centers

Shahid Shafi, MD, MPH, Nadine Rayan, MHS, Sunni Barnes, PhD, Neil Fleming, PhD, Larry M. Gentilello, MD, *and* **David Ballard, MD, PhD, MSPH, FACP,** *Dallas, Texas*

BACKGROUND: The Trauma Quality Improvement Program has shown that risk-adjusted mortality rates at some centers are nearly 50% higher than at others. This "quality gap" may be due to different clinical practices or processes of care. We have previously shown that adoption of processes called core measures by the Joint Commission and Centers for Medicare and Medicaid Services does not improve outcomes of trauma patients. We hypothesized that improved compliance with trauma-specific clinical processes of care (POC) is associated with reduced in-hospital mortality.

METHODS: Records of a random sample of 1,000 patients admitted to a Level I trauma center who met Trauma Quality Improvement Program criteria (age ≥16 years and Abbreviated Injury Scale score ≥3) were retrospectively reviewed for compliance with 25 trauma-specific POC (T-POC) that were evidence-based or expert consensus panel recommendations. Multivariate regression was used to determine the relationship between T-POC compliance and in-hospital mortality, adjusted for age, gender, injury type, and severity.

RESULTS: Median age was 41 years, 65% were men, 88% sustained a blunt injury, and mortality was 12%. Of these, 77% were eligible for at least one T-POC and 58% were eligible for two or more. There was wide variation in T-POC compliance. Every 10% increase in compliance was associated with a 14% reduction in risk-adjusted in-hospital mortality.

CONCLUSION: Unlike adoption of core measures, compliance with T-POC is associated with reduced mortality in trauma patients. Trauma centers with excess in-hospital mortality may improve patient outcomes by consistently applying T-POC. These processes should be explored for potential use as Core Trauma Center Performance Measures. (*J Trauma.* 2012;72: 870–877. Copyright © 2012 by Lippincott Williams & Wilkins)

LEVEL OF EVIDENCE: II.

KEY WORDS: Trauma quality improvement; core measures; trauma processes of care.

Injured patients treated at designated trauma centers are more likely to survive than those treated at nondesignated hospitals.[1] However, Trauma Quality Improvement Program has demonstrated that risk-adjusted mortality rates are highly variable across designated trauma centers, with some centers achieving significantly better (or worse) outcomes than others.[2–5] The reasons for this variation are unclear. Donabedian principles of quality improvement suggest that structures and processes of care (POC) determine outcomes. Hence, if these centers have similar administrative and organizational structures ensured by their designation as trauma centers, there must be differences in patient care processes that result in variations in patient outcomes.

Variations in clinical practices across a spectrum of disease are well known. Extensive variations in clinical practices across and within trauma centers have also been reported.[6,7] For example, we have recently shown that risk-adjusted operative procedure rates for injuries to liver, spleen, and kidneys vary widely between trauma centers.[7] In addition, we found that centers that more frequently selected operative rather than nonoperative management of patients with the same severity of injury to these organs, and with the same hemodynamic status after injury, had significantly higher risk-adjusted mortality, suggesting that more is not always better. This was an example of a clinical process that both increased mortality and wasted precious healthcare resources. Hence, to ensure a high degree of clinical effectiveness and efficiency, it is necessary to identify clinical practices that are associated with best patient outcomes.

In an attempt to reduce variations in care and increase adoption of evidence-based practices, "core measures" were developed by the Centers for Medicare and Medicaid Services and the Joint Commission for the management of acute myocardial infarction, congestive heart failure, ventilator-associated pneumonia, and surgical care improvement (SCIP). Several studies suggest that compliance with core measures improves patient outcomes.[8] However, we have recently demonstrated that compliance with these measures, including SCIP, does not correlate with risk-adjusted outcomes of trauma patients.[9] Hence, there is a need to develop trauma-specific measures of best practices.

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The purpose of this study was to determine whether there were trauma-specific POC (T-POC) that may reduce mortality in trauma patients and to analyze the potential impact of improved adoption of T-POC on patient outcomes. The hypothesis for this study was that clinical care processes for management of trauma patients are variable and that improved compliance with T-POC was associated with reduced in-hospital mortality.

MATERIALS AND METHODS

This is a 3-year retrospective study (January 1, 2006 to December 31, 2008) of patients treated at a large, urban Level I trauma center, approved by the Institutional Review Board. During the study period, the trauma registry included 7,581 patients. Inclusion criteria consisted of the following:

1. Adults, defined as age ≥16 years.
2. Moderate to severe injuries defined as at least Abbreviated Injury Scale score ≥3 injuries.
3. Primary outcomes (in-hospital mortality, complications, and length of stay [LOS]) must be known.

Exclusion criteria consisted of the following:

1. Delayed admission defined as time from injury to arrival in emergency department (ED) ≥1 day.
2. Those deemed dead on arrival.
3. Gunshot wounds to the head or penetrating injuries outside the torso (torso defined as neck, chest, and abdomen).
4. Primary mechanism of injury of burns, poisoning, drowning, hanging, submersion, and asphyxiation.

Application of inclusion/exclusion criteria identified 2,242 patients. From these, 1,000 patients were chosen as the study population using a simple random methodology. Six patients were later eliminated due to incomplete information which could not be obtained. Thus, the final study population consisted of 994 patients. Any relevant information that was incomplete or missing in the registry data, such as date and time of admission, and certain laboratory values (specifically prothrombin time with international normalized ratio) were obtained from administrative databases or patient charts.

Trauma-Specific Processes of Care

Clinical guidelines from several professional groups were reviewed to select 25 T-POC for inclusion in this study (Appendix). These included the American College of Surgeons Committee on Trauma,[10] Eastern Association for the Surgery of Trauma,[11] Society for Critical Care Medicine,[12] the Brain Trauma Foundation,[13] the Glue Grant Consortium,[14] and the SCIP Project.[15] Because it was not possible to measure all care processes, only those that could directly impact outcomes of trauma patients and were measureable were selected. Selected T-POC encompassed all aspects of trauma care, including initial evaluation, resuscitation, operative care, critical care, rehabilitation, and injury prevention. We focused on four specific groups of patients: traumatic brain injuries (TBI), hemorrhagic shock (systolic blood pressure 90 mm Hg or less), pelvic fractures, and long bone extremity fractures (femur or tibia). TBI was chosen as it is the most common cause of death and disability in trauma patients, while hemorrhage is the second leading cause of death. Fractures were chosen as they represent a common injury in trauma patients. These fractures are an uncommon cause of death but are the second most common cause of disability after TBI.

Patient Eligibility

Information from the trauma registry dataset was used to identify patients who were eligible for each T-POC. Eligibility was determined by a combination of mechanism of injuries, specific injuries and severity, comorbidities, and procedures (Appendix). All data definitions were based on the National Trauma Data Standard Data Dictionary (NTDS, version 1.2.5) whenever possible.[16] Definition of complications was supplemented by our previous work.[17] In addition, definitions of the American Association for the Surgery of Trauma were used to identify solid organ injuries and related procedures.[18] Injuries were classified into blunt and penetrating based on the matrix of E-code groupings proposed by the Centers for Disease Control. Specific injuries, complications, and procedures were identified using ICD-9 codes which were validated by trauma registry staff. A software tool was developed that analyzed information contained in the trauma registry to identify patients who were eligible for T-POC. The accuracy of the tool in identifying patients eligible for T-POC was validated by manual review of registry data in a simple random sample of 72 patients (7% of the study population). For each T-POC, at least one patient who was deemed eligible and one who was deemed ineligible by the tool were identified and their trauma registry data were manually reviewed to determine whether they were correctly classified by the tool. If any discrepancy was identified, the tool was modified and revalidated until no further discrepancies were identified. After completion of the validation, the tool identified 774 patients (out of 994) who were eligible for at least one T-POC.

Data Collection

A written data dictionary was developed that defined each T-POC and a source hierarchy to determine whether patients received T-POC for which they were eligible. Four nurse abstractors reviewed electronic medical records after undergoing training on data abstraction guidelines. During training, each nurse abstractor reviewed the 10 patient charts. Discrepancies among reviewers were discussed and definitions were modified to minimize ambiguity during data abstraction. To further validate the quality of data abstracted, an independent nurse reviewer abstracted data from a 10% simple random sample of charts (82 patients). Inter-rater reliability was then calculated using kappa statistic. Inter-rater reliability was high (kappa >0.9), except for two T-POC whose definition was modified after initial data collection.

Data Analysis

The primary predictors of interest were the 25 T-POC. Patients who were eligible for each T-POC are reported as percentage of total study population. Patients who received each T-POC are reported as percentage of total number of patients who were eligible for that T-POC. Where relevant, times to T-POC from the time of arrival in ED are reported as medians.

For each patient, a compliance score was calculated based on the opportunity model used by Centers for Medicare and Medicaid Services for reporting compliance with their core measures.[9] For example, if a patient was eligible for 10 T-POC and received 8 of them, then his T-POC compliance score was 80. Similarly, if a patient was eligible for six T-POC and received all six, then his T-POC compliance score was 100. Primary outcome of interest was in-hospital mortality. Relationship between T-POC compliance score and risk-adjusted mortality was measured using logistic regression. Patients who were not eligible for any T-POC were excluded from this part of the analysis. Risk adjustment models included age; gender; mechanism of injury; injury severity score; first systolic blood pressure in ED; total Glasgow Coma Scale (GCS) in ED; and Abbreviated Injury Scale for injuries to head, chest, and abdomen. The final model for mortality was also used to estimate the number of lives that may be saved by improvements in compliance rates. Compliance score was also entered as a predictor in the model. All statistical analysis was performed using SAS (SAS Inc, Cary, NC).

RESULTS

Patient characteristics are summarized in Table 1 and reflect a typical urban trauma patient population. Median age was 41 years, 65% were men, 53% were Caucasians, 88% sustained a blunt mechanism, and 55% were uninsured. Overall in-hospital mortality was 12%, complication rate was 22%, and median LOS was 5 days. Of the study population, 774 patients (77%) were eligible for at least 1 of the 25 T-POC (Table 2). Of these, 197 (25%) were eligible for only one T-POC, 159 (21%) for two T-POC, 132 (17%) for three T-POC, and the rest of 286 (37%) were eligible for four or more T-POC. Compliance rates with various T-POC ranged from 10% to 99% (Table 2 and Fig. 1). Compliance in 90% or more eligible patients was achieved in only three T-POC: blood transfusion in hypotensive patients, endotracheal intubation with low GCS, and laparotomy for gunshot wounds to the abdomen. For patients who were eligible for at least one T-POC, the median Compliance Score was 60 (interquartile range, 29–100), suggesting that half of the patients only received 60% of the care they needed (Fig. 2). Less than a third of the patients had a 90% or higher compliance score (Fig. 2). In the multivariable model (controlling for all potential confounders listed in Methods section), there was a significant association between compliance score and mortality (odds ratio, 0.9862; 95% confidence interval, 0.9758–0.9967; $p = 0.01$) meaning that for every 1% increase in compliance score, the risk of mortality decreased by 1.38%. In other words, each 10% increase in compliance score was associated with an almost 14% reduction in risk-adjusted mortality. Hence, increasing compliance with these 25 POC from the observed rate of 57% to 100% has the potential to save 52 lives.

TABLE 1. Patient Characteristics and Crude Outcomes

Age (yr, Median With IQR)	**41 (27, 60)**
Male gender	65%
Blunt mechanism	88%
Ethnicity—minority	47%
Insurance—none, including Medicaid	54%
Injury Severity Score (median with IQR)	16 (10, 24)
Systolic blood pressure (mm Hg, median with IQR)	133 (114, 152)
Glasgow Coma Scale (median with IQR)	15 (14, 15)
Head injuries	49%
Chest injuries	46%
Abdominal injuries	28%
Mortality rate (crude)	12%
Complication rate (crude)	22%
Length of stay (d, median with IQR)	5 (3, 9)

IQR, interquartile range.

DISCUSSION

The findings of this study demonstrate large variations in clinical practices resulting in inadequate compliance with several commonly recommended clinical POC that are necessary for optimal management of trauma patients. These data also suggest that significant improvements in patient mortality may be achieved by improving compliance with these T-POC.

There are several implications of our findings. Trauma quality improvement efforts to date have focused on the availability of optimal resources. This approach has been highly successful as evidenced by the expansion of trauma systems and designated trauma centers. From 1991 to 2002, the number of trauma centers in the country has more than doubled, from 471 to 1,154.[19] Existing criteria for trauma center designation are based on expert consensus but not on patient outcomes. However, centers meeting these criteria have demonstrated improved outcomes.[1] Despite two decades of experience with trauma center designation process, it remains unclear which specific institutional structures and POC, or their combination, contribute to patient outcomes. In addition, designation criteria primarily focus on institutional structures with little emphasis on POC. The underlying assumption suggests that if resources are available, patients will receive the care they need. However, the findings of this study suggest otherwise. The results show that despite availability of adequate resources, almost half of the patients did not receive the care they should have. Hence, the focus of trauma quality improvement needs to shift from provision of "optimal resources" to provision of "optimal care."

Practice management guidelines have been developed by several professional societies and quality improvement forums to improve the quality of care. However, there has been little emphasis on measuring compliance with these guidelines which has resulted in inconsistent practices. Currently, there are no mechanisms in place to measure adoption of these guidelines in daily clinical practices. Hence, our observations are not surprising. The overall median compliance score of 60 is consistent with previous reports on management of other acute and chronic diseases showing that, on average, Americans receive about half of recommended medical care processes.[20] For example, it has been shown that less than half of patients with acute myocardial infarction who were eligible for thrombolytic therapy received it during hospitalization.[21] Only 45% of patients who suffered heart attacks received beta-blockers, whereas only 28% smokers received advice on smoking cessation. Never-

TABLE 2. Process of Care

Process	Eligible Number (%)	Compliant Number (%)	Inter-Rater Agreement (Kappa)	Time to Process (Median)
Head CT scan	295 (30)	218 (74)	1.00	16 min
CT angiography neck for blunt cerebrovascular injuries	262 (26)	37 (14)	0.97	
PRBC transfusion	117 (12)	116 (99)	0.92	16 min
Blood gas measurement	117 (12)	72 (62)	1.00	11 min
Endotracheal intubation	90 (9)	86 (96)	0.93	5 min
FFP or PCC	37 (4)	15 (41)	0.24*	5 h
ED thoracotomy	8 (<1)	2 (25)	1.00	
Laparotomy in abdominal gunshot wounds	11 (1)	10 (91)	1.00	39 min
Laparotomy in blunt abdominal trauma	20 (2)	10 (50)	Too few to evaluate	51 min
External pelvic compression (binder, sheet, other devices) in ED	23 (2)	6 (26)	1.00	34 min
Angioembolization	23 (2)	4 (17)	1.00	2 h
Preoperative antibiotics	83 (8)	68 (82)	1.00	
Craniotomy	74 (7)	11 (15)	1.00	3.5 h
Intracranial pressure monitor	100 (10)	10 (10)	1.00	5.5 h
I&D in operating room	17 (2)	10 (59)	1.00	3 h
Intravenous antibiotics	17 (2)	15 (88)	1.00	1 h
Definitive fracture fixation	89 (9)	78 (89)	1.00	Day 1
Operative pelvic fixation	94 (9)	38 (40)	1.00	Day 2
Initiation of DVT prophylaxis (chemical or filter)	224 (22)	145 (65)	1.00	Day 2
Initiation of nutrition (Enteral or TPN)	276 (28)	194 (70)	1.00	Day 3
Low stretch ventilation (≤6 mL/kg)	0	NA	NA	
VAP—specimen obtained before antibiotic use	45 (5)	39 (87)	1.00	
SBI before discharge from hospital	322 (32)	143 (44)	0.970	
Physical therapy/rehabilitation evaluation	138 (14)	96 (70)	1.00	Day 2
Abdominal CT scans for blunt solid organ injuries	121 (12)	92 (76)	0.48*	

ARDS, acute respiratory distress syndrome; CT, computed tomography; DVT, deep venous thrombosis; FFP, fresh frozen plasma; I&D, irrigation and debridement; INR, international normalized ratio; IRR, inter-rater reliability; PCC, prothrombin complex concentrate; PRBC, packed red blood cells; SBI, alcohol screening and brief intervention; SBP, systolic blood pressure; TPN, total parenteral nutrition; VAP, ventilator-associated pneumonia.

* Eligibility criteria for these process measures were modified in the data entry tool during the chart review. Kappa calculations did not account for these modifications.

theless, some of the deficits in delivery of trauma care that are identified in this study are truly astounding, especially considering the fact that the study focused on basic clinical practices in trauma care, not cutting-edge research. For example, only half of the patients with severe blunt abdominal injuries who were hypotensive underwent a laparotomy. Over a third of the patients with open fractures of femur and tibia did not undergo an operative irrigation and debridement of their fractures. Only a quarter of patients with pelvic fracture who were hypotensive received external pelvic compression. Nine of 10 patients with documented intracranial injury on computed tomography and a low GCS who were intubated were managed without an intracranial pressure monitor. Lack of provision of basic clinical care reflects a significant quality gap. In addition, variable practice patterns result in over- or underutilization of specific therapies and increase healthcare costs with no improvement in patient outcomes.[21]

It is clear that the availability of "optimal resources" does not ensure delivery of "optimal care." Delivery of optimal care requires translation of scientific knowledge into everyday practice.[22] It is a complex process with several interacting components. Hence, it is unlikely that a single intervention can improve clinical practices. Clearly, it is important to monitor and report compliance with common clinical processes. The use of standardized order sets and computerized decision support systems has been shown to improve compliance with recommended POC.[23,24] Another approach is the use of checklists.[25] A checklist of clinical care processes that a patient is eligible for based on his injuries and injury severity may enable providers to monitor these processes at the bedside regularly and use them as a performance improvement tool. Further multi-institutional studies, and perhaps interventional trials, need to be undertaken to identify best POC at trauma centers. The resultant set of POC, when viewed together, will permit a robust assessment of quality of care provided to injured patients. These processes, which may be called Trauma Core Measures, will maximize the patients' likelihood of survival, minimize the risk of complications, and may reduce healthcare resource utilization. In addition, the findings will spur coalitions of stakeholders to improve the quality of care across all trauma centers and may be used to improve the criteria used for verification and designation of trauma centers.

This study has a few limitations that must be acknowledged. It is a retrospective analysis with all its inherent limitations. The findings reflect the experience of a single

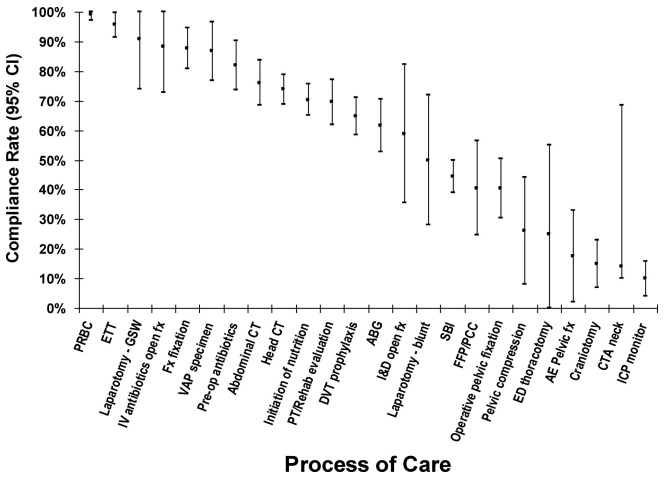

Figure 1. Compliance rate (percentage with 95% confidence interval). ABG, arterial blood gas; AE, angioembolization; CT, computed tomography; CTA, computed tomographic angiography; DVT, deep venous thrombosis; FFP, fresh frozen plasma; ETT, endotracheal tube; Fx, fracture; ICP, intracranial pressure; I&D, irrigation and debridement; IV, intravenous; PCC, prothrombin complex concentrate; PRBC, packed red blood cells; PT, physical therapy; SBI, alcohol screening and brief intervention; VAP, ventilator-associated pneumonia.

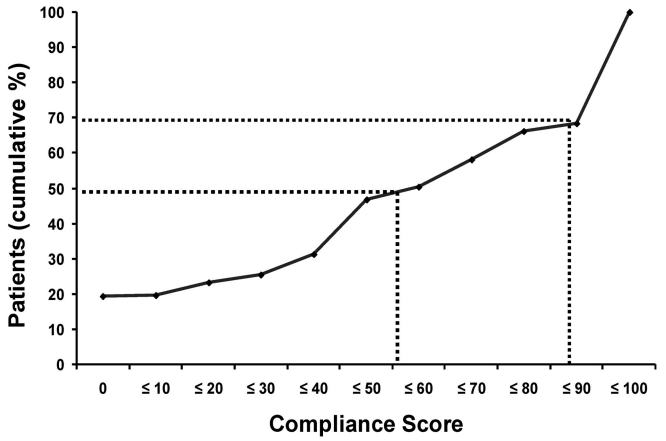

Figure 2. Compliance score.

urban institution with its own unique characteristics. Compliance with specific processes was determined based on the documentation provided in the charts, and lack of documentation may not mean lack of compliance. In addition, we were not able to analyze the reasons for noncompliance. For example, it is possible that patients who were eligible for endotracheal intubation but did not undergo intubation might have been designated as do not resuscitate or may have had sustained nonsurvivable injuries in which case the intervention would be futile. Because of these reasons, it is probably safe to say that "optimal" care may not necessarily mean 100% compliance. Large multicenter studies are needed to define "acceptable" or "optimal" compliance rate for each specific process. The study did not have enough power to determine specific clinical processes that were independent predictors of patient outcomes. Nor was the study design appropriate to determine the impact of specific interventions on patient outcomes as that would require a randomized controlled trial. In other words, our findings do not identify "best practices" in trauma. However, they do suggest that compliance with currently recommended care has the potential to improve patient outcomes but that needs to be proven in a prospective study. The processes studied were chosen by the study investigators only. An important consideration in the selection process was our ability to obtain information in a retrospective chart review within the resources available. It is possible that a longer list of processes may be more appropriate to measure the quality of care. It should be noted that McGlynn et al. [20] measured an average of 15 processes per patient whereas we measured 25 processes in this study. Moreover, the purpose of this study was not only to validate which POC work the best but also to determine whether our practices were consistent with published guidelines. We would also like to emphasize that although the statistical analysis suggests that additional lives could be saved with increased compliance with T-POC, we do not know the causes of death in this patient population. In fact, peer review during this time period did not find any preventable or potentially preventable deaths. Thus, impact on mortality and other outcomes, such as complications, costs, and LOS, needs further study. However, we think that this discrepancy provides further impetus to elucidate details of care provided to the patients, especially given our finding of improvement in patient outcomes associated with increased compliance with recommended care. A more detailed analysis of how the care is delivered to individual patients using a structured and standardized approach based on current practice guidelines will make the peer review process more informative.

In conclusion, the findings of this study demonstrate that compliance with several generally recommended clinical processes for management of patient with moderate to severe traumatic injuries remains inadequate. Improved compliance with these processes has the potential to significantly reduce mortality. The focus of quality improvement in trauma care needs to shift from "optimal resources" to "optimal care." In short, we can do better.

AUTHORSHIP

S.S., N.R., and N.F. designed this study. S.S., N.R., and S.B. collected and analyzed the data. All authors participated in manuscript preparation.

DISCLOSURE

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APPENDIX

TABLE A1. Processes of Care

Care Aspect	Process	Eligible Patients
1. Initial evaluation	Head CT scan	Blunt mechanism AND GCS score <15 upon initial assessment
2. Initial evaluation	CT angiography neck for blunt cerebrovascular injuries	Blunt mechanism AND any one of the following fractures Le Forte II or III facial OR cervical spine OR base of skull
3. Resuscitation	PRBC transfusion	Hypotensive (SBP ≤90 mm Hg) upon arrival
4. Resuscitation	Blood gas measurement	Hypotensive (SBP ≤90 mm Hg) upon arrival
5. Resuscitation	Endotracheal intubation	GCS score ≤8 upon initial assessment
6. Head injuries	FFP or PCC	Intracranial bleed AND INR ≥1.5
7. Resuscitation	ED thoracotomy	Pulse present upon arrival AND died in ED
8. Hemorrhage control	Laparotomy in abdominal gunshot wounds	Gunshot wound abdomen AND SBP ≤90 mm Hg AND any abdominal injury
9. Hemorrhage control	Laparotomy in blunt abdominal trauma	Blunt mechanism AND SBP ≤90 mm Hg AND Abdominal AIS score ≥4
10. Hemorrhage control	External pelvic compression (binder, sheet, other devices) in ED	Pelvic fracture AND SBP ≤90 mm Hg
11. Hemorrhage control	Angioembolization	Pelvic fracture AND SBP ≤90 mm Hg
12. Operative care	Preoperative antibiotics	Patients undergoing laparotomy
13. Head injuries	Craniotomy	GCS score ≤8 AND intracranial bleed on head CT
14. Head injuries	Intracranial pressure monitor	GCS score ≤8 AND intracranial bleed on head CT AND endotracheal intubation
15. Fracture management	I&D in operating room	Open fracture femur OR tibia
16. Fracture management	Intravenous antibiotics	Open fracture femur OR tibia
17. Fracture management	Definitive fracture fixation	Open or closed fracture femur OR tibia AND not in ICU
18. Fracture management	Operative pelvic fixation	Operative pelvic fracture AND no intracranial bleed AND no acute lung injury
19. Critical care	Initiation of DVT prophylaxis (chemical or filter)	No Intracranial bleed AND any one of the following: femur fracture, tibia fracture, intubated
20. Critical care	Initiation of nutrition (Enteral or TPN)	Intubated patients
21. Critical care	Low stretch ventilation (≤6 mL/kg)	ARDS
22. Critical care	VAP—specimen obtained before antibiotic use	Pneumonia AND intubated
23. Injury prevention	SBI before discharge from hospital	Nondependent drug use
24. Rehabilitation	Physical therapy/rehabilitation evaluation	Fracture femur OR tibia OR pelvis AND not in ICU
25. Evaluation	Abdominal CT scans during hospital stay	Patients with blunt injuries to liver, spleen, kidneys managed nonoperatively

ARDS, acute respiratory distress syndrome; CT, computed tomography; DVT, deep venous thrombosis; FFP, fresh frozen plasma; I&D, irrigation and debridement; INR, International Normalized Ratio; IRR, inter-rater reliability; PCC, prothrombin complex concentrate; PRBC, packed red blood cells; SBI, alcohol screening and brief intervention; SBP, systolic blood pressure; TPN, total parenteral nutrition; VAP, ventilator-associated pneumonia.

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DISCUSSION

Dr. Mark R. Hemmila (Ann Arbor, Michigan): Dr. Shafi and co-authors have presented excellent work in examining the trauma-specific processes of care and their ability to reduce in-hospital mortality.

The take-home point of this paper is that for every percentage increase in compliance with these processes of care, they found a 14% reduction in mortality.

I'm a firm believer that the more we can reduce unnecessary variation in care, the more likely we are to see improved results for our patients, keeping in mind that there will always be occasional exceptions to these processes based on circumstances.

Examining processes of care and also performance of external data validation represent two difficult but necessary areas for TQIP in which much heavy lifting will be required.

Digging a little deeper, the most striking result to me was the wide variation in compliance with these trauma specific processes of care. Specifically, rates of compliance were low for ICP monitor and craniotomy of brain injured patients and were also low for pelvic compression and angioembolization in pelvic fracture patients. Is this because the software algorithm identified patients with a set of injuries that suggests the potential for intervention but in reality intervention would be futile (i.e. a bad brain injury in an elderly patient) or is it reflective of lapses in optimal care? Along these lines, I have the following three questions:

First, your results suggest that within a trauma system there are still many patients with preventable or potentially preventable mortality. For the same cohort do you have any data on how many patients were peer reviewed and found to be within this category?

Secondly, there may be factors why aggressive neurosurgical intervention was not pursued. For example a patient may appear to be a candidate for life saving intervention but the surgeons and family are concerned about a non-functional outcome. Does this explain low compliance with the use of neurosurgical interventions?

And third, is there a subgroup of three to five processes of care from your study when applied prospectively would potentially result in improved outcomes? In other words, which of these trauma-specific processes of care when followed is likely to have the greatest impact?

Thank you for the opportunity to discuss this paper. I think this is excellent work. It involves a lot of nitty-gritty digging into the data. I look forward to more work from Dr. Shafi on this topic.

Dr. Ajai K. Malhotra (Richmond, Virginia): I really enjoyed that work and the presentation. But I'm curious, is this trauma center maybe not a good trauma center? Did you go back and look at the caterpillar curve from the TQIP data to see where it stood? Is it one of the higher-performing centers, lower-performing centers, or intermediate centers?

Dr. Walter Biffl (Denver, Colorado): It's a nice presentation, but for those of us who do work in quality in our institutions, we're all well aware of the shortcomings of the administrative data bases.

While a TQIP is certainly a rigorous one, my primary question is how are you proposing that these data be used?

I mean, looking at the low compliance factors on your list, there is certainly not consensus among those in this room or among large organizations how ED thoracotomy should be applied, who should be screened for cerebrovascular injury, who should have pelvic angioembolization, etc. So you need to be careful about publishing these data and saying a center is not doing their job if they're not complying with certain measures.

Dr. Thomas J. Esposito (Maywood, Illinois): I was just wondering if any of the particular compliance violations were anymore egregious than others with regard to mortality? And, secondly, did you assess the impact on morbidities as well?

Dr. Carnell Cooper (Baltimore, Maryland): In the last statement in your abstract, you suggest that trauma centers of excess mortality may improve with application of this process. Does it suggest that only if you have excess mortality this is helpful? Is it not helpful in improving if you are from an average trauma center?

Dr. Shahid Shafi (Grapevine, Texas): That wasn't so bad, actually. Dr. Hemmila asked me three questions:

How many of these deaths were preventable? Now, let me preface my answer – and I have five minutes I can go on – that this study has, of course, certain limitations. This is a single center experience. And we all know that the practices vary from institution to institution and from within an institution from surgeon to surgeon.

In fact, I was looking at the program. There are at least two other papers, one is by Dr. Hoyt who is going to talk

about the variation in practices for DVT screening, and there was another paper which talks about variation in practices about pain management for rib fractures. So those variations are common.

What I showed is a reflection of a single center, but the data are quite compelling, especially the relationship between the compliance with processes and the outcomes.

How many of these deaths were preventable? During the same three year period, there were 500 or close to 600 deaths at the trauma center. Only 17 of those deaths were classified as potentially preventable. The rest of them were classified as non-preventable. Now, but we all know that at some point every death becomes inevitable. And this is actually the talk of another paper. Here we will talk about the failure to rescue.

So if you have complication, you fail to rescue and then the patient inevitably goes into multiple organ failure and dies, is that a non-preventable death? So I think that we need to look at that classification more carefully.

Low compliance with neurosurgical interventions is pretty obvious in our data set. In every trauma center that I have worked at, that has always been discussed between trauma surgeons and neurosurgeons as to what needs to be done. The Brain Trauma Foundation Guidelines look pretty clear to us, but they don't look as clear to neurosurgeons. So, all I can say is that just reflects local practices. I don't know why. In this retrospect analysis we were not able to identify the causes of why certain things were not done.

A critical question that you asked, and I think Dr. Esposito also asked the same question, is which of these 25 processes of care are the critical ones? Well, again, I think the concept that I am trying to convey is that there is no magic bullet. I think what we have to do is provide good quality care day in and day out. And that requires going through multiple processes and making sure that every one of them is crossed.

I think that the 25 processes that were chosen should be looked at simply as an indicator of the quality of care – not that DVT prophylaxis will save life but if you're doing DVT prophylaxis diligently in more than 90% of your patients you are probably doing everything else right too.

So in this particular data set, we did not have the power to identify which specific processes of care are associated with mortality. Now, we have recently received funding from National Trauma Institute to expand the study to three centers and hopefully in a couple of years we will be able to give you some more information.

Dr. Malhotra asked if this is a poorly-performing trauma center. Dr. Peitzman, what can I say? I mean, I guess I'm up here and I'm willing to say that we are not doing as well as we think we are. The question is, are you willing to do the same? But this particular center is actually, I don't know their OT ratio. They are not a participant in TQIP, yet. But they obviously allowed me access to their data.

Dr. Biffl asked about the specific processes of care. I totally agree with you. There is no consensus on which processes actually matter. But, again, I think concept is to look at compliance as an indicator of quality of care and not an end in itself.

The last question Dr. Esposito asked was have you looked at morbidities? We have started looking at morbidities but our analysis is still quite preliminary. And it actually seems like the more lives you save the complications go up. So the increase in compliance seems to be associated with the increase in complication rates and length of stay, but we are not sure about that yet.

I'm missing the last question. This was about improvements. Which process is more important? I already addressed that. Oh, yes, how would you use it? The reason I started looking at it is because if TQIP tells a center that you're not doing well, we must also tell them what you can do to improve yourself. So this was an example of what can be done to improve yourself. But certainly every center will probably improve their outcomes based on better compliance.

Barriers to compliance with evidence-based care in trauma

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BACKGROUND: We have preciously demonstrated that trauma patients receive less than two-thirds of the care recommended by evidence-based medicine. The purpose of this study was to identify patients least likely to receive optimal care.

METHODS: Records of a random sample of 774 patients admitted to a Level I trauma center (2006–2008) with moderate to severe injuries (Abbreviated Injury Scale score ≥3) were reviewed for compliance with 25 trauma-specific processes of care (T-POC) endorsed by Advanced Trauma Life Support, Eastern Association for the Surgery of Trauma, the Brain Trauma Foundation, Surgical Care Improvement Project, and the Glue Grant Consortium based on evidence or consensus. These encompassed all aspects of trauma care, including initial evaluation, resuscitation, operative care, critical care, rehabilitation, and injury prevention. Multivariate logistic regression was used to identify patients likely to receive recommended care.

RESULTS: Study patients were eligible for a total of 2,603 T-POC, of which only 1,515 (58%) were provided to the patient. Compliance was highest for T-POC involving resuscitation (83%) and was lowest for neurosurgical interventions (17%). Increasing severity of head injuries was associated with lower compliance, while intensive care unit stay was associated with higher compliance. There was no relationship between compliance and patient demographics, socioeconomic status, overall injury severity, or daily volume of trauma admissions.

CONCLUSION: Little over half of recommended care was delivered to trauma patients with moderate to severe injuries. Patients with increasing severity of traumatic brain injuries were least likely to receive optimal care. However, differences among patient subgroups are small in relation to the overall gap between observed and recommended care. (*J Trauma.* 2012;72: 585–593. Copyright © 2012 by Lippincott Williams & Wilkins)

LEVEL OF EVIDENCE: II.

KEY WORDS: Quality improvement; practice guidelines; innovation diffusion.

In 2006, the Committee on Trauma of the American College of Surgeons implemented the Trauma Quality Improvement (TQIP) program to measure and improve the quality of care in trauma. TQIP demonstrated that risk-adjusted patient outcomes varied between designated trauma centers despite availability of optimal resources.[1,2] Donabedian[3] principles of quality improvement suggest that outcomes depend on structures and processes. Hence, if structural characteristics of trauma centers are similar based on their designation status, variations in patient outcomes must be related to differences in processes of care.

In an attempt to reduce variations in care and increase adoption of evidence-based practices, "core measures" were developed by the Centers for Medicare and Medicaid Services and the Joint Commission. These core measures focus on management of acute myocardial infarction, congestive heart failure, ventilator-associated pneumonia, and surgical care improvement. However, adoption of evidence-based practices in healthcare remains a challenge. In a nationwide study, McGlynn et al.[4] showed that only 55% of patients received recommended care. Also, we have previously shown that surgical care improvement measures have little relevance to outcome of trauma patients.[5] Several trauma professional societies have developed evidence- or consensus-based practice management guidelines.[6–10] However, compliance with these guidelines is neither measured nor reported in routine trauma practice. Recently, we demonstrated that these guidelines are often not followed as less than two-thirds of the patients receive the recommended care. In that study of 25 trauma-specific processes of care (T-POC), compliance rate with each varied from as little as 10% to close to 100%. Most importantly, we demonstrated that every 10% increase in compliance rate was associated with a 14% reduction in risk-adjusted in-hospital mortality.[11]

Barriers to adoption of recommended care are poorly understood.[4] In the case of trauma patients, little is known about reasons why patients receive suboptimal care. The purpose of this study was to identify patients who are at high risk of receiving suboptimal care. The hypothesis for this article was that trauma patients least likely to receive recommended care (evidence or consensus based) can be prospec-

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tively identified. Such patients may then be targeted for increased adoption of optimal trauma care.

PATIENTS AND METHODS

This was a 3-year retrospective study (January 1, 2006, through December 31, 2008) of patients treated at a large, urban Level I trauma center. During the study period, the trauma registry included a total of 7,581 patients. We examined the relationship of trauma patient characteristics such as demographics, socioeconomic status, injury type and severity, and certain trauma center characteristics with delivery of care recommended by several professional societies (details given below). The methods for patient selection and data collection have been described previously and are summarized below.[11]

Trauma-Specific Processes of Care

Twenty-five T-POC were selected for inclusion in this study after reviewing clinical practice guidelines from the American College of Surgeons Committee on Trauma, Eastern Association for the Surgery of Trauma, Society for Critical Care Medicine, the Brain Trauma Foundation, the Glue Grant Consortium, and Surgical Care Improvement Project (Appendix).[6–10,12] As it was not possible to measure all care processes, only 25 T-POC were selected by the investigators based on their likelihood of directly impacting patient outcomes and our ability to measure them from retrospective chart reviews. Selected T-POC encompassed all features of trauma care, including initial evaluation, resuscitation, operative care, critical care, rehabilitation, and injury prevention. We focused on four specific groups of types of injuries: traumatic brain injuries (TBI), hemorrhagic shock (systolic blood pressure $\leq$90 mm Hg), pelvic fractures, and long bone extremity fractures (femur or tibia). TBI was selected as it is the most common cause of death and disability in trauma patients. Hemorrhage was chosen as it is the second leading cause of death and most amenable to timely interventions. Long bone extremity fractures were chosen because they represent a common injury and are the second most common cause of disability after TBI.

Patient Selection

TQIP criteria were used to identify patients for the study.[2] Inclusion criteria consisted of the following:

1. Adults, defined as age $\geq$16 years.
2. Moderate to severe injuries, defined as at least one injury Abbreviated Injury Scale (AIS) score $\geq$3.

Exclusion criteria consisted of the following:

1. Delayed admissions, defined as time from injury to arrival in emergency department $\geq$1 day.
2. Those deemed dead upon arrival.
3. Gunshot wounds to the head or penetrating injuries outside the torso (torso defined as neck, chest, and abdomen).
4. Primary mechanism of injury of burns, poisoning, drowning, hanging, submersion, and asphyxiation.

Application of inclusion/exclusion criteria identified 2,242 patients. From these, 1,000 patients were randomly selected for chart reviews. Six patients were later eliminated due to incomplete information that could not be obtained. The remaining 994 patients were then reviewed for their eligibility for T-POC.

Patient Eligibility for T-POC

Data from the trauma registry were used to identify patients who were eligible for each T-POC. Eligibility was determined by a combination of injury mechanism, type and severity, comorbidities, and procedures (Appendix). Whenever possible, all variable definitions were based on those in the National Trauma Data Standard Data Dictionary (Version 1.2.5).[13] Our previous work was used to supplement the definition of complications.[14] Classifications from the American Association for the Surgery of Trauma were used to identify solid organ injuries and related procedures.[15] Injuries were classified into blunt and penetrating based on the matrix of E-code groupings recommended by the Centers for Disease Control.[16] Injury mechanism, type, complications, and procedures were identified using ICD-9 codes and validated by the trauma registry staff. A software tool was created which used information contained in the trauma registry to identify patients who were eligible for specific T-POC. The accuracy of the tool in detecting patients eligible for specific T-POC was validated by a manual review of registry data in a random sample of 72 patients. For each T-POC, at least one patient who was considered eligible and one who was considered ineligible by the tool were identified, and their trauma registry data were manually examined to determine accuracy of classification by the tool. If any disagreement was identified, the tool was modified and revalidated until no further discrepancies were identified. Once validation was complete, the tool was applied to the previously identified 994 patients to identify those who were eligible for the selected T-POC. A total of 774 patients were eligible for at least one T-POC and constituted the study population.

Data Collection

A data dictionary was developed that defined each specific T-POC and included a source hierarchy to determine whether patients received T-POC for which they were eligible. Four nurse abstractors reviewed patient charts (all electronic) after undergoing training on data abstraction guidelines. During training, each nurse abstractor reviewed 10 test patient charts. Discrepancies between reviewers were discussed and definitions were clarified to minimize ambiguity during data abstraction. To enhance validation of data collection quality, an independent nurse reviewer abstracted data from randomly selected charts for 82 patients. Interrater reliability designed to test agreement between auditors was then determined using κ statistic. Agreement was high ($\kappa > 0.9$), except for two T-POC whose definition was modified after initial data collection.

Data Analysis

Outcome

Patients in this study were eligible for a total of 2,603 T-POC which constituted the unit of observations. The primary outcome of interest was compliance with each of the T-POC. Multiple imputation was used to estimate missing data related to race, systolic blood pressure, Glasgow Coma Scale (GCS) score, median income, and insurance status.

Predictors

Several predictors of compliance with T-POC were explored. These are listed below:

Sociodemographics—age, gender, race/ethnicity, insurance status, and median yearly household income estimated using zip code of residence.

Injury characteristics—mechanism of injury, Injury Severity Score, systolic blood pressure (SBP) and GCS score upon presentation, number of T-POC each patient was eligible for, length of stay, AIS head, chest, and abdomen, intensive care unit (ICU) utilization, and ventilator support.

Trauma center characteristics—trauma patient volume on the day of admission, time of week (weekend Friday through Sunday vs. weekdays), and time of presentation (day 7 AM to 7 PM vs. nights 7 PM to 7 AM).

Analytics

A generalized estimating equation with a logit link was used to model the relationship between compliance with T-POC measures and several predictors. Generalized estimating equations take into account the correlation among outcomes or in this case within a patient as several patients were eligible for more than one T-POC. Univariate analysis was carried out first and then a multivariate model was developed to identify independent predictors of compliance with T-POC after adjusting for potential confounding factors. All analysis was conducted using SAS version 9.3 (SAS Institute, Cary, NC). Test statistics with an associated probability of ≤ 0.05 were considered statistically significant unless otherwise noted. Data are summarized as means ($\pm$standard deviations) and medians (interquartile range) for continuous variables and proportions for categorical variables.

RESULTS

Patient characteristics are summarized in Table 1 and reflect a typical urban trauma patient population. Of 774 patients in the study, the number of T-POC each patient was eligible for ranged from 1 to 17, with a median of 3 processes per patient. Of these, 197 patients (25%) were eligible for only one T-POC, 159 (21%) for two, 132 (17%) for three, while the rest of 286 patients (37%) were eligible for four or more T-POC. Compliance rates with various T-POC ranged from 10% to 99% as reported in our previous study.[11] Of the total 2,603 T-POC that the study population was eligible for, only 1,515 were actually provided to the patients for an overall compliance rate of 58%. The compliance was highest with T-POC related to initial resuscitation and lowest for those related to management of head injuries (Fig. 1). The rate of compliance increased with increasing number of T-POC patients were eligible for (Fig. 2). However, compliance declined when the number of T-POC per patient exceeded five processes.

The univariate analysis (Table 2) showed that the following predictors were associated with increased likelihood of compliance with T-POC: having health insurance, ICU

TABLE 1. Patient Characteristics (N = 774)

Characteristics	
Age, yr, median (IQR)	38 (25–57)
Male gender	68%
Blunt mechanism	86%
Race/ethnicity*	
White	50%
Minorities	50%
Injury Severity Score, median (IQR)	17 (10–26)
Injury Severity Score >24	31%
Systolic blood pressure, mm Hg, median (IQR)	130 (109–150)
Systolic blood pressure ≤90 mm Hg	16%
Glasgow Coma Scale, median (IQR)	15 (12–15)
Glasgow Coma Scale ≤8	22%
Length of stay, d, median (IQR)	5 (3–10)
Intensive care unit stay	51%
Mortality	15%

IQR, interquartile range.
* Note that one patient had missing race/ethnicity.

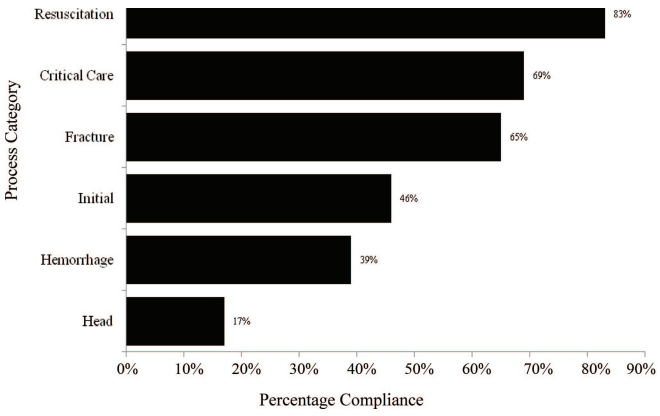

Figure 1. Compliance by T-POC by aspect of trauma care.

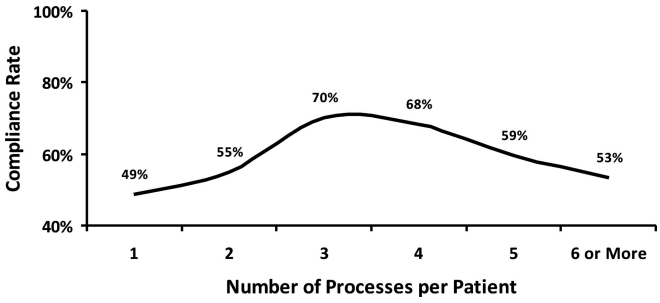

Figure 2. Compliance rate by number of T-POC per patient.

TABLE 2. Potential Predictors of Compliance: Univariate Analysis

Predictor	Process Compliance Rate	OR (95% Confidence Interval)
Age (yr)		
>65	58%	0.99 (0.75–1.32)
≤65	58%	
Gender		
Female	56%	0.87 (0.70–1.08)
Male	59%	
Ethnicity		
Caucasians	60%	1.13 (0.93–1.38)
Minorities	57%	
Mechanism of injury		
Blunt	59%	1.25 (0.95–1.65)
Penetrating	54%	
Insurance status*		
Insured	62%	1.25 (1.02–1.54)
Uninsured	56%	
Household income when compliant $US, median (IQR)	50, 823 (38, 988–62, 499)	1.02 (0.97–1.07)
Household income when noncompliant $US, median (IQR)	48, 133 (38, 989–60, 094)	
AIS head*		
1–2	67%	0.89 (0.85–0.93)
3–4	52%	
5–6	49%	
AIS thorax*		
1–2	55%	0.91 (0.87–0.97)
3–4	58%	
5–6	40%	
AIS abdomen		
1–2	52%	1.00 (0.94–1.07)
3–4	62%	
5–6	59%	
Glasgow Coma Scale*		
>8	65%	1.07 (1.05–1.09)
≤8	48%	
Injury Severity Score*		
>24	53%	0.98 (0.98–0.99)
≤24	62%	
Systolic blood pressure (mm Hg)*		
>90	62%	1.55 (1.25–1.94)
≤90	51%	
Intensive care unit stay*		
Yes	64%	1.70 (1.40–2.05)
No	51%	
Ventilator use*		
Yes	68%	1.60 (1.29–1.99)
No	57%	
Time of admission		
Day—7 AM to 7 PM	59%	1.03 (0.84–1.25)
Night—7 PM to 7 AM	58%	
Day of admission		
Weekend (Fri-Sun)	59%	1.07 (0.88–1.30)
Weekday (Mon-Thur)	57%	
Total daily trauma admissions when compliant, median (IQR)	8 (6–10)	0.99 (0.95–1.02)
Total daily trauma admissions when noncompliant, median (IQR)	8 (6–10)	
Length of hospital stay when compliant*, median days (IQR)	7 (4–19)	1.03 (1.02–1.04)
Length of hospital stay when noncompliant*, median days (IQR)	3 (1–9)	

OR, odds ratio; IQR, interquartile range.
* $p < 0.05$.

TABLE 3. Independent Predictors of Compliance With T-POC: Multivariate Analysis

Predictor	OR (95% Confidence Interval)
Blunt traumatic injury	1.49 (1.12–1.20)
AIS injury of the head	0.86 (0.81–0.92)
Glasgow Coma Scale	1.04 (1.02–1.06)
Intensive care unit stay	1.49 (1.22–1.82)
Length of stay (d)	1.02 (1.02–1.03)

stay, ventilator support, increasing hospital length of stay, higher GCS, and increasing systolic blood pressure. Decreased compliance was associated with the following: increasing Injury Severity Score and increasing severity of head and chest injuries. There was no association between compliance with T-POC and age, gender, race/ethnicity, mechanism of injury, household income, abdominal injuries, trauma volume on the day of admission, weekend versus weekday admission, or day versus night admission.

Multivariate analysis (Table 3) revealed that the independent predictors of increased compliance with T-POC consisted of blunt mechanism of injury, increased total hospital length of stay, and ICU stay. Increasing severity of head injuries (increasing AIS, decreasing GCS) was associated with lower likelihood of compliance with T-POC.

DISCUSSION

We have previously shown that less than two-thirds of trauma patients with moderate to severe injuries receive optimal care.[11] In this study, we have attempted to explore the reasons for delivery of suboptimal care. Our findings suggest that patients with increasing severity of head injuries are at risk of not receiving the recommended care. On the other hand, patients with a blunt mechanism of injury and those who required ICU stay were more likely to receive optimal care. At the same time, it is encouraging to find that there was no impact of patient demographic or socioeconomic status on patient care. Also, the quality of care was consistent on day versus night, weekends versus weekdays, and was not impacted by the daily volume of trauma patients.

Patient Demographics

Our findings suggest that elderly were as likely to receive appropriate care as the young during their inpatient stay. Age is one of the single most important determinants of mortality in trauma patients. A few studies have noted less aggressive management in the elderly trauma patients as well as those with certain medical diseases.[17–19] This has been attributed to increasing complexity of care along with perceived lack of benefit in this age group leading to decreased adherence to care processes.[18,19] This is despite the fact that many researchers have shown that aggressively managing the severely injured elderly improves their outcomes.[17] With the number of elderly trauma patients predicted to rise to approximately 29% of all trauma hospital episodes, it is imperative to ensure compliance with recommended processes of care in the elderly.[20] We also did not find any association between gender, race/ethnicity, and household income or insurance status on compliance with recommended trauma care in this study. This finding is consistent with our previous study of lack of ethnic disparities in emergency care of trauma patients.[21] The observed equity in trauma care is in contrast to management of medical disease such as acute myocardial infarction, heart failure, and pneumonia where recent studies have demonstrated that suboptimal care is more likely in female patients, the elderly, and ethnic minorities.[4,18] These disparities have been attributed to various social and cultural factors, including language barriers.[18] Lack of such disparities in acute in-patient trauma care is a testament to successful implementation of trauma systems over the last 3 decades.

Injuries and Injury Severity

Complexity of care can certainly impact the likelihood of receiving optimal care.[18] Prior research in medical diseases has shown that patients with the most serious conditions are at a higher risk of receiving suboptimal care and suffer worse outcomes.[18,19] A study of in-patients with acute myocardial infraction, heart failure, and pneumonia revealed that those eligible for more processes of care were less likely to receive all of them.[18] Another study by Scott et al.[19] revealed decreased use of evidenced-based therapies in patients with multiple comorbid conditions such as diabetes, chronic obstructive pulmonary disease, and renal failure. In the case of trauma, one often encounters a patient with multiple blunt injuries such as head injuries, an open tibial fracture, and a complex liver injury. In such scenarios, it is conceivable that some of the recommended care, such as operative irrigation and debridement of open tibial fracture, is delegated to a lower priority and may not be performed in a timely fashion. However, our analysis suggests that the injured patients who require ICU care are almost 50% more likely to receive optimal care than patients who did not require ICU stay even though ICU patients are likely to have more severe and complex injuries. There are several possible explanations for better compliance with recommended care for trauma patients in an ICU environment. This may be attributable to protocol-driven care, management by trained intensivists, closer monitoring of patients, and more intense supervision of trainees.[22] For example, our trauma center implemented an adult ventilator order set in 2007 which may have been a factor in improving compliance with T-POC in patients on ventilators. A corollary to better care in the ICU is that patients who did not need to go to the ICU were less likely to receive optimal care. Hence, this patient group represents an opportunity for improving quality of care.

Head Injuries

Our findings suggest that patients with head injuries are a specific subgroup of trauma patients where increasing severity of injuries was associated with lower likelihood of receiving optimal care even when accounting for ICU stay. In fact, the lowest compliance in this study was observed in T-POC related to management of head injuries. These processes included craniotomy and use of intracranial pressure monitor in patients with an intracranial bleed and GCS score ≤ 8 and reversal of coagulopathy in patients with a coagulopathy and an intracranial bleed. This may be related to

perceived or real futility of care in patients with severe head injuries, clinical judgment about chances of functional recovery, overall prognosis, and response to initial treatment. Compliance with guidelines may also be influenced by local clinical practices. It is possible that neurosurgeons at this particular trauma center may not be convinced of the utility of the three intervention measures in this study. For example, although use of intracranial pressure monitor is recommended by the Brain Trauma Foundation, there is a lack of scientific evidence to support this recommendation.[23]

Trauma Center Characteristics

Little is known about hospital characteristics that may influence quality of patient care. Previous studies have suggested that patient volume, manpower availability, staff expertise, provider attitudes, and time of day of admission can influence patient outcomes. In the current single-center study, we were encouraged by the findings that the quality of care was consistent on weekends versus week days, on nights versus day time, and was not affected by the daily volume of trauma patients. Again, we believe that this is a testament to trauma center designation process that ensures availability of resources 24/7 at Level I trauma centers as well as an organized team approach composed of surgeons and nurses with added qualifications, interest, and expertise in the management of complex issues.

Conceptual Framework for Barrier to Adoption of Best Practices

Innovation diffusion spans a lag of several years from the discovery of a new treatment to its adoption in routine clinical practice.[24] Berwick[25] identified three clusters of influence on the diffusion rate of innovations: perception of the innovation, characteristics of individuals who adopt the change, and the contextual factors within an organization. Other factors that may hinder adoption of new guidelines include lack of awareness or familiarity, clinical disagreement with recommended care, ineffective communication, and high workload.[22] In the case of trauma, several of these factors may play a role concurrently. First, there is little consensus on the ideal processes of care most crucial to improving trauma outcomes. Identifying these processes of care is important, because clinicians must focus limited resources on implementation of practices that are most likely to improve patient outcomes. Second, compliance rates for these trauma-specific processes of care will also need to be monitored and communicated to the clinicians. Understanding areas of care, such as neurosurgical interventions that are a challenge for those adopting a change may identify opportunities for improvement, provides insight of the organizational culture of a trauma center and identify methods for adoption of new care processes. Finally, an understanding of the barriers to compliance of care in trauma centers will help us identify the contextual and managerial factors that may allow quality improvement policies that adapt to local cultures of trauma centers. This article is the first, and a small step, in our attempts to identify gaps in adoption of best practices in the care of the injured.

Study Limitations

This study has few limitations that must be acknowledged. It is a retrospective analysis with all its inherent limitations. The findings reflect the experience of a single urban institution with its own unique characteristics. Compliance with specific processes was determined based on retrospective chart review. Therefore, the findings may simply reflect lack of documentation and not lack of compliance. However, our analysis suggests that there were specific patient groups that did not receive recommended care, suggesting that it was more likely lack of care and not lack of documentation. An important limitation of this study is that we were not able to determine the reasons for noncompliance. For example, it may be appropriate to withhold certain interventions in patients with nonsurvivable injuries or a terminal preexisting condition. In these clinical scenarios, a lack of adherence to practice guidelines by clinicians may not constitute suboptimal care. In addition, this study did not have enough power to identify which specific clinical processes were most important independent determinants of patient outcomes. Hence, we do not know the clinical significance of not receiving optimal care on patient outcomes although a sensitivity analysis in our previous study suggested that each 10% increase in compliance with recommended care reduced mortality by 14%. Finally, this study only looked at in-patient care. There may be similar quality issues in prehospital and postdischarge care of trauma patients.

CONCLUSION

Our study shows that a little over half of recommended care was delivered to trauma patients with moderate to severe injuries who are at highest risk of dying. Two specific groups of patients at highest risk of receiving suboptimal care are those with head injuries and those who do not require ICU stay. Although, the exact estimate of this deficit in quality of care may be debated due to the limitations of the study, it is clear that a gap exists between our knowledge of trauma care and its practice. This gap represents a significant quality chasm in the care of the injured which must be addressed. Further research, including an in-depth analysis of provider perceptions of recommended care and reasons for noncompliance, is needed to develop appropriate interventions.

AUTHORSHIP

N.R. conducted literature searches and, with N.F. and L.M.G., designed this study. R.K. collected data, which were interpreted by N.R., S.B., N.F., R.K., and L.M.G. S.B. conducted statistical analyses. N.R., S.B., D.B., and L.M.G. wrote the manuscript, for which N.R., S.B., and L.M.G. created figures.

DISCLOSURE

The authors declare no conflicts of interest.

APPENDIX

TABLE A1. Trauma-Specific Processes of Care

	Care Aspect	Process of Care	Eligible Patients
1	Initial evaluation	Head CT scan	Blunt mechanism AND GCS score <15 upon initial assessment
2	Initial evaluation	CT angiography neck for blunt cerebrovascular injuries	Blunt mechanism AND any one of the following fractures: Le Forte II or III facial OR cervical spine OR base of skull
3	Resuscitation	PRBC transfusion	Hypotensive (SBP ≤90) upon arrival
4	Resuscitation	Blood gas measurement	Hypotensive (SBP ≤90) upon arrival
5	Resuscitation	Endotracheal intubation	GCS score ≤8 upon initial assessment
6	Head injuries	FFP or PCC	Intracranial bleed AND INR ≥1.5
7	Resuscitation	ED thoracotomy	Pulse present upon arrival AND died in ED
8	Hemorrhage control	Laparotomy in abdominal gunshot wounds	Gunshot wound abdomen AND SBP ≤90 AND any abdominal injury
9	Hemorrhage control	Laparotomy in blunt abdominal trauma	Blunt mechanism AND SBP ≤90 AND abdominal AIS score ≥4
10	Hemorrhage control	External pelvic compression (binder, sheet, other devices) in ED	Pelvic fracture AND SBP ≤90
11	Hemorrhage control	Angioembolization	Pelvic fracture AND SBP ≤90
12	Operative care	Preoperative antibiotics	Patients undergoing laparotomy
13	Head injuries	Craniotomy	GCS score ≤8 AND intracranial bleed on head CT
14	Head injuries	Intracranial pressure monitor	GCS score ≤8 AND intracranial bleed on head CT AND endotracheal intubation
15	Fracture management	I&D in operating room	Open fracture femur OR tibia
16	Fracture management	Intravenous antibiotics	Open fracture femur OR tibia
17	Fracture management	Definitive fracture fixation	Open or closed fracture femur OR tibia AND not in ICU
18	Fracture management	Operative pelvic fixation	Operative pelvic fracture AND no intracranial bleed AND no acute lung injury
19	Critical care	Initiation of DVT prophylaxis (chemical or filter)	No Intracranial bleed AND any one of the following: femur fracture, tibia fracture, intubated
20	Critical care	Initiation of nutrition (enteral or TPN)	Intubated patients
21	Critical care	Low stretch ventilation (≤6 mL/kg)	ARDS
22	Critical care	VAP—specimen obtained before antibiotic use	Pneumonia AND intubated
23	Injury prevention	SBI before discharge from hospital	Nondependent drug use
24	Rehabilitation	Physical therapy/rehab evaluation	Fracture femur OR tibia OR pelvis AND not in ICU
25	Evaluation	Abdominal CT scans during hospital stay	Patients with blunt injuries to liver, spleen, kidneys managed nonoperatively

CT, computed tomography; PRBC, packed red blood cells; ED, emergency department; FFC, fresh frozen plasma; PCC, prothrombin complex concentrate; I&D, irrigation and debridement; DVT, deep venous thrombosis; TPN, total parental nutrition; ARDS, acute respiratory distress syndrome; VAP, ventilator-associated pneumonia; SBI, Alcohol Screening and Brief Intervention.

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DISCUSSION

Dr. L.D. Britt (Norfolk, Virginia): Dr. Croce, I want to thank you and the Program Committee for inviting me to be a discussant on this very important study that could have broad and highly unfavorable implications.

I want to commend Dr. Rayan and Dr. Shafi, along with the other authors, for tackling a sensitive but complex problem with respect to patients receiving the recommended evidence-based care. I will use my time allotted to me to ask seven questions and have one commentary.

Question Number 1. Were you able to determine the associated morbidity and mortality of the core quota patients who did not receive the recommended care?

Number 2. I recognized the inherent limitations associated with obtaining data from a chart review; however, were you able to glean from the medical records if the lack of implementation of the recommended patient management paradigm was solely the result of the physician provider not being compliant with his or her own specialty practice guidelines?

Number 3. What were the legal repercussions resulting from any deviation in the standard of care?

Number 4. Based on your findings that approximately 50% of the recommended care was delivered to trauma patients with moderate to severe injuries, what programmatic or system changes were made as a result?

Number 5. Your findings highlighted that the least likely to receive optimal care were patients with severe traumatic injuries. With 25 trauma-specific processes of care you demonstrated that the highest compliance involved resuscitation, (83%) and the lowest neurosurgical intervention at 17%.

Let me be clear here: Is this an indictment of the neurosurgeons at your institutions with respect to those specialists not adhering to the practice guidelines/algorithms endorsed by their own specialty association – example, AANS?

Number 6. With your investigation underscoring the fact that there was no relationship between compliance and patient demographics and socio-economic status, what are the possible relationships?

And then, last, the substantial time and effort that we are all putting into evidence-based practice and comparative effectiveness research, this study certainly dampens the enthusiasm for decision making and practice management-based evidence. However, this is a single institution experience. Will you share with the audience how you justify these findings actually being generalizable? Doesn't this reflect just a local problem?

My comments and then I'm done. The Rand Corporation reported several years ago that with respect to simple measures, Americans receive only half of the recommended medical care.

The Institute of Medicine also reported that 90 million adults – there are only 315 million people in this country – have trouble understanding and acting on health information. While distressing, I can understand the genesis of such findings based on less than optimal health literacy. However, the findings of this study are the most egregious and shocking.

Again, I want to thank the authors for unveiling something that has been strongly suspected. I encourage them to expand this project and apply a more prospective, randomized analysis to their study design.

And I want to thank the Association for the privilege of the floor.

Dr. Steven R. Shackford (San Diego, California): That was a nice presentation. I would caution you, though, to be a little less strident in your commentary about quality gaps without knowing what the difference between outcomes were in the compliant and non-compliant groups. In other words, you need to compare outcomes in the patients that were fully compliant with guidelines to the TRISS calculated observed and expected outcomes and do the same for your non-compliant group. Another point I want to make is that several years ago Fred Rogers and I looked at our compliance with VTE guidelines from the American College of Chest Physicians on a surgical service. In 8,000 patients, we found that our compliance with the ACCP guidelines for thromboprophylaxis in surgery patients was the highest in reported literature, around 83%. This was published in *Surgery.* Despite this compliance our VTE rate went up. Understand that these practice guidelines are, for the most part, supported by Level II evidence. They are, many times, developed using the Delphi technique.

In short, the most important thing that you can show us is that outcomes were better in the fully compliant group compared to the group that was not compliant. Process improvement is about better outcomes, not about compliance with process.

Dr. John R. Clarke (Philadelphia, Pennsylvania): I think this is another wakeup to what Dr. Britt reported. The Rand Corporation indicates that across the country we only "practice what we preach" about 50% of the time.

You noted that there was a decrease in compliance with increased complexity and I wonder if you looked at a very

simple relationship, which is the percentage of compliance as a function of the number of guidelines for which the patient was eligible.

Nadine Rayan, MHA (Dallas, Texas): First of all I want to thank Dr. Britt for his discussion and insightful questions.

I don't know if I can address all of these in the time I've been allocated but will make my best attempt. I would also like to thank those in the audience who asked questions. I also want to clarify that I am not actually a doctor, but have my masters in health administration.

Our results with compliance rates for all recommended processes of care were consistent with what was found in the literature. For example, we reviewed a study by McGynn and her colleagues which looked at 30 acute and chronic conditions. Only 55 percent of the recommended care was delivered to patients. This wasn't a trauma study and the results were demonstrated in patients with other chronic conditions but we believe that this may be an issue across the board.

Dr. Britt and the other discussants make a good point that processes may not always be directly related to outcomes. However, our study was not designed to look at outcomes. To Dr. Britt's point, we do believe that in a randomized, controlled trial one could determine which processes would impact patient outcomes.

We are also aware that non-adherence to practice guidelines does not necessarily indicate that care was suboptimal. For example, it may be appropriate to withhold certain interventions from patients with non-survivable injuries or a terminal pre-existing condition. The retrospective nature of the study does not allow us to identify reasons for non-compliance. Dr. Britt also asked if this was an indictment against our neurosurgeons. No, this was not meant to speak against any surgeons at our institute. It is important to note that increased complexity of care associated with severely injured patients, such as those with head injuries, may result in lower compliance. It is hard to address all processes of care to those more severely injured.

Dr. Clarke asked if we compared compliance rates with the number of processes for which that patient was eligible and we did look at this. We conducted a univariate analysis, our initial analysis, and saw a relationship in compliance and the number of processes for which each patient was eligible. The more processes one was eligible for, the least likely they were to receive care. And this was consistent with the literature we reviewed as well.

However, we did not analyze this in our multivariate analysis, because the approach used in multivariate regression models accounts for clustering of processes by patients. However, we believe, a simple count of number of processes a patient is eligible for may not be the best measure of complexity of care. There were also many other indicators of patient severity we looked at. We made this decision with the help of our biostatisticians, who I know would be able to articulate the rationale better than I would.

Thank you all very much.

Compliance with Recommended Care at Trauma Centers: Association with Patient Outcomes

Shahid Shafi, MD, MPH, FACS, Sunni A Barnes, PhD, Nadine Rayan, MHA, Rustam Kudyakov, MD, Michael Foreman, MD, FACS, H Gil Cryer, MD, PhD, FACS, Hasan B Alam, MD, FACS, William Hoff, MD, FACS, John Holcomb, MD, FACS

BACKGROUND: State health departments and the American College of Surgeons focus on the availability of optimal resources to designate hospitals as trauma centers, with little emphasis on actual delivery of care. There is no systematic information on clinical practices at designated trauma centers. The objective of this study was to measure compliance with 22 commonly recommended clinical practices at trauma centers and its association with in-hospital mortality.

STUDY DESIGN: This retrospective observational study was conducted at 5 Level I trauma centers across the country. Participants were adult patients with moderate to severe injuries (n = 3,867). The association between compliance with 22 commonly recommended clinical practices and in-hospital mortality was measured after adjusting for patient demographics and injuries and their severity.

RESULTS: Compliance with individual clinical practices ranged from as low as 12% to as high as 94%. After adjusting for patient demographics and injury severity, each 10% increase in compliance with recommended care was associated with a 14% reduction in the risk of death. Patients who received all recommended care were 58% less likely to die (odds ratio = 0.42; 95% CI, 0.28−0.62) compared with those who did not.

CONCLUSIONS: Compliance with commonly recommended clinical practices remains suboptimal at designated trauma centers. Improved adoption of these practices can reduce mortality. (J Am Coll Surg 2014;219:189−198. © 2014 by the American College of Surgeons)

Trauma center designation criteria used by state health departments and the American College of Surgeons focus on ensuring availability of specific resources to care for the injured. This approach has been successful, as patients treated at designated trauma centers are more likely to survive than are those treated at nondesignated hospitals.[1,2] However, the Trauma Quality Improvement Program has shown that risk-adjusted mortality rates vary across designated trauma centers; some achieve considerably better (or worse) outcomes than others.[3-6] The reasons for these variations, despite adequate resource availability, are unclear. Donabedian principles for quality improvement suggest that, besides patient characteristics, institutional structures and clinical practices determine patient outcomes.[7] Variability in risk-adjusted patient outcomes, despite availability of optimal resources, suggests that outcomes variations might be related to differences in clinical practices. Currently, trauma center designation criteria do not require compliance with specific practices.

Variations in clinical practices across trauma centers, such as nonoperative management of solid-organ injuries and prophylaxis against venous thromboembolism, are well known.[8-11] Professional societies and research consortia have developed trauma-specific management guidelines and recommendations, but their adoption appears inadequate.[12] Increased compliance with guidelines has been shown to improve patient outcomes in other clinical scenarios, such as the use of β-blockers in acute MI.[13] There is no systematic information on compliance with

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recommended care at designated trauma centers. The hypothesis for this study was that compliance with commonly recommended practices at trauma centers is suboptimal, with adverse impacts on in-hospital mortality and length of stay (LOS).

METHODS

A retrospective multicenter study of a sample of patients from 5 Level I trauma centers was undertaken for 3 years at Center A (January 1, 2006 through December 31, 2008) and 2 years at Centers B, C, D, and E (January 1, 2009 through December 31, 2010). Findings from Center A only have been published previously.[14]

Selection of common clinical practices

The study focused on the following common injuries: traumatic brain injuries, hemorrhagic shock, pelvic fractures, and long-bone extremity fractures. We reviewed practice management guidelines developed by the American College of Surgeons, the Eastern Association for the Surgery of Trauma, Society of Critical Care Medicine, Glue Grant, Brain Trauma Foundation, and the Surgical Care Improvement Project.[15-20] From these, several clinical practices that are measurable and can affect outcomes were selected using a 2-step approach used by the Centers for Medicare & Medicaid Services and the Centers for Disease Control and Prevention for developing Surgical Infection Prevention guidelines in 2002.[21] In the first step, a single investigator (SS) reviewed the guidelines and selected several processes for this study encompassing all aspects of trauma care, including initial evaluation, resuscitation, operative care, critical care, rehabilitation, and injury prevention. In the second step, the rest of the investigators reviewed those processes and developed the final list of 22 processes by consensus (Table 1).

Patient selection

Inclusion criteria were age 16 years or older and moderate to severe injuries, defined as $\geq$1 injury (Abbreviated Injury Scale $\geq$3) to head, neck, face, thorax, abdomen, spine, or extremities for blunt mechanism and to neck, thorax, or abdomen for penetrating mechanism.

Exclusion criteria included time from injury to arrival in emergency department of $\geq$1 day; burns, poisoning, drowning, hanging, submersion, or asphyxiation; gunshot wounds to the head; and dead on arrival in emergency department.

A total of 9,633 patients met inclusion and exclusion criteria. Using simple random selection, stratified by centers, 1,000 patients were obtained from Center A (total eligible 2,242), 757 from Center B (total eligible 3,876), 756 from Center C (total eligible 1,884), and 760 from Center D (total eligible 1,037). At Center E, 594 patients were eligible, and all were included in the study. The final study population was 3,867 patients.

Data sources

Patient data were obtained from 3 sources.

Trauma registry

Trauma registry data were obtained from each center as well as the National Trauma Data Bank to extract the following[22]: demographics, including age, sex, race/ethnicity, insurance; mechanism of injury; anatomic description of injuries using ICD-9 and Abbreviated Injury Scale; injury severity using Glasgow Coma Scale on presentation, Injury Severity Score (ISS), systolic blood pressure on presentation, Abbreviated Injury Scale codes; procedures; and outcomes, including in-hospital mortality and hospital LOS.

These data were used to determine patient eligibility, develop risk-adjustment models using Trauma Quality Improvement Program methodology,[4,23] and identify patient eligibility for specific clinical processes. We developed and pretested a software algorithm to identify patients eligible for specific processes based on their injuries. A second programmer validated this algorithm independently.

Medical records

Medical records of patients eligible for each process were reviewed to determine if they actually received the care. Trained abstractors collected data using a standardized pretested tool.

Compliance with clinical processes

For each patient, a compliance score was calculated based on the opportunity model used by the Centers for Medicare & Medicaid Services for reporting compliance with their core measures.[24] For example, if a patient was eligible for 10 clinical processes and received 8, the compliance score was 80. Similarly, if a patient was eligible for 6 processes and received all 6, the compliance score was 100.

Statistical analysis

All analyses were conducted using SAS software (version 9.3, SAS Institute), with $p \leq 0.05$ considered statistically significant.

Risk-adjustment methodology

Generalized estimating equations methods using the GENMOD procedure in SAS were used to build models that accounted for clustering of patients within the same

Table 1. Selected Clinical Practices, Patient Eligibility, and Compliance

Clinical practices	Eligibility	Eligible patients, n (% of 3,867)		Compliance, n (% of eligible)		Compliance 95% CI
		n	%	n	%	
Head CT scan in suspected blunt traumatic brain injury	Blunt mechanism AND Glasgow Coma Scale <15 on arrival	1,203	31	1,063	88	86.86–90.45
CT angiography of neck for blunt cerebrovascular injuries	Blunt mechanism AND any 1 of the following fractures: Le Forte II or III facial OR cervical spine OR vase of skull	1,158	30	448	39	35.94–41.57
Resuscitation using blood	Hypotensive (systolic blood pressure ≤90 mmHg) on arrival	281	7	186	66	60.63–71.76
Shock assessment using blood gas measurement	Hypotensive (systolic blood pressure ≤90 mmHg) on arrival	281	7	189	67	61.98–73.02
Endotracheal intubation	Glasgow Coma Scale ≤8 on initial assessment	497	13	468	94	92.10–96.23
Correction of coagulopathy using fresh frozen plasma or prothrombin complex concentrate	Intracranial bleed AND INR ≥1.5	155	4	118	76	69.34–82.92
Emergency department thoracotomy	Pulse present on arrival AND died in emergency department	24	1	3	13	0–26.77
Laparotomy in abdominal gunshot wounds	Gunshot wound abdomen AND systolic blood pressure ≤90 mmHg AND any abdominal injury	21	1	19	90	76.78–100
Laparotomy in blunt abdominal trauma	Blunt mechanism AND systolic blood pressure ≤90 mmHg AND abdominal Abbreviated Injury Scale ≥4	44	1	27	61	49.17–79.40
External pelvic compression in emergency department (binder, sheet, other devices)	Pelvic fracture AND systolic blood pressure ≤90 mmHg	65	2	9	14	5.22–22.47
Angioembolization for hemorrhage control in pelvic fractures	Pelvic fracture AND systolic blood pressure ≤90 mmHg	61	2	13	21	10.01–29.99
Preoperative antibiotics before laparotomy	Patients undergoing laparotomy	257	7	193	75	70.08–80.70
Craniotomy in severe traumatic brain injuries	Glasgow Coma Scale ≤8 AND intracranial bleed on head CT	326	8	66	20	15.86–24.63
Intracranial pressure monitoring in severe traumatic brain injuries	Glasgow Coma Scale ≤8 AND intracranial bleed on head CT AND endotracheal intubation	257	7	100	39	32.91–44.91
Irrigation and debridement of open fractures in operating room	Open fracture femur OR tibia	175	5	158	90	85.85–94.72
Intravenous antibiotics in open fractures	Open fracture femur OR tibia	178	5	167	94	90.25–97.39
Definitive fracture fixation	Open or closed fracture femur OR tibia AND not in ICU	740	19	674	91	89.29–93.36
Operative pelvic fixation	Pelvic fracture AND no intracranial bleed AND no acute lung injury	430	11	166	39	36.65–46.35
Initiation of prophylaxis against venous thromboembolism by day 3 (chemical or filter)	No intracranial bleed AND survived 3 or more days AND any 1 of the following: femur fracture, tibia fracture, intubated	1,174	30	940	80	77.85–82.42
Initiation of nutrition by day 3 (enteral or parenteral)	Intubated patients AND survived 3 or more days	831	21	657	79	76.29–81.07
Physical therapy and rehabilitation evaluation before discharge	Fracture femur OR tibia OR pelvis, AND not in ICU AND discharged alive from the hospital	969	25	791	82	79.19–84.07
Abdominal CT scan for evaluation of blunt abdominal trauma	Patients with blunt injuries to liver, spleen, kidneys, AND nonoperative management	393	10	332	84	80.88–88.07

trauma center by controlling for the facility as a random effect, and assumed a binomial distribution for mortality and a negative binomial distribution for LOS. The predictors were based on our earlier work and Trauma Quality Improvement Program methodology.[4,25] These included age; sex; race/ethnicity; insurance status; mechanism of injury; presence of injuries to the head, chest, or abdomen; injury severity (measured using ISS, the Glasgow Coma Scale, and first systolic blood pressure on arrival); need for ICU; and ventilator use. Patients who died during hospitalization were excluded from the LOS models.

Identifying clinical processes associated with outcomes

After development of the risk-adjustment model for outcomes, compliance with each process (in binary format) was forced into the models to assess its risk-adjusted association with the respective outcomes. This design ensured that only patients eligible for each process were included in the analysis for that specific process. The association of compliance with each clinical process on outcomes is reported as adjusted odds ratio (OR) for in-hospital mortality and percent change in LOS, along with 95% CIs.

Measuring the association between compliance score and outcomes

Two approaches were used to measure the association between compliance with recommended practices and patient outcomes. First, compliance scores were included as a continuous variable in the risk-adjustment model for each of the outcomes. Second, compliance scores were used to create a new binary variable. Compliance scores of 100 indicated optimal care; compliance scores <100 indicated suboptimal care. This binary variable was then included in the model for mortality as one of the outcomes.

Measuring the potential improvement in mortality expected with full compliance or optimal care

The recycled prediction method was used to estimate the marginal impact of compliance on mortality.[26] Parameter estimates from the model described here (using a binary variable for optimal care) were used to estimate the expected mortality rate among the study population, assuming all patients received optimal care, and compared with the expected mortality rate, assuming none of the patients received optimal care. The difference between the 2 expected mortality rates was used to estimate the marginal effect of optimal care on mortality.

RESULTS

Study population

The study population was typical for Level I trauma centers (median age 47 years; 65% male; 94% blunt mechanism of injury; Table 2). These patients had a median ISS

Table 2. Patient Demographics, Injuries, Injury Severity, and Unadjusted Outcomes

Variable	All patients (n = 3,867)	Center A (n = 1,000)	Center B (n = 757)	Center C (n = 756)	Center D (n = 760)	Center E (n = 594)
Age, y, median (IQR)	47 (29–69)	41 (27–60)	41 (26–58)	62 (39–81)	58 (34–80)	42 (27–58)
Male sex, %	65	65	71	56	60	73
Blunt mechanism of injury, %	94	88	95	98	97	95
Minority ethnicity, %	31	47	40	16	5	43
Uninsured (including Medicaid), %	31	54	37	14	5	34
Injury Severity Score, median (IQR)	14 (9–24)	16 (10–24)	17 (9–25)	14 (9–22)	17 (10–25)	9 (9–17)
Hypotensive on arrival (systolic blood pressure ≤90 mmHg), %	9	12	11	8	3	9
Glasgow Coma Scale at presentation ≤8, %	21	18	23	18	29	16
Abdominal injury, %	23	28	24	12	24	25
Head injury, %	52	49	43	48	65	55
Chest injury, %	37	46	40	22	31	44
Mortality rate (crude), %	9	12	9	9	4	9
Length of stay, d, median (IQR)	6 (4–11)	5 (3–9)	8 (4–15)	6 (4–10)	6 (4–9)	8 (4–16)
ICU use, %	48	50	50	28	53	57
Compliance score, median (IQR)	83 (50–100)	67 (33–100)	73 (50–100)	100 (50–100)	100 (67–100)	100 (67–100)

Proportions for categorical variables; medians with interquartile range for continuous variables.
IQR, interquartile range.

of 14, and 24% had an ISS >24. A total of 334 patients died during hospitalization (crude mortality rate 8.6%).

Compliance with clinical processes

There were 3,292 patients eligible for ≥1 of the 22 clinical processes. Overall, median compliance score was 83 (interquartile range 50 to 100), indicating that half of the patients received <83% of the recommended care, and a quarter of the patients received <50% of the recommended care. Compliance also varied among the 5 centers (median compliance scores 67 to 100). Compliance with each individual process ranged from 13% for thoracotomy in emergency department to 94% for endotracheal intubation (Table 1, Fig. 1).

Relationship between compliance with recommended practices and mortality

Excluding rehabilitation-related processes when considering the result of death, 3,286 patients were eligible for ≤1 process; of these, 333 died. Crude odds of deaths associated with compliance score (grouped in deciles) was 0.89 (95% CI, 0.85−0.94), indicating a slightly more than 10% reduction in the risk of death associated with each 10% increase in compliance score. After adjusting for patient demographics and injury severity, the odds of death associated with compliance score remained statistically significant at 0.86 (95% CI, 0.82−0.90), indicating a 14% reduction in the risk of death associated with each 10% compliance score increase (Fig. 2).

Among 1,633 patients in the optimal care group, there were 54 deaths (crude mortality rate 3.3%). In the 1,653 patients in the suboptimal care group, there were 279 deaths (crude mortality rate 16.9%). The risk-adjusted OR of death associated with optimal care was 0.42 (95% CI, 0.28−0.62), indicating that 100% compliance was associated with a 58% reduction in mortality after accounting for patient differences. However, this protective effect can be spurious if patients in the optimal care group were also less severely injured than those in the suboptimal care group, if limited care was provided to patients with potentially nonsurvivable injuries, or if there were patient/family wishes for limiting care.

We used 3 approaches to minimize this effect. First, we excluded patients with the highest risks of death and measured the association between compliance score and mortality in the remaining lower-risk patients using 3 different thresholds (Fig. 2). Excluding patients with a risk of death >75%, there were 2,613 patients and 211 deaths (crude mortality rate 8.1%). In this group, the odds of death associated with compliance score remained unchanged (OR = 0.86; 95% CI, 0.82−0.91). Next, we excluded patients with a risk of death >50%. In the remaining 2,567 patients with 153 deaths (crude mortality rate 5.9%), the odds of death associated with compliance score were 0.88 (95% CI, 0.83−0.93). Finally, we

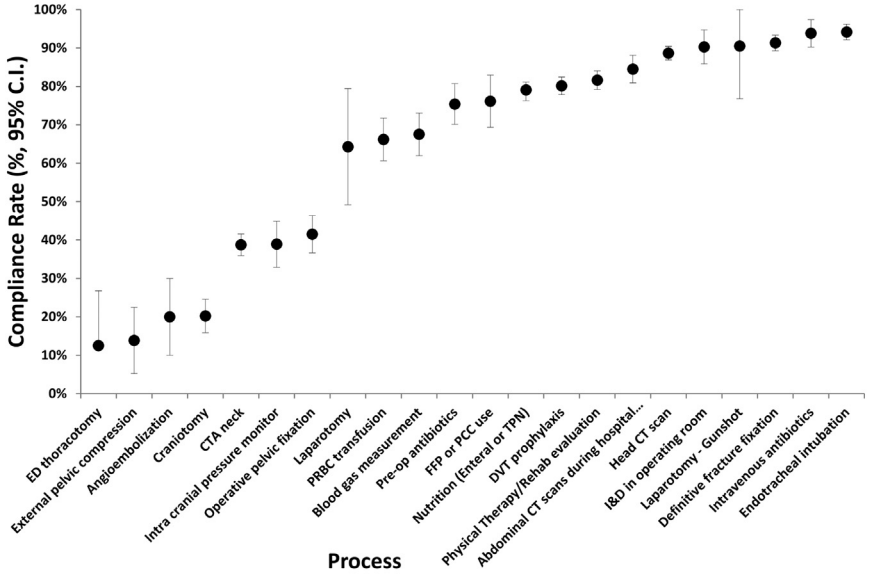

Figure 1. Compliance with processes of care: rates with 95% CI. CTA, CT angiography; DVT, deep venous thrombosis; ED, emergency department; FFP, fresh frozen plasma; I&D, incision and drainage; PCC, prothrombin complex concentrate; PRBC, packed RBC; TPN, total parenteral nutrition.

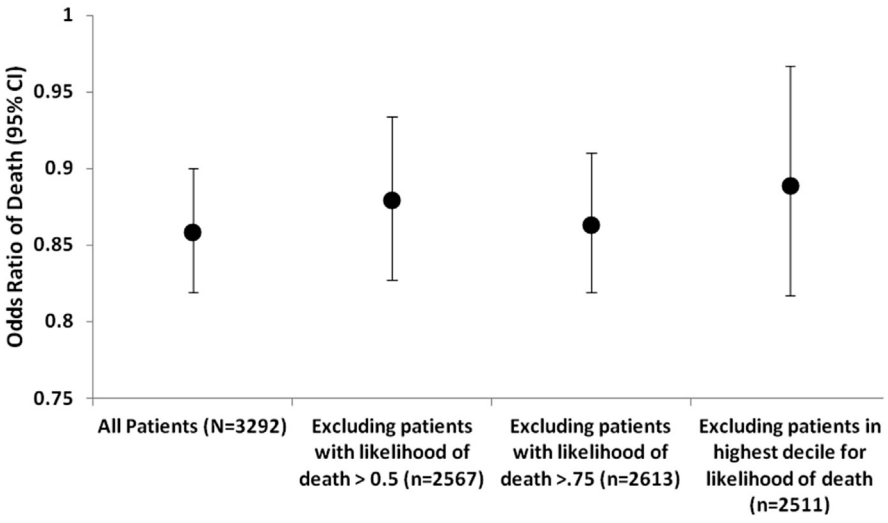

Figure 2. Relationship between compliance with processes of care and risk of death.

excluded patients in the top decile of likelihood of death. In the remaining 2,511 patients with 109 deaths (crude mortality rate 4.3%), the odds of death associated with compliance score were 0.89 (95% CI, 0.82−0.97), suggesting a persistent protective effect among the least-injured patients.

In the second approach, we estimated compliance propensity based on patient demographics and injury characteristics. Patients were then divided into quartiles by their compliance propensity. Within each quartile, we measured the association between observed compliance score and the risk of death. This analysis showed a persistent protective effect of compliance among patients in the highest (OR = 0.82; 95% CI, 0.78−0.87) and lowest quartiles (OR = 0.86; 95% CI, 0.78−0.96) of propensity of compliance, but not in the middle 2 quartiles (OR = 0.98; 95% CI, 0.89−1.08 and OR = 1.04; 95% CI, 0.93−1.16).

In the third approach, we calculated expected survival probability for each patient using TRISS methodology.[27] The observed-to-expected mortality ratio was 0.49 in the optimal care group and 0.89 in the suboptimal care group. The lower ratio in the optimal care group indicates that, after accounting for injury severity using TRISS methodology, patients receiving all indicated care were less likely to die than those who did not.

Relationship between compliance score and length of stay

After adjusting for patient characteristics, each 10% increase in compliance score was associated with a small but statistically significant increase in hospital LOS (4.1%; 95% CI, 3.2−5.1).

Relationship between individual clinical processes and patient outcomes

The analysis showed that compliance with 11 processes was associated with a reduction in the risk of mortality, and none was associated with an increased risk of death. Diagnostic interventions associated with reduced mortality included (Fig. 3) head CT scan in patients with blunt traumatic brain injury; shock assessment using arterial blood gas in hypotensive patients; and abdominal CT scan in patients with severe blunt abdominal injuries managed nonoperatively.

Therapeutic interventions associated with reduced mortality included correction of coagulopathy in patients with traumatic brain injuries; laparotomy in hypotensive patients with blunt abdominal injuries; preoperative antibiotics in patients undergoing laparotomy; IV antibiotics in patients with open long-bone fractures; definitive fixation of long-bone fractures; pelvic fracture fixation; venous thromboembolism prophylaxis in high-risk patients; and nutrition in intubated patients.

Similarly, compliance with 11 processes was associated with increased hospital LOS; 1 process was associated with decreased LOS (Fig. 4).

Potential reduction in mortality with optimal care

The recycled prediction analysis showed that the expected mortality rate in the entire study population of 3,286 patients with optimal care would be 6.7% (expected number of deaths = 220) and the expected mortality rate with suboptimal care would be 11.3% (expected number of deaths = 371). This 41% reduction would translate into 151 fewer deaths associated with optimal care.

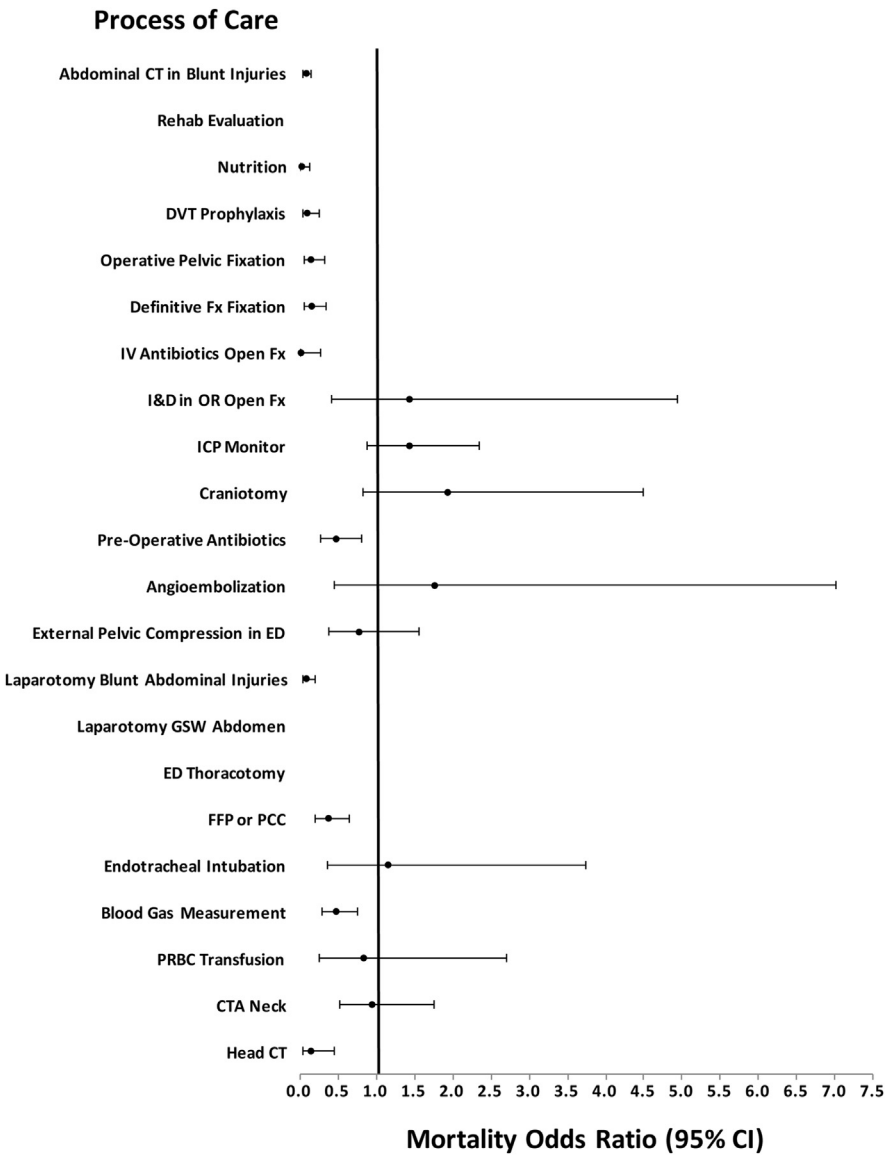

Figure 3. Risk-adjusted association between individual clinical practices and in-hospital mortality (all patients). No point estimates or confidence intervals appear for emergency department thoracotomy because all 24 patients who were eligible for this process died. No point estimates or confidence intervals appear for laparotomy gunshot wound (GSW) abdomen because only 2 of 20 eligible patients did not comply with the process. Values for Rehab Evaluation exclude patients who died during their hospital stay. CTA, CT angiography; DVT, deep venous thrombosis; ED, emergency department; FFP, fresh frozen plasma; Fx, fraction; I&D, incision and drainage; OR, operating room; PCC, prothrombin complex concentrate; PRBC, packed RBC.

DISCUSSION

There are 3 key findings from this study. First, the adoption of recommended practices at trauma centers remains suboptimal, with a median compliance of 83%. Second, improved compliance was associated with reduced mortality, and this finding was consistent across multiple methodological approaches used in this study. Third, a few specific processes can serve as trauma-specific core measures for quality improvement.

This multicenter study showed a considerable gap between knowledge and practices at trauma centers and is consistent with research of other diseases. Studying several acute and chronic conditions, McGlynn and colleagues[28] showed that patients, on average, received about

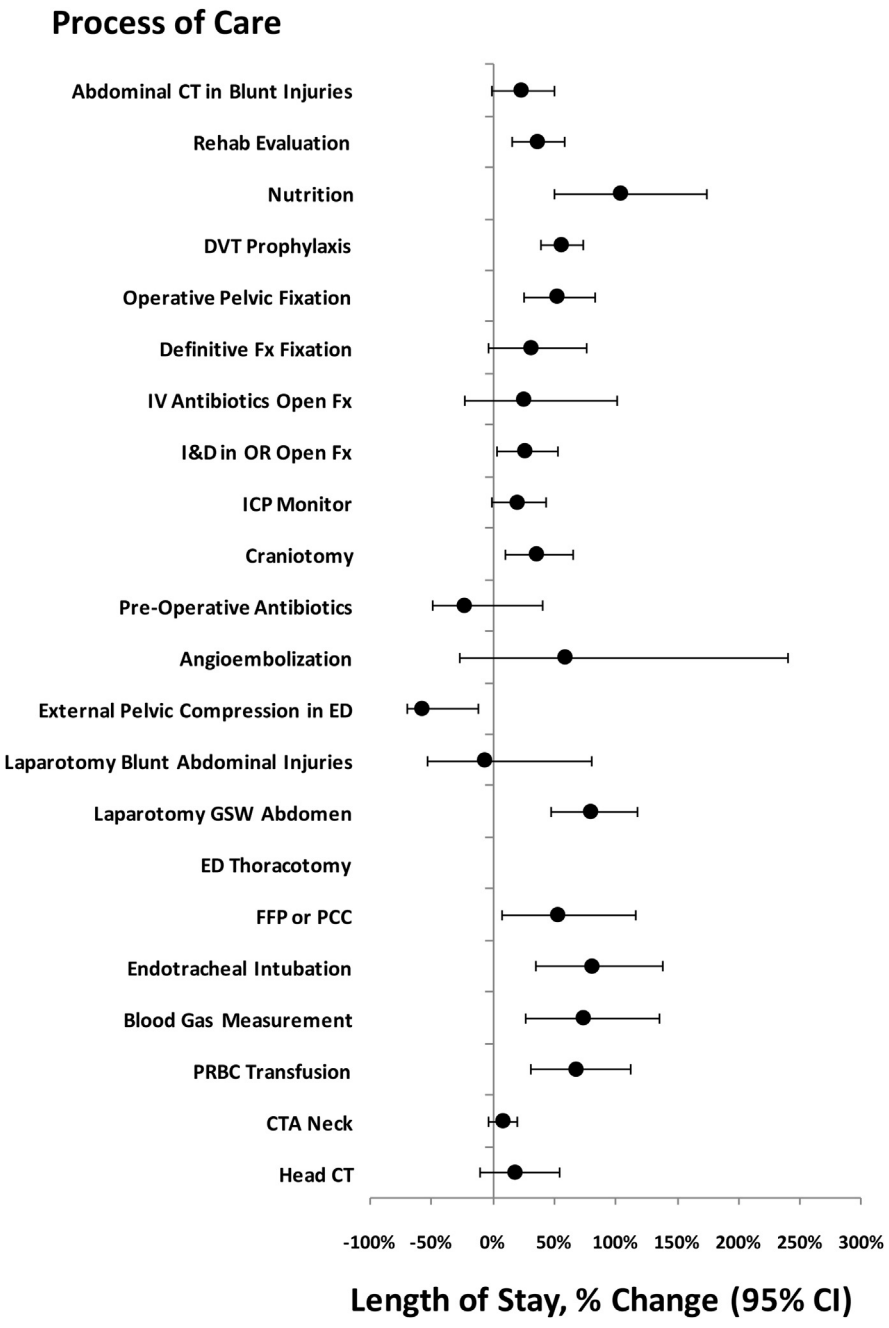

Figure 4. Risk-adjusted association between individual clinical practices and length of stay. Excluding patients who died during their hospital stay. No point estimates or confidence intervals appear for emergency department (ED) thoracotomy because all 24 patients who were eligible for this process died. CTA, CT angiography; DVT, deep venous thrombosis; ED, emergency department; FFP, fresh frozen plasma; Fx, fraction; GSW, gunshot wound; I&D, incision and drainage; OR, operating room; PCC, prothrombin complex concentrate; PRBC, packed RBC.

half of the indicated care. Similarly, a study of patients with acute MI showed that less than half of eligible patients received thrombolytic therapy and β-blockers; only a quarter received advice on smoking cessation.[29] In another study, minimally invasive technique for breast biopsy was used in only about two thirds of the patients, despite recommendations by multiple expert consensus panels.[30] In a recent survey of 54 Level I and II trauma

centers, half reported adopting ≤14 of 32 recommended guidelines.[12] During the last 3 decades, the focus of the trauma systems development by the American College of Surgeons and state health departments has been on ensuring availability of optimal resources using the trauma center designation process. Our findings suggest that there is now a need to focus on delivery of optimal care.

The reasons for suboptimal adoption of recommended care at trauma centers have not been studied, but this phenomenon is not unique to trauma.[31,32] Barriers to adoption include lack of consensus on best practices, lack of awareness, institutional culture, clinical disagreement with recommended care, ineffective communication, and high workload.[33] Importantly, this study identified several specific processes associated with reduced mortality. These processes, if validated in an appropriately designed study, could be adopted as "Trauma Core Measures." Our findings suggest that nationwide compliance with these clinical processes could save several thousand lives.

An interesting finding of this study was that optimal care was associated with increased LOS. This is contrary to the prevailing belief that improved quality of care reduces resource use. However, in trauma, it is plausible that improved care quality leads to salvage of severely injured patients who then require prolonged hospitalization. This finding has substantial cost implications, as increased LOS leads to increased costs. Additional analysis is needed to measure other important outcomes, such as functional status and quality of life among the survivors.

This study has a few limitations. First, this is a retrospective analysis with its inherent limitations. Specifically, compliance was determined based on chart review. Lack of compliance might simply reflect lack of documentation. Also, reasons for noncompliance could not be ascertained. Several factors, such as patient/family preferences, can affect the ability to comply with recommended care. Second, the study is limited to only 5 Level I trauma centers. However, these centers serve a large, diverse population, with a substantial rural/urban, geographic, ethnic, and socioeconomic mix. Third, the sample size was inadequate to study the association between certain processes of care and outcomes. Fourth, it is possible that none of the 22 processes was causally associated with improved outcomes, and compliance with them is simply an indicator of a high-quality trauma center. The findings of this study do not validate specific interventions. Lastly, other outcomes, such as costs, complications, and functional status, might be more appropriate in certain scenarios, but were not included in this analysis.

CONCLUSIONS

Our findings suggest suboptimal compliance with several commonly recommended clinical processes at designated trauma centers. Improved adoption of some of these practices might be associated with a reduction in mortality but can increase LOS. Additional studies should identify specific practices that should constitute core measures in trauma. Finally, advancement in trauma quality improvement requires a shift from provision of optimal resources to provision of optimal care.

Author Contributions

Study conception and design: Shafi, Barnes, Foreman, Cryer, Alam, Hoff, Holcomb
Acquisition of data: Shafi, Rayan, Kudyakov
Analysis and interpretation of data: Barnes
Drafting of manuscript: Shafi, Barnes
Critical revision: Foreman, Cryer, Alam, Hoff, Holcomb

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X Chromosome-Linked IRAK-1 Polymorphism Is a Strong Predictor of Multiple Organ Failure and Mortality Postinjury

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Objective(s): Clinical research characterizing the mechanisms responsible for sex-based outcome differences postinjury remain conflicting. We sought to characterize an X chromosome-linked IRAK-1 (IL-1 receptor-associated kinase) polymorphism as an alternative mechanism responsible for sex differences postinjury. IRAK-1 is key intermediate in the toll-like receptor (TLR) pathway thought to drive inflammation postinjury.

Methods: A prospective cohort study was performed over a 24-month period. Bluntly injured patients requiring intensive care unit admission were enrolled, whereas patients with isolated brain and spinal cord injuries were excluded. Outcomes of interest included multiple organ failure (MOF, Marshall MOD score > 5) and mortality. Logistic regression was utilized to determine the independent risk of poor outcome associated with the IRAK-1 variant after controlling for important differences.

Results: In an enrolled cohort of 321 patients, the IRAK-1 variant was common (12.5%). Patients with and without the variant were similar in age, injury severity, and 24hr blood transfusion. After controlling for important confounders, the IRAK1 variant was independently associated with more than eightfold (OR = 8.4, $P = 0.005$, 95% CI: 1.9–37.1) and 11-fold (OR = 11.8, $P = 0.037$, 95% CI: 1.1–121) greater risk of MOF and mortality, respectively. These differences were most prominent in men, whereas women heterozygous for the variant demonstrated worse outcome in a dose-dependent fashion.

Conclusions: The IRAK1 polymorphism is a strong independent predictor of MOF and mortality postinjury and represents a common variant with prognostic potential. These data demonstrate the importance of TLR signaling postinjury and supports that a genetic mechanism may drive sex outcome differences postinjury.

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Although significant advances in the care of the injured patient have occurred over the last decade, those who survive their initial injury continue to be plagued with the development of coagulopathy, multiple organ failure (MOF), nosocomial infection (NI), and their attributable morbid effects.[1–6] A persistent finding has been that men and women respond differently after traumatic injury with significant protection afforded to the female sex.[7,8] Controversy exists regarding the clinical explanation and underlying mechanisms responsible for this female protective effect.[7,9,10]

A large body of laboratory evidence suggests that a sex-hormone–based mechanism (estrogen being protective) is responsible for these postinjury differences.[8,11–14] In prior work, our group has shown that the protective effect afforded to women following severe injury is independent of age and the hormonal status of the female, suggesting other mechanisms may be involved clinically.[7,15] Men and women also are different genetically, primarily due to the method of inheritance of, and the genes which reside on, the X chromosome. Secondary to the known mosaic expression of the X chromosome, women would be less affected by unfavorable X chromosome-linked genetic variants.[16] Importantly, increasing evidence also has demonstrated that the Toll-like receptor (TLR) signaling cascade plays an essential role in the early activation of the innate immune response after traumatic injury.[17–24] The IL-1 receptor-associated kinase (IRAK-1) is a protein constituent member of the TLR signaling cascade, which resides on the X chromosome and has been demonstrated to have 2 haplotypes. The IRAK-1 variant haplotype has been demonstrated to be relatively common and associated with worse outcome in septic patients, thought to be secondary to an excessive innate immune response brought about by upregulated NF-κB signaling.[25–27] No evidence currently exists regarding the significance of this TLR pathway variant, which resides on the X chromosome on pertinent outcomes after traumatic injury. We sought to characterize the IRAK-1 variant as an alternative mechanism responsible for sex-based outcome differences postinjury. We hypothesized that the IRAK-1 variant would be common and independently associated with poor clinical outcome after traumatic injury.

METHODS

A prospective observational cohort study was performed over a 24-month time period (2011–2012) with the overarching goal to further characterize the mechanisms responsible for sex (male vs female) based outcome differences after traumatic injury. Inclusion criteria for the study included blunt injured patients older than 17 years requiring ICU (intensive care unit) admission. Patients older than 90 years, with isolated traumatic brain injury, preexisting immune-suppression, or those with an anticipated survival of less than 24 hours were excluded from enrollment. Blood samples were obtained within 6 hours of injury for serial cytokine measurements, coagulation assessment [international normalized ratio (INR), thromboelastography (TEG) analysis], and DNA isolation and haplotype discrimination. Clinical outcomes assessed included the development of MOF, NI, and mortality.

The IRAK-1 variant haplotype was determined by genotyping the single nucleotide polymorphism on the X chromosome where a T→ C substitution [rs1059703] at position 1595 in exon12 results in a nonsynonymous mutation (532, L→ S). Probe and primer combinations were designed for genotyping this polymorphism and polymerase chain reaction (PCR) was performed using an Applied

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Biosystems 7300 Real-Time PCR system using methods previously described.[25,27] Allelic discrimination was verified by direct DNA sequencing of a small subgroup of patients of each haplotype (men and women: wild-type, variant, and heterozygous, respectively) to assure the PCR-based assay was sufficiently accurate.

MOF was evaluated using the well-validated Marshall Multiple Organ Dysfunction Score.[5,28,29] A Marshall Multiple Organ Dysfunction Score of more than 5 beyond 48 hours of injury and ICU admission was classified as MOF. Primary infectious outcomes of interest include ventilator-associated pneumonia, blood stream infection (excluding those associated with an intra-abdominal abscess), and urinary tract infections.[30] These were selected in attempts to use those infectious outcomes that can be used as a marker for the degree of relative immune suppression. The development of these NIs was based on positive culture evidence.

Blood samples were serially obtained at 6 hours, 24 hours, and 72 hours of injury, and serum was separated and frozen at −80°C until assayed for cytokine analysis. Cytokine expression including IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IFN-α, and IFN-γ were measured from patients' serum using a Luminex 100 IS System and commercially available human specific beadsets. Thromboelastography (TEG) was performed within the first 6 hours of injury and at 24 hours using on a TEG 5000 Thromboelastograph Hemostasis Analyzer and standard TEG parameters were recorded including *r* value, k time, α angle, maximal amplitude (MA), G value, and fibrinolysis at 30 minutes (LY30) as previously described.[31–35]

First, patients with and without the IRAK-1 polymorphism underwent unadjusted comparison of demographics, injury characteristics, resuscitation and transfusion requirements, and clinical outcomes. Multivariable logistic regression analysis was then utilized to determine the independent risks of our clinical outcomes associated with the IRAK-1 variant. Covariates adjusted for in the regression model included age, sex (male vs female), race, body mass index (BMI), injury severity score (ISS), presenting systolic blood pressure (SBP), presenting Glasgow Coma Score (GCS), intubation status, presenting coagulopathy (INR > 1.5), 24-hour crystalloid, and blood component transfusion requirements. Because of the X chromosome location of the polymorphism, we then characterized the risk of our clinical outcomes across whether the IRAK-1 variant existed in a homozygous manner (male-1 variant allele, female -2 variant alleles) or heterozygous manner (women—1 variant allele) to determine if a dose-response relationship existed. Finally, we characterized serial cytokine expression and TEG parameters for patients with and without the IRAK-1 polymorphism.

All data were summarized as mean ± SD, median (interquartile range), or percentage (%). Student *t* test or Mann-Whitney statistical test was used to compare continuous variables, whereas χ^2 test or Fischer exact test was used for categorical variables. $P \leq 0.05$ was considered statistically significant. The institutional review board at the University of Pittsburgh approved this study.

RESULTS

Over the study time period, 321 patients met inclusion and exclusion criteria and constituted the study cohort. The overall study cohort had a mean age of 50 ± 16 years, 70% male, and a median ISS of 16 [10, 21]. The cohort had an average ICU length of stay of 5.3 ± 6 days, and an overall incidence of MOF, NI, and mortality for the cohort was 8.1%, 27.0%, and 4.4%, respectively. The prevalence of the IRAK-1 polymorphism across men and women in the study cohort was 21.5% when heterozygous women (n = 29) were also included. For the purposes of the principal analyses, only homozygous patients (men—1 variant allele, women—2 variant alleles) were considered to have the IRAK-1 variant (12.5%). In the IRAK-1 variant group, 5 patients were female and were homozygous for the variant allele.

When IRAK-1 variant patients were compared with those with the normal haplotype, patients were similar in demographics, presenting vital and GCS, overall injury severity, and 24-hour resuscitation and transfusion requirements. (Table 1). IRAK-1 patients were more commonly male and had a significantly higher rate of MOF and mortality in unadjusted comparison.

TABLE 1. Unadjusted Comparison of IRAK-1 Variant and Normal Haplotype Demographics, Injury Characteristics, and Outcomes

	IRAK-1 Variant (n = 40)	Normal Haplotype (n = 281)	*P*
Age (yrs)	47 ± 22	50 ± 19	0.388
Sex (%Male)	87.5%	67.3%	0.009
Race			
Caucasian	72.5%	81.5%	
African American	7.5%	2.5%	
Other/Unknown	20%	16.0%	0.176
ED SBP (mm Hg)	130 ± 25	129 ± 28	0.817
ED GCS	15 [14, 15]	15 [14, 15]	0.645
Injury Severity Score (ISS)	17 [13, 20]	16 [10, 21]	0.442
Intubation status (% yes)	16.7%	11.2%	0.573
Body mass index (BMI)	26.9 ± 4	29.2 ± 7	0.078
ICU days	6.3 ± 7	5.2 ± 6	0.289
Length of stay	12 ± 10	11 ± 9	0.337
24-h crystalloid (cc)	3770 ± 2900	3290 ± 2160	0.249
24-h blood transfusion (cc)	447 ± 820	437 ± 1010	0.956
24-h plasma transfusion (cc)	179 ± 653	218 ± 790	0.786
24-h platelet transfusion (cc)	114 ± 326	70 ± 227	0.328
NI	33.3%	26.1%	0.381
Pneumonia	21.2%	18.3%	0.683
MOF, %	18.2%	5.4%	0.006
Mortality, %	12.5%	3.2%	0.007

ED indicates emergency department.

Our logistic regression model was an excellent predictor of mortality with an area under the curve of 0.94 via receiver operating characteristic curve analysis. The model was also a strong predictor of MOF and adequate predictor of NI with area under the curve of 0.90 and 0.70, respectively. After controlling for all important confounders, the IRAK-1 variant was not a significant independent risk factor for the development of NI (OR = 1.6, $P = 0.315$, 95% CI: 0.62–4.3). When both MOF and mortality were analyzed, the IRAK-1 variant was significantly associated with over an eightfold greater independent odds of MOF (OR = 8.4, $P = 0.005$, 95% CI: 1.9–37.1) and over an 11-fold greater independent odds of mortality (OR = 11.8, $P = 0.037$, 95% CI: 1.1–121) (Fig. 1).

To characterize significance of homozygous or heterozygous status of the IRAK-1 variant, we first looked at the incidence of MOF and mortality across the haplotype designation (Table 2). This unadjusted comparison revealed a dose-response relationship with heterozygous women having an intermediate incidence of MOF and mortality relative to the normal haplotype and homozygous IRAK-1 variant. When the haplotype (CT and CC relative to the normal haplotype TT) of the IRAK-1 variant was analyzed concurrently in the regression model, as compared to the odds of poor outcome associated with the normal haplotype, both the heterozygous haplotype and homozygous IRAK-1 variant were significant independent risk factors for MOF (*P*'s 0.012 and 0.003, respectively). Only the homozygous IRAK-1 variant (CC) remained a significant independent risk factor for mortality when both variant haplotypes were included in the model.

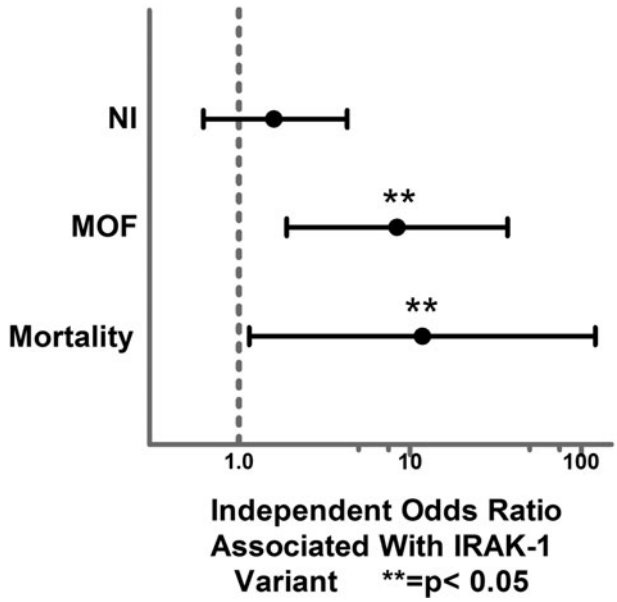

FIGURE 1. Forest plot depicting the independent Odds Ratio for the development of NI, MOF, and mortality associated with the IRAK-1 variant.

When serial cytokine measurements were characterized, early IL-6 and IL-10 levels were significantly correlated in a positive direction with the propensity to develop MOF and mortality; however, there was no significant relationship with serial cytokine expression and the IRAK-1 variant or IRAK-1 haplotype (TT, CT, CC).

When coagulopathy was further characterized, we first excluded those patients who presented on oral anticoagulation or antiplatelet medications (n = 91). We then looked the continuous TEG parameter variables and the extreme quartile (>75th percentile or < 25th percentile) associated with coagulopathy for each TEG parameter (*r* value, k time, α-angle, MA, G value, and LY30). When these were compared across TEG measurements drawn in the first 6 hours from injury, there were no significant differences found across those with and without the IRAK-1 variant. When TEG measurements that were performed at 24 hours out from injury were analyzed, there were significantly higher k time, α-angle, MA, and G values ($P = 0.029$, $P = 0.021$, $P = 0.45$, $P = 0.043$, respectively) in those patients with the IRAK-1 variant. As this represents a potential evolving coagulopathy over the initial 24 hours postinjury, we verified these significant differences in a regression model, which also controlled for differences in demographics, injury severity, and 24-hour resuscitation and transfusion requirements (age, sex, ISS, 24-hour blood, plasma, platelet, and crystalloid). After controlling for important differences across the groups, the IRAK-1 variant remained significantly associated in 3 out of the 4 TEG parameters with more than a threefold greater independent risk of coagulopathic tendency (α-angle, $P = 0.018$; MA, $P = 0.047$; and G value, $P = 0.012$, Fig. 2).

DISCUSSION

As few effective interventions exist, which alter the morbidity and mortality that inherently follows traumatic injury, investigation into novel mechanisms, which result in a protective effect may provide a route to reduce these sequelae postinjury. The ultimate elucidation of the mechanisms responsible for sex-based outcome differences will provide insight and understanding of novel therapeutic targets, which have significant potential to improve outcomes in both men and women postinjury.

TLRs are an evolutionarily conserved family of protein receptors, which are central to NF-κB cellular signaling and the initiation of the innate immune response to infection.[36–38] Accumulating evidence suggests that TLRs also recognize endogenous ligands that arise from cellular damage that are unrelated to infection.[21–24] Compelling evidence has revealed that the TLR receptor, specifically TLR4, is required and plays a critical role in the early activation and upregulation of the innate immune response, the resultant systemic inflammatory response, and the secondary organ dysfunction, which is known to complicate and follow traumatic injury.[17–20] Concurrently, it is known that women would potentially be less affected by an unfavorable X chromosome-linked genetic polymorphism due to the mosaic expression pattern of the X chromosome, which has been shown in other disease processes to be protective for women.[16,39–41]

The results of the current prospective analysis suggest that an IRAK-1 polymorphism, which is a TLR signaling pathway constituent that also resides on the X chromosome that is known to result in increased NF-κB cellular signalling, is strongly associated with the

TABLE 2. Unadjusted Rates of MOF and Mortality Across Haplotype of *IRAK-1* Gene

	Normal Haplotype (TT, n = 281)	Variant Haplotype Heterozygous (CT, n = 29)	Variant Haplotype Homozygous (CC, n = 40)	*P*
MOF	4.7%	11.1%	18.2%	0.012
Mortality	2.4%	10.3%	12.5%	0.004

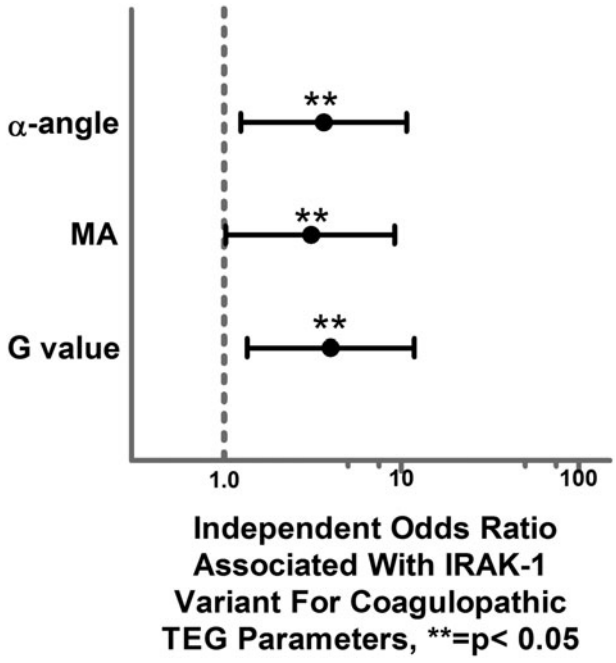

FIGURE 2. Forest plot depicting the independent odds ratio for the development of coagulopathic TEG parameters at 24 hours from injury associated with the IRAK-1 variant.

development of MOF and mortality in a prospectively enrolled cohort of injured patients that required ICU admission. Further confirmation of the significance of these findings is demonstrated by prevalence of the polymorphism in a single-center injured population and the dose-response relationship determined by the haplotype (heterozygous or homozygous expression) of the IRAK-1 variant. The current results verify that the IRAK-1 polymorphism represents a common variant with prognostic potential and demonstrates the importance of TLR signaling postinjury and further supports that a genetic mechanism may in part drive sex-based outcome differences postinjury.

These results compliment prior studies demonstrating a detrimental association of the IRAK-1 variant in patients with sepsis.[25,26] Arcaroli and colleagues studied the same polymorphism to identify the IRAK-1 variant haplotype and characterized its association with clinical outcomes in a septic population (n = 155). The IRAK-1 variant was relatively common in this septic population (prevalence = 21.3%) and was associated with increased nuclear translocation of NF-κB (synonymous with NF-κB activation), more severe organ dysfunction, and independently associated with a higher risk of mortality, in this cohort of patients. Similarly, evidence exists that racial disparities exist in the strength of sex-based outcome differences, which correlates with the known prevalence of the IRAK-1 variant across different racial groups.[42]

Importantly, the current results are not simply an extension of sex-based outcomes, which have been previously demonstrated after traumatic injury in multiple studies.[7,15,43–51] In the current 321 patient cohort, there were no significant differences in the clinical outcomes (NI, MOF, or mortality) across male and female sex nor was sex a significant covariate in any of the regression models. Prior studies demonstrating sex-based outcome differences postinjury have utilized larger retrospective and prospective injured populations and have demonstrated a significantly lower magnitude of risks of poor outcome across men and women with few able to characterize the risk of MOF and attributable complications.[7,43–51] The strength of the current findings, demonstrated in a relatively small cohort of patients, provides insight into the magnitude of effect the IRAK-1 variant may have on clinical outcomes postinjury. Despite the strength of these clinical outcome findings, the underlying mechanism responsible remains less clearly characterized.

It is somewhat surprising that no differences in serial cytokine expression between patients with and without the IRAK-1 variant were found. It is known that IL-6 levels are a strong predictor of the development of MOF[52,53] and despite the strong association between the IRAK-1 variant and MOF, no differences were found for IL-6 or other cytokine expression. Importantly, the full spectrum of circulating mediators that might contribute to the immune response driven by TLR and IRAK-1 were not able to be measured for the analysis. The most current paradigm holds that increased innate immune activity leads to proinflammatory mediators and subsequent organ dysfunction and attributable morbidity and mortality.[54] The lack of any significant measurable differences in the proteomic (cytokine) response may call into question this paradigm in this particular circumstance. It may be that either early proteomic or innate immune response differences exist but were not able to be appropriately measured by standard cytokine analysis which was undertaken, or that the clinical outcome differences found follow an alternative paradigm or model in this specific situation.

We attempted to characterize the early and evolving coagulopathy for the cohort as it has been shown to be a significant risk factor for poor outcome and we have previously demonstrated significant differences in the risk of coagulopathy across male and female sex postinjury.[55–60] Although no TEG parameter differences were demonstrated early (6 hours) postinjury, a strong relationship with evolving coagulopathy over the first 24 hours was demonstrated. It may be that patients at high risk for MOF have a tendency toward coagulopathy or these early differences in coagulation may be in part driving the risk of MOF. Mechanistic possibilities include that the TLR signaling cascade by way of the IRAK-1 polymorphism in some way drives this evolving coagulopathy. It has been previously demonstrated that hypoperfusion and activated protein C are principal drivers of trauma-induced coagulopathy.[61,62] Importantly, hemorrhagic shock and traumatic injury are principal drivers of TLR activation.[63,64] It may be that the principal drivers of these occurrences overlap. Importantly, there were no significant differences in the initial 24-hour transfusion or resuscitation requirements across patients with and without the IRAK-1 variant. The TEG parameters, which were found to be significantly abnormal after adjustment, were the α-angle, MA, and G value. The α-angle characterizes the rate of thrombin generation; conversion of fibrinogen to fibrin; and the interactions among fibrinogen, fibrin, and platelets. Both the MA and G value TEG parameters characterize the overall clot strength with contributors to clot strength including platelet and fibrinogen function. The current results verify there is an association with the IRAK-1 variant in this cohort with evolving coagulopathy based on serial TEG measurements. However, the current analysis is unable to provide causal information regarding these developments and the interaction of MOF, coagulopathy, and the early innate immune response postinjury.

The current analysis does have several limitations that deserve discussion. First, this study was performed at a single, level I trauma center and may not be generalizable or pertinent to other centers with differing admission demographics, injury characteristics, or management practices. Although the data collected for the prospective cohort analysis was extensive, potential unknown or unmeasured confounding variables may be responsible for the associations described and the conclusions formulated. The study group represents a smaller cohort than previous sex studies but is substantially larger than some of the prior sepsis studies for which the IRAK-1 variant has been characterized clinically.[15,25] There was a lower than expected incidence of the

selected pertinent outcomes of the study including MOF and mortality, which can have an exaggeratory effect on the odds ratios presented in certain circumstances. Importantly, it has been previously demonstrated that a large portion of the most critically injured patients suffer mortality relatively early, commonly within the first 48 hours.[65] Because of the requirement of informed consent, the most critically ill patients had a lower consent rate significantly reducing the incidence of mortality for the study cohort. Although the within 6-hour early cytokine expression measurements that were performed represent a relatively early time point compared with most other studies, this may still represent a delayed measurement for cytokine expression, which drives the development of MOF and mortality. Finally, our current understanding of the early coagulopathy, which complicates trauma, is just beginning to expand because of the complex nature of the process. The most appropriate analysis of TEG parameters remains controversial with variability in the methods of comparison across studies.[31–35] We utilized the extreme quartile, either more than 75% or less than 25% depending on the specific parameter for logistic regression modeling. This possibly may result in an underestimation or overestimation of coagulopathic tendency for specific patients.

CONCLUSIONS

The IRAK-1 polymorphism is a strong independent predictor of MOF and mortality postinjury and represents a common variant with prognostic potential. These data demonstrate the importance of TLR signaling postinjury and supports that an X –chromosome-linked genetic mechanism may drive sex-based outcome differences postinjury.

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DISCUSSANTS

R. Maier (Seattle, WA):

It's not often that you discuss a paper that may change an entire paradigm of how we approach a disease. I believe this paper has that potential.

One of the holy grails of trauma care is elucidating why 2 similar people hit by the same truck with identical injuries have different outcomes. One of the major differences identified over the last decades is that women and men behave differently to identical injuries.

However, we have failed to fully explain why. As you heard, hormonal differences were the obvious cause invoked initially and confirmed in models. But, in the human setting, the mice results do not hold up. The obvious conclusion is that women are stronger than men and are better able to tolerate severe injury. Today, the authors further provide a unique insight of a potential genetic basis for this observed gender bias in outcome, and, in part, explain why previous studies focused on a proteomic gender difference, primarily hormonal, have not been reproducible.

The authors focus on the IRAK gene, and its polymorphisms as an X chromosome-linked gene. Men, as described, are much more likely to express any polymorphism, and express it homozygously, compared to the double-X chromosome female. In their study, they were then able to show their individuals having a homozygous dysfunctional polymorphism had a markedly increased risk of MOF and mortality using multiple different analyses.

The analysis is very clean and very exciting. The data focus on the genetic cause of the difference rather than chasing genomic products, or proteomes, which we have done for decades and have failed. Attempts at modifying protein levels have not succeeded in improving survival.

I have 3 questions for the authors.

The current paradigm is based on the variable response in hormonal levels between men and women. The authors do not mention whether they measured hormonal levels in their various cohorts of IRAK polymorphisms. Is there a linkage between select hormonal expressions and polymorphism particularly in the heterozygous female? Is hormonal protein production variable based on these various cohorts?

Second, the authors similarly demonstrate that, while IRAK is known to control inflammatory mediator production, they were unable to discern any differences in inflammatory mediator levels between the various polymorphism cohorts. Do they have an explanation? With the increased IRAK activation they demonstrate, there should have been a marked increase in proinflammatory mediators. Why were they unable to demonstrate any increase?

Is there another level of control that's more important than the gene? Is there evidence of increased inflammatory protein production that is not being released or is not being processed to a functional state?

The third question is an extension of this principle. The only functional assay examined was coagulation. Again, they demonstrate that coagulopathy was worse in patients with this genetic polymorphism variant. However, were they able to identify any of the recognized mediators of trauma-induced coagulopathy and whether the genetic polymorphism variant produced a difference in levels of those mediators? Was there any correlation between the genetic cohorts and the biology of coagulation?

Response From B. Zuckerbraun:

Thank you. It has been a privilege to present this work and represent our lead author, Dr Jason Sperry. In regard to associating the IRAK1 variant with differences in hormonal production, at the level of simple analysis we did not see any measurable differences of statistical significance between patients with the IRAK1 variant and those without, again suggesting that the mechanisms of action related to IRAK1 and innate immune signaling may extend beyond and be independent of hormone production.

Dr Sperry is looking intensely at the temporal relationship of hormones, including estrogens and testosterones, which may suggest that there is a difference that occurs over time and that the relationship may be quite complex, without relationship to the IRAK1 variant.

With regard to looking at our association of IRAK1 variant and the cytokine response, we were a bit surprised when we did not see a difference, especially knowing that cytokines, such as IL-6, have been clearly shown to be associated with outcomes such as MOF. But as you mentioned, perhaps we shouldn't be so surprised for a number of reasons. The complexity of cytokines or chemokine measurements or other inflammatory mediators extends beyond that of simple measurements of absolute values and may have more to do with trends. In addition, values of these parameters may change drastically depending on when they are measured, specifically in relationship to the timing of injury, in relationship to the timing of resuscitation efforts, so on and so forth, and, again, may suggest that overinterpretation of the importance of these inflammatory parameters on a causal association with MOF or poor outcome measurements is not accurate.

In relationship to trauma-induced coagulopathy, again, I think there's a little bit of an oversimplification of looking at this as a single phenotypic entity, and perhaps the coagulopathy that's seen early is very different than a coagulopathy that you may see at 24 hours, which, again, may be a manifestation of a measurement of overall immune dysfunction that is occurring at this time point. However, we have not looked specifically at any of the known factors associated with coagulopathy other than looking at the coagulopathy as determined by thromboelastography itself.

DISCUSSANTS

D. Soybel (Hershey, PA):

I think this type of work is important and thought-provoking. Along those lines, I wondered if you could clarify for us just a little bit about the biology of the IRAK1 variant. First of all, you mentioned it's a nonsynonymous variant. My question in that regard is, is this a deletion or is it an alteration of the receptor so that it is more or less responsive to interleukin 1?

Second, this IRAK1 variant, I believe, has also been associated with autoimmune diseases like lupus that are more likely to occur in women. So, I sort of wonder if there is some broader issue involved, such as susceptibility to persistent inflammation or nonresolving inflammation. If so, what your thoughts are about why it persists in the population if it's so maladaptive.

Response From B. Zuckerbraun:

The interesting thing about this IRAK1 variant is there are overall 8 polymorphisms seen in this variant. Invariably, almost all 8 are always seen in the presence of this variant. The one that we measured on this specific exon is just the one that's been described previously.

What that results in is definitely this hyper-exaggerated inflammatory response, which has been demonstrated in vitro. For example, increased NF-κB signaling has been measured as part of that exaggerated response. What the genetic variant does to the protein levels or function has been less clear. Whether or not that alters the phosphorylating ability of the kinase or perhaps alters the ubiquitination sites of the protein. But functionally, it is clear that the variant does phenotypically result in exaggerated downstream signaling.

In addition, pertaining to your second question, it seems to be expressed in a recessive fashion in some circumstances, which may predispose to some of the ongoing inheritance of this variant. As far as the variant's role in other inflammatory diseases that is an ongoing question. We highlighted the response in sepsis and the results of this manuscript illustrate in patients that are surviving their initial traumatic insults are experiencing complications, including mortality and organ dysfunction related to that immune dysfunction of trauma. So, this is clearly a gene variant that has been associated with genetic predisposition to susceptibility to inflammatory responses in the setting of infection or other immune-mediated diseases makes is all I can comment on.

May this variant be protective for other disease processes where that hyperexaggerated immune response be beneficial? For example, in some cancers. It is known that the polymorphism exists quite commonly in certain populations. For example, 80% of Chinese populations have the polymorphism. So, it remains to be seen.

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T. Buchman (Atlanta, GA):

I have a question as to the degree to which your analysis depends on the assumption that women who are heterozygous are expressing at 50%, if you will. Women are natural mosaics with regard to X chromosome inactivation. For example, as seen in the hair color of a calico cat or a tortoise shell cat, inactivation may not be 50% with respect to a given allele. There's a bell-shaped curve to it. Given the relatively small numbers in your study, I wonder about the extent to which your analysis might be affected by the heterozygotes whose X chromosomes are unevenly inactivated, that is they are at one end of the bell curve or the other.

Response From B. Zuckerbraun:

That's a great point, and we don't know the answer to that question.

T. Buchman (Atlanta, GA):

Did you consider doing a methylation analysis of a closely linked gene to see, in a given subject, whether the X chromosome inactivation pattern was even or distributed at one end of the curve or the other?

Response From B. Zuckerbraun:

We could do that; we have not. We had done our analysis excluding heterozygotes from each group. Regardless of that, there were still differences between the normal haplotypes and the homozygous patients. But you bring up a good point that heterozygotes represent perhaps a very mixed population along that spectrum, based on differences in mosaic expression to varying extents throughout the body.

DISCUSSANTS

A. Harken (Oakland, CA):

I'm sure you, in chasing the mechanism, were disappointed that the inflammatory cytokines did not correlate with the IRAK polymorphism, but I guess my questions is, I think of a hormone as being a small amount of material working at a distance and a cytokine as a sometimes relatively large amount of material working locally.

You guys have a huge amount of experience in this. Can you help us or me interpret how we should think of circulating cytokines, just measuring them and relating them to anything that's happening locally?

Response From B. Zuckerbraun:

Clearly, the background of others in our group, including Dr Vodovotz and Dr Billiar, really would suggest that the cytokine and chemokine relationships are much more complex than the absolute measurements. They are looking at these in the context of trends and as part of dynamic Bayesian networks and looking at it that way may reveal a more rigorous relationship or causal relationship, or associations at the very least, with outcomes.

So, I think the real question is, can you apply that to clinical care? Can you perform these analyses, the measurements, and then the interpretation of these measurements to affect care real time? I think that will continue to be a challenge.

The beauty of the genetic variant is that although, again, there's still some challenge in measuring that in real time, but the genetic variant is not changed in relationship to the timing postinjury, so on and so forth. If it could be measured early, you could perhaps target that higher at-risk population early in the course of their care or down the road as this genetic predisposition is going to be omnipresent throughout their hospital stay and life.

X Chromosome-Linked IRAK-1 Polymorphism Is a Strong Predictor of Multiple Organ Failure and Mortality Postinjury

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Objective(s): Clinical research characterizing the mechanisms responsible for sex-based outcome differences postinjury remain conflicting. We sought to characterize an X chromosome-linked IRAK-1 (IL-1 receptor-associated kinase) polymorphism as an alternative mechanism responsible for sex differences postinjury. IRAK-1 is key intermediate in the toll-like receptor (TLR) pathway thought to drive inflammation postinjury.

Methods: A prospective cohort study was performed over a 24-month period. Bluntly injured patients requiring intensive care unit admission were enrolled, whereas patients with isolated brain and spinal cord injuries were excluded. Outcomes of interest included multiple organ failure (MOF, Marshall MOD score > 5) and mortality. Logistic regression was utilized to determine the independent risk of poor outcome associated with the IRAK-1 variant after controlling for important differences.

Results: In an enrolled cohort of 321 patients, the IRAK-1 variant was common (12.5%). Patients with and without the variant were similar in age, injury severity, and 24hr blood transfusion. After controlling for important confounders, the IRAK1 variant was independently associated with more than eightfold (OR = 8.4, $P = 0.005$, 95% CI: 1.9–37.1) and 11-fold (OR = 11.8, $P = 0.037$, 95% CI: 1.1–121) greater risk of MOF and mortality, respectively. These differences were most prominent in men, whereas women heterozygous for the variant demonstrated worse outcome in a dose-dependent fashion.

Conclusions: The IRAK1 polymorphism is a strong independent predictor of MOF and mortality postinjury and represents a common variant with prognostic potential. These data demonstrate the importance of TLR signaling postinjury and supports that a genetic mechanism may drive sex outcome differences postinjury.

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Although significant advances in the care of the injured patient have occurred over the last decade, those who survive their initial injury continue to be plagued with the development of coagulopathy, multiple organ failure (MOF), nosocomial infection (NI), and their attributable morbid effects.[1–6] A persistent finding has been that men and women respond differently after traumatic injury with significant protection afforded to the female sex.[7,8] Controversy exists regarding the clinical explanation and underlying mechanisms responsible for this female protective effect.[7,9,10]

A large body of laboratory evidence suggests that a sex-hormone–based mechanism (estrogen being protective) is responsible for these postinjury differences.[8,11–14] In prior work, our group has shown that the protective effect afforded to women following severe injury is independent of age and the hormonal status of the female, suggesting other mechanisms may be involved clinically.[7,15] Men and women also are different genetically, primarily due to the method of inheritance of, and the genes which reside on, the X chromosome. Secondary to the known mosaic expression of the X chromosome, women would be less affected by unfavorable X chromosome-linked genetic variants.[16] Importantly, increasing evidence also has demonstrated that the Toll-like receptor (TLR) signaling cascade plays an essential role in the early activation of the innate immune response after traumatic injury.[17–24] The IL-1 receptor-associated kinase (IRAK-1) is a protein constituent member of the TLR signaling cascade, which resides on the X chromosome and has been demonstrated to have 2 haplotypes. The IRAK-1 variant haplotype has been demonstrated to be relatively common and associated with worse outcome in septic patients, thought to be secondary to an excessive innate immune response brought about by upregulated NF-κB signaling.[25–27] No evidence currently exists regarding the significance of this TLR pathway variant, which resides on the X chromosome on pertinent outcomes after traumatic injury. We sought to characterize the IRAK-1 variant as an alternative mechanism responsible for sex-based outcome differences postinjury. We hypothesized that the IRAK-1 variant would be common and independently associated with poor clinical outcome after traumatic injury.

METHODS

A prospective observational cohort study was performed over a 24-month time period (2011–2012) with the overarching goal to further characterize the mechanisms responsible for sex (male vs female) based outcome differences after traumatic injury. Inclusion criteria for the study included blunt injured patients older than 17 years requiring ICU (intensive care unit) admission. Patients older than 90 years, with isolated traumatic brain injury, preexisting immune-suppression, or those with an anticipated survival of less than 24 hours were excluded from enrollment. Blood samples were obtained within 6 hours of injury for serial cytokine measurements, coagulation assessment [international normalized ratio (INR), thromboelastography (TEG) analysis], and DNA isolation and haplotype discrimination. Clinical outcomes assessed included the development of MOF, NI, and mortality.

The IRAK-1 variant haplotype was determined by genotyping the single nucleotide polymorphism on the X chromosome where a T→ C substitution [rs1059703] at position 1595 in exon12 results in a nonsynonymous mutation (532, L→ S). Probe and primer combinations were designed for genotyping this polymorphism and polymerase chain reaction (PCR) was performed using an Applied

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Biosystems 7300 Real-Time PCR system using methods previously described.[25,27] Allelic discrimination was verified by direct DNA sequencing of a small subgroup of patients of each haplotype (men and women: wild-type, variant, and heterozygous, respectively) to assure the PCR-based assay was sufficiently accurate.

MOF was evaluated using the well-validated Marshall Multiple Organ Dysfunction Score.[5,28,29] A Marshall Multiple Organ Dysfunction Score of more than 5 beyond 48 hours of injury and ICU admission was classified as MOF. Primary infectious outcomes of interest include ventilator-associated pneumonia, blood stream infection (excluding those associated with an intra-abdominal abscess), and urinary tract infections.[30] These were selected in attempts to use those infectious outcomes that can be used as a marker for the degree of relative immune suppression. The development of these NIs was based on positive culture evidence.

Blood samples were serially obtained at 6 hours, 24 hours, and 72 hours of injury, and serum was separated and frozen at −80°C until assayed for cytokine analysis. Cytokine expression including IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IFN-α, and IFN-γ were measured from patients' serum using a Luminex 100 IS System and commercially available human specific beadsets. Thromboelastography (TEG) was performed within the first 6 hours of injury and at 24 hours using on a TEG 5000 Thromboelastograph Hemostasis Analyzer and standard TEG parameters were recorded including *r* value, k time, α angle, maximal amplitude (MA), G value, and fibrinolysis at 30 minutes (LY30) as previously described.[31–35]

First, patients with and without the IRAK-1 polymorphism underwent unadjusted comparison of demographics, injury characteristics, resuscitation and transfusion requirements, and clinical outcomes. Multivariable logistic regression analysis was then utilized to determine the independent risks of our clinical outcomes associated with the IRAK-1 variant. Covariates adjusted for in the regression model included age, sex (male vs female), race, body mass index (BMI), injury severity score (ISS), presenting systolic blood pressure (SBP), presenting Glasgow Coma Score (GCS), intubation status, presenting coagulopathy (INR > 1.5), 24-hour crystalloid, and blood component transfusion requirements. Because of the X chromosome location of the polymorphism, we then characterized the risk of our clinical outcomes across whether the IRAK-1 variant existed in a homozygous manner (male-1 variant allele, female -2 variant alleles) or heterozygous manner (women—1 variant allele) to determine if a dose-response relationship existed. Finally, we characterized serial cytokine expression and TEG parameters for patients with and without the IRAK-1 polymorphism.

All data were summarized as mean ± SD, median (interquartile range), or percentage (%). Student *t* test or Mann-Whitney statistical test was used to compare continuous variables, whereas χ^2 test or Fischer exact test was used for categorical variables. $P \leq 0.05$ was considered statistically significant. The institutional review board at the University of Pittsburgh approved this study.

RESULTS

Over the study time period, 321 patients met inclusion and exclusion criteria and constituted the study cohort. The overall study cohort had a mean age of 50 ± 16 years, 70% male, and a median ISS of 16 [10, 21]. The cohort had an average ICU length of stay of 5.3 ± 6 days, and an overall incidence of MOF, NI, and mortality for the cohort was 8.1%, 27.0%, and 4.4%, respectively. The prevalence of the IRAK-1 polymorphism across men and women in the study cohort was 21.5% when heterozygous women (n = 29) were also included. For the purposes of the principal analyses, only homozygous patients (men—1 variant allele, women—2 variant alleles) were considered to have the IRAK-1 variant (12.5%). In the IRAK-1 variant group, 5 patients were female and were homozygous for the variant allele.

When IRAK-1 variant patients were compared with those with the normal haplotype, patients were similar in demographics, presenting vital and GCS, overall injury severity, and 24-hour resuscitation and transfusion requirements. (Table 1). IRAK-1 patients were more commonly male and had a significantly higher rate of MOF and mortality in unadjusted comparison.

TABLE 1. Unadjusted Comparison of IRAK-1 Variant and Normal Haplotype Demographics, Injury Characteristics, and Outcomes

	IRAK-1 Variant (n = 40)	Normal Haplotype (n = 281)	*P*
Age (yrs)	47 ± 22	50 ± 19	0.388
Sex (%Male)	87.5%	67.3%	0.009
Race			
Caucasian	72.5%	81.5%	
African American	7.5%	2.5%	
Other/Unknown	20%	16.0%	0.176
ED SBP (mm Hg)	130 ± 25	129 ± 28	0.817
ED GCS	15 [14, 15]	15 [14, 15]	0.645
Injury Severity Score (ISS)	17 [13, 20]	16 [10, 21]	0.442
Intubation status (% yes)	16.7%	11.2%	0.573
Body mass index (BMI)	26.9 ± 4	29.2 ± 7	0.078
ICU days	6.3 ± 7	5.2 ± 6	0.289
Length of stay	12 ± 10	11 ± 9	0.337
24-h crystalloid (cc)	3770 ± 2900	3290 ± 2160	0.249
24-h blood transfusion (cc)	447 ± 820	437 ± 1010	0.956
24-h plasma transfusion (cc)	179 ± 653	218 ± 790	0.786
24-h platelet transfusion (cc)	114 ± 326	70 ± 227	0.328
NI	33.3%	26.1%	0.381
Pneumonia	21.2%	18.3%	0.683
MOF, %	18.2%	5.4%	0.006
Mortality, %	12.5%	3.2%	0.007

ED indicates emergency department.

Our logistic regression model was an excellent predictor of mortality with an area under the curve of 0.94 via receiver operating characteristic curve analysis. The model was also a strong predictor of MOF and adequate predictor of NI with area under the curve of 0.90 and 0.70, respectively. After controlling for all important confounders, the IRAK-1 variant was not a significant independent risk factor for the development of NI (OR = 1.6, $P = 0.315$, 95% CI: 0.62–4.3). When both MOF and mortality were analyzed, the IRAK-1 variant was significantly associated with over an eightfold greater independent odds of MOF (OR = 8.4, $P = 0.005$, 95% CI: 1.9–37.1) and over an 11-fold greater independent odds of mortality (OR = 11.8, $P = 0.037$, 95% CI: 1.1–121) (Fig. 1).

To characterize significance of homozygous or heterozygous status of the IRAK-1 variant, we first looked at the incidence of MOF and mortality across the haplotype designation (Table 2). This unadjusted comparison revealed a dose-response relationship with heterozygous women having an intermediate incidence of MOF and mortality relative to the normal haplotype and homozygous IRAK-1 variant. When the haplotype (CT and CC relative to the normal haplotype TT) of the IRAK-1 variant was analyzed concurrently in the regression model, as compared to the odds of poor outcome associated with the normal haplotype, both the heterozygous haplotype and homozygous IRAK-1 variant were significant independent risk factors for MOF (*P*'s 0.012 and 0.003, respectively). Only the homozygous IRAK-1 variant (CC) remained a significant independent risk factor for mortality when both variant haplotypes were included in the model.

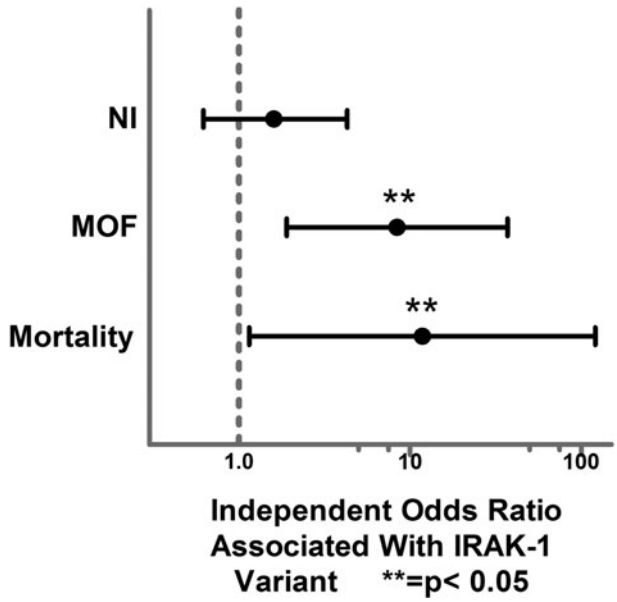

FIGURE 1. Forest plot depicting the independent Odds Ratio for the development of NI, MOF, and mortality associated with the IRAK-1 variant.

When serial cytokine measurements were characterized, early IL-6 and IL-10 levels were significantly correlated in a positive direction with the propensity to develop MOF and mortality; however, there was no significant relationship with serial cytokine expression and the IRAK-1 variant or IRAK-1 haplotype (TT, CT, CC).

When coagulopathy was further characterized, we first excluded those patients who presented on oral anticoagulation or antiplatelet medications (n = 91). We then looked the continuous TEG parameter variables and the extreme quartile (>75th percentile or < 25th percentile) associated with coagulopathy for each TEG parameter (*r* value, k time, α-angle, MA, G value, and LY30). When these were compared across TEG measurements drawn in the first 6 hours from injury, there were no significant differences found across those with and without the IRAK-1 variant. When TEG measurements that were performed at 24 hours out from injury were analyzed, there were significantly higher k time, α-angle, MA, and G values ($P = 0.029$, $P = 0.021$, $P = 0.45$, $P = 0.043$, respectively) in those patients with the IRAK-1 variant. As this represents a potential evolving coagulopathy over the initial 24 hours postinjury, we verified these significant differences in a regression model, which also controlled for differences in demographics, injury severity, and 24-hour resuscitation and transfusion requirements (age, sex, ISS, 24-hour blood, plasma, platelet, and crystalloid). After controlling for important differences across the groups, the IRAK-1 variant remained significantly associated in 3 out of the 4 TEG parameters with more than a threefold greater independent risk of coagulopathic tendency (α-angle, $P = 0.018$; MA, $P = 0.047$; and G value, $P = 0.012$, Fig. 2).

DISCUSSION

As few effective interventions exist, which alter the morbidity and mortality that inherently follows traumatic injury, investigation into novel mechanisms, which result in a protective effect may provide a route to reduce these sequelae postinjury. The ultimate elucidation of the mechanisms responsible for sex-based outcome differences will provide insight and understanding of novel therapeutic targets, which have significant potential to improve outcomes in both men and women postinjury.

TLRs are an evolutionarily conserved family of protein receptors, which are central to NF-κB cellular signaling and the initiation of the innate immune response to infection.[36–38] Accumulating evidence suggests that TLRs also recognize endogenous ligands that arise from cellular damage that are unrelated to infection.[21–24] Compelling evidence has revealed that the TLR receptor, specifically TLR4, is required and plays a critical role in the early activation and upregulation of the innate immune response, the resultant systemic inflammatory response, and the secondary organ dysfunction, which is known to complicate and follow traumatic injury.[17–20] Concurrently, it is known that women would potentially be less affected by an unfavorable X chromosome-linked genetic polymorphism due to the mosaic expression pattern of the X chromosome, which has been shown in other disease processes to be protective for women.[16,39–41]

The results of the current prospective analysis suggest that an IRAK-1 polymorphism, which is a TLR signaling pathway constituent that also resides on the X chromosome that is known to result in increased NF-κB cellular signalling, is strongly associated with the

TABLE 2. Unadjusted Rates of MOF and Mortality Across Haplotype of *IRAK-1* Gene

	Normal Haplotype (TT, n = 281)	Variant Haplotype Heterozygous (CT, n = 29)	Variant Haplotype Homozygous (CC, n = 40)	*P*
MOF	4.7%	11.1%	18.2%	0.012
Mortality	2.4%	10.3%	12.5%	0.004

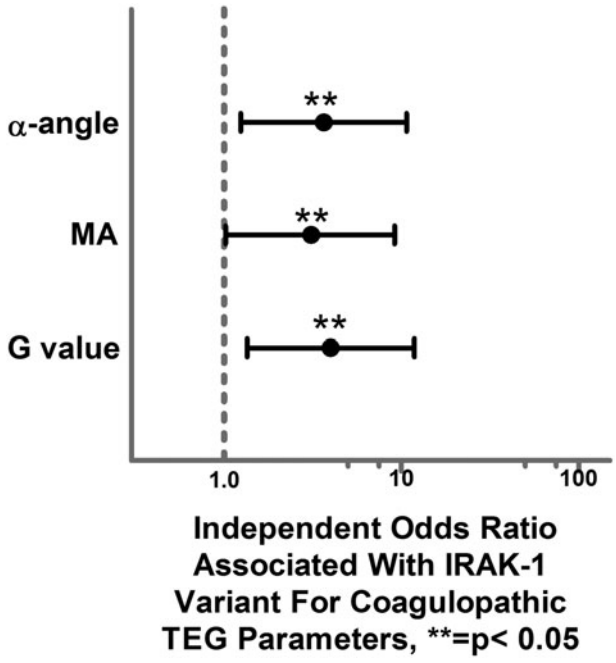

FIGURE 2. Forest plot depicting the independent odds ratio for the development of coagulopathic TEG parameters at 24 hours from injury associated with the IRAK-1 variant.

development of MOF and mortality in a prospectively enrolled cohort of injured patients that required ICU admission. Further confirmation of the significance of these findings is demonstrated by prevalence of the polymorphism in a single-center injured population and the dose-response relationship determined by the haplotype (heterozygous or homozygous expression) of the IRAK-1 variant. The current results verify that the IRAK-1 polymorphism represents a common variant with prognostic potential and demonstrates the importance of TLR signaling postinjury and further supports that a genetic mechanism may in part drive sex-based outcome differences postinjury.

These results compliment prior studies demonstrating a detrimental association of the IRAK-1 variant in patients with sepsis.[25,26] Arcaroli and colleagues studied the same polymorphism to identify the IRAK-1 variant haplotype and characterized its association with clinical outcomes in a septic population (n = 155). The IRAK-1 variant was relatively common in this septic population (prevalence = 21.3%) and was associated with increased nuclear translocation of NF-κB (synonymous with NF-κB activation), more severe organ dysfunction, and independently associated with a higher risk of mortality, in this cohort of patients. Similarly, evidence exists that racial disparities exist in the strength of sex-based outcome differences, which correlates with the known prevalence of the IRAK-1 variant across different racial groups.[42]

Importantly, the current results are not simply an extension of sex-based outcomes, which have been previously demonstrated after traumatic injury in multiple studies.[7,15,43–51] In the current 321 patient cohort, there were no significant differences in the clinical outcomes (NI, MOF, or mortality) across male and female sex nor was sex a significant covariate in any of the regression models. Prior studies demonstrating sex-based outcome differences postinjury have utilized larger retrospective and prospective injured populations and have demonstrated a significantly lower magnitude of risks of poor outcome across men and women with few able to characterize the risk of MOF and attributable complications.[7,43–51] The strength of the current findings, demonstrated in a relatively small cohort of patients, provides insight into the magnitude of effect the IRAK-1 variant may have on clinical outcomes postinjury. Despite the strength of these clinical outcome findings, the underlying mechanism responsible remains less clearly characterized.

It is somewhat surprising that no differences in serial cytokine expression between patients with and without the IRAK-1 variant were found. It is known that IL-6 levels are a strong predictor of the development of MOF[52,53] and despite the strong association between the IRAK-1 variant and MOF, no differences were found for IL-6 or other cytokine expression. Importantly, the full spectrum of circulating mediators that might contribute to the immune response driven by TLR and IRAK-1 were not able to be measured for the analysis. The most current paradigm holds that increased innate immune activity leads to proinflammatory mediators and subsequent organ dysfunction and attributable morbidity and mortality.[54] The lack of any significant measurable differences in the proteomic (cytokine) response may call into question this paradigm in this particular circumstance. It may be that either early proteomic or innate immune response differences exist but were not able to be appropriately measured by standard cytokine analysis which was undertaken, or that the clinical outcome differences found follow an alternative paradigm or model in this specific situation.

We attempted to characterize the early and evolving coagulopathy for the cohort as it has been shown to be a significant risk factor for poor outcome and we have previously demonstrated significant differences in the risk of coagulopathy across male and female sex postinjury.[55–60] Although no TEG parameter differences were demonstrated early (6 hours) postinjury, a strong relationship with evolving coagulopathy over the first 24 hours was demonstrated. It may be that patients at high risk for MOF have a tendency toward coagulopathy or these early differences in coagulation may be in part driving the risk of MOF. Mechanistic possibilities include that the TLR signaling cascade by way of the IRAK-1 polymorphism in some way drives this evolving coagulopathy. It has been previously demonstrated that hypoperfusion and activated protein C are principal drivers of trauma-induced coagulopathy.[61,62] Importantly, hemorrhagic shock and traumatic injury are principal drivers of TLR activation.[63,64] It may be that the principal drivers of these occurrences overlap. Importantly, there were no significant differences in the initial 24-hour transfusion or resuscitation requirements across patients with and without the IRAK-1 variant. The TEG parameters, which were found to be significantly abnormal after adjustment, were the α-angle, MA, and G value. The α-angle characterizes the rate of thrombin generation; conversion of fibrinogen to fibrin; and the interactions among fibrinogen, fibrin, and platelets. Both the MA and G value TEG parameters characterize the overall clot strength with contributors to clot strength including platelet and fibrinogen function. The current results verify there is an association with the IRAK-1 variant in this cohort with evolving coagulopathy based on serial TEG measurements. However, the current analysis is unable to provide causal information regarding these developments and the interaction of MOF, coagulopathy, and the early innate immune response postinjury.

The current analysis does have several limitations that deserve discussion. First, this study was performed at a single, level I trauma center and may not be generalizable or pertinent to other centers with differing admission demographics, injury characteristics, or management practices. Although the data collected for the prospective cohort analysis was extensive, potential unknown or unmeasured confounding variables may be responsible for the associations described and the conclusions formulated. The study group represents a smaller cohort than previous sex studies but is substantially larger than some of the prior sepsis studies for which the IRAK-1 variant has been characterized clinically.[15,25] There was a lower than expected incidence of the

selected pertinent outcomes of the study including MOF and mortality, which can have an exaggeratory effect on the odds ratios presented in certain circumstances. Importantly, it has been previously demonstrated that a large portion of the most critically injured patients suffer mortality relatively early, commonly within the first 48 hours.[65] Because of the requirement of informed consent, the most critically ill patients had a lower consent rate significantly reducing the incidence of mortality for the study cohort. Although the within 6-hour early cytokine expression measurements that were performed represent a relatively early time point compared with most other studies, this may still represent a delayed measurement for cytokine expression, which drives the development of MOF and mortality. Finally, our current understanding of the early coagulopathy, which complicates trauma, is just beginning to expand because of the complex nature of the process. The most appropriate analysis of TEG parameters remains controversial with variability in the methods of comparison across studies.[31–35] We utilized the extreme quartile, either more than 75% or less than 25% depending on the specific parameter for logistic regression modeling. This possibly may result in an underestimation or overestimation of coagulopathic tendency for specific patients.

CONCLUSIONS

The IRAK-1 polymorphism is a strong independent predictor of MOF and mortality postinjury and represents a common variant with prognostic potential. These data demonstrate the importance of TLR signaling postinjury and supports that an X –chromosome-linked genetic mechanism may drive sex-based outcome differences postinjury.

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DISCUSSANTS

R. Maier (Seattle, WA):

It's not often that you discuss a paper that may change an entire paradigm of how we approach a disease. I believe this paper has that potential.

One of the holy grails of trauma care is elucidating why 2 similar people hit by the same truck with identical injuries have different outcomes. One of the major differences identified over the last decades is that women and men behave differently to identical injuries.

However, we have failed to fully explain why. As you heard, hormonal differences were the obvious cause invoked initially and confirmed in models. But, in the human setting, the mice results do not hold up. The obvious conclusion is that women are stronger than men and are better able to tolerate severe injury. Today, the authors further provide a unique insight of a potential genetic basis for this observed gender bias in outcome, and, in part, explain why previous studies focused on a proteomic gender difference, primarily hormonal, have not been reproducible.

The authors focus on the IRAK gene, and its polymorphisms as an X chromosome-linked gene. Men, as described, are much more likely to express any polymorphism, and express it homozygously, compared to the double-X chromosome female. In their study, they were then able to show their individuals having a homozygous dysfunctional polymorphism had a markedly increased risk of MOF and mortality using multiple different analyses.

The analysis is very clean and very exciting. The data focus on the genetic cause of the difference rather than chasing genomic products, or proteomes, which we have done for decades and have failed. Attempts at modifying protein levels have not succeeded in improving survival.

I have 3 questions for the authors.

The current paradigm is based on the variable response in hormonal levels between men and women. The authors do not mention whether they measured hormonal levels in their various cohorts of IRAK polymorphisms. Is there a linkage between select hormonal expressions and polymorphism particularly in the heterozygous female? Is hormonal protein production variable based on these various cohorts?

Second, the authors similarly demonstrate that, while IRAK is known to control inflammatory mediator production, they were unable to discern any differences in inflammatory mediator levels between the various polymorphism cohorts. Do they have an explanation? With the increased IRAK activation they demonstrate, there should have been a marked increase in proinflammatory mediators. Why were they unable to demonstrate any increase?

Is there another level of control that's more important than the gene? Is there evidence of increased inflammatory protein production that is not being released or is not being processed to a functional state?

The third question is an extension of this principle. The only functional assay examined was coagulation. Again, they demonstrate that coagulopathy was worse in patients with this genetic polymorphism variant. However, were they able to identify any of the recognized mediators of trauma-induced coagulopathy and whether the genetic polymorphism variant produced a difference in levels of those mediators? Was there any correlation between the genetic cohorts and the biology of coagulation?

Response From B. Zuckerbraun:

Thank you. It has been a privilege to present this work and represent our lead author, Dr Jason Sperry. In regard to associating the IRAK1 variant with differences in hormonal production, at the level of simple analysis we did not see any measurable differences of statistical significance between patients with the IRAK1 variant and those without, again suggesting that the mechanisms of action related to IRAK1 and innate immune signaling may extend beyond and be independent of hormone production.

Dr Sperry is looking intensely at the temporal relationship of hormones, including estrogens and testosterones, which may suggest that there is a difference that occurs over time and that the relationship may be quite complex, without relationship to the IRAK1 variant.

With regard to looking at our association of IRAK1 variant and the cytokine response, we were a bit surprised when we did not see a difference, especially knowing that cytokines, such as IL-6, have been clearly shown to be associated with outcomes such as MOF. But as you mentioned, perhaps we shouldn't be so surprised for a number of reasons. The complexity of cytokines or chemokine measurements or other inflammatory mediators extends beyond that of simple measurements of absolute values and may have more to do with trends. In addition, values of these parameters may change drastically depending on when they are measured, specifically in relationship to the timing of injury, in relationship to the timing of resuscitation efforts, so on and so forth, and, again, may suggest that overinterpretation of the importance of these inflammatory parameters on a causal association with MOF or poor outcome measurements is not accurate.

In relationship to trauma-induced coagulopathy, again, I think there's a little bit of an oversimplification of looking at this as a single phenotypic entity, and perhaps the coagulopathy that's seen early is very different than a coagulopathy that you may see at 24 hours, which, again, may be a manifestation of a measurement of overall immune dysfunction that is occurring at this time point. However, we have not looked specifically at any of the known factors associated with coagulopathy other than looking at the coagulopathy as determined by thromboelastography itself.

DISCUSSANTS

D. Soybel (Hershey, PA):

I think this type of work is important and thought-provoking. Along those lines, I wondered if you could clarify for us just a little bit about the biology of the IRAK1 variant. First of all, you mentioned it's a nonsynonymous variant. My question in that regard is, is this a deletion or is it an alteration of the receptor so that it is more or less responsive to interleukin 1?

Second, this IRAK1 variant, I believe, has also been associated with autoimmune diseases like lupus that are more likely to occur in women. So, I sort of wonder if there is some broader issue involved, such as susceptibility to persistent inflammation or nonresolving inflammation. If so, what your thoughts are about why it persists in the population if it's so maladaptive.

Response From B. Zuckerbraun:

The interesting thing about this IRAK1 variant is there are overall 8 polymorphisms seen in this variant. Invariably, almost all 8 are always seen in the presence of this variant. The one that we measured on this specific exon is just the one that's been described previously.

What that results in is definitely this hyper-exaggerated inflammatory response, which has been demonstrated in vitro. For example, increased NF-κB signaling has been measured as part of that exaggerated response. What the genetic variant does to the protein levels or function has been less clear. Whether or not that alters the phosphorylating ability of the kinase or perhaps alters the ubiquitination sites of the protein. But functionally, it is clear that the variant does phenotypically result in exaggerated downstream signaling.

In addition, pertaining to your second question, it seems to be expressed in a recessive fashion in some circumstances, which may predispose to some of the ongoing inheritance of this variant. As far as the variant's role in other inflammatory diseases that is an ongoing question. We highlighted the response in sepsis and the results of this manuscript illustrate in patients that are surviving their initial traumatic insults are experiencing complications, including mortality and organ dysfunction related to that immune dysfunction of trauma. So, this is clearly a gene variant that has been associated with genetic predisposition to susceptibility to inflammatory responses in the setting of infection or other immune-mediated diseases makes is all I can comment on.

May this variant be protective for other disease processes where that hyperexaggerated immune response be beneficial? For example, in some cancers. It is known that the polymorphism exists quite commonly in certain populations. For example, 80% of Chinese populations have the polymorphism. So, it remains to be seen.

DISCUSSANTS

T. Buchman (Atlanta, GA):

I have a question as to the degree to which your analysis depends on the assumption that women who are heterozygous are expressing at 50%, if you will. Women are natural mosaics with regard to X chromosome inactivation. For example, as seen in the hair color of a calico cat or a tortoise shell cat, inactivation may not be 50% with respect to a given allele. There's a bell-shaped curve to it. Given the relatively small numbers in your study, I wonder about the extent to which your analysis might be affected by the heterozygotes whose X chromosomes are unevenly inactivated, that is they are at one end of the bell curve or the other.

Response From B. Zuckerbraun:

That's a great point, and we don't know the answer to that question.

T. Buchman (Atlanta, GA):

Did you consider doing a methylation analysis of a closely linked gene to see, in a given subject, whether the X chromosome inactivation pattern was even or distributed at one end of the curve or the other?

Response From B. Zuckerbraun:

We could do that; we have not. We had done our analysis excluding heterozygotes from each group. Regardless of that, there were still differences between the normal haplotypes and the homozygous patients. But you bring up a good point that heterozygotes represent perhaps a very mixed population along that spectrum, based on differences in mosaic expression to varying extents throughout the body.

DISCUSSANTS

A. Harken (Oakland, CA):

I'm sure you, in chasing the mechanism, were disappointed that the inflammatory cytokines did not correlate with the IRAK polymorphism, but I guess my questions is, I think of a hormone as being a small amount of material working at a distance and a cytokine as a sometimes relatively large amount of material working locally.

You guys have a huge amount of experience in this. Can you help us or me interpret how we should think of circulating cytokines, just measuring them and relating them to anything that's happening locally?

Response From B. Zuckerbraun:

Clearly, the background of others in our group, including Dr Vodovotz and Dr Billiar, really would suggest that the cytokine and chemokine relationships are much more complex than the absolute measurements. They are looking at these in the context of trends and as part of dynamic Bayesian networks and looking at it that way may reveal a more rigorous relationship or causal relationship, or associations at the very least, with outcomes.

So, I think the real question is, can you apply that to clinical care? Can you perform these analyses, the measurements, and then the interpretation of these measurements to affect care real time? I think that will continue to be a challenge.

The beauty of the genetic variant is that although, again, there's still some challenge in measuring that in real time, but the genetic variant is not changed in relationship to the timing postinjury, so on and so forth. If it could be measured early, you could perhaps target that higher at-risk population early in the course of their care or down the road as this genetic predisposition is going to be omnipresent throughout their hospital stay and life.

Session: VI: Plenary Papers 9-12
Paper 11: 8:40-9:00 AM

THE EARLY EVOLVING SEX HORMONE ENVIRONMENT IS ASSOCIATED WITH SIGNIFICANT CLINICAL OUTCOME AND INFLAMMATORY RESPONSE DIFFERENCES POST-INJURY

Samuel J. Zolin BS, Yoram Vodovotz Ph.D., Raquel M. Forsythe* MD, Rosengart Matthew* MD,MPH, Rami Namas MD, Andrew P. Peitzman* MD, Timothy R. Billiar* MD, Jason L. Sperry* MD,MPH, University of Pittsburgh

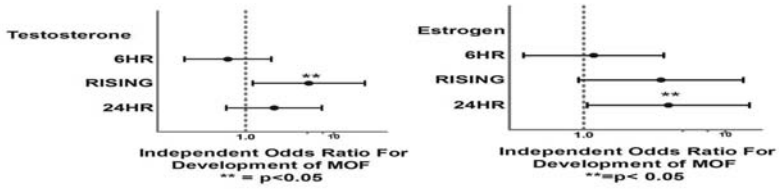
Invited Discussant: Reuven Rabinovici, MD

OBJECTIVE(S): Clinical research characterizing the mechanisms responsible for gender based outcome differences post-injury remain conflicting. Currently lacking is an understanding of the early sex-hormone milieu of the injured patient (< 6 hour from injury) and the effects these early hormone differences have on clinical outcomes and the innate immune response following injury. We hypothesized that the early sex-hormone environment would be associated with significant differences in clinical outcomes and the early innate immune response.

METHODS: A prospective cohort study was performed over an 18 month period. Blunt injured patients requiring ICU admission were enrolled while patients with isolated brain and spinal cord injuries were excluded. Blood samples were collected within 6 hours and at 24 hours post-injury and were analyzed for total testosterone (TT) and estradiol (EST) concentrations. Hormone variables were dichotomized into HIGH and LOW groups and into those patients with increasing hormone levels between 6hr and 24hr measurements (RISING). Outcomes of interest included Multiple Organ Failure (MOF, Marshall MODscore > 5), nosocomial infection (NI), mortality and serial cytokine/mediator measurements. Multivariate logistic regression was utilized to determine the independent risks associated with early sex hormone measurements after controlling for differences in demographics, injury characteristics, shock severity and resuscitation requirements.

RESULTS: In 288 prospectively enrolled patients, 68% were male with a median ISS of 16 [IQR 10,21]. Prevalence of MOF, NI and mortality was 12.5% , 29.9% and 4.1%, respectively. After controlling for important confounders, HIGH TT levels at 6hrs were associated with elevated IL-6 levels and cytokine/mediator measurements (22 out of 26 measured). RISING TT levels were significantly associated with over a 5-fold and 2-fold higher independent risk of MOF and NI, respectively (OR 5.2, p=0.02, 95%CI 1.2-22.3, OR 2.1, p= 0.03, 95%CI 1.02-4.2). At 24hrs HIGH TT was no longer associated with poor outcome while HIGH EST was significantly associated with almost a 4-fold higher independent risk of MOF (OR 3.9, p=0.04, 95% CI 1.05-13).

CONCLUSIONS: Early elevations and increasing testosterone levels over the initial 24hrs are associated with an exaggerated inflammatory response and a significantly greater risk of MOF and NI. HIGH Estrogen levels at 24hrs are independently associated with a greater risk of MOF. Inflammation is known to result in the peripheral conversion of androgens to estrogens. The current analysis suggests an early evolving testosterone to estrogen hormonal environment is associated with a significantly higher independent risk of poor outcome following traumatic injury.



Poster 18

SEX BASED THROMBOELASTOGRAPHY DISPARITIES POST-INJURY: INDEPENDENTLY DIFFERENT EARLY ON BUT WHY?

Tiahuna Zhou, BS, Samuel Zolin, Timothy Billiar, MD,
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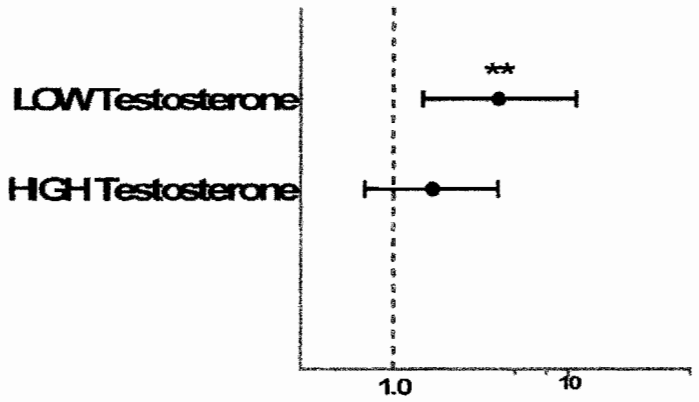
Presenter: Tiahuna Zhou, BS

Objectives: The beneficial use of thromboelastography (TEG) to adequately detect coagulopathy and direct blood component transfusion during resuscitation has been demonstrated. Despite this evidence, little is known regarding male and female differences in TEG measurements and the mechanisms responsible for disparities post-injury. We hypothesized there would be significant differences in TEG parameters with females being more hypercoaguable due to sex hormone differences.

Methods: Data were derived from a prospective cohort study designed to characterize mechanisms responsible for sex based outcome differences post injury. Blunt injured patients requiring ICU admission were included. Isolated TBI, cord injury or patients on anticoagulation were excluded. TEG parameters (r, k-time, alpha angle, MA, G, LY30) and sex hormone levels (estradiol, total testosterone) were obtained <6 hrs and at 24 hrs post injury.

Results: Males and females in the study cohort (n=208) were similar in injury severity, presenting vitals, GCS, 24 hour resuscitation/transfusion needs and presenting INR. Regression analysis demonstrated female sex was independently associated with hypercoaguable TEG parameters at 6 hours (R, k-time, MA, G) and at 24 hours (k-time, alpha angle) after controlling for important confounders. TEG based hypercoagulability in females was present irrespecitve of age (>/< 50yoa) and early estrogen levels (high/low). TEG based hypercoagulability in females was no longer apparent when early testosterone levels were elevated.

Conclusions: Independent disparities exist in TEG parameters across males and females post-injury. These differences were apparent early and remained persistent with females demonstrating a hypercoaguable phenotype. The data suggest that early testosterone rather than age or estrogen levels may be play a role in these independent TEG based disparities across males and females post injury.



Independent OR For TEG Hypercoagulability Associated With Female Sex,**=p< 0.05

The early evolving sex hormone environment is associated with significant outcome and inflammatory response differences after injury

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BACKGROUND: Clinical research characterizing the mechanisms responsible for sex-based outcome differences after injury remains conflicting. Currently lacking is an understanding of the early sex hormone milieu of the injured patient and the effects these early hormone differences have on clinical outcomes and the innate immune response following injury.

METHODS: A prospective cohort study was performed over a 20-month period. Blunt injury patients requiring intensive care unit admission were enrolled. Samples were collected within 6 hours and at 24 hours after injury and were analyzed for total testosterone (TT) and estradiol concentrations. Outcomes of interest included multiple-organ failure (MOF; Marshall Multiple Organ Dysfunction Score [MODScore] > 5), nosocomial infection (NI), mortality, and serial cytokine/chemokine measurements. Multivariate logistic regression was used to determine the independent risks associated with early sex hormone measurements.

RESULTS: In 288 prospectively enrolled patients, 69% were male, with a median Injury Severity Score (ISS) of 16 (interquartile range 10–21). Elevated TT levels at 6 hours were associated with elevated interleukin 6 levels and cytokine/chemokine measurements (18 of 24 measured). Rising TT levels were significantly associated with more than a fivefold and twofold higher independent risk of MOF and NI, respectively (odds ratio [OR], 5.2; $p = 0.02$; 95% confidence interval [CI], 1.2–22.3; and OR, 2.1; $p = 0.03$; 95% CI, 1.02–4.2). At 24 hours, TT levels were no longer associated with poor outcome, while estradiol levels were significantly associated with nearly a fourfold higher independent risk of MOF (OR, 3.9; $p = 0.04$, 95% CI, 1.05–13).

CONCLUSION: Early elevations and increasing testosterone levels over initial 24 hours after injury are associated with an exaggerated inflammatory response and a significantly greater risk of MOF and NI. High estrogen levels at 24 hours are independently associated with an increased risk of MOF. The current analysis suggests that an early evolving testosterone to estrogen hormonal environment is associated with a significantly higher independent risk of poor outcome following traumatic injury. (*J Trauma Acute Care Surg*. 2015;78: 451–458.

LEVEL OF EVIDENCE: Prognostic/epidemiologic study, level II.

KEY WORDS: Testosterone; estrogen; multiple-organ failure; nosocomial infection; regression.

An important and persistent finding has been that males and females respond differently following traumatic injury and hemorrhagic shock, with a relative protection afforded to females.[1,2] An increasing body of evidence from animal models has revealed that sex hormones and or their derivatives play an intricate role in the pathologic response to trauma hemorrhage. Estrogen and testosterone in disparate ways have been shown to influence the hemodynamic, immunologic, organ system, and cellular responses to traumatic insult in animals.[1–10]

The hormonal milieu of the proestrus female rodent has been shown to be protective following trauma and hemorrhage, while male sex steroids are associated with deleterious effects.[11–13] The strength of these laboratory findings has even led some to consider estrogen-based therapy as a possible therapeutic intervention following traumatic injury in human patients.[12,14]

Despite this mounting evidence, clinical studies have been unable to consistently reproduce these laboratory findings.[15–22] Recent prospective evidence, where sex hormone levels were measured 48 hours following injury, provides compelling evidence for estrogen (17β-estradiol [EST]) levels being associated with a greater risk of mortality, a conclusion which contradicts the majority of the experimental animal literature.[23] Similar findings for noninjured but critically ill patients have also been reported.[24,25] It remains unknown whether elevated endogenous estrogens out from the time of injury (>48 hours) are simply a

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marker or play a causal role for poor outcome.[23–25] Currently lacking is an understanding of the early sex hormone milieu of the injured patient (<6 hours from injury through 24 hours after injury) and the effects early sex hormones have on clinical outcomes and the immune response trajectory soon after injury.

In the present study, we sought to characterize the early sex hormone environment and its independent association with important clinical outcomes and the early innate immune response after injury. We hypothesized that estrogen would be associated with beneficial effects, while testosterone moieties would be associated with poor outcome.

METHODS

A prospective observational cohort study was performed over a 20-month period (February 2011 to October 12) with the overarching goal of characterizing the mechanisms responsible for sex (male vs. female)–based outcome differences following traumatic injury. Inclusion criteria for the overall cohort study included blunt injury patients 17 years or older requiring intensive care unit (ICU) admission who arrived within 6 hours of injury to obtain early blood samples. Patients older than 90 years, those with isolated traumatic brain injury (no other injury identified other than brain injury), those with preexisting immunosuppression, those with an anticipated survival of less than 24 hours, or those patients where consent was unable to be obtained were excluded from enrollment. Blood samples were collected within 6 hours and again at 24 hours after injury and were analyzed for sex hormones (total testosterone [TT] and estradiol) and serial cytokine concentrations by specifically trained staff.[26] Clinical outcomes assessed included the development of multiple-organ failure (MOF, Marshall Multiple Organ Dysfunction Score [MODScore] > 5), nosocomial infection (NI), and in-hospital mortality.

Under the auspices of a waiver of initial consent (up to 48 hours), blood was obtained from enrolled patients upon arrival or soon after within 6 hours from the time of injury and again at 24 hours after injury in most patients. A 48-hour window was approved to obtain consent for the use of samples from the time of admission. All samples and data were destroyed if consent was unable to be obtained within the 48-hour widow. Plasma was separated from whole blood and stored at −70°C for batched analysis. TT and EST levels were measured using high sensitivity ELISA kits following the manufacturer's directions (Testosterone ELISA kit, catalog #ADI-900-176; 17β-estradiol ELISA kit, catalog # ADI-900-174; ENZO Life Sciences, Inc., Farmingdale, NY). Human inflammatory MILLIPLEX MAP Human Cytokine/Chemokine Panel-Premixed kits (catalog # HCYTOMAG-60K and # HCYTOMAG-60K; Millipore Corporation, Billerica, MA) and Luminex 100 IS (Luminex, Austin, TX) were used to measure plasma levels of cytokines and chemokines (interleukin 1β [IL-1β], IL-1RA, IL-2, sIL-2Rα, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-17, interferon γ, IP-10, MIG, MIP-1α, MIP-1β, MCP-1, GM-CSF, Eotaxin, tumor necrosis factor α, NO2/NO3, and interferon α) per the manufacturer's directions.

Sex hormone variables for TT and EST were first dichotomized at their median values into high and low groups. Sex hormone levels were further categorized over time into groups of less than 6 hours (<6HR), 24 hours (24HR), and additionally a group where hormone measurements were increasing between 6HR and 24HR measurements (rising). Finally, EST/TT ratios were also characterized and used for the cytokine and outcome analyses.

MOF was evaluated using the well-validated Marshall MODScore.[27–29] A MODScore greater than 5 beyond 48 hours from injury was classified as MOF. Primary nosocomial infectious outcomes of interest included ventilator-associated pneumonia, blood stream infection (excluding those associated with an intra-abdominal abscess), and urinary tract infections.[30] These were selected in attempts to use those infectious outcomes, which can be used as a marker for the degree of relative immune dysregulation/suppression. The development of these NIs was based on positive culture evidence. Diagnosis of a ventilator-associated pneumonia required a quantitative culture threshold of equal to or greater than 10^4 colony-forming units (CFU) per milliliter from bronchoalveolar lavage specimens. Diagnosis of catheter-related blood stream infections requires positive peripheral cultures with an identical organism obtained from either a positive semiquantitative culture (>15 CFU per segment) or a positive quantitative culture (>10^3 CFU per segment) from a catheter segment specimen. Urinary tract infections required greater than 10^5 organisms per milliliter of urine.

First, male and female patients underwent unadjusted comparison of demographics, injury characteristics, resuscitation and transfusion requirements, clinical outcomes, and sex hormones. Correlation analysis was then performed between sex hormone levels and cytokine/chemokine measurements following variable log transformation. Finally, multivariable logistic regression analysis was then used to determine the independent odds of our clinical outcomes associated with sex hormone levels (high vs. low) after adjusting for important confounders. Covariates adjusted for in the regression model included age (>50 years or ≤50 years), sex, Injury Severity Score (ISS), emergency department systolic blood pressure (SBP), emergency department Glasgow Coma Scale score (GCS score > 8 or GCS score ≤ 8), intubation status (yes/no), presenting coagulopathy (international normalized ratio > 1.3, yes/no), 6-hour or 24-hour crystalloid and blood component transfusion requirements (packed red blood cells, fresh frozen plasma, platelets), body mass index, and oral contraceptive use (yes/no).

Mortality was used as the primary outcome to determine our sample size because this is the most stringent outcome to occur relative to the development of MOF and NI. Based on trauma admissions to the ICU at the University of Pittsburgh and with the use of similar inclusion and exclusion criteria, for a similarly injured cohort as proposed, the mortality rate overall was 10%. Based on these projections, an allocation ratio of 0.10 (survivor vs. nonsurvivor) was used for sample size estimation. Based on the previous literature[23,24] where serum levels of EST in both males and females were found to be significantly associated with mortality; survivors-[EST] = 32.4 (50) pg/mL vs. nonsurvivors-[EST] = 66.9 (70) pg/mL, with an $\alpha = 0.05$ and a $\beta = 0.20$, our projected sample size using a two-sided Mann-Whitney U-test was 320 patients.

All data were summarized as mean (SD), median (interquartile range [IQR]), or percentage. Student's *t* or Mann-Whitney

statistical tests were used to compare continuous variables, while X^2 or Fisher's exact test was used for categorical variables. A $p \leq 0.05$ was considered statistically significant. The institutional review board at the University of Pittsburgh approved this study.

RESULTS

During a 20-month period, more than 2,000 patients were screened, with 288 patients being prospectively enrolled and consented who met all inclusion and exclusion criteria and underwent early (<6HR) blood sampling from the time of injury (Fig. 1). This cohort of patients was 69% male, with a mean (SD) age of 50 (18) years, and constituted a moderately injured study cohort, with a median ISS of 16 (IQR, 10–21). More than 31% of the patients required blood transfusion in the first 24 hours, with the prevalence of MOF, NI, and in-hospital mortality being 13.6%, 29.9%, and 3.1%, respectively. Importantly, 24HR sample collection was attempted for all enrolled patients but were only able to be obtained in 237 patients, representing an 82% patient sample follow-up rate.

Males and females were statistically similar in age, injury severity, presenting injury characteristics, transfusion and resuscitation requirements, and important clinical outcomes (Table 1). Interestingly, there were no statistical differences in <6HR sex hormone measurements for either EST or TT as continuous variables across males or females. Males were, however, more likely to have pneumonia as a subtype of NI, despite NIs overall not being different across the groups.

When <6HR sex hormone levels were dichotomized into high and low groups based on the median of the measurement distribution, there was no statistical differences in early EST levels, early TT levels, or the EST/TT ratio across males and females (Table 2). To verify that EST and TT measurements were not concurrently elevated and colinear, we verified that more than 36% of the patients had either high TT with low EST measurements or vice versa irrespective of male or female sex ($p < 0.001$). We similarly found no significant differences in <6HR sex hormones (EST, TT) across age (≤50 years vs. >50 years, EST, $p = 0.444$; TT, $p = 0.958$) or when further stratified by male or female sex.

When high and low sex hormone and IL-6 cytokine levels were compared, both early (6 hours) and 24-hour high TT were significantly associated with elevated IL-6 levels ($p = 0.015$, $p = 0.004$), while no significant relationship was found between IL-6 levels and EST. When correlation analysis of TT, EST, and 24 cytokine/chemokine levels were performed following log transformation for normality considerations, no significant correlations were found for <6HR EST, rising EST, or 24HR EST with any of the measured cytokine/chemokine levels. Similar results were found when correlation analysis was performed between cytokine/chemokine levels and EST/TT ratio at any time point. Interestingly, when <6HR TT levels were correlated with cytokine/chemokine levels, 3 of the 24 measurements demonstrated a significant correlation (IP10, MIP-1α, and MIP-1β). When rising TT levels were analyzed, the majority (16 of 24) of the cytokine/chemokine panels were significantly correlated (positive correlation) with rising TT levels. When 24HR TT levels underwent correlation analysis, the majority (16 of 24) of cytokine/chemokine measurements again were significantly correlated (positive correlation) but with higher Pearson correlation coefficients in all cases, consistent with a greater

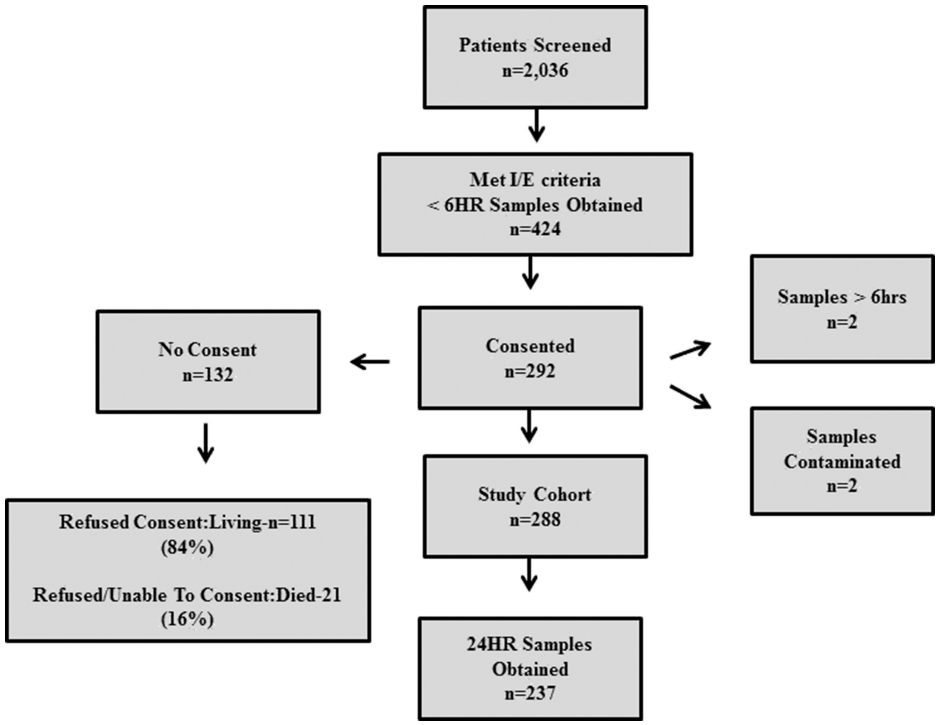

Figure 1. Study cohort enrollment diagram.

TABLE 1. Unadjusted Comparison of Male and Female Demographics, Injury Characteristics, Resuscitation Needs, and Clinical Outcomes

	Males (n = 197)	Females (n = 91)	*P*
Age, mean (SD), y	54 (18)	50 (18)	0.060
ED SBP, mean (SD), mm Hg	131 (27)	126 (29)	0.085
ED hypotensive (SBP < 90 mm Hg), %	8.1	10.0	0.601
ED GCS score, median (IQR)	15 (14–15)	15 (15–15)	0.446
ED GCS score < 8, %	16.8	15.6	0.800
ISS, median (IQR)	17 (10–22)	14 (10–19)	0.450
ISS > 16, %	51.3	45.6	0.386
ED intubation status, yes, %	13.7	11.2	0.573
Presenting coagulopathy (international normalized ratio > 1.3), %	17.9	24.6	0.221
Body mass index, mean (SD)	29.1 (7)	28.3 (7)	0.153
ICU days, mean (SD)	6.1 (6)	5.0 (6)	0.183
Length of stay ICU	11.7 (9)	11.2 (9)	0.685
24-h crystalloid, mean (SD), mL	3,593 (2,527)	3,354 (1,963)	0.428
24-h blood transfusion, mean (SD), mL	509 (1,244)	445 (895)	0.493
24-h plasma transfusion, mean (SD), mL	264 (978)	158 (520)	0.335
24-h platelet transfusion, mean (SD), mL	92 (284)	41 (187)	0.109
Massive transfusion (≥10 U packed red blood cells in 24 h), %	5.6	2.2	0.204
NI, %	30.5	27.8	0.645
Pneumonia, %	23.4	13.3	0.050*
MOF, %	14.7	11.1	0.408
Mortality, %	4.1	1.1	0.183
6-h TT, mean (SD), pg/mL	38.4 (44)	33.6 (16)	0.315
6-h EST, mean (SD), pg/mL	44.2 (38)	41.2 (22)	0.849

magnitude of correlation (Supplemental Digital Content 1, http://links.lww.com/TA/A524).

Our regression models were excellent predictors of our primary outcomes based on the area under the curve from receiver operating characteristic curves and demonstrated adequate diagnostics (Table 3). After controlling for important confounders, logistic regression analysis demonstrated no significant independent relationship between <6HR TT levels or <6HR EST and the development of MOF, NI, or in-hospital mortality (Figs. 2 and 3). When the analysis focused on hormone levels, which increased between the early and 24-hour period, rising TT levels were significantly associated with more than a fivefold and a twofold higher independent odds of MOF and NI, respectively (odds ratio [OR], 5.2; $p = 0.02$; 95% confidence interval [CI], 1.2–22.3; and OR, 2.1; $p = 0.03$; 95% CI, 1.02–4.2). Rising EST levels were associated with a threefold higher odds of MOF, but this relationship failed to reach statistical significance (OR, 3.0; $p = 0.089$; 95% CI, 0.85–10). Interestingly, at the 24HR time point, TT levels were no longer significantly associated with the development of MOF or NI, while EST levels were significantly associated with almost a fourfold higher independent odds of MOF (OR, 3.9; $p = 0.04$; 95% CI, 1.05–13) at this time point.

TABLE 2. Dichotomized Sex Hormone Levels (High vs. Low) for Early (<6HR) Sex Hormone Level Compared Across Males and Females

Early (<6HR) Sex Hormone Measurements (n = 288)	Male (n = 197)	Female (n = 90)	*p*
High EST	52.2%	49.2%	0.639
High TT	48.9%	50.3%	0.830
High EST/TT ratio	48.7%	53.3%	0.469

DISCUSSION

Significant advances in trauma care delivery and post-injury management practices have occurred during the last decade, yet patients who survive their initial injury continue to be plagued with the development of sepsis and MOF and their attributable morbidity and mortality.[27,31–35] Despite a significant increase in our basic understanding of these detrimental outcomes, a dearth of effective interventions exist. An important and persistent literature finding, with possible therapeutic potential, has been that males and females respond differently following traumatic injury and hemorrhagic shock.[1,17,18] A growing body of evidence from animal models suggests that this dimorphic

TABLE 3. Logistic Regression Model Diagnostics

Logistic Regression Model Outcome	AUC via ROC Curve Analysis	Hosmer-Lemeshow
Mortality	0.969	0.840
MOF	0.898	0.559
NI	0.760	0.463

AUC, area under the curve; ROC, receiver operating characteristic.

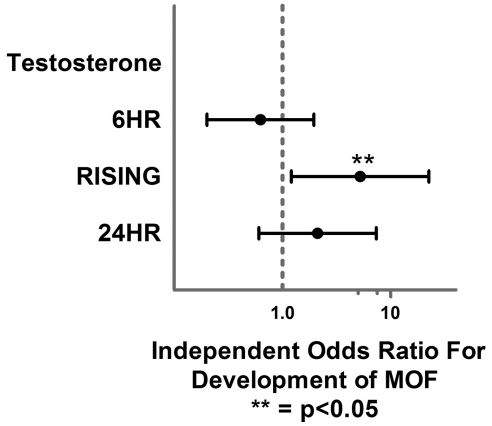

Figure 2. Forest plot depicting independent odds of MOF associated with early, increasing, and 24-hour TT levels.

response following trauma and hemorrhage is hormonally based (estrogen, testosterone, or their derivatives).[4,6,36] Despite these advancements in our understanding, clinical studies have been unable to consistently reproduce these laboratory findings and have provided clinical evidence that contradicts the majority of animal literature.[23–25] Lacking until this time has been a clear understanding of the early sex hormone environment, which potentially has effects on clinical outcomes, and the early immune response trajectory, which follows traumatic injury. The results of the current analysis demonstrate that despite a paucity of sex-specific differences in a moderate-sized blunt injury cohort of patients, the evolving sex hormone environment after injury is associated with both clinical outcome and innate immune response differences soon after injury. Despite sex hormones varying little across male and female sex early on (<6HR), these sex hormone–specific associations were strong and independent of important confounders. Early and rising testosterone levels were found to be significantly associated with an exaggerated cytokine/chemokine response and detrimental clinical outcomes, which diminished in strength over time up until 24 hours after injury. Concurrently, estrogen levels were found to be strongly associated with detrimental clinical outcome at the delayed 24-hour period alone.

These results correspond and add further understanding to the previous literature, which has demonstrated that estrogen is associated with mortality and poor outcome irrespective of male or female sex at 48 hours out from injury or sepsis.[23–25] These results provide insight into the possible mechanisms by which the sex-based outcome differences after injury come about.[2] Essential to understanding these associations is the fact that peripheral conversion of androgens to estrogens can occur via increased aromatase activity and may be stimulated by the early cytokine response, which complicates traumatic injury.[37–39] The current results suggest that early testosterone may be associated with an exaggerated innate immune response and an early evolving testosterone to estrogen hormonal environment is associated with a significantly higher independent risk of poor outcome following traumatic injury.

The potential implications of these results may bridge the current "bench to bedside divide"[2] in our understanding of experimental animal evidence, suggesting testosterone's detrimental effects following hemorrhagic shock and the clinical evidence in humans demonstrating the negative associations of estrogen. Although the current results cannot imply causation and although peripheral conversion of testosterone to estrogen was not measured, the strength of the independent findings in a relatively small, moderately injured cohort of patients does provide strength to the validity of these associations and provides the impetus to further study these relationships to determine if a therapeutic benefit can be derived from sex hormone therapy following injury.

The current analysis has several limitations that deserve discussion. First, the potential for selection and survivor bias exist, despite all attempts to minimize such difficulties, because of the nonrandomized enrollment. Although the data collected for the prospective cohort analysis were extensive, potential unknown or unmeasured confounding variables may be responsible for the associations described and the conclusions formulated. Prehospital medicines that may interfere with sex hormone measurements were prospectively collected and controlled for in the analysis, but unknown or undocumented medicines remain a potential confounder for the analysis. There was a lower-than-expected incidence of the selected pertinent outcomes of the study including MOF and mortality, which can have an exaggeratory effect on the ORs presented in certain circumstances. Despite showing a robust association with MOF and NI, no relationship was found between sex hormone levels and mortality. Importantly, it has been previously demonstrated that a large portion of the most critically injured patients experience mortality relatively early, commonly within the first 24 hours to 48 hours.[40] Because of the requirement of informed consent, the most critically ill patients had a lower consent rate significantly reducing the incidence of mortality for the study cohort. Although the <6HR early cytokine expression measurements that were performed represents a relatively early time point compared with most other studies, this may still represent a delayed measurement for cytokine/chemokine expression, which drives the development of MOF, NI, and mortality. The time of sample obtainment in the 6-hour inclusion criteria window was

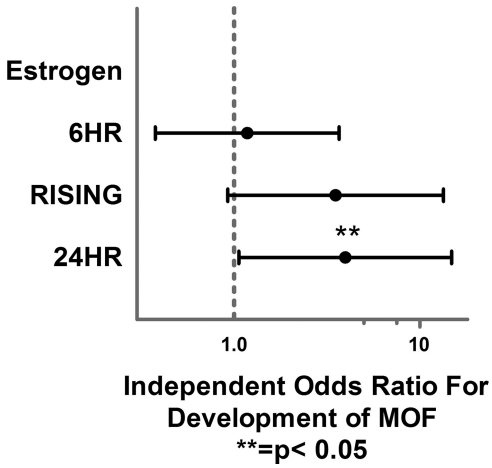

Figure 3. Forest plot depicting independent odds of MOF associated with early, increasing and 24-hour total EST levels.

not recorded and potentially may confound these early measurements and result in a time bias. There also existed a reduction or drop off in the number of samples collected from the enrolled 288 patients at the <6HR period to 237 samples at the 24HR period. The potential exists that the 18% of measurements could alter the reported results and conclusions of the study. Interestingly, there existed no differences in early sex hormone measurements across males and females. Similarly, there were no differences found across age (<50 years or ≥50 years) when compared. Despite this lack of hormone differences, there existed strong clinical associations for the sex hormone levels themselves. The study may be underpowered to see these sex- and age-based hormonal differences. The menstrual cycle status or the menopausal status was not obtained from females in the study cohort. Differences in these cycles and periods in females may result in spurious modeling and alter the significance of these findings and limit the applicability to other studies. Finally, this study was performed at a single Level I trauma center and may not be generalizable or pertinent to other centers with differing admission demographics, injury characteristics, or management practices.

In conclusion, early (<6 hours) elevations and increasing testosterone levels over the initial 24 hours are associated with an exaggerated inflammatory response and a significantly greater independent odds of MOF and NI. By 24 hours after injury, however, testosterone is no longer significantly associated with poor outcome. Early elevations and increasing estrogen levels were not associated with differences in the early inflammatory response or a significant greater odds of poor outcome, but estrogen levels at 24 hours after injury are independently associated with a greater odds of MOF. These results suggest that an early evolving testosterone to estrogen hormonal environment over the initial 24 hours after injury has the potential to predict clinical outcome trajectory. These sex hormone changes may in part be responsible for sex-based outcome differences following traumatic injury. Higher-level studies are required to determine if these sex hormone changes play a causal role in these outcome differences and whether therapeutic potential exist via their actions.

AUTHORSHIP

S.J.Z. and J.L.S. designed the study and performed the literature search, data collection, and data analysis. S.J.Z., Y.V. J.B.B., M.R.R., R.M.F., and J.L.S. participated in the initial manuscript preparation. All authors contributed to the data interpretation and critical revision of the manuscript.

DISCLOSURE

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DISCUSSION

Dr. Reuven Rabinovici (Boston, Massachusetts): Before I discuss this paper I would like to congratulate the presenter, Samuel Zolin, who is a medical student, for being courageous enough to present his work at AAST. I hope he will continue to conduct trauma-related research and wish him success in these efforts.

Now, as a husband and father of three daughters I pretty quickly noticed that there are differences between males and females. As we all know, some of these differences are quite obvious. However, whether males and females respond differently to traumatic injury is not one of those.

In fact, in detailed review of the papers investigating this topic reveals nothing short of chaos and demonstrates conflicting reports on all aspects of potential trauma-related gender differences. Citing the many contradictory papers is beyond the scope of my discussion today. However, to spice it up I will quote some of the reported conclusions.

One study concluded that, "…these data suggest that gender has no relationship to mortality in blunt trauma patients…", while another study summarized that, "…females aged between 13 and 64 years exhibit significantly lower mortality than males after trauma-associated shock." Another study determined "…gender does not play a role in post-traumatic mortality or in the incidence of acute complications after any degree of traumatic brain injury." In contrast, other authors reported that female gender is independently associated with reduced mortality and decreased complications after TBI. And the list goes on and on.

So it is within this context that the authors aim to further investigate this complex topic by establishing the testosterone and estradiol profile during the first 24 post-injury hours in ICU blunt trauma patients. They also recorded multiple organ failure, nosocomial infection, and mortality rates as well as the serum levels of proinflammatory mediators. Lastly, they used multivariate logistic regression to identify which of the above influences is associated with the hormonal profile they described.The authors report that elevated testosterone within the initial six hours was associated with increased rate of multiple organ failure and nosocomial infection as well as an enhanced inflammatory response. They also stated that the 24-hour estradiol were associated with higher risk of multiple organ failure. I have four questions to the authors.

1. How do your data help put in order in the disorder I just described?
2. You did not find inter-gender and age-related hormonal differences. How do you explain that?
3. You did not find correlation between the hormonal profile and mortality, the ultimate outcome parameter. How do you explain that?

And, lastly, do you have any information to suggest whether the hormonal profile you described is responsible for or a marker of outcome?

In summary, this is a well-designed study. However, it seems to add more confusion to an already complex and much-debated topic. I congratulate the group from Pittsburgh for their continuous efforts to identify yet another difference between males and females and thank the association for the privilege of the floor.

Dr. Carl J. Hauser (Boston, Massachusetts): Thank you. Very nice paper. I'd like to ask whether the authors think that testosterone is an acute phase respondent here or whether one of the enzymes in the peripheral aromatase pathway may be, for instance, a negative acute phase respondent, perhaps under the influence of IL-6.

Dr. David Livingston (Newark, New Jersey): Yes, very nicely done. Very nicely presented as a student. Great job. We showed that being female and young you seem to be a lot more resistant to shock. That's really what a lot of the experimental studies did.

Did you look at differences in blood utilization, lactate, and base deficit? Were there any between some of your younger females and males eventually leading on to organ failure?

And, as you nicely showed, the milieu is kind of very complicated but at least early on, the females appear to be more resistant to shock.

Mr. Samuel J. Zolin (Pittsburgh, Pennsylvania): Thank you very much for these questions. With regard to what exactly this adds to our knowledge base and what it might clarify regarding the early sex hormone environment and outcomes, this study provided a much earlier measurement of serum sex hormones than previous work had done.

As I mentioned, previous work at 48 hours following injury had demonstrated poor outcome with differences in hormonal status.

We demonstrated that an evolving hormonal profile over the first 24 hours following injury may be associated with poor outcome.

Measurement of hormone level at earlier time points, as we did in this study, may reduce the confounding effect that peripheral aromatization may play, although we did not make any direct measurement of peripheral aromatization within subjects in this study.

To answer the line of questioning regarding testosterone's role as either being a marker of poor outcome versus a driver of poor outcome following injury, I believe further research is needed.

While we demonstrated significant associations between testosterone level at different time points and markers of the innate immune response and poor clinical outcome, this was a strictly observational study.

While we attempted to obtain blood samples from patients as soon as possible following injury in order to characterize their baseline hormonal status, which should give a more clear picture of the role of these hormones as markers or causative factors, it is possible that changes in hormone level begin very early after injury, perhaps even earlier than we accounted for.

It's worth recalling that animal studies do support a causative role for testosterone in poor outcome following trauma.

An interventional study of androgen modulation following trauma in humans would likely provide the most conclusive evidence regarding testosterone's role in outcome following injury.

With regard to the question of why there was no demonstrated difference between men and women with respect to sex hormone levels in this study, this is one of the first studies to analyze the early sex hormone environment following injuries in humans.

It may be that alternations of sex hormone levels following injury occur very soon after injury, even earlier than the six-hour window from injury to sampling that we used.

It may also be that lack of information regarding menstrual and menopause status of females in this study was a confounder.

Finally, regarding the question of mortality as an outcome, our analysis demonstrated no statistically significant difference with regard to the incidence of mortality based on hormone level.

This aspect of our study may have been influenced by the fact that there was a lower-than-expected consent rate for those with early mortality in our study population.

It is possible that with a higher incidence of in-hospital mortality, a hormone-based relationship would have become apparent.

Of course, it is also possible that a hormone-associated relationship with mortality following injury does not exist in humans.

Thank you again for your attention and for the opportunity to present today.

Session XVII: Miscellaneous
Paper 72 10:20 AM

EXCEPTION FROM INFORMED CONSENT FOR EMERGENCY RESEARCH: CONSULTING THE TRAUMA "COMMUNITY"

Carrie Sims*, MD, MS, Joshua Isserman, Latha Mary Sundaram , Nikolai Tolstoy, Sarah Greer, MD, MPH, Jose Pascual, MD, PhD, Patrick Reilly*, M.D., Daniel Holena, MD*, University of Pennsylvania

Invited Discussant: Joseph P. Minei

Introduction: Because consent for research is impossible in emergencies, the FDA established an Exception from Informed Consent (EFIC) Policy mandating "community consultation". This study investigates trauma "community" attitudes regarding EFIC and willingness to participate.

Methods: In the context of an upcoming trial, trauma pts, family and community members were asked to rank statements regarding EFIC and willingness to participate in emergency research (WILLINGNESS) using a 5 point Likert Scale. Higher total scores reflected a more positive attitude regarding EFIC (range 4-20, neutral=12) and WILLINGNESS (range 19-95, neutral=57). The influence of demographics, education, and interpersonal violence were evaluated by Kruskall-Wallis and Mann Whitney ($p<0.05$).

Results: Overall, the 309 participants (trauma pts=172, family=73, community=64) were positive about EFIC (median 16 (IQR 14-18)) and demonstrated high WILLINGNESS scores (median 75 (IQR 69-81)). EFIC and WILLINGNESS were not influenced by age, sex, race, or education. Victims of interpersonal violence and their family members had lower EFIC scores than those with other mechanisms (median 16 (IQR 14-18) vs 16 (IQR 13-16), $p=0.04$), but similar WILLINGNESS. Although EFIC scores were similar between groups, trauma pts had significantly lower WILLINGNESS than family (median 74 (IQR 68-77) vs 77 (IQR 70-85), $p=0.03$) and community members (median 76 (IQR 70-840, $p=0.01$).

Conclusion: Trauma pts, their families, and the surrounding geographic community expressed a high degree of support for EFIC and willingness to participate in emergency research, although support was influenced by injury mechanism and group status. Consultation efforts for emergency research should extend beyond the geographic "community" to include trauma victims and their families.

52.17. Insurance Type, Not Race, Predicts Mortality After Pediatric Trauma. S. S. Short, D. Liou, M. B. Singer, D. Margulies, M. Bukur, A. Salim, E. J. Ley; Cedars-Sinai Medical Center, Los Angeles, California

CLINICAL TRIALS/OUTCOMES 5: DIAGNOSTIC MEASURES AND QUALITY

53.1. The Limitations of an IPTH-Based Protocol for the Prevention of Symptomatic Hypocalcemia After Thyroidectomy. Y. M. Carter, H. Chen, R. S. Sippel; University of Wisconsin Department of Surgery, Madison, WI

53.2. Validation of The Surgical Apgar Score in a Veteran Population. M. Melis,[1] A. Pinna,[1] A. S. Rosman,[3] D. Neihaus,[2] S. Okochi,[2] J. K. Saunders,[1] E. Newman,[1] T. H. Gouge[1]; [1]New York University School of Medicine and NYHHS VAMC, New York, NY; [2]New York Harbor Healthcare System VAMC, New York, NY; [3]Mount Sinai School of Medicine and James J. Peters VAMC, Bronx, NY

53.3. Negative Imaging is Not a Contraindication to Surgical-Parathyroidectomy. H. Wachtel, M. C. Wismer, E. K. Bartlett, P. K. Shah, K. K. Shah, R. R. Kelz, G. C. Karakousis, D. L. Fraker; Hospital of The University of Pennsylvania, Philadelpha, PA

53.4. Evaluation of Surgical APAGAR Scale in the Prediction of Postoperative Complications and Mortality: a Prospective Analysis in a Fourth Level Center in Bogota. M. Castro, E. L. Espitia, N. Tarazona, L. C. Dominguez; Pontificia Universidad Javeriana - Hospital Universitario San Ignacio, Bogota, Colombia

53.5. Decreased Sensitivity and Positive Predictive Value of Ultrasound Findings in the Diagnosis of Pediatric Cholecystitis. J. Tsai,[2] P. C. Minneci,[1] J. Sulkowski,[1] J. Cooper,[1] K. J. Deans[1]; [1]Center For Surgical Outcomes Research, Department of Surgery, Nationwide Children's Hospital, Columbus, OH; [2]Department of Surgery, The Children's Hospital of Philadelphia, Philadelphia, PA

53.6. Efficacy of Localization Studies and Intraoperative Parathormone Monitoring in the Surgical Management of Hyperfunctioning Ectopic Parathyroid Glands. M. B. Albuja-Cruz,[1] B. J. Allan,[2] C. M. Thorson,[1] P. Parikh,[1] J. I. Lew[1,2]; [1]Division of Endocrine Surgery, Miami, FL; [2]Division of General Surgery, Miami, FL

53.7. Novel Computational Model to Reassess Cancer Patient Survival at Time of Follow-up Visits. L. Ross, A. Nowacki, A. Siperstein; Cleveland Clinic, Cleveland, Ohio

53.8. Intraoperative PTH Monitoring Criteria in Secondary Hyperparathyroidism. C. M. Webb, H. Wachtel, E. K. Bartlett, P. K. Shah, K. K. Shah, R. Kelz, G. C. Karakousis, D. L. Fraker; Department of Surgery, Perelman School of Medicine At The University of Pennsylvania, Philadelpha, PA

53.9. Radioactive Iodine Scanning is Not Beneficial but its Use Persists for Euthyroid Patients. R. Panneerselvan, D. F. Schneider, R. S. Sippel, H. Chen; University of Wisconsin School of Medicine and Public Health - Department of Surgery, Madison, WI

53.10. The Ethics of Exception for Emergency Research. T. L. Chin,[1] E. E. Moore,[1,2] A. Ghasabyan,[1,2] J. N. Harr,[1] A. Banerjee,[1] M. Coors[1]; [1]University of Colorado Denver, Aurora, CO; [2]Denver Health Medical Center, Denver, CO

53.11. The Community Speaks: Analyzing Attitudes About the AVERT Shock Trial and Exception From Informed Consent (EFIC) in Emergency Research. S. E. Greer,[1] R. M. Speck,[1] L. M. Sundaram,[2] J. Isserman,[1] P. G. Nathanson,[2] S. Sonnad,[1] C. A. Sims[1]; [1]Hospital of The University of Pennsylvania, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA

53.12. Qualitative Assessment of Dimensions of Trauma Care. S. W. Lu,[1] D. Helitzer,[2] A. Sussman[2]; [1]University of New Mexico HSC, Albuquerque, NM; [2]University of New Mexico HSC, Albuquerque, NM

53.13. Resource Utilization Following Bowel Resection Versus Reduction of Intussusception. N. Sharp, P. Thomas, S. D. St. Peter; Children's Mercy Hospital- University of Missouri Kansas City, Kans, MO

53.14. Monocyte Levels Differ Between Diabetic and Non-Diabetic Patients With Peripheral Arterial Disease After Lower Extremity Revascularization. M. Kollisch-Singule,[1,2] E. Jaruga-Killeen,[3] B. Sadowitz,[1,2] M. J. Costanza,[1,2] K. Amankwah[1,2]; [1]Upstate Medical University, Syracuse, NY; [2]Syracuse VA Medical Center, Syracuse, NY; [3]Orlando Regional Medical Center, Orlando, FL

53.15. Elevated Heparin Induced Antibodies are More Common in Diabetic Patients With Vascular Disease. M. S. Patel,[1] T. Street,[1] M. Haydar,[3] J. Bismuth,[1] E. J. Arbid,[2,3] M. G. Davies,[1] J. J. Naoum[1,2,3]; [1]The Methodist Hospital and DeBakey Heart and Vascular Center, Houston, TX; [2]Lebanese American University, Beirut; [3]University Medical Center Rizk Hospital, Beirut

53.16. The Impact of Intensivists' Base Specialty Training on Care Process and Outcomes of Critically Ill Trauma Patients. K. Matsushima, E. Goldwasser, E. Schaefer, J. Then, E. Bradburn, S. Armen, D. Galvan, M. Indeck; Penn State Milton S. Hershey Medical Center, Hershey, PA

53.17. Counting the Lives Lost: How Many Black Trauma Deaths are Attributable to Disparities? V. K. Scott,[1] Z. G. Hashmi,[1] E. B. Schneider,[1] X. Hui,[1] N. Y. Enwerem,[1] D. T. Efron,[1] E. E. Cornwell,[2] A. H. Haider[1]; [1]Center For Surgery Trials and Outcomes Research (CSTOR), The Johns Hopkins School of Medicine, Baltimore, MD; [2]Department of Surgery, Howard University College of Medicine, Washington, DC

Exception from informed consent for emergency research: Consulting the trauma community

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BACKGROUND: Research investigating the resuscitation and management of unstable trauma patients is necessary to improve care and save lives. Because informed consent for research is impossible in emergencies, the Federal Drug Administration has established an Exception from Informed Consent (EFIC) Policy that mandates "community consultation" as a means of protecting patient autonomy. We hypothesized that the trauma community represents a heterogeneous population whose attitudes regarding EFIC and willingness to participate in emergency research are influenced by status as a patient, family, or geographic community member.

METHODS: In the context of an upcoming trial, trauma patients as well as family and community members were asked to rank statements regarding EFIC and willingness to participate in emergency research using a 5-point Likert-type scale. Higher total scores reflected a more positive attitude regarding EFIC (range, 4–20; neutral = 12) and willingness (range, 21–105, neutral = 63). The influence of demographics, education, and interpersonal violence were evaluated by Kruskal-Wallis and Mann-Whitney U-tests ($p < 0.05$).

RESULTS: Overall, the 309 participants (trauma patients, n = 172; family, n = 73; community, n = 64) were positive about EFIC (median, 16; interquartile range, [IQR], 14–18) and demonstrated high willingness scores (median, 82; IQR, 76–88.5). EFIC and willingness were not influenced by age, sex, race, or education. Victims of interpersonal violence and their family members had lower EFIC scores than those with other mechanisms (median [IQR], 16 [14–18] vs. 16 [13–16]; $p = 0.04$) but similar willingness. Although EFIC scores were similar between groups, trauma patients had significantly lower willingness than family (median [IQR], 74 [68–77] vs. 77 [70–85]; $p = 0.03$) or community members (median [IQR], 76 [70–84]; $p = 0.01$).

CONCLUSION: Trauma patients, families, and the geographic community expressed a high degree of support for EFIC and willingness to participate in emergency research, although support was influenced by injury mechanism and group status. Consultation efforts for emergency research should extend beyond the geographic community to include trauma victims and their families. (*J Trauma Acute Care Surg*. 2013;74: 157–166. Copyright © 2013 by Lippincott Williams & Wilkins)

LEVEL OF EVIDENCE: Epidemiologic, level III.

KEY WORDS: Emergency research; EFIC; consent; community.

Trauma surgeons need valid evidence to assure patients, families, and the society that patients with life-threatening injuries receive safe and effective interventions. Given the physiologic complexity of trauma, the best way to demonstrate therapeutic benefit and safety is through human subject research. Ideally, such clinical research should only be conducted when the patient has had the opportunity to carefully evaluate the risks and benefits and can provide informed consent before enrollment.[1] In trauma, however, many research questions concern the initial resuscitation and management of unstable patients who, by virtue of their injuries, are incapable of providing informed consent. Moreover, identifying potential subjects a priori or their legally authorized representatives (LARs) in a timely fashion may not be practical in most circumstances.[2,3]

Conducting emergency research, therefore, poses a substantial ethical challenge: how to balance respect for human autonomy with the potential for medical progress. Recognizing this potential conflict, the Federal Drug Administration (FDA) established an Exception from Informed Consent (EFIC) policy in 1996.[4] Under the most current guidelines, emergency research using EFIC may only be conducted in unexpected, life-threatening circumstances where (1) the patient is incapacitated, (2) available treatments are unproven or unsatisfactory, (3) the proposed intervention may benefit the patient directly, and (4) the therapy must be initiated before consent from the LAR is feasible. To further safeguard patient autonomy, the FDA requires several other protective measures including the mandate for "community consultation" (Table 1).[5]

The FDA requirement of community consultation, however, remains poorly defined and variably interpreted by both researchers and institutional review boards (IRBs).[6–15] Because each potential emergency research protocol draws on a uniquely different "community," it is essential that researchers consult with the "community" from which the subjects will be drawn to truly

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TABLE 1. Summary of 21CRF50.24—EFIC Requirements for Emergency Research

Subjects may receive the experimental therapy without consent only if all of the following conditions are met:

1. Available treatments are unproven or unsatisfactory.
2. The research cannot otherwise be performed to determine whether the therapy is safe and effective.
3. It is not feasible to obtain informed consent from the subject or the subject's legal representative, and there is no reasonable way to identify potential subjects prospectively.
4. Participation in the research holds out the prospect of direct benefit to the subjects.
5. The risks and benefits of the experimental procedure/treatment are reasonable compared with those associated with the subject's medical condition and standard therapy.
6. The proposal has been reviewed and approved by the IRB.

Additional protections include the following:

1. Consultation with the community and public disclosure of the study design, including risks and benefits, before initiation of the study.
2. The establishment of an independent data and safety monitoring board during the study.
3. Public disclosure of study results upon completion of the study.
4. FDA review and approval of the protocol design and study information.
5. An attempt to contact the subjects LAR within the therapeutic window before enrollment if at all feasible. The subject's LAR must be informed of the subject's inclusion as soon as feasible and asked for consent. If the LAR is not reasonably available, a family member must be approached as soon as possible and asked whether he or she objects to the subject's participation in the clinical investigation.

engage in the consultation process. While defining the community of interest may be the most obvious first step, it is perhaps one of the most challenging requirements of emergency research.[16]

Defining the trauma community may be particularly challenging. Geographically, patients may come from local neighborhoods, or they be transported a great distance. The community members may themselves be diverse group of patients, family, friends and neighbors—each with a different experience of the traumatic event as well as different races, religions, socioeconomic backgrounds, and exposure to violence. The unique experience of being a patient or family member may also provide an important perspective on research that involves the trauma population. We hypothesized that attitudes regarding EFIC and willingness to participate in emergency research would be influenced by demographic variables including status as a patient, family, or geographic neighbor in the trauma community.

PATIENTS AND METHODS

Our study was conducted in the context of community consultation for an upcoming trial investigating the use of vasopressin during the resuscitation of hemorrhagic shock (AVERT Shock Trial).[17,18] After obtaining IRB approval, trauma patients and family members at an urban Level I trauma center were asked to participate in a semistructured interview before discharge. Using a convenience sample, interviews were conducted during 6 months, including weekends. Patients (and family members) older than of 18 years identified as "ready for discharge" were invited to participate. Patients with moderate-to-severe traumatic brain injuries and those with psychiatric disorders requiring inpatient evaluation were excluded, although their families were invited to participate. During the interview, details of the AVERT Shock Trial and its use of EFIC were explained. In addition to collecting demographic data, participants were asked about their experiences with clinical research, the importance of emergency research, opinions about consent, reason for hospital admission, and exposure to life-threatening trauma requiring blood transfusion. Using a modified version of the Clinical Research Involvement Scales,[19] participants were asked rank statements regarding EFIC in the context of the AVERT Shock Trial and their general willingness to participate in emergency research using a 5-point Likert-type scale (1 = strongly disagree to 5 = strongly agree). Four questions investigated attitudes regarding EFIC and 21 assessed willingness to participate in emergency research. Higher total scores reflected a more positive attitude.

In addition, community-based organizations in the neighborhood surrounding our Level I center were contacted and invited to host a focus group. Focus groups were moderated by a professional facilitator and observed by IRB members. A research team member was also present to address questions. In addition to describing the details of AVERT Shock, discussions were directed at their knowledge and perception of emergency research, their opinion of the EFIC policy, and suggestions for conducting the proposed research. Following the discussion, participants completed the survey tool used by patients and family members.

Data were entered into a Microsoft Excel database (2007, Microsoft Corp., Redmond, WA) and imported into SPSS (version 19.0, SPSS Inc., Chicago, IL) for analysis. Data were analyzed using χ^2 and Kruskal-Wallis tests as appropriate with a two-tailed significance set at $p < 0.05$. For χ^2 tests found to be significant in analyses that contained more than two groups, pairwise comparisons were performed using z test of proportions with Bonferroni's correction. Reliability analyses were performed to examine the internal consistency of the EFIC and willingness scores.

RESULTS

Of the 336 subjects invited, 309 completed the survey for a 92% response rate. Participants included 172 trauma patients, 73 family members, and 64 community members. Community members attended one of six focus groups sponsored by a Baptist church, a mosque, three community organizations, and a recreational center. The three groups (patients, family, community) did not differ in age, religion, or educational level. Significant differences in sex, race, and employment status were present (Table 2). Patients were significantly more likely to be male (65%), white (20%), and unemployed/disabled (56%).

There were no significant differences in previous experience with medical research. Roughly 20% had previously participated in a clinical study, and only 4% to 6% had been asked to consent on behalf of another person (Table 3). When asked who would be the best person to consent on their behalf for an emergency research trial, the majority (93–96%) listed a family member. If that person were unavailable, community members were significantly more likely to indicate a religious leader (45%), another family member (28%), or a community leader (16%). Patients and family members were divided in

TABLE 2. Demographics of the Study Population

Variable	All Participants (N = 309), n (%)	Patients (n = 172), n (%)	Family Members (n = 73), n (%)	Community (n = 64), n (%)	*p*
Male sex*†	162 (52)	111 (65)	23 (32)	28 (44)	<0.001
Age, y					
18–24	64 (21)	38 (22)	15 (20)	11 (17)	0.337
25–34	57 (18)	35 (20)	16 (22)	6 (9)	
35–49	83 (27)	46 (27)	18 (25)	19 (30)	
50–64	76 (25)	35 (20)	19 (26)	22 (35)	
≥65	29 (9)	18 (10)	5 (7)	6 (9)	
Race					
African American*‡	191 (62)	96 (56)	39 (53)	55 (87)	<0.001
White*‡	102 (33)	69 (20)	28 (38)	5 (8)	
Other	16 (5)	7 (4)	6 (8)	3 (5)	
Religion					
None	55 (18)	27 (16)	16 (22)	12 (19)	0.116
Judeo-Christian	203 (66)	118 (69)	46 (63)	39 (61)	
Islam	40 (13)	24 (14)	7 (10)	9 (14)	
Other	11 (3)	3 (1)	4 (5)	4 (6)	
Declined	2 (1)	0 (0)	0 (0)	0 (0)	
Education					
Some high school	37 (12)	24 (14)	7 (10)	6 (9)	0.657
High school graduate	128 (41)	75 (44)	28 (38)	25 (39)	
Some college or technical school	82 (27)	44 (26)	21 (29)	17 (27)	
College degree	39 (13)	19 (11)	10 (14)	10 (16)	
Professional degree	23 (7)	10 (6)	7 (10)	6 (9)	
Employment status					
In school	18 (6)	11 (6)	4 (5)	3 (5)	<0.001
Part time*†	41 (13)	11 (6)	13 (18)	17 (27)	
Full time*†‡	111 (36)	54 (31)	36 (49)	21 (33)	
Unemployed/disabled*†	137 (44)	96 (56)	20 (27)	21 (33)	
Declined	2 (1)	0	0	2 (3)	

χ^2 test was used to evaluate significance of variables between groups. Significance further analyzed with pairwise comparisons reported at $p < 0.05$ following z test of proportions with Bonferroni's correction.
*Patient versus community.
†Patient versus family.
‡Family versus community.

their responses. Roughly one third listed "doctor" as their second choice, while another third stated that it would not be proper for anyone other than a family member to consent on their behalf.

Subjects did not differ with regard to their experience with trauma resulting in significant blood loss (Table 3). Although most participants either "agreed" or "strongly agreed" that trauma was a significant concern in their community (85–92%), community members were statistically more likely to endorse this concern. The overwhelming majority of participants

TABLE 3. Experience and Attitudes Regarding Emergency Research and Trauma as a Function of Trauma Community Subgroups

Variable	Patient (n = 172), n (%)	Family (n = 73), n (%)	Community (n = 64), n (%)	*p*
Previous participation in clinical research	36 (21)	14 (19)	16 (24)	0.694
Given permission for others to participate in clinical research	10 (6)	3 (4)	4 (6)	0.830
If you were a victim of trauma and could not consent, who would you want to consent for you to participate in emergency research?				
Family member	160 (93)	70 (96)	60 (94)	0.039
Community leader/member	10 (6)	2 (3)	0 (0)	
Religious leader	2 (1)	1 (1)	4 (6)	
If your family were not available, who would be the best person to consent for you to participate in emergency research?				
Family member*†	13 (8)	2 (3)	18 (28)	<0.001
Community leader/member*	6 (3)	3 (4)	10 (16)	
Religious leader*†	20 (12)	5 (7)	29 (45)	
Friend	19 (11)	12 (16)	5 (8)	
Doctor*†	57 (33)	30 (41)	1 (2)	
Responsible person	5 (3)	1 (1)	1 (2)	
Nobody, it would not be right for someone other than my family to consent for me to participate in this type of research even if it could potentially be helpful to me or save my life.*†	52 (30)	20 (27)	0 (0)	
Have you or someone you know experienced trauma resulting in significant blood loss and the need for blood transfusions?				
Yes	70 (41)	33 (45	27 (43)	0.369
No	102 (59)	40 (55)	36 (57)	
Trauma, such as injuries related to violence or accidents, is a serious concern in my community.				
Strongly disagree	2 (1)	0 (0)	3 (5)	0.026
Disagree*†	11 (6)	2 (3)	0 (0)	
Neutral	13 (8)	4 (5)	3 (5)	
Agree	88 (51)	32 (44)	25 (39)	
Strongly agree*‡	58 (34)	35 (48)	33 (52)	
More research that could benefit trauma patients should be performed.				
Strongly disagree	0 (0)	0 (0)	0 (0)	0.003
Disagree	0 (0)	0 (0)	0 (0)	
Neutral	2 (1)	3 (4)	3 (5)	
Agree*‡	117 (68)	38 (52)	27 (42)	
Strongly agree*†‡	53 (31)	32 (44)	34 (53)	
Do you think the AVERT Shock Trial is an important study for us to perform?				
Yes	166 (96)	72 (99)	61 (95)	0.279
No	3 (2)	1 (1)	0 (0)	
Undecided	3 (2)	0 (0)	3 (5)	

χ^2 test was used to evaluate significance of variables between groups. Significance further analyzed with pairwise comparisons reported at $p < 0.05$ following z test of proportions with Bonferroni correction.
*Patient versus community.
†Family versus community.
‡Patient versus family.

supported the need for more research in trauma (95–99%) and indicated that the AVERT Shock Trial was an important study to perform (95–99%).

When asked to rank their support of six statements related to the EFIC policy as it pertained to the AVERT Shock Trial, participants were generally supportive (Table 4). If a LAR could not be contacted, 67% of participants agreed that it would be acceptable to enroll a family member in this study without consent; 77% indicated that it would be acceptable to enroll them personally, and 84% believed that a member of the community could be enrolled. Eighty-five percent agreed that it would be acceptable to conduct the AVERT Shock Trial in their community. Only 63% agreed that it was important to involve the community in making decisions on behalf of potential patients.

Although most subjects supported the use of EFIC, there were statistically significant differences in the degree of support. Community members tended to be more supportive of EFIC as it pertained to family members and themselves but less supportive of enrolling others without consent. They were also more emphatic regarding the importance of involving the community (Table 5).

Based on the average interitem correlation, Cronbach's α was 0.8 for EFIC score and 0.9 for willingness, indicating an excellent degree of internal consistency. Of note, question 7 and question 9 were excluded from the willingness score because of lack of internal consistency.

Three factors were found to influence the degree of positive support for EFIC: race, admission following interpersonal

TABLE 4. EFIC Questions Regarding the AVERT Shock Trial

	Disagree, %	Neutral, %	Agree, %
I think it would be acceptable to enroll my family members in this study without consent if they had a traumatic injury resulting in blood loss and I could not be contacted.	20	13	67
I think it would be acceptable for me to be enrolled in this study without my written consent if I had a traumatic injury resulting in blood loss and my legal representative (spouse, children, or guardian) could not be contacted.	13	10	77
I think it is acceptable for a member of my community to be enrolled in this study without consent if they had a traumatic injury resulting in blood loss and their legal representative (spouse, children, or guardian) could not be contacted.	4	12	84
I think it is acceptable for this study to be performed in my community.	4	11	85
I think the opt-out bracelet and online registry is an acceptable way to identify which potential patients do not want to be enrolled in the study.	15	11	74
To perform this type of research, it is important to involve the community in making decisions on behalf of the patients who might be enrolled.	25	13	62

TABLE 5. Willingness Score

WILLINGNESS Questions	Patient	Family	Community	*p*
1. I think it would be acceptable to enroll my family members into this study without consent if they had a traumatic injury resulting in blood loss and I could not be contacted.	3.5 (1.1)	3.8 (1.1)	3.8 (1.2)	0.026*
2. I think it is acceptable for me to be enrolled into this study without my written consent if I had a traumatic injury resulting in blood loss and my legal representative (spouse, children, or guardian) could not be contacted.	3.7 (1.0)	4.1 (0.9)	4.2 (1.1)	<0.001*†
3. I think it is acceptable for a member of my community to be enrolled into this study without consent if they had a traumatic injury resulting in blood loss and their legal representative could not be contacted.	4.1 (0.6)	4.2 (0.8)	3.8 (1.1)	0.037*
4. I think it is acceptable for this study to be performed in my community.	4.1 (0.6)	4.2 (0.7)	4.3 (0.8)	0.094
5. I think the opt-out bracelet and online registry is an acceptable way to identify which potential patients do not want to be enrolled in the study.	3.7 (0.9)	3.6 (1.1)	4.1 (0.9)	0.005*‡
Willingness questions				
1. I believe that medical research (in trauma care) is important.	4.4 (0.5)	4.4 (0.6)	4.6 (0.6)	0.005*
2. My participation in a medical study would be a very good thing.	3.9 (0.7)	4.1 (0.8)	4.2 (0.8)	0.025*
3. I or my family would benefit from medical research on trauma.	4.1 (0.7)	4.3 (0.6)	4.4 (0.7)	0.01*
4. My community would benefit from medical research on trauma.	4.0 (0.7)	4.3 (0.6)	4.4 (0.8)	<0.001*†
5. My participation in a medical study on trauma would be more trouble than it is worth.§	2.3 (0.9)	2.1 (1.0)	2.3 (1.1)	0.304
6. Participation in medical study on trauma seems risky.§	2.8 (1.0)	2.8 (1.0)	2.7 (1.2)	0.746
7. I think my doctor would approve of my involvement in emergency research.	3.6 (0.9)	3.9 (0.9)	4.0 (0.8)	0.006*
8. My immediate family is supportive of my involvement in emergency research.	3.6 (1.0)	3.9 (1.0)	3.7 (0.9)	.082

TABLE 5. *(Continued)*

WILLINGNESS Questions	Patient	Family	Community	*p*
9. If my pastor, imam, or rabbi supported emergency research, I would be inclined to get involved.	3.2 (1.1)	3.3 (1.1)	3.4 (0.9)	0.579
10. My community feels that my participation in emergency research is good.	3.6 (0.8)	3.6 (0.8)	3.7 (0.8)	0.544
11. I like to do good for others.	4.3 (0.5)	4.3 (0.6)	4.7 (0.5)	<0.001*‡
12. Trauma, such as injuries related to violence or accidents, is a serious concern in my community.	4.1 (0.9)	4.4 (0.7)	4.3 (0.9)	0.013*
13. I would benefit from the medical care associated with an emergency study.	4.0 (0.5)	4.2 (0.6)	4.1 (0.7)	0.343
14. More research that could benefit trauma patients should be performed.	4.3 (0.5)	4.4 (0.6)	4.5 (0.6)	0.019*
15. Involving patients in a medical research study without asking their permission first is acceptable in emergency circumstances.	3.4 (1.1)	3.7 (1.1)	3.9 (1.0)	0.009*
16. It is okay to get permission from the family member if a patient cannot give permission themselves to participate in a research study.	4.1 (0.7)	4.2 (0.7)	4.3 (0.8)	0.008*
17. It is okay to enroll patients in emergency research studies if there is no family member present and there is not time to contact anyone.	3.6 (1.1)	3.8 (1.0)	4.1 (0.8)	0.001*
18. It is okay for medical researchers to include me in a study that might help me if I am unconscious or too sick to give permission myself.	3.6 (1.0)	3.9 (0.9)	4.1 (0.7)	0.015*
19. It is okay for my family members to give permission for me to be in a study that might help me if I am unconscious or too sick to give permission myself.	4.1 (0.7)	4.2 (0.8)	4.1 (0.7)	0.257
20. It is okay for medical researchers to include me in a study that might NOT help me but might help future patients if I am unconscious or too sick to give permission myself.	3.5 (1.0)	3.6 (1.0)	3.6 (1.1)	0.292
21. It is okay for my family members to give permission for me to be in a study that might not help me but might help future patients, if I am unconscious or too sick to give permission myself.	3.6 (1.0)	3.7 (1.0)	3.8 (0.9)	0.599
22. It is okay for emergency research that does not ask for patient's consent to be done in my community if the study might help the patient.	3.7 (0.9)	4.0 (0.8)	3.6 (0.9)	0.014‡
23. It is okay for emergency research that does not ask for patient's consent to be performed in my community if the study might help future patients.	3.7 (0.8)	3.9 (0.7)	3.5 (1.0)	0.033‡

Participants ranked each statement using a 5-point Likert-type scale (strongly disagree = 1; disagree = 2, neutral = 3, agree =4, strongly agree = 5)

Groups compared using the Kruskal-Wallis test. Significant differences underwent pairwise comparisons with adjusted significance of $p < 0.05$ reported.

*Patient versus community.

†Patient versus family.

‡Family versus community.

§Questions that were reverse coded when included in the willingness score.

violence, and the participant's opinion regarding the importance of the AVERT Shock Trial (Table 6). Participants also endorsed factors known to positively influence willingness to participate clinical research with a median willingness score of 82 (interquartile range, [IQR], 76–88.5). Family members had statistically higher willingness scores, as did those who believed in the importance of conducting the AVERT Shock Trial (Table 6).

DISCUSSION

Although traumatic injury remains the leading cause of death for people younger than 40 years, very few of the "standard" interventions used to treat critically injured patients have been rigorously investigated and only a handful of clinical trials have used EFIC.[20–24] Our study investigated trauma community attitudes about emergency research and the use of EFIC in the context of the upcoming AVERT Shock Trial.

Overwhelmingly, the trauma community we consulted considered the AVERT Shock Trial an important study that could be acceptably conducted in their community. Importantly, the key concept of enrollment without consent was endorsed by most participants. Surprisingly, subjects in our study were more supportive of enrolling a community member without consent than they were in applying EFIC to either themselves or a family member. In contrast, previous investigators have noted reluctance on the part of participants to assume responsibility for supporting EFIC on behalf of others.[25,26] In a survey study of 1,901 community members, Biros et al.[27] found that only 35% supported using EFIC in emergency research. Support increased to 70%, however, when respondents were asked to evaluate emergency research in the context of their own care. Participants in our study may be more supportive of implementing EFIC as applied to the general community because while they universally perceive trauma research

TABLE 6. Univariate Analysis of EFIC and Willingness Scores

Variable	EFIC Score, Median (IQR)	*p*	Willingness Score, Median (IQR)	*p*
Group	16 (14–18)		82 (76–88.5)	
Patient (n = 172)	16 (14–16)	0.50	81 (75–84)	0.005
Family (n = 73)	16 (14–19)		84 (76–92.5)	
Community (n = 64)	16 (13–18.75)		83.5 (77–91)	
Sex				
Male (n = 162)	16 (14–17)	0.58	81 (75–87)	0.507
Female (n = 147)	16 (14–18)		82 (76–90)	
Age, y				
18–24 (n = 64)	16 (14–18)	0.466	82 (76–86.75)	0.205
25–34 (n = 57)	16 (14–18)		81 (73.5–86)	
35–49 (n = 83)	16 (14–18)		82 (76–91)	
50–64 (n = 76)	16 (14–18)		83.5 (76.25–91.75)	
≥65 (n = 29)	16 (13.5–16)		80 (75–84)	
Race				
African American (189)	16 (14–17.5)	0.044	81 (75–86)	0.118
White (n = 102)	16 (15–18)		83 (76–92)	
Other (n = 16)	14 (13–16)		84.5 (75.5–88.5)	
Religion				
None (n = 55)	16 (14–18)	0.339	80 (74.5–88)	0.413
Judeo-Christian (n = 203)	16 (14–18)		82 (75.25–86.75)	
Islam (n = 40)	16 (14–17.75)		81 (75.25–86.75)	
Other (n = 11)	16 (14.25–18)		84.5 (76.75–89.25)	
Education				
Some high school (n = 37)	16 (14–18)	0.50	80 (76–87)	0.314
High school graduate (n = 128)	16 (14–17)		82 (75–86)	
Some college or technical school (n = 82)	16 (14–18)		84 (76–90)	
College degree (n = 39)	16 (13–19)		82 (74–93)	
Professional degree (n = 23)	16 (15–20)		84 (79–91)	
Employment status				
Attending school (n = 18)	16 (13–16.5)	0.649	81 (74.5–89.25)	0.683
Part time (n = 41)	16 (13–19)		84 (76–91.5)	
Full time (n = 111)	16 (14–18)		82 (75–91)	
Unemployed/disabled (n = 137)	16 (14–17)		82 (76–85)	
Previous participation in medical research				
Yes (n = 66)	16 (14–17)	0.367	84 (75–90.25)	0.314
No (n = 243)	16 (14–17)		82 (76–87)	
Given permission for other to participate in medical research				
Yes (n = 17)	16 (14–18)	0.636	84 (70.5–90.5)	0.843
No (n = 292)	16 (14–18)		82 (76–88)	
Experience with significant trauma requiring a transfusion				
Yes (n = 130)	16 (14–18)	0.339	82.5 (75.75–90)	0.543
No (n = 178)	16 (14–18)		82 (75.75–86)	
Admitted following violent event				
Yes (n = 76)	16 (13–16)	0.035	81 (73–84)	0.078
No (n = 167)	16 (14–18)		82 (76–89)	
The AVERT Shock Trial is an important study to perform				
Yes (n = 299)	16 (14–18)	0.007	82 (76–89)	0.016
No (n = 4)	13.5 (9–16)		82 (74.5–91)	
Neutral (n = 6)	12 (10–14)		69 (60–77.25)	

Groups compared using the Kruskal-Wallis test. Significant differences underwent pairwise comparisons with adjusted significance of $p < 0.05$ reported.

as important, they felt less strongly that they would personally benefit from care associated with emergency research (Table 5).

Our community consultation process may have also had a positive influence on the community's support of emergency research. Public disclosure and consultation activities that actively educate and engage participants have been shown to positively influence attitudes about EFIC research.[7,28,29] Moreover, educating the community about the need for the proposed research and the lack of effective lifesaving therapies may also improve the community's perception of EFIC.[25] Our use of focus groups and semistructured interviews served to facilitate a dialogue in which the trauma community learned more about hemorrhagic shock, the lack of effective therapies, and the specifics of the AVERT Shock Trial. In addition, the research team was able to solicit views and recommendations from the trauma community. Although the community does not need to "approve" the study, the process of consultation is valuable in that the community is able to serve more as "partners rather than simply as subjects."[9]

As hypothesized, the demographics, experiences, and attitudes of our trauma community varied depending on group assignment. When compared with trauma patients, community members were significantly more likely to be female, African American, and employed. Although age, sex, education, and income previously have been shown to influence attitudes about emergency research, these factors did not impact either the EFIC or willingness scores in our study.[25,27,30,31] While race was found to be statistically significant, African American and white subjects both reported high EFIC and willingness scores that were not statistically different.

Group assignment was also associated with differences in willingness but not in the composite EFIC score. When compared with trauma patients, community members were more likely to believe that trauma was a serious concern in their community, felt more strongly that trauma research should be performed, and were more likely to believe that their community would directly benefit from trauma research. Importantly, community members were also more likely to believe in the importance of consulting the community before initiating the AVERT Shock Trial. When individual question regarding EFIC and willingness were analyzed, community members were significantly more positive than patients in their support

of EFIC research. They were, however, significantly less likely to endorse this type of research on behalf of their community. This reluctance to provide "consent" on behalf of strangers may stem from an altruistic desire as a community leader to protect the public, or it may represent a more democratic notion in which the community derives its authority from the will of its individual members.

Universally, participants supported contacting a family member to obtain consent for research in emergency situations. The concept of consent by proxy for research was initially introduced in the Declaration of Helsinki and later expanded to include an emphasis on respect for persons in the Belmont Report (1979).[1,32] Because a family member is the most likely person to know the values and attitudes of the injured patient, it seems ethically appropriate to seek out family for surrogate decision making in emergency situations. Under the EFIC policy, consent for participation, or continued enrollment, must be discussed with the patient's LAR as early as feasibly possible. In most cases, a parent or spouse is considered the LAR and should be approached first. However, if the LAR is unavailable, federal policy requires that any available family member should be asked whether they object to the patient's participation. Our research suggests that the mandate to seek out family as surrogate decision makers in emergency research represents an important protection that is widely supported by the trauma community.

If family were unavailable, however, the community and patients/family expressed strikingly different opinions regarding an alternative proxy. Community members were significantly more likely to designate a religious leader or a community member or write in a specific family member. Trauma patients and their families, in contrast, were equally divided between designating a doctor and declaring that consent could only be obtained from a family member. It is important to note that the category "doctor" was not included as an option in the survey. Under the option of "other," respondents specifically wrote in terms such as "doctor," "trauma doctor," and "researcher." This trust in the medical profession may be directly related to the recent trauma and resuscitation experience.[33] Because trauma patients and family members in our study had just survived an unexpected emergency, they may be more keenly aware of the difficulties associated with emergency care and more likely to trust that their physicians have their best interest in mind.

Trust, however, may not be universal. Patients, and their families, who were admitted following interpersonal violence were statistically less supportive of EFIC than those admitted following nonviolent mechanisms. Experiencing violence shakes one's core belief that the world is safe and victims frequently suffer a loss of trust in people that can continue for years.[34] In a recent prospective study of 1,386 trauma patients seen in follow-up, more than 40% of those treated after a violent assault experienced symptoms of posttraumatic stress.[35] While it is unclear how many patients or family members in our study were experiencing posttraumatic stress, the recent exposure to violence may have negatively impacted their sense of trust as well as their support of EFIC. Further research investigating the impact of posttraumatic stress on attitudes about emergency research may provide useful insight regarding how to conduct meaningful community consultation with this vulnerable population.

There are a number of limitations to this study. First, this study was performed in context of the upcoming AVERT Shock trial, and attitudes were clearly influenced by the participant's opinion regarding the importance of this particular study. It is unclear if a different study with perhaps less overall support would report similarly high EFIC and willingness scores. Second, while we did find statistical differences between groups in the trauma community, these differences may not necessarily reflect clinical important distinctions. Specifically, because there was such a high degree of support for the AVERT Shock Trial and willingness to participate, statistically significant differences between the groups reflected variations in the degree of positive support rather than any one group expressing disapproval. Again, if there was less support for the AVERT Shock Trial overall, the differences observed between the groups may actually become important distinctions. Third, the opinions of the participants may not reflect the views of the general public. Participants in the focus groups may represent a self-selected group of people with altruistic motives. Similarly, trauma patients and their family members may be more biased toward trusting the medical profession that just saved their lives when compared with the general public. Fourth, although we used a convenience inpatient sample at various times throughout the week, not all admitted trauma patients or their family members were interviewed. The families of patients who died as well as those who declined or who were never contacted may have very different opinions about emergency research and EFIC. In addition, given the face-to-face semistructured nature of the interview, participants may have responded in a way that they thought the researcher would approve. Finally, while both the focus group format and the individual assessment allowed for in-depth questions and dialogue, this method of community consultation was time intensive, with each focus group lasting 1.5 hours to 2 hours and individual patient/family interviews taking 20 minutes to 40 minutes. The potential issue of bias may be mitigated by randomly consulting a larger sample size in a more anonymous fashion. Recently, random digit dialing has been successfully used to investigate community attitudes regarding the use of hypertonic saline in trauma.[36] Although this method clearly allows access to a larger population, it is expensive, has a fairly low response rate (33–45%), and may not capture the trauma patient's unique perspective. Further research is needed to determine the optimal balance between individualized consultation and the widespread dissemination of information.

CONCLUSION

Trauma patients, their families, and the surrounding geographic community expressed a high degree of support for the AVERT Shock Trial, EFIC, and emergency research in general. Although injury mechanism and group status were found to have a significant influence on EFIC and willingness scores, their impact merely changed the degree of positive support. By allowing participants the opportunity to ask questions and provide direct feedback to the research team via focus groups

and semistructured interviews, the trauma community became "partners" in the research process. This method of community consultation may have contributed to the overwhelming support of the AVERT Shock Trial. Future consultation efforts for emergency research in trauma should consider using community participatory methods that extend beyond the geographic community to include patients and their families.

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AUTHORSHIP

C.A.S., J.A.I., S.G., and P.R. designed this study. C.A.S., J.A.I., L.M.S., and N.T. contributed to data collection. C.A.S., D.H., and S.S. performed data analysis. C.A.S., J.A.I., D.H., S.G., S.S., J.P., and P.R. prepared the article.

DISCLOSURE

The authors declare no conflicts of interest.

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DISCUSSION

Dr. Joseph P. Minei (Dallas, Texas): I applaud the efforts of Dr. Sims and her colleagues from Penn in their analysis of their community consultation efforts as part of the requirements for exception from informed consent as they prepare for the AVERT shock study.

This analysis is quite timely as we have heard multiple studies at this meeting, including the full-day PROMMTT symposium, studies referring to the Resuscitation Outcomes Consortium (ROC), and a substantial portion of the FITTS Lecture by Dr. Hoyt that discusses these issues.

As Dr. Hoyt noted, there is no right way to perform community consultation. In this study Dr. Sims directed community consultation to three groups: trauma patients at the time of hospital discharge, their families, and then the community at large.

Their results show that while there were statistical differences in some aspects of support for the concept of exception from informed consent and willingness to participate in the specific study between the groups, these result differences were not clinically meaningful in the context of the study. Most patients supported the concept and the study, and differences were really in the degree of acceptance. I have a few questions.

How do you think the trauma patients at the time of discharge or their family members reflect the larger trauma community? These patients did well enough to be discharged home.

How do you account for the biases introduced into the analysis? Perhaps, those who did poorly would have different points of view. For instance, a family member of a patient who did poorly may feel that only if he were in a research study, he might have done better or survived. Similarly, those that did well may feel invincible and feel that they do not need any study after sustaining trauma and surviving it.

You conclude that this trauma community should be included in future attempts at community consultation. How can this be justified when this group does not have significant differences from others, requires significant resources to complete, and has clear bias?

The ROC, which arguably has the greatest experience in this regard—and for full disclosure, I am a member of the ROC—has used random digit dialing in the majority of their community consultation.

This technique limits bias, is statistically valid, similar to presidential polling, and does not require human resources. The downside is that it must be outsourced and is quite expensive. It costs approximately $15,000 per random digit dialing for a geographic area.

This technique has been widely accepted by multiple and varied institutional review boards (IRBs) from across North America. Do you think your technique should be used instead of the ROC's technique or in addition to the ROC's technique? How should it be used?

Should there be a validated menu of techniques that IRBs should accept for community consultation? Should the trauma community accept a technique for community consultation and use that exclusively in future studies as a means of educating naive IRBs?

I appreciate the authors getting me the publication in a timely manner. I enjoyed your work, and I wish you good luck with your AVERT shock study in the future. Thank you.

Dr. Arthur Cooper (New York, New York): Important study and great discussion. Thank you both to the author and the discussant. You know the kernel of the debate here really resides in the definition of community. You know what community are we actually seeking to, you know, obtain consultation from, and, you know, when this exception was originally proposed and adopted as rule by the federal government almost 20 years ago, community was very clearly intended to mean the geographic community around which, you know, or within which the patient lives.

It strikes me that the trauma community, as you have pointed out, is somewhat different, but that if one were to apply the demographics of the trauma community that was injured to the geographic population in the particular area, in other words, target the high-risk for trauma groups within a geographic area, you might be able to get a little bit closer to the original intent that the fed had proposed. I just wondered if you would care to comment on that.

Dr. Carrie Sims (Philadelphia, Pennsylvania): Thank you, Drs. Minei and Cooper for your insightful comments. Trauma is an abstract concept and something that most people do not plan for, so as such, trauma patients and their families really bring a unique perspective to the trauma experience and one that has been missing in the consultative process.

Ideally, consultation should include representatives from the community from which the subjects have been drawn from or will be drawn from. So I think Dr. Cooper's point about who and what the community is, is really the kernel of the question.

Having just experienced the trauma, one could argue that the trauma population, the family, and the patients themselves may be the best representatives to discuss what the trauma community truly is.

If anything, our city suggests that patients may be less willing to participate in the emergency research compared with the outside trauma community or the geographic community.

Again, as I mentioned, that if our study has less support or if there were a more controversial topic, maybe, those differences would actually become not only statistically significant but clinically significant.

Whether our consultation process is generalizable, I think our consultation process should potentially be used in other, in conjunction with other means such as the random digit dialing.

I think the problem with the random digit dialing is that it really does have a very low response rate. In Dr. Bulger's article, 40% of the patients had or 40% of those contacted actually responded, so it is quite time intensive and expensive. For a small study such as the one we were going to do, it would be prohibitively expensive.

Our consultation process, however, was valuable in several ways. First, it really did allow the researcher to have a very in-depth conversation and detailed discussion with potential representatives of our community and that these participants really became partners in designing the research in the sense that they were able to provide suggestions and feedback and thoughts regarding the study design and really good recommendation on how to go about disclosing. So as researchers, we got a better understanding of our community's concerns as well as other things that we should be more sensitive to.

Whether there should be a standardized approach, I think Dr. Jill Barren has done a very nice job in putting together a compendium of a menu of consultative pathways.

I am not sure every study will be cookie cutter and every community will be cookie cutter. Certainly our population may be very different from the population seen at the University of Washington.

So this might be a nice adjunct for smaller cities that have potentially higher risk. Larger cities in which there is definitely greater ability to pay for a larger consultative process may benefit.

I do think coming together as a trauma community to decide what we believe is a good way to represent community and to get their feedback would be highly valuable to IRBs across the country.

Thank you very much again for your thoughtful comments. Mahalo.

Introduction: Exception from Informed Consent (EFIC) presents a challenge to researchers, in part due to concern about patient reluctance to participate. It is unknown what community characteristics affect respondents' attitudes toward EFIC or their willingness to participate in emergency research. We hypothesized that race and proximity to high crime neighborhoods would negatively influence the perception of EFIC and decrease willingness to participate.
Methods: As part of an EFIC community consultation process, trauma patients, their families and community members living within the city limits of Philadelphia were asked to rank statements regarding EFIC and willingness to participate in emergency research using a 5-point Likert-type scale. Higher total scores reflected a more positive attitude regarding EFIC (range, 6-30; neutral = 18) and willingness (range, 23-115, neutral = 69). Subject zip code information was utilized to calculate proximity to the top 5 most violent zip codes in Philadelphia. The association between violence proximity and scores, race, group, and mechanism of injury was evaluated using linear regression modeling, t-test, Kruskal-Wallis and omnibus tests where appropriate ($p<0.05$).
Results: A total of 179 subjects participated and included trauma patients (n=99), their families (n=33) and community members (n=47). Overall, the cohort reported high EFIC perception and willingness to participate scores (median 24, IQR 13-30 and median 89, IQR 52-115 respectively). Community members were more likely to live in a distribution near violent neighborhoods than either patients or their families ($p=0.023$), but median proximity to these neighborhoods was no different between patients, their families and community members. Proximity to high crime areas correlated with violent mechanism of injury ($p=0.021$), but was not associated with race, the perception of EFIC or the willingness to participate in emergency research.
Conclusion: Proximity to high crime zip codes does not appear to decrease willingness or worsen the perception of EFIC. While researchers have been concerned that consulting high crime and urban communities could be a roadblock to implementing EFIC in emergency research, our data suggest that this may not be the case. Given the importance of EFIC research in the care of injured patients, this data should embolden future research pursuits.

Does proximity to violence negatively influence attitudes toward exception from informed consent in emergency research?

Zoë Maher, MD, Elena Kosar Grill, Brian Patrick Smith, MD, *and* **Carrie A. Sims, MD,** *Philadelphia, Pennsylvania*

BACKGROUND:	Trauma research has been limited by perceived patient reluctance to participate in exception from informed consent (EFIC) studies. We hypothesized that race, socioeconomic status, and proximity to violence influence willingness to participate in and perception of EFIC research among at-risk populations.
METHODS:	Trauma patients, families, and community members ranked statements regarding attitude toward EFIC in the context of an upcoming trial and willingness to participate in emergency research using a 5-point Likert scale during a community consultation. Higher total scores reflected a more positive attitude regarding EFIC (range, 6–30; neutral, 18) and willingness (range, 23–115; neutral, 69). Subject zip code was used to calculate median income, as an estimate for socioeconomic status, and proximity to the five most violent city zip codes. Linear regression, Spearman's correlation, and Kruskal-Wallis tests ($p < 0.05$) were used to evaluate relationships between estimated socioeconomic status, race, mechanism of injury, proximity to violence, and attitudes toward EFIC.
RESULTS:	A total of 179 subjects participated including trauma patients (n = 99), families (n = 33), and community members (n = 47). Overall, participants were supportive of EFIC and reported high scores in willingness to participate (median, 24; interquartile range, 21–25; median 89, interquartile range, 82–95, respectively). Proximity to violence did correlate with race ($p = 0.03$) but was not associated with violent mechanism of injury, perception of EFIC, or willingness to participate in emergency research. Estimated socioeconomic status and race did not correlate with perception of or willingness to participate in EFIC.
CONCLUSION:	Based on our data, there is no correlation between either proximity to violence or estimated socioeconomic status and willingness to participate in EFIC research. Given this lack of correlation, researchers should partner with at-risk communities to conduct EFIC studies without concern for limited participation. (*J Trauma Acute Care Surg*. 2015;79: 364–371. Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.)
LEVEL OF EVIDENCE:	Epidemiologic/prognostic study, level III.
KEY WORDS:	Community consultation; exception from informed consent; proximity to violence; emergency research; consent.

Accidental and violence-related injuries are two of the most significant causes of preventable death and disability in the United States.[1] In fact, unintentional injury resulted in more than 29 million visits to the emergency department in 2011 and is the leading cause of death in those younger than 45 years.[1] To improve and optimize care of the injured, early interventions must be critically evaluated and prospectively studied. One of the major challenges associated with conducting trauma research, however, is that of obtaining informed consent from critically ill patients.[2] Recognizing the need to improve emergency research while continuing to protect patient autonomy, the Food and Drug Administration and the Department of Health and Human Services established guidelines for exception from informed consent (EFIC) for emergency research in 1996.[3] Under these strict guidelines, emergency research may be conducted in the absence of explicit consent provided the subjects have a life-threatening condition, the research holds the prospect of direct benefit, and consent is not feasible. In addition, the Food and Drug Administration guidelines require that a community consultation be conducted before initiation of the trial.[4]

Currently, there are no standards regarding the conduct of a "community consultation" process, and the approach has varied widely in the literature.[5–14] In many cases, the consultation has included a "community" survey documenting factors that influence both attitudes and willingness to participate in emergency research. While many community consultation studies have demonstrated a generally positive view toward EFIC and a proclivity toward consent, factors such as sex, race, socioeconomic status, and educational level may contribute to lower rates of willingness.[5,7,14–16] Willingness to participate may also be influenced by exposure to violence.[17] Given the burden of life-threatening trauma and the unique demographics associated with urban trauma centers, it is imperative that we understand this "community's" perception of EFIC research and develop guidelines to assist researchers and institutional

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review boards in the process of community consultation. We hypothesized that race, socioeconomic status, and closer proximity to violence would make urban trauma patients, their families, and community members less willing to participate in and more skeptical of EFIC research.

PATIENTS AND METHODS

As a component of an institutional review board–approved community consultation for the AVERT Shock Trial (a trial investigating the use of vasopressin during the resuscitation of hemorrhagic shock)[18] voluntary, in-person interviews were conducted. A convenience sample of trauma patients, their families, and community members were approached in one of three settings: in the hospital before discharge, clinic follow-up visit, or at a community focus group. Discharge-ready patients and their family members who were mentally and physically capable of completing a 20-minute interview were approached daily between the hours of 9:00 AM and 9:00 PM. Patients and family members who did not participate while hospitalized were invited to participate during their first outpatient appointment. Community members were invited to participate in a one of six 2-hour structured focus groups sponsored by a Baptist church, a mosque, three community organizations, and a recreational center located in the West Philadelphia neighborhood. After informed consent was obtained, participants were asked by trained research staff, including a research coordinator, research assistants, and research physicians, to respond to a 42-item modified Clinical Research Involvement Scale (CRIS). The CRIS is a validated and reliable instrument designed to measure community attitudes toward participation in biomedical research studies.[19] The items included demographic, dichotomous (yes/no), and 5-point Likert scale–ranked statements regarding attitudes toward EFIC and general willingness to participate in emergency research (see Supplemental Digital Content 1, http://links.lww.com/TA/A610). Ranked responses were reverse coded where appropriate such that higher scores reflected a more positive attitude regarding EFIC.

The AVERT attitude score was calculated from the sum of answers to six Likert scale attitude questions (range, 6–30; neutral, 18) and reflected attitude toward enrollment in the AVERT Shock Trial under EFIC. The willingness score was calculated from the sum of answers to 23 Likert scale willingness questions (range, 23–115, neutral, 69) and reflected general attitude toward emergency research. Cronbach's α measure of internal consistency was then calculated for the attitude and willingness scores and was found to be acceptable.

Subjects who lived in a zip code outside the City of Philadelphia and those with incomplete records were eliminated from the analysis. Subject zip codes were used to estimate median income using 2011 Census Bureau data as a marker for socioeconomic status.[20,21] Subject zip codes were also used to calculate proximity to the five most violent zip codes in Philadelphia. These "violent hotspots" were identified using

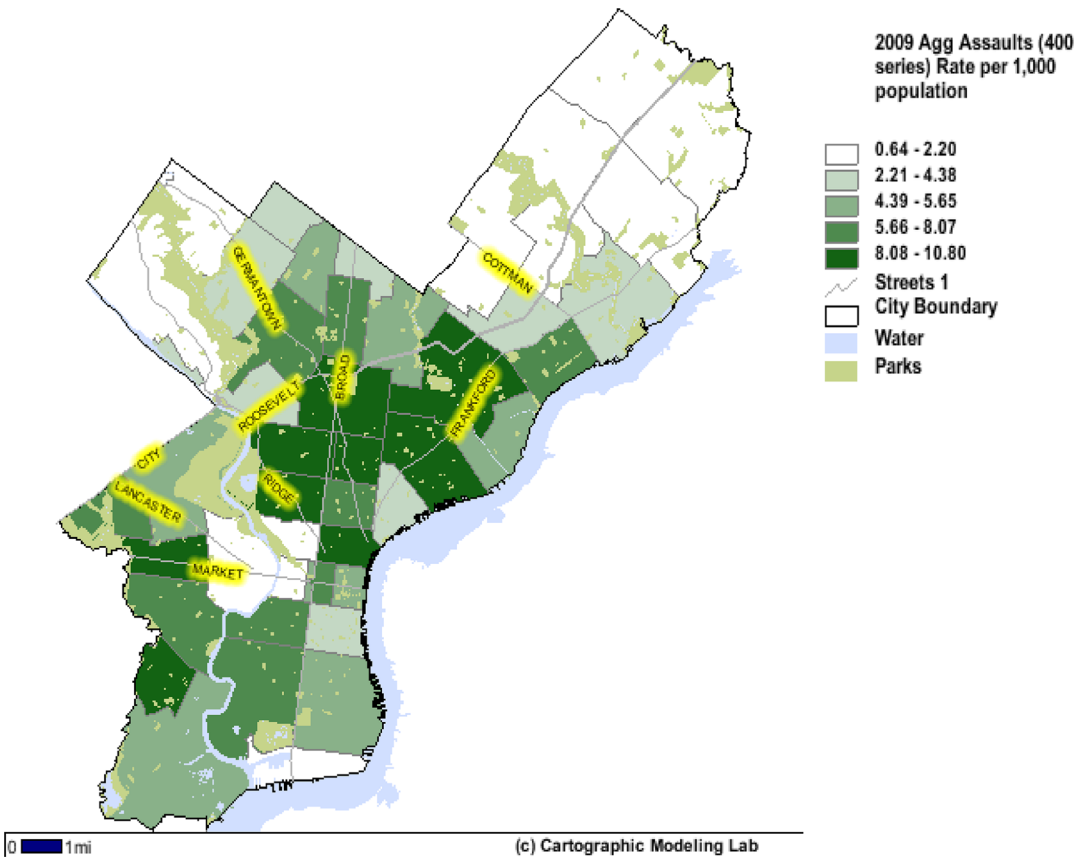

Figure 1. Aggravated assault rate per 1,000 population by Philadelphia zip code.

data provided by the Philadelphia Police Department to the University of Pennsylvania Cartographic Modeling Laboratory.[22] These hotspots were defined as the five Philadelphia zip codes with the highest aggravated assault rate per 1,000 (Fig. 1).[22] The shortest distance between subject zip code and violent hotspot zip codes was used in "proximity to violence" analysis.

Data were analyzed using SPSS (version 20.0, SPSS Inc., Chicago, IL). Multivariable linear regression, Spearman's correlation, and Kruskal-Wallis tests ($p < 0.05$) were used to evaluate relationships between role as patient, family member, or community member; race; sex; age; estimated socioeconomic status; mechanism of injury (for patient and family members); proximity to violence; attitudes toward EFIC in the context of the AVERT Shock Trial; and willingness to participate in emergency research.

RESULTS

A total of 179 subjects including trauma patients (n = 99), families (n = 33), and community members (n = 47) were included in the analysis. There was an overall response rate of 92%. Respondents were primarily African American (83%), evenly split between male (54%) and female (46%), and distributed across age ranges (Table 1). Respondents lived an average of 2.74 miles (0–10.2 miles) from the nearest violent hotspot and 3.7 miles (0–15.7 miles) from our Level 1 trauma center, with an estimated median income of $32,313 (range, $14,586–$93,222).[20] Respondents lived in 30 of the 49 zip codes found in Philadelphia.

Overall, participants were supportive of EFIC as reflected by AVERT attitude scores (median, 24; interquartile range [IQR], 21–25) and willingness scores (median, 89; IQR 82–95; Table 2). Importantly, median participant scores were well above neutral rankings; a score of 18 for AVERT attitude and 69 for willingness. There was no correlation between AVERT attitude or willingness scores and race, sex, age, estimated median income, or status as inpatient or outpatient. A correlation was found between AVERT attitude score and role, with community members having a more positive attitude toward EFIC than families or patients (median, 25, 24, 23; $p < 0.01$, Table 2). However, there was no correlation between willingness scores and role.

"Proximity to violence" did not correlate with perception of EFIC, willingness to participate in emergency research, or violent mechanism of injury but was associated with African American race ($p = 0.03$) and socioeconomic status ($p < 0.01$) (Table 3). In addition, there was no correlation found between living within a "violent hotspot" and perception of EFIC in the context of the AVERT trial or willingness to participate in emergency research. Similarly, no correlation was detected between having experienced or known someone who had experienced a significant trauma resulting in blood loss and AVERT attitude or general willingness scores. However, a correlation was found between mechanism of injury and AVERT attitude score, with patients or families of patients injured by a nonviolent mechanism having a more positive attitude toward EFIC than those injured by assault, gunshot, or stab wound ($p < 0.01$) (Table 2). In contrast, there was no

TABLE 1. Demographics of the Study Population

	All Participants	Patients	Family Members	Community	
	n = 179	n = 99	n = 33	n = 47	*p*
Race, n (%)					NS
African American	149 (83)	78 (79)	28 (85)	43 (91)	
White	25 (14)	18 (18)	3 (9)	4 (9)	
Hispanic	5 (3)	3 (3)	2 (6)	0 (0)	
Age, n (%)***					0.03
18–24	41 (23)	25 (25)	9 (26)	7 (15)	
25–34	40 (20)	25 (25)	10 (29)	5 (11)	
35–49	52 (29)	28 (28)	9 (27)	15 (33)	
50–64	34 (19)	14 (14)	4 (12)	16 (35)	
65+	12 (7)	7 (7)	2 (6)	3 (6)	
Male sex, n (%)**†	97 (54)	66 (67)	10 (30)	21 (46)	<0.01
Miles to hospital (range)	3.70 (0–15.7)	3.92 (0–15.7)	4.30 (0–15.6)	2.78 (0–10.1)	NS
Estimated median income, n (%)***					0.02
0–25,000	32 (18)	20 (20)	6 (18)	6 (13)	
25,001–31,000	54 (30)	21 (21)	4 (12)	29 (63)	
31,001–35,000	53 (30)	31 (31)	13 (38)	9 (20)	
35,000–100,000	40 (22)	27 (27)	11 (32)	2 (4)	
Miles to violence hotspot (range)	2.74 (0–10.2)	2.96 (0–10.2)	3.17 (0–10.2)	1.95 (0–10.2)	NS
Have you or someone you know experienced a trauma resulting in significant blood loss or need for fluid resuscitation? n (%)	Yes, 73 (41)	37 (37)	15 (44)	21 (46)	NS

*Family versus community.
**Patients versus community.
†Patients versus family.
NS, not significant.
Spearman's correlation was used to evaluate relationships between variables. Pairwise comparison was then used to further evaluate significance.

TABLE 2. Univariate Analysis of AVERT Attitude and EFIC Willingness Scores

	AVERT Attitude Score		EFIC Willingness Score	
	Neutral, 18		Neutral, 69	
	Median (IQR)	*p*	Median (IQR)	*p*
All participants (n = 179)	24 (21–25)		89 (82–95)	
Role†		<0.01		NS
Patients (n = 99)	23 (21–24)		88 (81–92)	
Family members (n = 33)	24 (21–25)		91 (82–98)	
Community (n = 47)	25 (22–28)		90 (82–99)	
Race		NS		NS
African American (n = 149)	23 (21–25)		88 (82–95)	
White (n = 25)	24 (22–26)		92 (81–98)	
Hispanic (n = 5)	22 (21–26)		91 (82–95)	
Age		NS		NS
18–24	23 (21–24)		88 (82–94)	
25–34	23 (21–26)		90 (80–98)	
35–49	24 (22–26)		88 (82–100)	
50–64	24 (21–26)		92 (82–99)	
65+	23 (21–24)		85 (80–92)	
Sex		NS		NS
Male	23 (82–96)		89 (82–96)	
Female	24 (82–95)		89 (82–95)	
Violent mechanism		<0.01		NS
No (n = 119)	24 (22–26)		89 (82–98)	
Yes (n = 60)	22 (21–24)		88 (81–93)	

*Patients versus community.

Kruskal-Wallis test used to evaluate for significance of variables between groups. Pairwise comparison was then used to further evaluate significance.

correlation found between mechanism of injury and willingness to participate in emergency research.

DISCUSSION

The community consultation process is a central and valuable requirement for conducting emergency research under EFIC.[3,4] Through our community consultation process, we sought to further understand the factors that influence attitudes toward EFIC in the context of the AVERT Shock Trial among urban trauma patients, families, and community members by engaging with both our geographic and at-risk patient communities. In contrast to previous reports, we found that sex, race, and estimated socioeconomic status did not influence views on emergency research. Moreover, we found that living in or near a violent hotspot had no influence on attitudes toward the AVERT Shock Trial or general willingness to participate in emergency research using EFIC. Collectively, these findings should embolden researchers and institutional review boards to partner with at-risk communities to conduct emergency research.

Since people living closest to areas of concentrated violence are more likely to be the victims of violent injury,[21] we anticipated an association between proximity to violence and violent mechanism of injury. In the City of Philadelphia, the rate of aggravated assault per 1,000 residents ranges from 0.64 in the safest zip code to 10.79 in the most violent.[22] Among respondents living within one of the five most violent zip codes, 51.6% either had known someone or had themselves been the victim of a traumatic injury requiring blood product or fluid resuscitation. In contrast, only 38.5% of respondents living outside these violent hotspots had a similar experience ($p = 0.05$).

Proximity to violence, however, had no influence on the perception of emergency research in our study. Using proximity as a corollary for exposure to violence, we theorized that those living closer to violent hotspots would be more likely to develop collateral consequences of chronic direct or indirect exposure to trauma.[23] Stress theory suggests that exposure to community violence closely correlates with emotional, social and behavioral maladaptation, including anxiety, posttraumatic stress disorder, and social and educational disengagement.[24] In a study using data representing more than 20,000 adolescents from the National Longitudinal Study of Adolescent Health, Warner and Swisher[25] found that violent victimization among youth was associated with decreased survival expectation, placing them at increased risk for social disengagement. As such, we hypothesized the potential presence of these maladaptive influences would make respondents less willing to participate in emergency research. In the course of this community consultation, however, we found no direct correlation between proximity to violence and willingness to participate in emergency research. While it is possible that our respondents were not negatively influenced by proximity to violence, it is also possible that the community consultation process minimized the effect of these influences on the respondent's attitudes and willingness. The concept that education and community involvement can improve diverse participation in clinical trials is critical to emergency research and was the central theme of a recent national meeting on the topic.[26]

Contrary to our hypothesis, community members living closest to violent hotspots actually demonstrated a positive attitude toward EFIC and a trend toward increased willingness (Table 2). In our study, community members lived an average of 1.95 miles from the nearest violent hotspot. In fact, 35% of community members surveyed actually lived within a violent hotspot and, therefore, theoretically had the highest exposure to violence. Perhaps, this increased exposure to as well as the

TABLE 3. Proximity to Violence or Socioeconomic Status Correlation

Proximity to violent hotspot	Race	**0.03**
	Estimated income	**<0.01**
	Role	NS
	Mechanism of injury	NS
	Attitude score	NS
	Willingness score	NS
Estimated socioeconomic status	Attitude score	NS
	Willingness score	NS

Spearman's correlation was used to evaluate relationships between two variables.

direct community consequences of violence made community members feel a stronger responsibility to find effective solutions for the problem. Hill et al.[27] found this to be the case in a study investigating the relationship between exposure to violence and coping strategies among African American mothers, where activism was identified as a commonly used strategy.

Violent mechanism of injury, however, represents a direct exposure to violence and may acutely influence a subject's attitude and willingness to participate in emergency research. We have previously reported a correlation between violent injury mechanism and decreased support of EFIC in a more heterogeneous group of respondents.[17] We have built on this previous work and have identified these same trends in our urban resident cohort. Although violent mechanism of injury negatively influenced attitudes toward EFIC in the context of the AVERT Shock Trial, it did not diminish the general willingness to participate in emergency research (Table 2). This lack of association reinforces the assertion that our urban "community's" exposure to violence should not negatively bias our willingness to partner with those most at risk for violent injury.

That being said, there is an undeniable history of unethical medical research conducted in disenfranchised communities, and it is not surprising that race and socioeconomic status have been previously shown to correlate with mistrust of the medical establishment. In particular, the infamous Tuskegee Syphilis Experiment still influences opinion about research today.[28,29] As a result, African American race has been independently associated with distrust of medical investigators, even after controlling for social class.[28] Moreover, African American race seems to significantly influence the willingness to participate in EFIC research.[30] When presented with a theoretical study, Baren et al.[5] found that African American parents were significantly less likely to consent to emergency research than white parents. Given that the two most impoverished zip codes are also violent hotspots in which the majority of residents are African American (Figs. 2 and 3), we were surprised to discover that our study demonstrated no direct correlation between race and willingness to participate in emergency research. Similarly, socioeconomic status did not correlate with attitude toward or willingness to consent to emergency research.

Finally, sex has been previously implicated as a factor influencing willingness to participate in emergency research, a correlation that we did not observe.[31,32] Although 70% of our trauma patient population is male, half of the total respondents in our study were female family members and community meeting participants.[11,33] Female family members may be more likely to visit patients in the hospital or accompany them to outpatient appointments, or females may be more likely to voluntary as survey participants. Regardless, if one goal of the community consultation process is to generate discourse with the individuals who will be providing emergency consent, it is appropriate for females to be equally represented because mothers frequently function as legally authorized representatives in our trauma population (unpublished data).

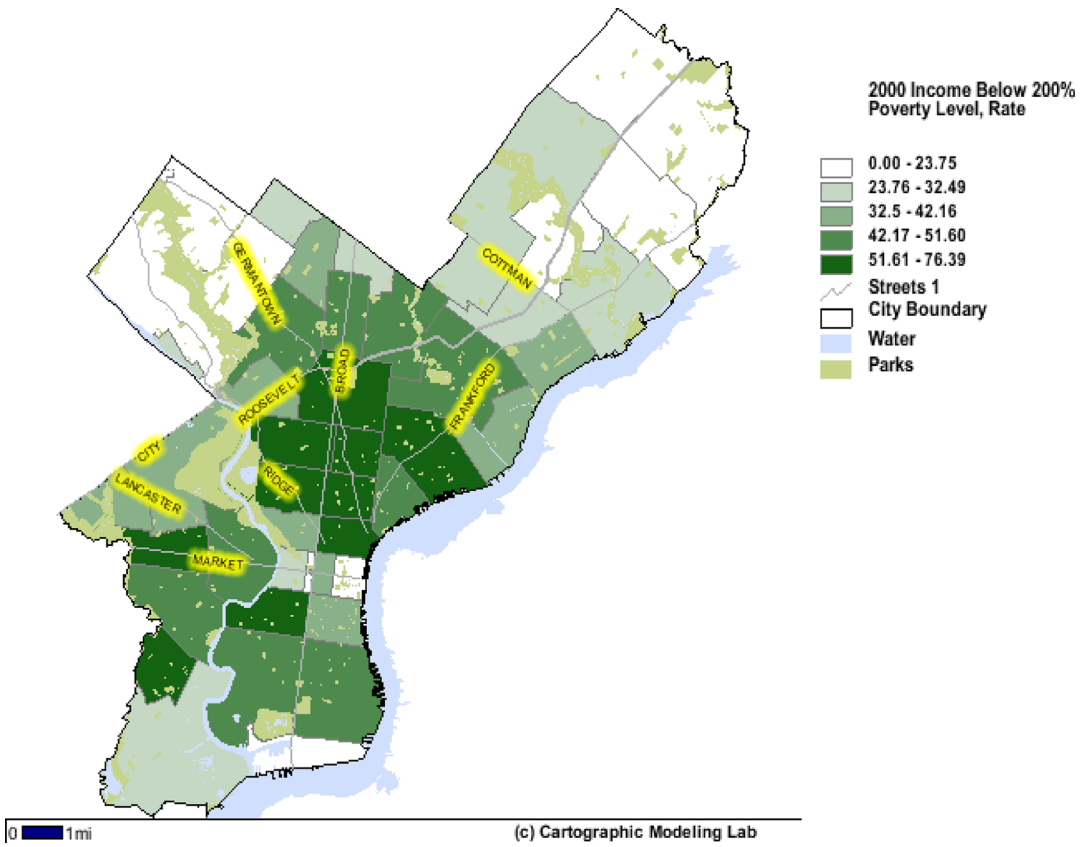

Figure 2. Percentage of residents 200% below poverty line by Philadelphia zip code.

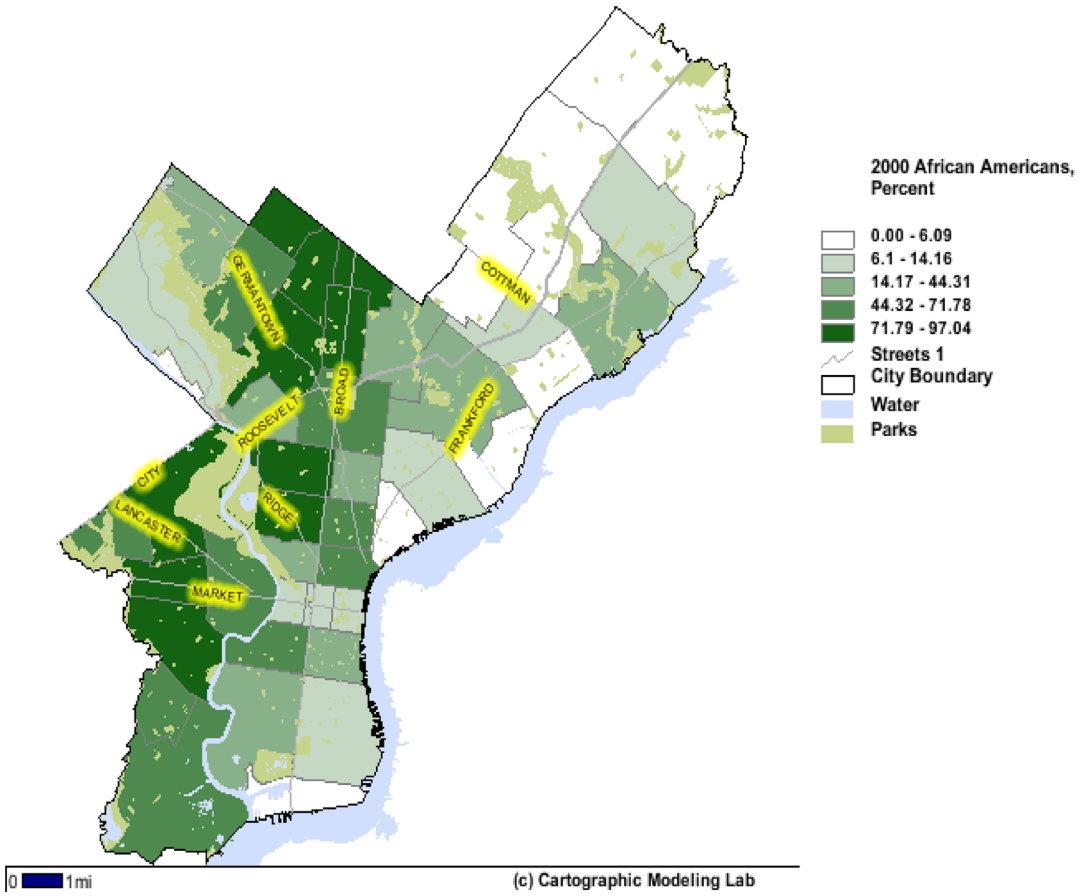

Figure 3. Percentage of African American residents by Philadelphia zip code.

So, why is there discordance between our findings and those of many previous well-conducted studies?[5,7,31] The answer may lie in the approach we took to conduct our community consultation. Our process included a significant educational component aimed at building understanding and trust between respondent and researcher, an essential component of the relationship required for successful EFIC investigations.[7,11,33,34] Specifically, each subject completed the survey in the context of either a semistructured interview while in the hospital or at the clinic or following an hour-long community-based focus group. As Thomas found, "Engaging in dialogue with at-risk groups often empowers them to create effective solutions for addressing concerns."[35] In this study, we engaged with an urban community at risk for inclusion in a trial investigating a novel therapy for hemorrhagic shock. Although at the outset of this investigation, we theorized that race, socioeconomic status, and exposure to violence would lead to lower levels of willingness to collaborate with researchers, we found this not to be the case. We believe our positive findings resulted from a community consultation process that engaged with and educated the geographic and at-risk community. By understanding the potential benefits of emergency research in the context of one of the community's pervasive public health issues, we believe respondents were able to look beyond underlying mistrust of the medical establishment toward a more considered opinion about trauma research.

In addition, although previous studies have found associations between income and consent decisions in EFIC trials, these trials relied on either read survey tools or those administered during acute care in the emergency department.[5,7] In contrast, our community consultation process included significant one-on-one education about the need for emergency research, the process of EFIC research, and details regarding the upcoming AVERT Shock Trial. While this process is certainly more resource intensive, it may serve to overcome the well-documented issue of illiteracy for individuals engaged in community consultation[36–38] while minimizing the impact of socioeconomic status[7,14] and providing an opportunity for trust to develop between researcher and subject.

There are a number of limitations to this study. Performance of a community consultation in the context of a widely supported trial certainly could influence attitudes toward EFIC. In addition, there is a selection bias for patients and families, as we collected a convenience sample of those who were well enough to participate in a 20-minute interview.[39] This would exclude the patients and families of patients who were acutely ill or who died as a result of their injury. These patients and families may not have been as supportive of emergency research as those who were well enough to be discharged from the hospital. Voluntary convenience sampling could also lead to selection bias.[39,40] Patients and families willing to participate

in the interviews may have been those generally more in support of research. Similarly, members of the community who were willing to participate in focus groups may have been generally more in support of research. Members of the community with mistrust of the medical establishment and more negative attitudes toward research, researchers, and medicine in general could have been either underrepresented or overrepresented. While the responses of our community members were statistically more positive than those of our patients and family members, all groups attitude and willingness scores were well above neutrality. Although it is true that when people are informed of an EFIC study, they are more likely to have a positive attitude,[41] one of the mandates of a community consultation is to inform the community and solicit their educated advice and recommendations regarding the proposed research. In addition, estimation of proximity to violence was based on zip code data of the respondent and the zip codes of violent hotspots. This could lead to either overestimation or underestimation of the subject's actual proximity to violent hotspots. The survey response rate for actual income was less than 20%; therefore, we estimated income based on the median income for a zip code. It is certainly possible that the median income for the respondent's zip code could misrepresent their actual income. Lastly, this is a retrospective post hoc analysis, and therefore, the data are not powered to prevent a Type 2 error.

Based on our data, we found no correlation between race, socioeconomic status, or proximity to violence and willingness to participate in EFIC research. Given this lack of correlation, researchers should partner with at-risk communities to conduct EFIC studies without concern that the consultation will be negatively biased. Moreover, because EFIC research is absolutely essential to develop lifesaving treatments, our research can also serve as part of the growing body of literature providing guidance for institutional review boards as they navigate the community consultation process.[42] Although there remains ongoing and valid concern regarding the most effective method for engaging the community, we believe that community education and thoughtful partnerships with those most at risk are investments worth making. It is clear that the time has come for collaboration between at-risk communities and researchers in the pursuit of better emergency care.

AUTHORSHIP

Z.M., E.K.G., and C.A.S designed this study. C.A.S. performed the data collection. Z.M., E.K.G., and B.P.S. performed the data analysis. Z.M., E.K.G., B.P.S., and C.A.S performed the data interpretation. Z.M., E.K.G., and C.A.S prepared the article.

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DISCLOSURE

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Impact of Department of Defense Funded Research at the

NATIONAL TRAUMA INSTITUTE

Funding Research ■ Changing Practice ■ Creating Awareness

Disclosure

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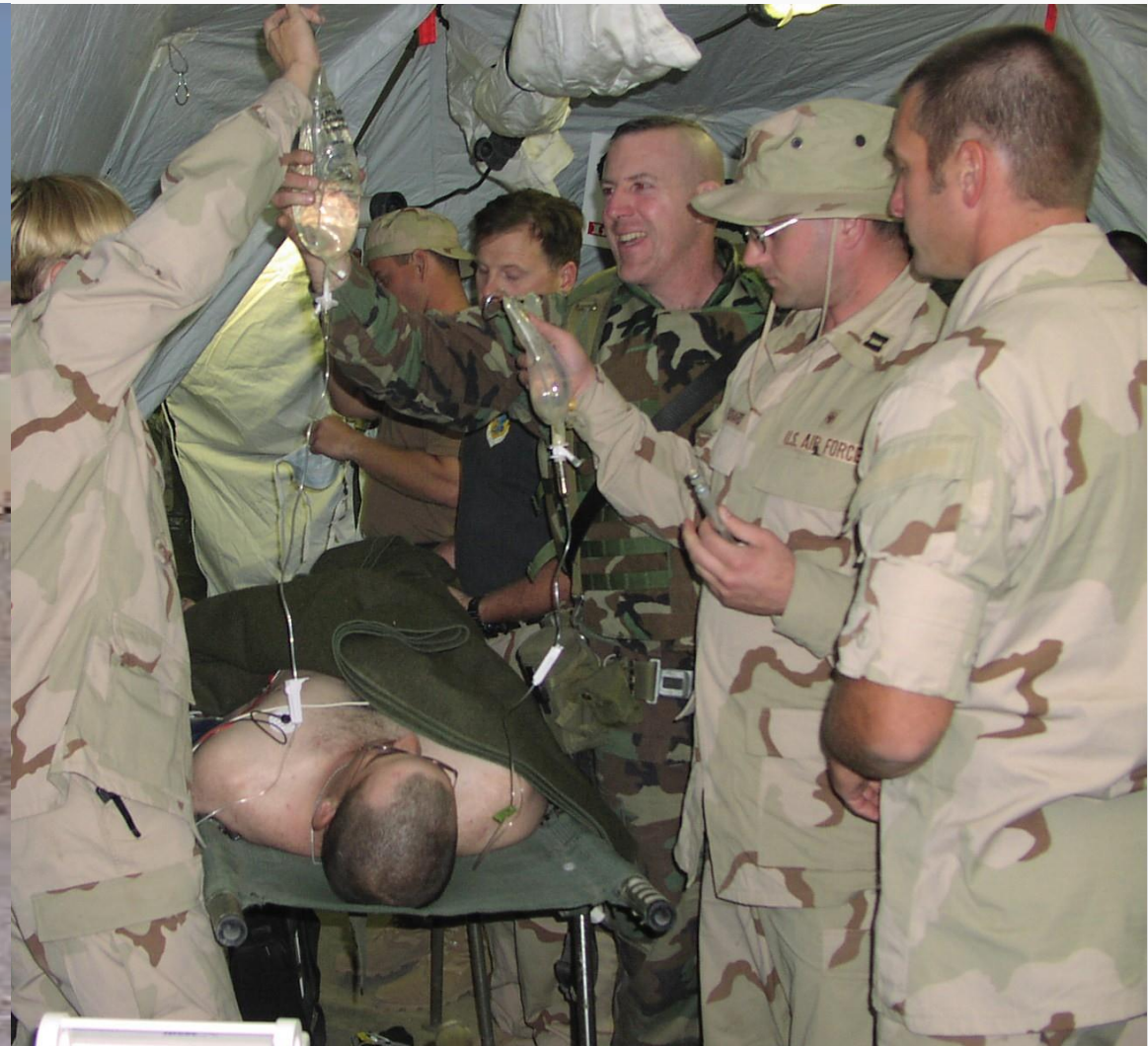
The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the view of the Department of the Army or the Department of Defense.

Management of Blunt Abdominal Trauma (BAT) and Splenic Injury



Splenic Injury Prospective Outcomes Trial: An American Association for the Surgery of Trauma Multi-Institutional Study

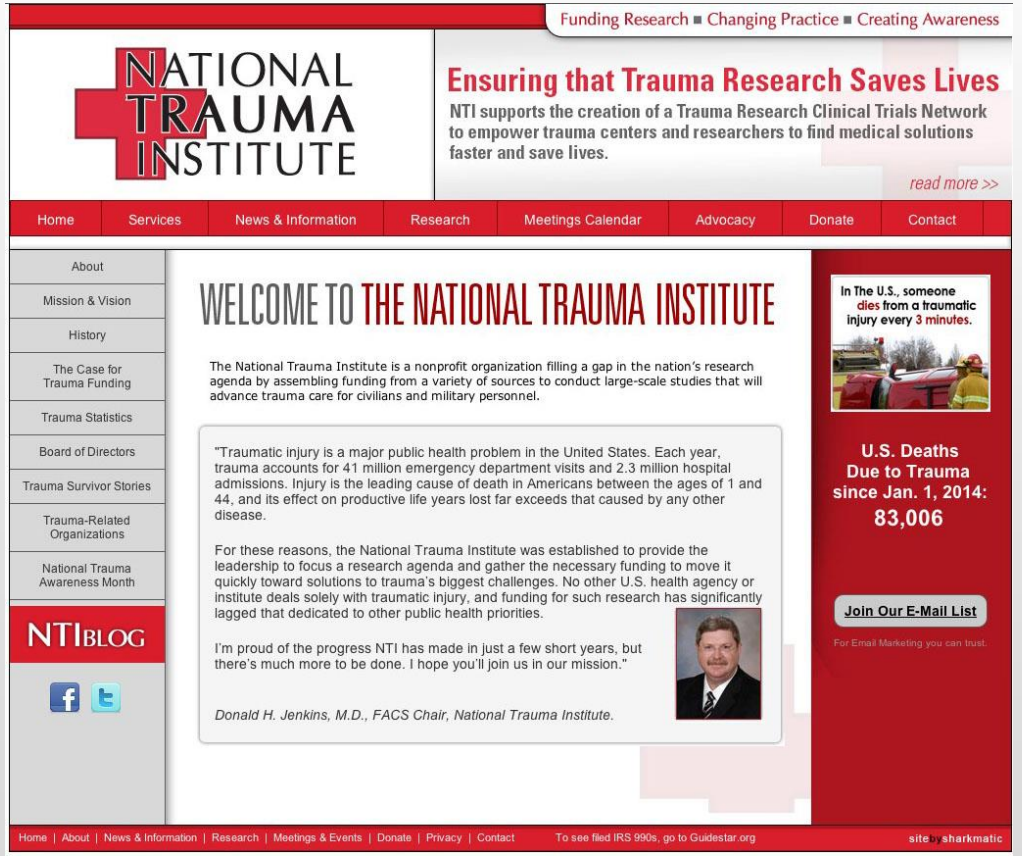
- Principal Investigator: Ben Zarzaur, MD, MPH at University of Tennessee Health Science Center
- First multi-institutional, long-term prospective study of patients with blunt splenic injury
- Funded by the DoD through the National Trauma Institute for $299,422 (NTI-NCH-10-020 & W81XW-11-1-0841)

Findings and Clinical Impact of *Splenic Injury Prospective Outcomes Trial: An AAST Multi-Institutional Study*

- After the first 24 hour of nonoperative management, risk of splenectomy is rare:
  - 3.1% during inpatient phase of care
  - 0.27% during 180 days after discharge
- The benefits of splenic preservation techniques (angiography and embolization) are unclear.
- This study highlighted the need for further large scale multicenter trials that randomize to either management with angiography and embolization or nonoperative management. (*J Trauma Acute Care Surg*. 2015.79;3, 335-342 and AAST Plenary Paper in 2014)

National Trauma Institute Mission

- To generate funds for clinical trauma research
- To discover new funding opportunities
- To advocate for trauma research across federal entities as well as other agencies
- To distribute those funds to clinical investigators, but to do no research ourselves



National Trauma Institute Origins

NATIONAL TRAUMA INSTITUTE

- 2003: Began as local organization of 3 Level 1 Trauma Centers (TRISAT); based within University of Texas Health Science Center at San Antonio (UTHSCSA)
- Product of both civilian and military trauma centers
- 2003-2006: Worked within UTHSCSA to achieve federal appropriations
  - $4.2M total awarded for local trauma research/education & training; recruitment of first civilian burn center director at BAMC, funding salary for 5 years
- 2006: Reorganized as national non-profit entity
  - New Mission: to address lack of federal trauma research funding
  - New Leadership: National Board of Directors

NTI Board of Directors includes members of…

- American Association for the Surgery of Trauma
- Eastern Association for the Surgery of Trauma
- Western Trauma Association
- Shock Society
- American College of Emergency Physicians
- Orthopedic Trauma Association
- American Association of Neurological Surgeons
- US Army Institute of Surgical Research
- US Navy
- US Army
- US Air Force

NTI Research Priorities

- Hemorrhage
  - Non-compressible (truncal/torso)
  - Blood Products
  - Resuscitation
  - Shock and bleeding
  - Coagulopathy
  - Systemic and local hemostatic therapy
- Airway and Ventilation
- Infection
  - Eliminating hospital acquired infections in the ICU
  - Antibiotic utilization
- Disaster Preparedness
  - Mass casualty
  - Transportation of the critically ill
- Burn
  - New skin
  - Off the shelf skin
- Technology development

NTI Trauma Studies Funding Rounds

FIRST

- Issued first Request for Proposals (RFP) October 1, 2009 with $1.4M available funds
- 85 pre-proposals
- 15 full proposals reviewed on February 5, 2010
- 7 selected for funding March, 2010

SECOND

- Issued second RFP June 10, 2010 with $2.46M available funds
- 92 pre-proposals
- 21 full proposals reviewed on August 30, 2010
- 9 selected for funding January, 2011

NTI Funded Studies

16 Lead Sites

43 Participating Sites

NTI Research in 35 cities in 22 states

Funded Awards

PI Name	Institution	Study	$ Awarded	Participating Sites
Martin Croce	UTenn HSC	Multicenter Prospective Evaluation of the Ventilator Bundle in Injured Patients	$225,000	5
Joel Baseman	UTHSC - San Antonio	*Mycoplasma Pneumoniae* in the ICU	$190,000	5
Fred Pieracci	U Co. Denver	A Multicenter, Randomized, Double-blind Comparison of Intravenous Iron Supplementation to Placebo for the Anemia of Traumatic Critical Illness	$188,541	3
Shahid Shafi	Baylor Hosp, Dallas	Comparative Effectiveness of Clinical Care Processes in Resuscitation and Management of Moderate to Severe Traumatic Injuries	$225,000	3
Jason Sperry	U. Pittsburgh	Characterization of the Effects of the Early Sex-Hormone Environment Following Injury	$225,000	Single Center
Mitchell Cohen	UC-SF	Timing and Mechanism of Traumatic Coagulopathy	$225,000	2
Carrie Sims	U. Penn.	Vasopressin Supplementation during the Resuscitation of Hemorrhagic Shock	$125,000	Single Center
Ben Zarzaur	AAST/PI: UTenn HSC	Splenic Injury Prospective Outcomes Trial	$299,422	11

Funded Awards (continued)

PI Name	Institution	Study	$ Awarded	Participating Sites
Jay J Doucet	UC San Diego	Detection and Management of Non-Compressible Hemorrhage by Vena Cava Ultrasonography	$230,000	3
Jean-Francois Pittet	U AL Birmingham	Effect of Antioxidant Vitamins on Coagulopathy and Nosocomial Pneumonia after Severe Trauma	$300,000	Single Center
Mark Cipolle	Christiana HCS, DE	The Safety and Efficacy of Platelet Transfusion in Patients Receiving Antiplatelet Therapy that Sustain Intracranial Hemorrhage	$130,500	Single Center
Henry Cryer	UCLA	Transfusion of Stored Fresh Whole Blood in a Civilian Trauma Center: A Prospective Evaluation of Feasibility and Outcomes	$200,000	Single Center
Suresh Agarwal	Boston Med Center	Acute Lung Injury Ventilation Evaluation (ALIVE) Trial	$295,172	5
Robert Maxwell	UTenn HSC, Chattanooga	Methicillin-Resistant Staphylococcus aureus in a Trauma Population: Does Decolonization Prevent Infection?	$180,000	1
Martin A Schreiber	Oregon Health & Science University	Thrombelastography (TEG®) based dosing of enoxaparin for thromboprophylaxis: a prospective randomized trial	$675,761	3
Lena M. Napolitano	U Mich Health System, Ann Arbor	Hepcidin and Anemia in Trauma	$154,109	Single Center

Initial Scientific Contributions

- Sixteen peer-reviewed publications
- Two publications in press
- One manuscript submitted/under review
- Sixteen national, 2 regional and 6 local presentations
- Ten of the 13 completed studies have published or submitted a manuscript (76%)
- Two PIs received additional funding through NTI applications to the Joint Warfighter Medical Research Program ($500K each)
- Twelve PIs trained junior researchers, fellows, residents or students on their study

Timing and Mechanism of Traumatic Coagulopathy

- Principal Investigator: Mitchell Cohen, MD, at University of California San Francisco
- Funded by the DoD through the National Trauma Institute for $224,950 (W81XWH-10-1-0924 & NTI-TRA-09-034)
- Prospective, multi-institutional observational study to characterize coagulation parameters in the severely injured, to use systems biology to identify the central mediators involved in coagulopathic phenotypes and to develop a predictive model to support diagnosis and treatment

Findings and Clinical Impact of *Timing and Mechanism of Traumatic Coagulopathy*

- Identified clinical significant platelet dysfunction after trauma in the presence of a reassuring platelet count and clotting, with profound implications for mortality. Arachidonic acid and collagen responsiveness are independent predictors of mortality (*J Trauma Acute Care Surg*. 2012; 73: 13-19)
- Consideration of empiric antifibrinolytic therapy is warranted in trauma patients presenting with acidosis, hypothermia, coagulopathy, or relative thrombocytopenia. These criteria facilitate empiric treatment of hypofibrinolysis for clinicians without access to thromboelastography (*J Trauma Acute Care Surg*. 2012; 73: 87-93)

Findings and Clinical Impact of *Timing and Mechanism of Traumatic Coagulopathy* (continued)

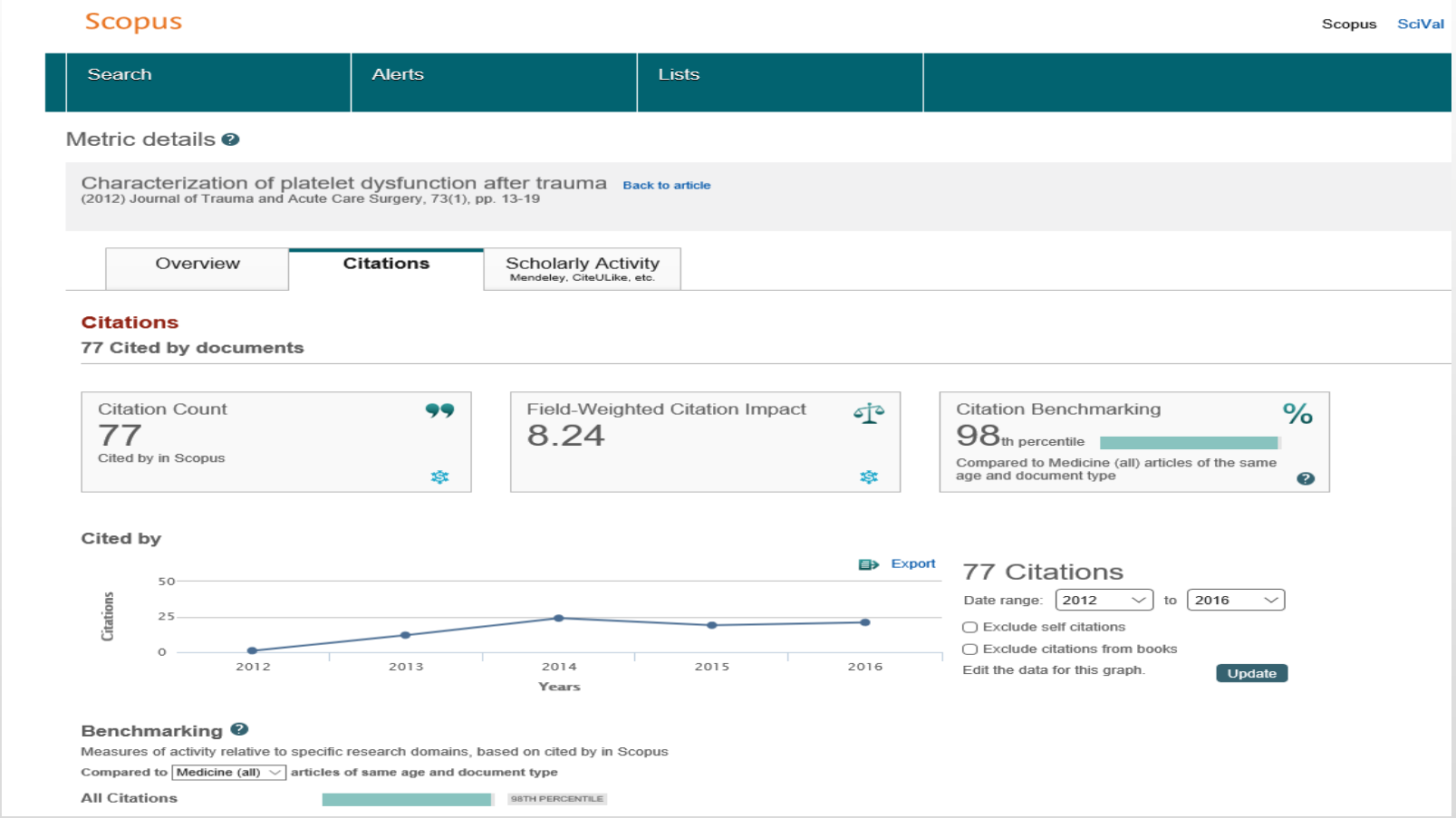
- Extracellular histone levels are elevated in response to traumatic injury, correlate with fibrinolysis and activation of anticoagulants and are predictive of mortality from admission to 6 hours.
- Concomitant elevation of activated protein C (aPC) abrogates this effect, suggesting a possible role for aPC in mitigating sterile inflammatory response through the proteolysis of circulating histones (*J Trauma Acute Care Surg*. 2012; 73: 1389-1394 & WTA 2012 Plenary Paper)
- Characterization of the cause death in severely injured patients requiring massive transfusion suggest targeted surgical and resuscitative strategies to increase the physiologic reserve time and potential survivability (*J Trauma Acute Care Surg*. 2013;75: S255-262)

Findings and Clinical Impact of *Timing and Mechanism of Traumatic Coagulopathy* (continued)

- Although 1:1:1 reconstituted whole blood (RWB) had a superior clotting profile relative to 2:1:1 RWB, platelet modified whole blood (MWB) exhibited even better global hemostasis than 1:1:1 RWB.
- Characterization of factor-level and functional clotting differences between WB variants is imperative for understanding the clinical benefits of hemostatic resuscitation. (*J Trauma Acute Care Surg*. 2014;77: 818-827 and WTA 2014 Plenary Paper)

Scientific Impact of *Timing and Mechanism of Traumatic Coagulopathy* (PI: Mitchell J. Cohen, MD)

The most cited publication from this study is the 2013 *JOT* manuscript *Characterization of platelet dysfunction after trauma*. It has been cited 77 times.



The Science of Conducting Trauma Research

- National Trauma Institute Research Group et al. The National Trauma Institute: Lessons learned in the funding and conduct of sixteen trauma research studies. *J Trauma Acute Care Surg*. 2016 (epub ahead).
- Smith SL, Price MA, Fabian TC, Jurkovich G, Pruitt BA, Jr., Stewart RM, Jenkins DH. The National Trauma Research Repository: Ushering in a new era of trauma research. *SHOCK: 2016 Military Supplement*. Accepted for publication.

- A robust, searchable and scalable repository for data resulting from current and future clinical and basic trauma research
- All federally funded clinical trauma investigators will be eligible to contribute their data.
- Coordination between agencies and civilian academic and professional trauma organizations through data sharing will further data utilization, cooperation and collaboration.

National Trauma Research Repository

10 Years of Advocating for Trauma Research

- Works with Congressional offices to seek sponsors and supporters to augment the Defense Health Agency budget for trauma research
- NTI works with principle investigators (PIs) and institutions to obtain funding through a competitive proposal process
- NTI has generated and/or managed $55M in trauma research funding since 2003

Coalition for National Trauma Research (CNTR)

- In 2014, CNTR formed to advocate for adequate, sustained federal funding for trauma clinical research studies, a national research agenda and infrastructure
- CNTR successfully advocated for additional $10M in DoD budget for FY2016 for a clinical trauma research network
- Advocating for additional $10M in the DoD budget for FY2017 (supported by 15 senators and 69 representatives from 25 states)
- Received notification of first DoD award to CNTR for *Multi-institutional Multidisciplinary Injury Mortality Investigation in the Civilian Pre-Hospital Environment (MIMIC*) to investigate potentially preventable deaths in the prehospital setting in 6 states in partnership with the National Association of Medical Examiners and Johns Hopkins Bloomberg School of Public Health

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NATIONAL TRAUMA INSTITUTE

Funding Research ■ Changing Practice ■ Creating Awareness

Lessons learned in funding 16 trauma studies

Disclosure

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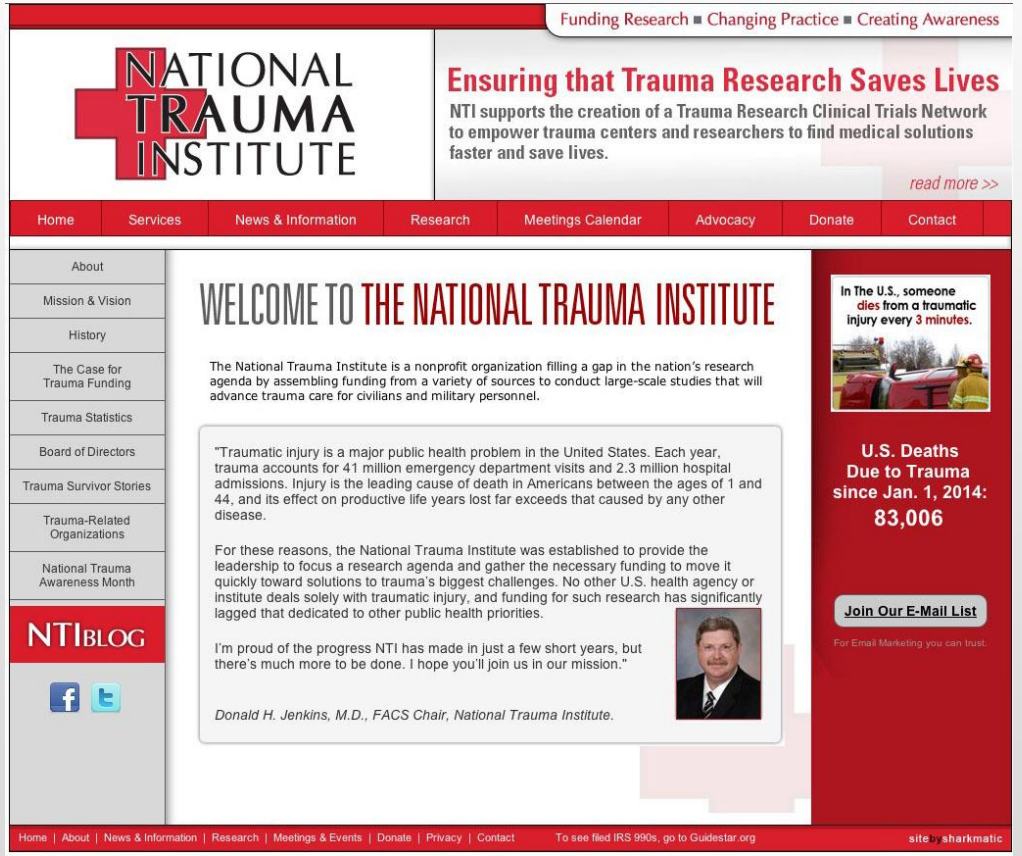
National Trauma Institute Mission

- To generate funds for clinical trauma research
- To discover new funding opportunities
- To advocate for trauma research across federal entities as well as other agencies
- To distribute those funds to clinical investigators, but to do no research ourselves



National Trauma Institute Origins

NATIONAL TRAUMA INSTITUTE

- 2003: Began as local organization of 3 Level 1 Trauma Centers (TRISAT); based within University of Texas Health Science Center at San Antonio
- Product of both civilian and military trauma centers
- 2003-2006: Worked within UTHSCSA to achieve earmarks/federal appropriations
  - $4.2M total awarded for local trauma research/education & training; recruitment of first civilian burn center director at BAMC, funding salary for 5 years
- 2006: Reorganized as national non-profit entity
  - New Mission: to address lack of federal trauma research funding
  - New Leadership: National Board of Directors

NTI Board includes members of…

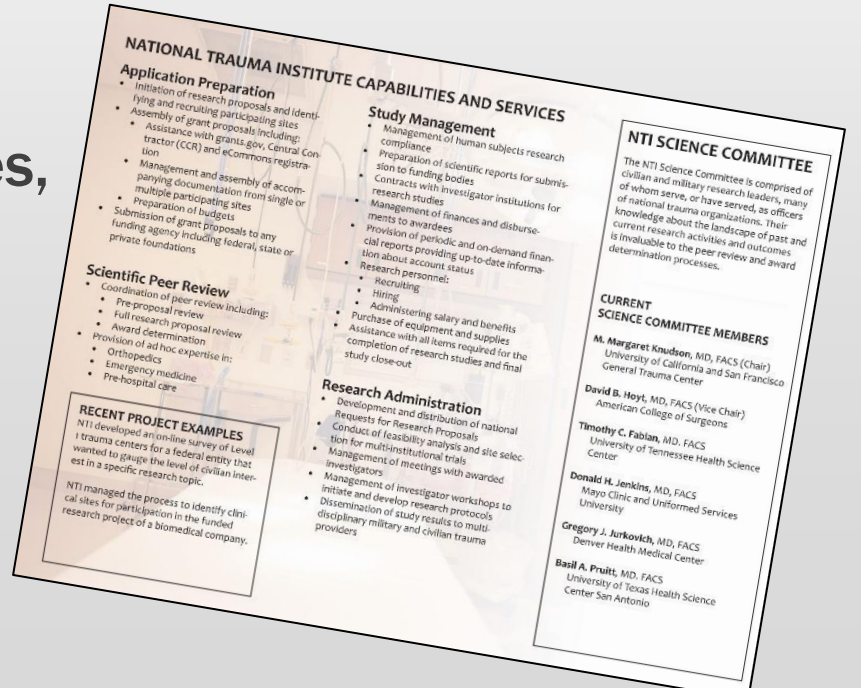
- American Association for the Surgery of Trauma
- Eastern Association for the Surgery of Trauma
- Western Trauma Association
- Shock Society
- US Army Institute of Surgical Research
- American College of Emergency Physicians
- Orthopedic Trauma Association
- American Association of Neurological Surgeons
- US Navy
- US Army
- US Air Force

NTI Research Priorities

- Hemorrhage
  - Non-compressible (truncal/torso)
  - Blood Products
    - Freeze-dried blood
    - Blood substitute
  - Resuscitation
    - Optimal resuscitation, whether to resuscitate, what to resuscitate with
    - Prediction models of resuscitation requirements
    - Fluid treatments
    - Immunomodulatory effects of resuscitation
  - Shock and bleeding
  - Coagulopathy
  - Systemic and local hemostatic therapy
- Infection
  - Eliminating hospital acquired infections in the ICU
  - Antibiotic utilization
- Disaster Preparedness
  - Mass casualty
  - Transportation of the critically ill
- Burn
  - New skin
  - Off the shelf skin
- Airway and Ventilation
  - Ventilation-induced injury
- Technology development

NTI Award and Contract Management

- Work with principle investigators (PIs) and institutions to obtain IRB and DoD (HRPO) approvals following funding rounds
- Execute sub-contracts with each lead organization and, in some cases, all participating institutions
- Manage funds and payments to sub-awardees
- Manage federal monitoring and compliance activities, gather periodic reports from sub-awardees and integrate into required federal reports



NATIONAL TRAUMA INSTITUTE CAPABILITIES AND SERVICES

Application Preparation
- Initiation of research proposals and identifying and recruiting participating sites
- Assembly of grant proposals including:
  - Assistance with grants.gov, Central Contractor (CCR) and eCommons registration
  - Management and assembly of accompanying documentation from single or multiple participating sites
  - Preparation of budgets
- Submission of grant proposals to any funding agency including federal, state or private foundations

Scientific Peer Review
- Coordination of peer review including:
  - Pre-proposal review
  - Full research proposal review
  - Award determination
- Provision of ad hoc expertise in:
  - Orthopedics
  - Emergency medicine
  - Pre-hospital care

RECENT PROJECT EXAMPLES

NTI developed an on-line survey of Level I trauma centers for a federal entity that wanted to gauge the level of civilian interest in a specific research topic.

NTI managed the process to identify clinical sites for participation in the funded research project of a biomedical company.

Study Management
- Management of human subjects research compliance
- Preparation of scientific reports for submission to funding bodies
- Contracts with investigator institutions for research studies
- Management of finances and disbursements to awardees
- Provision of periodic and on-demand financial reports providing up-to-date information about account status
- Research personnel:
  - Recruiting
  - Hiring
  - Administering salary and benefits
- Purchase of equipment and supplies
- Assistance with all items required for the completion of research studies and final study close-out

Research Administration
- Development and distribution of national Requests for Research Proposals
- Conduct of feasibility analysis and site selection for multiinstitutional trials
- Management of meetings with awarded investigators
- Management of investigator workshops to initiate and develop research protocols
- Dissemination of study results to multidisciplinary military and civilian trauma providers

NTI SCIENCE COMMITTEE

The NTI Science Committee is comprised of civilian and military research leaders, many of whom serve, or have served, as officers of national trauma organizations. Their knowledge about the landscape of past and current research activities and outcomes is invaluable to the peer review and award determination processes.

CURRENT SCIENCE COMMITTEE MEMBERS

M. Margaret Knudson, MD, FACS (Chair)
University of California and San Francisco General Trauma Center

David B. Hoyt, MD, FACS (Vice Chair)
American College of Surgeons

Timothy C. Fabian, MD, FACS
University of Tennessee Health Science Center

Donald H. Jenkins, MD, FACS
Mayo Clinic and Uniformed Services University

Gregory J. Jurkovich, MD, FACS
Denver Health Medical Center

Basil A. Pruitt, MD, FACS
University of Texas Health Science Center San Antonio

NTI Trauma Studies Funding Rounds

FIRST

- Issued first Request for Proposals (RFP) October 1, 2009 with $1.4M available funds
- 85 pre-proposals
- 15 full proposals reviewed on February 5, 2010
- 7 selected for funding March, 2010

SECOND

- Issued second RFP June 10, 2010 with $2.46M available funds
- 92 pre-proposals
- 21 full proposals reviewed on August 30, 2010
- 9 selected for funding January, 2011

NTI Funded Studies

16 Lead Sites

43 Participating Sites

NTI Research in 35 cities in 22 states

Funded Award Details

PI Name	Institution	Study	$ Awarded	Participating Sites
Martin Croce	UTenn HSC	Multicenter Prospective Evaluation of the Ventilator Bundle in Injured Patients	$225,000	5
Joel Baseman	UTHSC - San Antonio	*Mycoplasma Pneumoniae* in the ICU	$190,000	5
Fred Pieracci	U Co. Denver	A Multicenter, Randomized, Double-blind Comparison of Intravenous Iron Supplementation to Placebo for the Anemia of Traumatic Critical Illness	$188,541	3
Shahid Shafi	Baylor Hosp, Dallas	Comparative Effectiveness of Clinical Care Processes in Resuscitation and Management of Moderate to Severe Traumatic Injuries	$225,000	3
Jason Sperry	U. Pittsburgh	Characterization of the Effects of the Early Sex-Hormone Environment Following Injury	$225,000	Single Center
Mitchell Cohen	UC-SF	Timing and Mechanism of Traumatic Coagulopathy	$225,000	2
Carrie Sims	U. Penn.	Vasopressin Supplementation during the Resuscitation of Hemorrhagic Shock	$125,000	Single Center
Ben Zarzaur	AAST/PI: UTenn HSC	Splenic Injury Prospective Outcomes Trial	$299,422	11

Funded Award Details (continued)

PI Name	Institution	Study	$ Awarded	Participating Sites
Jay J Doucet	UC San Diego	Detection and Management of Non-Compressible Hemorrhage by Vena Cava Ultrasonography	$230,000	3
Jean-Francois Pittet	U AL Birmingham	Effect of Antioxidant Vitamins on Coagulopathy and Nosocomial Pneumonia after Severe Trauma	$300,000	Single Center
Mark Cipolle	Christiana HCS, DE	The Safety and Efficacy of Platelet Transfusion in Patients Receiving Antiplatelet Therapy that Sustain Intracranial Hemorrhage	$130,500	Single Center
Henry Cryer	UCLA	Transfusion of Stored Fresh Whole Blood in a Civilian Trauma Center: A Prospective Evaluation of Feasibility and Outcomes	$200,000	Single Center
Suresh Agarwal	Boston Med Center	Acute Lung Injury Ventilation Evaluation (ALIVE) Trial	$295,172	5
Robert Maxwell	UTenn HSC, Chattanooga	Methicillin-Resistant Staphylococcus aureus in a Trauma Population: Does Decolonization Prevent Infection?	$180,000	1
Martin A Schreiber	Oregon Health & Science University	Thrombelastography (TEG®) based dosing of enoxaparin for thromboprophylaxis: a prospective randomized trial	$675,761	3
Lena M. Napolitano	U Mich Health System, Ann Arbor	Hepcidin and Anemia in Trauma	$154,109	Single Center

Methods

- The NTI Executive and Science Committees identified key study management topics
- A semi-structured interview with 30 open-ended questions was developed addressing:
  - Project management
  - Regulatory review
  - Financial management
  - Investigator development
  - Scientific productivity
- 15 of 16 principal investigators (PIs) participated in the interviews

Methods (Continued)

- PI responses were de-identified and analyzed in aggregate
- NTI Science Committee meeting minutes and policies were reviewed
- NTI project management data and reports were reviewed
- Descriptive statistics and qualitative analysis performed

Results

- Study Oversight by NTI
  - PIs submitted quarterly progress reports to the NTI Science Committee
  - Science committee met regularly to review progress
  - Science committee members met with PIs regarding any concerns such as slow progress on IRB & HRPO and participant accruals to develop a corrective plan
  - Three studies were closed due to low enrollment or other start-up delays
  - NTI submitted quarterly progress reports to federal funding source

Institutional Approval Challenges

- Studies were reviewed and approved by local Institutional Review Boards (IRBs) and then by the DoD Human Research Protection Office (HRPO)
- Funding contracts could not be issued until HRPO approval was obtained
- Time to IRB & HRPO approval varied widely
  - 6 studies had IRB approval by the time they were selected for funding
  - Among the remaining 10 studies, the mean number of days from funding selection to IRB approval was 210 days
  - Randomized clinical trials had the highest mean days (262 days)
  - Mean number days from selection to HRPO approval was 401 days
- 40% of PIs reported challenges in obtaining approval.

Study Progression – Measured in Calendar Days

Study	Study Type	Selection to IRB Approval	IRB Approval to DoD HRPO Approval	DoD HRPO Approval to Enrollment	NTI Prime Contract to Site Enrollment	Enrollment & Data Collection
1	RCT	537	424	133	950	Ongoing
2	PO	*	**	0	125	693
3	PO	*	**	194	242	751
4	HV	*	**	280	437	105
5	RCT	128	220	115	226	673
6	PO	76	92	8	29	686
7	PC	51	234	0	138	943
8	PO	*	**	88	40	382
9	RCT	155	779	7	704	140
10	PO	88	67	116	128	794
11	RCT	248	115	30	246	829
12	PO	307	111	30	211	688
13	RR	*	**	NA	NA	NA
14	RCT	173	249	68	253	381
15	RCT	*	**	141	482	828
16	RCT	332	222	276	593	104
	Mean Days	210	251	99	320	571
	Minimum	51	67	0	29	104
	Maximum	537	779	280	950	943
	Standard Deviation	142	221	91	254	280

IRB: Institutional Review Board; DoD HRPO: Department of Defense Human Research Protection Office; HV: Healthy Volunteer; PC: Prospective Cohort; PO: Prospective Observational; RCT: Randomized Clinical Trial; RR: Retrospective Review; NA: Not Applicable; *Denotes studies that had IRB approval at the time of selection notification; **Unknown

Screening & Enrollment

- Enrollment periods ranged from 104 – 943 days (Mean=551; SD=280 days)
- 28,725 patients were screened and 5,579 were participants were enrolled (as of January 2016)
- 33% of PIs reported screening & enrollment were at or above targets
- 67% of PIs reported screening & enrollment were below targets
  - Lower incidence of disease
  - Sicker patients or shorter lengths of stay
  - Patient or family refusal to consent
  - Insufficient staffing during evenings, nights & weekends

Multi-site Study Coordination

- 10 studies were multi-site (ranging from 2-11 sites)
- 4 studies had site attrition
  - IRB/HRPO delays or required protocol changes
  - PI was deployed
  - Insufficient funding
- PIs emphasized importance of monitoring screening & enrollment at multiple sites with regular meetings and reporting

Study Financial Management

- Nine PIs reported study funding was insufficient (6 of these budgets had been reduced by the NTI Science Committee)
- Budgeted staffing ranged from 0.6 to 2.4 full time equivalents (FTEs)
- Budgeted PI time ranged from 0.0 to 0.25 FTEs
- Nine PIs augmented study funding from other sources
- Seven PIs achieved 24/7 coverage with funding from other sources

Current Status of Studies

- Ten studies completed initial data analysis
- Two studies are analyzing initial data
- One study is still enrolling
- Three studies were closed due to low enrollment or start-up issues

Initial Scientific Contributions

- Sixteen peer-reviewed publications
- Two manuscripts submitted/under review
- Fifteen national, 2 regional and 6 local presentations
- Ten of the 13 completed studies have published or submitted a manuscript (76%)
- Two PIs received additional funding through NTI applications to the Joint Warfighter Medical Research Program ($500K each)
- Twelve PIs trained junior researchers, fellows, residents or students on their study

Discussion – Lessons Learned

- Regulatory process management
- Multi-site coordination
- Funding adequacy
- Importance of an existing research infrastructure

Institution- or Process-Related Challenges

Challenges	Recommendations
Multiple human subject review processes at two or more levels (IRB & HRPO)	Incorporate required IRB and HRPO language in protocol and consent documents in first submission to IRB; Academic departments should facilitate IRB application submissions. IRB approval should be obtained prior to submission of the funding application.
Completing community consultation process prior to the grant award	Identify funding from alternate source (e.g., departmental start-up or bridge funds) if funding is not provided by granting institution. Community consultation should be completed prior to submission of funding application.
Data sharing restrictions between trauma centers	Determine the potential for data sharing prior to including investigative sites in proposal

Investigator-Related Challenges

Challenges	Recommendations
Inadequate assessment of eligible patient density	Require that the PI document study patient accessibility and report recruitment success in clinical studies completed at the hospital over the past three years.
Underestimation of PI effort, personnel and other study costs	Application budgets should accurately include PI effort, staff and other costs at all sites. PIs should also document adequate research infrastructure and institutional support.
Site attrition among multi-site studies	Thorough assessment of site capacity and study requirements (by each site PI) should be completed at the time of site enrollment during the application process. PIs and funding organizations should establish quarterly progress goals and require explanation of failure to meet goals in quarterly progress reports.

Limitations

- Experiences drawn from this group of studies may not be representative of study management in all settings
- PIs were interviewed at various stages of the research process so their perspectives on barriers and facilitators may have been impacted
- Study management data were reviewed retrospectively with some equivocal or missing data

Next Steps

- Center for National Trauma Research (CNTR) formed to advocate for adequate, sustained federal funding for trauma clinical research studies and infrastructure
- CNTR successfully advocated for additional $10M in DoD budget for FY2016 for a clinical trauma research network
- Advocating for additional $20M in the DoD budget for FY2017 (currently supported by 15 senators and 69 representatives from a total of 25 states)
- NTI secured DoD funding to develop a National Trauma Research Repository

NATIONAL TRAUMA INSTITUTE

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