Trauma induced hypercoagulability in pediatric patients

Mark L. Ryan a, Robert M. Van Haren a, Chad M. Thorson a, David M. Andrews b, Eduardo A. Perez c, Holly L. Neville c, Juan E. Sola c, Kenneth G. Proctor a,*

a Dewitt-Daughtry Department of Surgery, Divisions of Trauma and Surgical Critical Care, University of Miami Miller School of Medicine, Miami, FL 33136, USA
b Department of Pathology, University of Miami Miller School of Medicine, Miami, FL 33136, USA
c Dewitt-Daughtry Department of Surgery, Division of Pediatric Surgery, University of Miami Miller School of Medicine, Miami, FL 33136, USA

ABSTRACT

Purpose: Coagulation changes in pediatric trauma patients are not well defined. To fill this gap, we tested the hypothesis that trauma evokes a hypercoagulable response.

Methods: A prospective observational study was conducted in hospitalized patients (age 8 months to 14 years) admitted for trauma or elective surgery. Informed consent was obtained from the parents and informed assent was obtained in patients 7 years of age or older. Coagulation changes were evaluated on fresh whole blood using thromboelastography (TEG) and on stored plasma using assays for special clotting factors.

Results: Forty three patients (22 trauma, median injury severity score = 9; and 21 uninjured controls) were evaluated. For trauma vs control, prothrombin time (PT) was higher by about 10% (p < 0.001), but activated partial thromboplastin time was not altered. TEG clotting time (R; p = 0.005) and fibrin cross-linking were markedly accelerated (K time, alpha angle; p < 0.001) relative to the control patients. D-Dimer, Prothrombin Fragment 1 + 2, and Plasminogen Activator Inhibitor-1 were all elevated, whereas Protein S activity was reduced (all p < 0.01). Importantly, a large fraction of TEG values and clotting factor assays in the pediatric control group were outside the published reference ranges for adults.

Conclusion: A hypercoagulable state is associated with minor trauma in children. More work is needed to determine the functional significance of these changes and to establish normal pediatric reference ranges.

© 2014 Elsevier Inc. All rights reserved.

Hemorrhage is a primary cause of potentially preventable trauma death in both adults and children [1,2]. Hypercoagulability is a homeostatic compensatory response to traumatic hemorrhage, and most patients manifest this condition on arrival to the emergency department. However, the opposite response is common, and is associated with hypothermia and acidosis in those who are the most severely injured [3–5]. Goal directed blood product therapy is probably most beneficial in these patients [6].

Traditionally, the coagulation status of a trauma patient is assessed using prothrombin time (PT), international normalized ratio (INR), and/or partial thromboplastin time (PTT). However, these standard tests do not routinely assess platelet function, fibrinolysis, or a hypercoagulable state, and these results do not necessarily correlate with who is actually bleeding [6,7]. Furthermore, findings in adult trauma patients cannot always be extrapolated to the pediatric population because there are age-related quantitative differences in the plasma concentrations of clotting factors [8,9]. In fact, pediatric reference ranges for many coagulation factors are not well defined [10–12].

Thromboelastography (TEG) is a rapid, point-of-care test that can assess the entire coagulation cascade. In pediatric patients, TEG has been used in cardiac surgery [13,14], massive transfusion [15], and in those with clotting factor deficiencies [16], but to our knowledge, there has been only one other study in pediatric trauma patients.

Vogel et al. retrospectively reviewed 86 severely injured patients (age < 14 years) and compared admission rapid thromboelastography (rTEG) to conventional coagulation tests and to early life-saving
interventions [17]. They observed that rTEG activated clotting time (ACT), k-time, and α-angle were strongly correlated to PTT, and that maximum amplitude (MA) was correlated to platelet count. When controlling for age, gender, and injury severity score (ISS), ACT, r-value, k-time, α-angle, and MA all predicted transfusion requirements [17]. There have been no other prospective studies on the subject. Therefore, we prospectively evaluated the hypothesis that pediatric trauma patients exhibit a hypercoagulable response similar to that in adults.

1. Materials and methods

This study protocol was approved by the Institutional Review Board (IRB) of the University of Miami and the Clinical Trials Office of Jackson Memorial Hospital (UM Protocol #20090816). Informed consent was obtained from the parents and informed assent was obtained in patients 7 years of age or older. This prospective, observational study included patients <15 years who were admitted to the Ryder Trauma Center at Jackson Memorial Hospital via ambulance or air rescue services between December 2009 and March 2011. Exclusion criteria were patients with burns, who were incarcerated, pregnant, suffering from a psychiatric condition, or had a history of coagulopathy or bleeding disorder.

Blood samples were obtained from the intravenous cannula placed at the time of admission using a “two-syringe technique”[18]. Three mL was drawn into the first syringe in order to prevent contamination with tissue thromboplastin. Three mL of blood was then drawn into a second syringe and transferred to 2 vacuum-sealed tubes containing 3.2% (0.105 M) sodium citrate. Samples stored in sodium citrate may be run within 4 h of blood draw, according to the manufacturer.

Previous studies in adult trauma patients have utilized either nothing, kaolin or tissue factor activating reagents in the vacuum sealed tubes. The use of activating reagents shortens the total analysis time. In this study, many of the pediatric trauma patients arrived at nights or on weekends. We chose sodium citrate to allow us enough time to come from home and run the sample of a new trauma patient. We chose to run the sample at exactly 60 min from the time of blood draw using a TEG 5000 Thromboelastograph Hemostasis System (Haemoscope Corporation, Niles, IL). Citrated whole blood (340 μL) was recalcified using 20 μL of 0.2 M calcium chloride prior to initiation of the test. The sample was run for 90 min to allow ample time to evaluate fibrinolysis. Data were interpreted using TEG Analytical Software Ver. 4.2.3. The remaining tube was centrifuged for 10 min at 3000 rotations per minute, at which point the plasma was extracted and stored at −70 °C for clotting factor analysis.

Sample size was estimated based on the probability of finding a 30% difference between trauma and control patients using a power and sample size calculator. A sample size of 20 with a 30% difference in R value (2 vs 2.6) yielded a significant difference with a power of 0.8. Once all samples had been collected from trauma patients, data were collected from age and sex matched patients who were uninjured, to account for the lack of reference range in the pediatric population. These patients were being seen in the pediatric surgery clinic prior to undergoing an elective operation or had presented to the emergency room with no evidence of systemic infection or trauma.

TEG parameters included: reaction time (R), k-time (K), alpha angle (α), maximum amplitude (MA), G-value (G), and coagulation index (CI) (Fig. 1). R is the time between initiation of test and initial fibrin formation, and represents the enzymatic portion of coagulation. K is the time needed to reach 20-mm clot strength, and represents clot kinetics. α is the measure of the TEG tracing’s slope, and represents fibrin cross-linking. MA is the measure of overall clot strength, and represents platelet aggregation. Coagulation Index (CI) is derived using the R, K, α, and MA of native whole blood using the equation:

\[
CI = -0.2454R + 0.0184K + 0.1655MA - 0.0241\alpha - 5.0220.
\]

The adult reference range for CI is −3.0 to 3.0. Values greater than 5.0 are associated with hypercoagulable conditions such as cancer and deep vein thrombosis [19].

The shear elastic modulus strength (G) reflects clot strength, is measured in dyn/cm², and is calculated using the formula

\[
G = \frac{2R}{\pi}.
\]
(5000MA/(100 – MA))/1000. G is the best predictor of massive transfusion and death in adult trauma patients [20]. LY60 describes the relative fibrinolysis at 60 min and is derived by subtracting the amplitude of the TEG tracing 60 min after MA (A60). This value is then divided by the MA value, and yields the percent fibrinolysis at 60 min (LY60). TEG reference range values for whole blood samples in sodium citrate were provided by the manufacturer, and are based upon analysis of 132 normal adults.

Batched plasma samples were thawed and assayed in the University of Miami/Jackson Memorial Hospital Department of Pathology for markers of hyper- and hypocoagulability, including dimer, PT, PTT, fibrinogen, protein C activity, protein S activity, antithrombin III activity, and prothrombin fragment 1 + 2.

Statistical comparisons of parametric data for the trauma and control groups were conducted via a student’s t test using SPSS Ver. 21.0 (IBM Corporation, Somers, NY).

2. Results

Over 15 months, blood samples were obtained from 22 children (14 males and 8 females) admitted to the trauma center via ambulance or air rescue. This study population would most appropriately be termed a “convenience sample” that was limited by the research staff not being available 24/7, the need to obtain consent, and trauma team not notifying research team of pediatric admission within two hrs. For these three reasons, it took over a year to enroll 22 patients. Twenty four patients were asked to participate; only two were excluded because the parents refused to provide consent.

The median age was 6.5; the youngest was 8 months and the oldest was 14 years. Twenty one presented with blunt injuries (motor vehicle collisions, pedestrian injuries, and falls), and one patient was admitted with a gunshot wound to the chest. A large number of these patients (45%) presented with traumatic brain injury (TBI) superimposed on their other injuries. Nevertheless, there were no mortalities, deep venous thromboses, pulmonary embolisms, or transfusions in this cohort. The median ISS was 9.

The uninjured control group was either hospitalized overnight or had outpatient procedures. This study population was comprised of 19 elective surgery patients and 2 emergency room patients. Consent was obtained in all 21 patients who were approached. There was a TEG hardware failure for two control samples so these two samples were excluded, but the stored plasma samples were assayed. All other samples were used.

Patients were assessed on initial presentation and at completion of their hospitalization. The demographic information for all patients is presented in Table 1.

In the trauma group, PT was significantly elevated relative to the control patients (16.7 ± 2.2 vs. 14.6 ± 0.7, p < 0.001), but no patients were clinically coagulopathic on admission (i.e., no significant hemorrhage was observed). Most (55%, n = 12) trauma patients had an elevated platelet count on admission (>400), and the median overall platelet count was 441.

<p>| Table 2 |</p>
<table>
<thead>
<tr>
<th>Test</th>
<th>Trauma</th>
<th>Uninjured control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Routine Labs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (s)</td>
<td>11.6–13.2</td>
<td>16.7 ± 2.2</td>
<td>100%</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>30.1–36.9</td>
<td>36.9 ± 13.4</td>
<td>29%</td>
</tr>
<tr>
<td><strong>platelets (thous/µL)</strong></td>
<td>150–450</td>
<td>406 ± 106</td>
<td>24%</td>
</tr>
</tbody>
</table>

**TEG parameters**

| R (min) | 9–27 | 7.3 ± 2.7 | 68% | 10.3 ± 3.9 | 42% | 0.005 |
| K (min) | 2–9 | 1.5 ± 0.7 | 71% | 2.8 ± 1.5 | 42% | <0.001 |
| α (°) | 22–58 | 67.4 ± 9.3 | 77% | 55.1 ± 12.5 | 58% | <0.001 |
| MA (mm) | 44–64 | 61.5 ± 12.4 | 55% | 56.5 ± 19 | 11% | NS |
| G (kdyn/cm²) | 3.6–8.5 | 8.8 ± 3.1 | 59% | 6.9 ± 2.1 | 21% | 0.023 |
| CI | –3.0–3.0 | 2.1 ± 1.4 | 29% | 0.5 ± 1.6 | 0% | 0.002 |
| LY60 (%) | 0–15 | 41 ± 3.1 | 0% | 4.9 ± 2.6 | 0% | NS |
| **Clotting Factor Assays** |
| d-Dimer (ng/ml) | 0–500 | 7507 ± 5468 | 100% | 405 ± 472 | 10% | <0.001 |
| Fibrinogen (mg/dl) | 217–278 | 261 ± 103 | 25% | 269 ± 51 | 24% | NS |
| Factor VIII (U/ml) | 0.74–1.24 | 2.0 ± 0.8 | 80% | 1.0 ± 0.3 | 24% | <0.001 |
| PC ACT (% activity) | 70–130 | 78 ± 19 | 33% | 92 ± 26 | 14% | 0.052 |
| PS ACT (% activity) | 66–133 | 44 ± 14 | 100% | 69 ± 18 | 10% | <0.001 |
| ATIII (%) | 80–120 | 95 ± 13 | 20% | 98 ± 16 | 15% | NS |
| FII + 2 (pmol/L) | 69–229 | 713 ± 365 | 100% | 117 ± 200 | 5% | 0.001 |
| PAI-1 (U/ml) | 0–194 | 9.6 ± 2.0 | 10% | 14 ± 2.0 | 0% | 0.017 |

Data reported as mean ± SD. RR, Reference Range. * p percent values higher than RR. † p percent values lower than RR. R, reaction time; K, time to clot firmness of 20 mm; α, angle; MA, maximum amplitude; G, shear elastic modulus strength; CI, Coagulation Index; LY60, percent fibrinolysis 60 min after MA; PC ACT, Protein C Activity; PS ACT, Protein S Activity; ATIII, Antithrombin III Activity; FII + 2, Prothrombin Fragment 1 + 2; PAI-1, plasminogen activator inhibitor-1.

There was no correlation between TEG parameters and age, sex, or the presence of TBI, so the data are not shown. TEG values in both study groups as well the reference ranges are shown in Table 2.

In the trauma group, R and K time were significantly greater relative to control (all p < 0.01), indicating a decreased latent period and accelerated fibrin build-up and cross linking.

There was no significant difference in MA or fibrinolysis (LY60), but the viscoelastic clot strength (G), which incorporates the MA in its calculation, was significantly higher in the trauma patients (p = 0.023), indicating greater overall clot strength. The CI, which incorporates all 4 measured variables, was significantly higher in the trauma group (p = 0.002). However, both groups had mean values that were within the reference range (−3.0 to 3.0).

The mean d-Dimer level was approximately 19 times higher in the trauma patients relative to control (p = 0.052 and p < 0.001, respectively), indicating inhibition of these anticoagulant proteins.

Table 1

<table>
<thead>
<tr>
<th>Demographics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trauma Patients</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>Age (years) *</td>
</tr>
<tr>
<td>Sex (M/F)</td>
</tr>
<tr>
<td>Mechanism (Blunt/Penetrating)</td>
</tr>
<tr>
<td>TBI (Y/N)</td>
</tr>
<tr>
<td>ISS *</td>
</tr>
</tbody>
</table>

* Data reported as median (range).

TBI, traumatic brain injury; ISS, injury severity score.
3. Discussion

To our knowledge this is the first prospective study (with consent) in pediatric trauma patients. The major new findings are that multiple TEG values indicate a hypercoagulable condition after minor trauma (ISS = 9) relative to uninjured patients. However, there are currently no reported TEG reference ranges established for pediatric blood samples stored in sodium citrate before or after injury, so more work is definitely needed.

Injury-induced hypercoagulability is a logical and homeostatic compensatory response to minimize blood loss and can be explained by significant elevations of D-Dimer, Prothrombin Fragment 1 + 2, and Plasminogen Activator Inhibitor-1, as well as by a reduction in Protein S Activity. All values were well outside of the reference range. Plasminogen activator inhibitor-1 had a significant difference, but remained within the reference range. In contrast, routine lab values were not helpful; PT was increased (suggesting hypercoagulability) whereas PTT was not different in trauma vs control. Altogether, it appears the coagulation system in children manifests a similar initial response to injury to that seen in adults [4].

These findings must be considered within the context of several limitations. It is important to note that this study does not address the “acute coagulopathy of trauma”, the dysregulated coagulation process that occurs in patients with high injury severity, prolonged hemorrhage, and/or massive transfusion. Hypercoagulability, as measured by prolongation of TEG R and K times along with decreased α and MA values, has been associated with the need for massive transfusion, morbidity, and mortality [21–23]. Recently Vogel et al. described this phenomenon in critically injured pediatric trauma patients, showing significant correlation between altered TEG and the need for lifesaving interventions, blood product transfusion, and overall mortality [17]. TEG directed hemostatic resuscitation in a pediatric trauma patient has been described by Nylund et al., who used it to guide plasma, platelet, and factor VIII administration in a severely coagulopathic child receiving a massive transfusion [15]. However, the “acute coagulopathy of trauma” is a dysfunctional process that is part of what Samuel D. Gross described in 1862 as the “rude unhinging of the machinery of life” [24]. It is clearly distinct from the homeostatic hypercoagulable response that is most common in trauma patients [4,25–27] and the response observed in this present study. None of these pediatric patients demonstrated any clinical evidence of coagulopathy aside from elevated PT on admission, nor did they require transfusion of blood or blood products.

Another limitation involves the lack of standardized reference range data for the TEG in pediatric patients. There are multiple reagents (kaolin, celite, tissue factor) that could have been used to accelerate the clotting process or study a specific component of the clotting cascade. The only reference ranges that have been established for pediatric patients are for whole blood and kaolin- and celite-activated specimens [18,28,29]. This study utilized recalciﬁed citrated whole blood samples, which do not yet have an established reference range in pediatric patients. Also, TEG values have been shown to vary with age. Children are hypercoagulable relative to adults until approximately 2 years of age, after which point, no signiﬁcant differences have been observed [30]. The inclusion of the control group partially addressed this limitation, but it was based on the assumption that samples obtained from uninjured patients in the emergency room or prior to elective surgery are comparable to those in normal baseline conditions.

Previously, trauma-induced hypercoagulability had only been demonstrated in adults. In a prospective series of 69 adults, Kaufmann et al. demonstrated hypercoagulability by TEG in 65% in patients with an ISS of 10–20. Patients with a hypercoagulable TEG had a mean ISS of 29 and were more likely to require transfusion [3]. Schreiber and colleagues found that 62% of adult trauma patients were hypercoagulable on admission, which decreased to 26% by hospital day 4 secondary to decreased thrombin activation [4]. Although they noted signiﬁcantly more hypercoagulability in females relative to males, we did not detect this in our subjects. Decreases in the G value have been previously associated with increased morbidly and mortality in trauma patients in association with acute coagulopathy of trauma [20]. In this present study, we demonstrated significant increases in viscoelastic clot strength in pediatric trauma patients, probably secondary to alterations several clotting factors. In other recent studies, we have observed hypercoagulable conditions in adult burn patients [31], trauma ICU patients [32], pre-operative and post-operative cancer patients [33,34], and after placement of central catheters [35,36].

TEG provides an overview of the functional kinetics of clot formation and fibrinolysis, but is not speciﬁc for the cause of any coagulation changes. We measured the levels and kinetics of multiple clotting factors in order to better elucidate this mechanism. Significant elevations were found in prothrombin fragment 1 + 2 as well as plasminogen activator inhibitor-1. Elevations in F1 + 2 occur secondary to increased thrombin generation and have previously been described in association with evidence of hypoperfusion and deinhibition of fibrinolysis in critically injured patients, while PAI-1 is normally decreased in the setting of acute coagulopathy of trauma, resulting in hyperfibrinolysis [37]. Decreased activity of coagulation inhibitor proteins C and S was also noted. Activation of Protein C has previously been described in patients with acute coagulopathy of trauma [38]. Protein S, normally bound to C4b-binding protein, has been shown to exhibit enhanced anticoagulant activity in the presence of activated protein C [39]. Reduced activity of these two proteins in this study provides an explanation for the hypercoagulability found on TEG. Our patients also demonstrated thrombocytosis following injury, which may also account for the rapid clot polymerization seen in the children studied [40].

Further research is required in order to determine the clinical signiﬁcance of these ﬁndings. Thrombotic complications are extremely rare in children, with an incidence of 5.3 per 10,000 hospital admissions, but carry signiﬁcant morbidity [41]. In 18 patients with non-catheter related deep venous thrombosis, there was signiﬁcant inhibition of fibrinolysis on TEG as well as increased thrombin activatable fibrinolysis inhibitor (TAI) relative to age, sex, and race matched controls [41].

In conclusion, relatively minor injury (ISS = 9) induces a hypercoagulable state in children that can be detected with TEG. More work is needed to determine the functional signiﬁcance of these changes and to establish normal pediatric reference ranges.

Acknowledgment

We appreciate the efforts of our clinical research coordinator, Ronald J. Manning, BSN, MPH. We also acknowledge the nursing and administrative staff at Ryder Trauma Center for their cooperation and assistance with the patients and their families.

References