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### 14. ABSTRACT
Hemostasis is arguably the highest treatment priority for the polytraumatized patient. Best hemostasis practices cannot be developed if we lack a more full understanding of the cell-based clotting system during trauma. In order to address this fundamental knowledge gap, this study will provide a comprehensive characterization of the most important cellular component of the coagulation system, the platelet. The study proposes a comprehensive characterization of platelet function over time in civilian victims of polytrauma that may inform development of new diagnostic, therapeutic, and clinical guidelines for hemorrhage control and treatment of coagulopathy in trauma. We have successfully developed a comprehensive panel of tests to include: 1. Platelet aggregation by aggregometry under conditions of low and high shear stress and in response to collagen and ADP stimulation, 2. Platelet-associated thrombin generation using calibrated automated thrombography, 3. Platelet-induced clot contraction and using viscoelastic measures such as TEG with Platelet Mapping™ and, 4. Flow cytometry for platelet receptor and white cell activation as well as circulating platelet and platelet-monocyte aggregates. These panels are being coupled with a comprehensive data base of trauma victim demographics, diagnostics, and therapeutics to define the relationships between platelet function and injury pattern/severity, tissue hypoperfusion, transfusion, traumatic brain injury, inflammation, and clinical outcomes.

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Introduction:

Hemostasis is arguably the highest treatment priority for the polytraumatized patient. Best hemostasis practices cannot be developed if we lack a thorough understanding of the cell-based clotting system during trauma. In order to address this fundamental knowledge gap, this study will provide a comprehensive characterization of the most important cellular component of the coagulation system, the platelet. What is known of the functional implications of polytrauma on platelet function offers conflicting and paradoxical results that are not readily interpretable or useful. The study proposes a comprehensive characterization of platelet function over time in civilian victims of polytrauma that may inform development of new diagnostic, therapeutic, and clinical guidelines for hemorrhage control and treatment of coagulopathy in trauma. Specifically, clearly defined relationships between platelet function and survival, transfusion, and coagulopathy may be found and then used to enhance the care of the victim of trauma. Only then can specific therapies targeting the critical actions of platelets during polytrauma be rationally designed.

Body:

The objective of this proposal is to clearly define platelet function in the polytrauma victim and to understand the impact of injury and treatment on platelet function. We hypothesize that platelet function is altered during multisystem injury and has significant implications for hemostasis. The proposed study will build a clear foundation for the development of sensitive and specific diagnostics and therapeutics aimed at optimizing hemostasis and survival in the polytrauma victim.

The objective will be met by comprehensive testing of platelet and coagulation function in a prospective cohort of polytrauma patients presenting to the Virginia Commonwealth University Medical Center (VCUMC) using blood sampled serially over the first 72 hours of admission. In order to test the stated hypothesis, the following aims will be met:

Aim I. Determine platelet function in response to trauma using a comprehensive panel of tests to include: 1. Platelet aggregation by aggregometry under conditions of low and high shear stress and in response to collagen and ADP stimulation 2. Platelet-associated thrombin generation using calibrated automated thrombography (CAT). 3. Platelet-induced clot contraction and using viscoelastic measures such as TEG with Platelet Mapping™ and, 4. Flow cytometry for platelet receptor activation such as CD41, CD62p, and PAC-1 as well as circulating platelet and platelet-monocyte aggregates.

Aim II. Define the relationships between platelet function and injury pattern/severity, tissue hypoperfusion, hypothermia, acidosis, transfusion (including massive transfusion), traumatic brain injury, and clinical outcomes including need for immediate surgery, and survival.

No such comprehensive evaluation of platelet function in the context of multisystem trauma has been performed. In order to accomplish these aims, the second year of this two year effort has been mainly spent performing the following tasks:

1) Developing a new comprehensive data-base linking patient demographics, injury patterns, diagnostics, and treatments with comprehensive platelet evaluation.
2) Enrolling polytrauma subjects and healthy volunteers 24 hours/day, 7 days a week.
3) Analyzing all study samples.
4) Gathering clinical data.
5) Entering all data into database.
6) Initiating statistical analysis

Enrollment took place from the summer of 2011 until January of 2013. Careful targeted enrollment took place from the summer of 2011 to December 2011 to ensure proper assay development and execution consistency. Of special note was the perfection of the flow-cytometry analysis of platelet receptors which are key to understanding functions. This includes:

**Platelet markers:**
- CD41- Platelet marker
- PAC-1- Early platelet activation marker. Directed against the fibrinogen binding site exposed by a conformational change in the GPIIb-IIIa complex of activated platelets (only binds to activated platelets and not to resting platelets)
- CD62p- (P-selectin) tests for degranulated platelets due to activation.

Robust 24 hour/day and 7 day a week enrollment began in January of 2012 and ended in January 2013.

We have also trained to use flow cytometry to detect platelet aggregates. This is important since elevated circulating platelet aggregates can be seen in patients with thrombotic disorders. Elevated Platelet aggregates have been reported in patients with arterial insufficiency, hypoxemia, inflammatory bowel disease, and cerebral ischemia. We expect that detection of platelet aggregates will be important in understanding the interaction between the coagulation and inflammatory systems in response to trauma.

In regards to this we developed a flow-cytometry protocol to examine receptor biochemistry for white blood cells to include:
- CD45-Leukocyte marker
- CD14- Monocyte marker
- CD61-Platelet leukocyte aggregate marker
- CD62L- Inflammation marker
- CD11b- Inflammation marker
- CD19- B cell marker
- CD3- T cell marker
- CD69-cell activation marker
- CD4- T-helper cell marker
- CD25- T-regulator cell marker
- CD161- Natural Killer T cell marker
- CD8- Cytotoxic T cell marker
- CD56- NK cell marker
- CD16- Fc gamma receptor III-A marker

Lastly, we have saved plasma from all subjects for future analysis of cytokines (IL-6n and TNF-apha) and for proteomic analysis. We feel strongly the combination of functional coagulation assays coupled with dynamic receptor identification will offer the most comprehensive information allowing for the best chance of understanding platelet and coagulation function in response to trauma and treatment.
A total of 124 subjects were enrolled during the duration of this project. Sixty three subjects were enrolled on year 1 (2011) and 61 were enrolled on year 2 (2012). These include ten healthy volunteers and 114 trauma subjects.

While full data-analysis is in its initial stages, important noticeable trends have emerged. One of these are noted abnormalities in fibrinogen levels in >48% of subjects during the time period 24-72 hours after injury (48% at 24 hrs, 86% at 48 hrs and 97% at 72hrs). Fibrinogen levels in these subjects are noted to be > 400 mg/dl. Intriguing is the fact that initial fibrinogen levels are abnormal in 5% of subjects initially after trauma and at 8 hours but then become abnormally elevated from 24-72 hours. While low fibrinogen levels have been noted acutely in trauma subjects and are believed to contribute to trauma-induced coagulopathy, less attention has been paid to elevated fibrinogen levels after trauma. A possible explanation for this may include a transitioning to an inflammatory state after 8 hours.

These high levels of fibrinogen are of a concern since they will interfere with current viscoelastic tests of the contribution of platelets to whole blood clot formation (such as Hemodyne's platelet contractile force measurement and thromboelastography). The degree to which certain injury patterns as well as treatment regimens such as transfusion contribute to these findings has not yet been examined. We anticipate that the combination of additional coagulation testing that we have proposed in the study will create new knowledge in this area to help explain these observations.

Key Research Accomplishments:
- Development of comprehensive trauma demographic, injury, diagnostics, treatment, and coagulation data base using REDCap database technology [http://project-redcap.org/]
- Development of 24 hour/7 Day a week enrollment capabilities.

Reportable Outcomes:
- Plasma repository for future analysis of inflammatory markers that may be associated with platelet function and trauma induced coagulopathy.
- Creation of comprehensive trauma demographic, injury pattern, diagnostics, treatment, and coagulation data base using REDCap database technology [http://project-redcap.org/]
- Doctoral student (PhD candidate) supported with thesis subject matter being platelet function in the polytrauma patient.
- Grant award obtained (Prehospital Use of Plasma in Traumatic Hemorrhage) from the U.S. Army based on experience provided by this grant.
- Office of Naval Research subcontract grant from Entegron Inc obtained from program developing multifunctional resuscitation fluids based in part on experience provided by this grant.
- Grant award obtained (Studies of the Effects of Perfluorocarbon Emulsions on Platelet Number and Function in Models of Critical Battlefield Injury) from the U.S. Army based on experience provided by this grant.
Conclusion:
A comprehensive temporal analysis of platelet and coagulation is still lacking in polytrauma patients. The current project has now stopped enrolling polytrauma subjects and normal volunteers and is initiating data analysis which will provide unique insights into the relationship between traumatic injuries, therapeutics and the resulting coagulation function which may be heavily modulated by inflammation. New knowledge gained from this project may provide information resulting in the development of new diagnostic and treatment strategies that improve outcomes in the victim of polytrauma.

References:
None

Supporting Data:
None
Appendix I: Clinical Study Protocol

Specific Aims and Research Strategy

This application addresses the FY10DMRDP polytrauma and blast injury/hemostasis project/task area. Hemostatic function is impaired by multisystem injury and impacts survival. Platelet function is a key mediator of hemostasis but surprisingly remains poorly described in trauma. The objective of this proposal is to clearly define platelet function in the polytrauma victim and to understand the impact of injury and treatment on platelet function. We hypothesize that platelet function is altered during multisystem injury and has significant implications for hemostasis and damage control resuscitation/surgery. The proposed study will build a clear foundation for the development of sensitive and specific diagnostics and therapeutics aimed at optimizing hemostasis and survival in the polytrauma and blast-injured victim.

The objective will be met by comprehensive testing of platelet and coagulation function in a prospective cohort of polytrauma patients presenting to the Virginia Commonwealth University Medical Center (VCUMC) using blood sampled serially over the first 72 hours of admission. In order to test the stated hypothesis, the following aims will be met:

Aim I. Determine platelet function in response to trauma using a comprehensive panel of tests to include: 1. Platelet aggregation by aggregometry under conditions of low and high shear stress and in response to collagen and ADP stimulation. 2. Platelet-associated thrombin generation using calibrated automated thrombography (CAT) in platelet-rich plasma. 3. Platelet-induced clot contraction and effect on clot structure by platelet contractile force, clot elastic modulus, scanning electron microscopy (SEM), and TEG with Platelet Mapping™. 4. Flow cytometry for platelet activation and glycoprotein IIb/IIIa expression by CD41 and CD42b receptors and circulating platelet and platelet-monocyte aggregates.

Aim II. Define the relationships between platelet function and injury pattern/severity, tissue hypoperfusion, hypothermia, acidosis, transfusion (including massive transfusion), traumatic brain injury, and clinical outcomes including need for immediate surgery, and survival.

No such comprehensive evaluation of platelet function in the context of multisystem trauma has been performed. It is difficult to perceive how significant advances in the trauma hemostasis will be made without such a fundamental but comprehensive characterization. The knowledge gained will be used to inform the development of diagnostic and therapeutic strategies to mitigate the development of traumatic coagulopathy and guide the development of damage control resuscitation using platelet-based strategies.

Research Design and Methods

Experimental Design

This study will be a prospective cohort study measuring comprehensive platelet function in civilian trauma patients. The study will take place at the Virginia Commonwealth University Medical Center (VCUMC) in Richmond, Virginia, an American College of Surgeons designated level I trauma center with >3,000 annual trauma admissions. The study will be conducted by the VCU Reanimation Engineering Shock Center (VCURES) and will be coordinated by the departments of Emergency Medicine, Pharmacy, and Surgery. Polytrauma patients will be identified by trauma team activation criteria. Once enrolled, patients will undergo serial testing of comprehensive platelet function upon arrival and at 8, 24, 48, and 72 hours of hospitalization in order to characterize the platelet response to acute injury. Platelet function will be measured in
terms of adhesion, aggregation, thrombin generation, and clot contraction. Subjects will be stratified and compared according to injury severity, degree of shock, traumatic brain injury, and criteria for Disseminated Intravascular Coagulation (DIC) (Taylor, 2001) in order to define the effects of polytrauma, shock, and traumatic brain injury (TBI) on platelet function. Subjects will be enrolled in three phases:

1. Phase One: With IRB approval, a pilot study of up to 10 patients, lasting no longer than one month, will be used to refine laboratory testing protocols.
2. Phase Two: Open enrollment of up to 120 polytraumatized patients with planned interim analysis taking place at 25 subjects. Simultaneous recruitment of 30 healthy volunteers will also take place.
3. Phase Three: Supplemental enrollment of up to 50 additional patients if required in order to meet requirements for subgroup analysis.

Data Analysis
Outcome variables including measured platelet function tests, hemodynamics, transfusion requirements, and survival will be described using means and standard deviation for normally distributed data and median with range for significantly skewed data. To describe the acute platelet response to polytrauma and the following 72 hours, repeated measures ANOVA will be used to detect significant changes in platelet function over time (Aim I). In order to identify important relationships, mixed model ANOVA and correlation analysis will be used to analyze platelet function parameters in terms of: injury severity by ISS, tissue hypoperfusion by base deficit and lactate, TBI, transfusion, DIC, and clinical outcome variables (Aim II).

Indices of platelet function will be used as independent variables in logistic regression analyses to ascertain their significance as predictors of relevant clinical outcomes including transfusion requirements, DIC, and survival to hospital discharge. Significant univariate relationships will be further evaluated using multivariate regression adjusting for covariates including injury severity, shock severity, and TBI.

The specific interaction between injury severity and tissue hypoperfusion has been found to be significantly related to coagulation function in trauma (Brohi et al., 2007). In order to evaluate for interactions between the subgroups, mixed model two-way ANOVA will be used for four planned analyses. The combined effects of injury severity, tissue hypoperfusion, transfusions (such as massive transfusion protocols), and TBI (as an effect modifier) on platelet function will be determined as follows:

1. Injury severity by ISS quartile and presence or absence of tissue hypoperfusion by base deficit ≥ 6 and lactate ≥ 3.
2. ISS severity and TBI stratified by GCS of 3-8 (severe), 9-12 (moderate), and 13-15 (mild). For each GCS category subjects will also be classified according to an anatomic descriptor based on neuro-imaging studies as having either; hemorrhage (hematoma, contusion, subarachnoid hemorrhage), diffuse axonal injury, or cerebral swelling. (Saatman et. al., 2008)
3. GCS category by tissue perfusion category.
4. Volume and type of transfused blood products used (with and without activation of massive transfusion protocols) in order to highlight the effects of transfusion on platelet function.

At least 30 subjects in each ISS quartile, totaling 120 subjects, will be required to detect significant and relevant changes in platelet function, including platelet contractile force, clot strength by TEG, aggregation, and thrombin generation with 80% power. A small pilot study of 10 subjects will be used initially to refine laboratory platelet testing protocols. Open enrollment
will then take place until 25 subjects are enrolled, when interim analysis will be performed in order to evaluate data quality and mitigate any risk to the study population. Open enrollment will then continue and will be capped at 120 subjects. An additional selective enrollment period will then take place of no more than 50 subjects in order to ensure that the distribution of the ISS quartiles is sufficient to reflect the polytraumatized patient population. An ISS of $\geq 15$ will be used initially during enrollment to define polytrauma. A control group of 30 healthy subjects will also be recruited to establish normal ranges for our local population. Therefore, in order to meet all requirements, up to a total of 180 polytraumatized subjects and 30 healthy controls will be enrolled.

**Subgroup Analysis**

*Injury Severity, Shock Severity, Traumatic Brain Injury, DIC, and Transfusion*

In order to examine the effects of injury severity, shock severity, traumatic brain injury, and DIC on platelet function during polytrauma, subjects will be categorized according to the following criteria for additional analysis:

1. **Injury Severity:** The Injury Severity Score (ISS), which is an established method of estimating injury severity by both anatomic distribution and severity of individual injury, will be used to stratify subjects into quartiles for comparison. An ISS of $>15$ indicates severe injury and coincides with a sharp increase in mortality and will therefore be used as the lower limit to define polytrauma (Baker, 1974). This definition will preclude multiple minor injuries such as contusions and lacerations that would not truly represent polytrauma while still including the severely injured. Similar comparisons have been made between ISS and plasma-phase coagulation function in trauma (Brohi, 2003) and will therefore facilitate comparison of our results to published literature. ISS will be calculated by standard algorithm and will be updated each time blood samples are drawn as injuries are discovered and in order to account for evolving injury. Full knowledge of all injuries is required to accurately calculate ISS. In many cases we expect that all injuries will not be readily identified upon initial patient evaluation leading to inclusion of mildly injured subjects that do not fit the criteria of polytrauma. Therefore, initial inclusion will be based on risk of polytrauma by hemodynamics and known injury at the time of arrival. In order to ensure that the cohort is representative of polytrauma, an additional selective supplemental enrollment period will take place focusing on enrolling the most severely injured patients only in order to achieve adequate numbers to evaluate polytrauma. These additional subjects will ensure that the cohort will have both a wide distribution of ISS for evaluation of the effect of injury severity and an adequate number of severely polytraumatized subjects for analysis.

2. **Shock Severity:** Two redundant biochemical markers of shock severity or tissue hypoperfusion will be used to classify subjects. A Base deficit of $\geq 6$ by blood gas analysis will be used to identify the presence of hypoperfusion. Base deficit will be corroborated by a blood lactate concentration of $\geq 3$ mmol/l in order to verify the shock state. Those subjects meeting both criteria will be classified as having tissue hypoperfusion. These measurements are more predictive for significant tissue hypoperfusion than hemodynamic variables such as blood pressure (Parks et al., 2006, Paladino et al., 2008,) and the use of both base deficit and lactate will add much-needed redundancy to the estimate of shock severity. Subjects will be divided according to presence or absence of tissue hypoperfusion and platelet function will be compared in these two subgroups. In addition, presence or absence of tissue hypoperfusion will be used as a covariate when examining the relationship between ISS and platelet function.

3. **Traumatic Brain Injury (TBI):** Traumatic brain injury with coagulopathy is linked to increased mortality, (Saggar et al, 2009) and has been shown to directly alter platelet aggregation by
impacting the cyclooxygenase pathway (Nekludov et al., 2007). TBI has also been shown to modify the relationship between markers of tissue hypoperfusion and mortality during polytrauma (Siegel et al., 1990). Therefore, TBI severity is an important variable to consider when measuring the platelet response to polytrauma. We will evaluate the effect of TBI on platelet function using the Glasgow Coma Scale (GCS) which is a common clinical score that predictive of both anatomic brain injury and outcomes in closed head injury, (Wardlaw et al., 2002). GCS is routinely measured in all trauma patients and will be recorded at each sample time in order to track the degree of brain injury to determine the contribution of TBI to platelet function during polytrauma. For each GCS category subjects will be further classified according to an anatomic descriptor based on neuro-imaging studies as either; hemorrhage (hematoma, contusion, subarachnoid hemorrhage), diffuse axonal injury, or cerebral swelling. (Saatman et al., 2008) Subjects will be stratified by TBI category and compared for significant differences in platelet function tests. GCS will also be used as a covariate when examining the effect of tissue hypoperfusion and injury severity on platelet function in order to control for its possible confounding effects.

4. Transfusion: The specific effect of transfused blood products on platelet function remains unknown and may represent an important contribution to overall platelet behavior during polytrauma. In addition, the use of protocolized transfusion in the form of massive transfusion protocols is becoming more frequent. Data regarding the volume, timing, and type of blood products transfused for each patient will be recorded. Use of the VCUMC massive transfusion protocol will also be recorded. We will use this information to analyze the relationship between blood transfusion, transfusion protocols, and platelet function. Platelet function will be compared in transfused and non-transfused subgroups and in those requiring activation of the massive transfusion protocol. In addition, within the transfused group, the effect of the number and type of blood products transfused on platelet function will be evaluated using regression analysis.

5. Disseminated Intravascular Coagulation (DIC) Scoring: There is much debate on mechanism of TIC and whether or not it is a process that is mechanistically distinct from the diagnosis of DIC. Regardless of the terminology used, platelets appear to play a critical role in the development of coagulopathy during early trauma and the later syndrome of consumptive coagulopathy with microvascular thrombosis known as DIC. The presence of TIC or overt DIC are associated with significant increases in mortality in critically ill patients (Voves et al., 2006) and platelet counts less than 100 are criteria for diagnosis of DIC according to the International Society on Thrombosis and Haemostasis guidelines (Taylor et al., 2001). There is no consensus for the diagnosis or classification of TIC in regards to combined testing of platelets, fibrinogen, and fibrinolysis. Therefore, we will use the current ISTH DIC criteria in order to describe the onset of microangiopathic thrombosis with fibrinolysis in our patient cohort, while keeping in mind that the mechanisms underlying TIC and DIC may differ. In addition, platelet function has not been described even in terms of DIC. Therefore, we will conduct additional post hoc analysis in order to compare platelet function in those subjects meeting DIC criteria (as an established classification system) vs. those who do not. This information will offer new insight into the pathologic mechanisms of coagulopathy during trauma and critical illness and will suggest new treatment options based on platelet function.

Subject Population and Timeline
The subject population will consist of adult (age ≥18 years) trauma patients presenting to the VCUMC after traumatic injury. VCUMC is a large urban level I designated trauma center with over 3,000 annual trauma admissions. The Emergency Department sees approximately 90,000 patients per year mostly consisting of residents of the city of Richmond and surrounding counties. An active aeromedical transport program also transports trauma patients from central,
eastern, and southern Virginia. The patient population is similar to other urban centers with the majority (70%) being blunt injuries from motor vehicle collisions, falls, and assaults. However, penetrating trauma including gunshot wounds and stab wounds do also make up a notable percentage of all trauma (20%), and are primarily derived from the city of Richmond. The local Emergency Medical Services for the city of Richmond and the adjacent counties of Henrico and Chesterfield are provided medical direction by faculty of the VCU Department of Emergency Medicine resulting in all major trauma cases being directed to VCUMC. As many as 100 trauma patients are admitted per month with 1/4 having ISS>15, and 13% having TBI.

**Subject Recruitment**

Patients presenting to the VCUMC Emergency Department meeting immediate trauma team activation criteria will be screened for study inclusion. Those with obvious significant injury including multiple trauma, abnormal vital signs, hypotension, or closed-head injury will be enrolled.

**Inclusion Criteria:**

- Age ≥18 years
- Acutely injured (within 3 hours) patients meeting predetermined mechanistic, vital sign, and physical-exam related criteria for immediate trauma team activation in the Emergency Department.

**Exclusion Criteria:**

- Conscious, well-appearing patients who receive trauma team evaluation by mechanistic criteria only and have no evidence of injury with stable vital signs on initial evaluation.
- Pregnancy (confirmed with urine pregnancy testing)
- Documented do not resuscitate order.
- Intentional self-inflicted injury
- Recent (within 2 weeks) use of anticoagulants including heparins, aspirin, clopidogrel, prasugrel, or warfarin as confirmed by patient report or the medical record.
- Prisoners
- Non-English speaking
- Refusal to participate.

In order to ensure that subjects are enrolled and samples are collected as soon as possible from the time of injury, physical exam findings, vital signs, and patient history will be used as criteria for initial study inclusion. ISS will not be used as entry criteria because knowledge of all injuries is required in order to properly calculate ISS. This information is often not available until after advanced imaging or surgical intervention. Therefore, using readily available physical exam, vital sign, and mechanistic criteria that have already been predefined for trauma team activation will allow for rapid identification of potential patients and will prevent unnecessary delays in sample collection.

**Blood Sampling Protocol**

Blood samples for evaluation of coagulation and platelet function will be obtained from non-heparinized peripheral IV catheters or direct peripheral venipuncture serially during the first 72 hours of patient admission to the hospital. The first sample of no more than 25 milliliters of whole blood will be drawn into standard sodium heparin and sodium citrate (9:1 ratio of blood to citrate) vacutainers. Some of the blood will be immediately fixed for analysis by flow cytometry to include
CD41- Platelet marker
PAC-1- Early platelet activation marker. Directed against the fibrinogen binding site exposed by a conformational change in the GPIIb-IIIa complex of activated platelets (only binds to activated platelets and not to resting platelets)
CD62p- (P-selectin) tests for degranulated platelets due to activation.

We have also trained to use flow cytometry to detect platelet aggregates. This is important since elevated circulating platelet aggregates can be seen in patients with thrombotic disorders. Elevated Platelet aggregates have been reported in patients with arterial insufficiency, hypoxemia, inflammatory bowel disease, and cerebral ischemia. We expect that detection of platelet aggregates will be important in understanding the interaction between the coagulation and inflammatory systems in response to trauma.

Flow-cytometry protocols to examine receptor biochemistry for white blood cells include:

- CD45-Leukocyte marker
- CD14- monocyte marker
- CD61-Platelet leukocyte aggregate marker
- CD62L- Inflammation marker
- CD11b- Inflammation marker
- CD19- B cell marker
- CD3- T cell marker
- CD69-cell activation marker
- CD4- T-helper cell marker
- CD25- T-regulator cell marker
- CD161- Natural Killer T cell marker

The remaining samples will be used to perform a comprehensive evaluation of platelet function to include:

- Platelet adhesion and aggregation under high shear in response to collagen/ADP using the PFA-100 (Dade International) platelet analyzer.
- Platelet aggregation in whole blood by impedance in response to collagen under low shear by aggregometry (Chronolog Whole Blood Aggregometer)
- Platelet-mediated thrombin generation using platelet-rich plasma standardized to platelet count in response to tissue factor stimulation by the Calibrated Automated Thrombogram (CAT) (Thrombinoscope Inc.)
- Platelet contractile force and clot elastic modulus of whole recalcified blood by the Hemostasis Analysis System™ (Hemodyne Inc.)
- The contribution of platelets to the whole blood clotting process and clot contraction by the TEG Platelet Mapping™ assay. (Haemoscope)
- Platelet-poor plasma will be assayed for prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen levels using the START-4 coagulation analyzer (Diagnostica Stago).
• Additional platelet-poor plasma will be stored at -80°C in order to obtain measurements of thrombin-antithrombin complex (TAT), specific coagulation factors and markers of thrombin generation including thrombin/antithrombin complex, and D-Dimer/fibrin degradation products (FDP) by enzyme-linked immunoassay (ELISA) for DIC scoring (Taylor 2001).

• Complete blood count with differential including platelet counts.

We will save plasma from all subjects for future analysis of cytokines and other mediators allowing for comprehensive information allowing for the best chance of understanding platelet and coagulation function in response to trauma and treatment.

All of the above tests will be performed in triplicate and will require at least 21.5 ml of whole blood to be drawn at each sample time. This volume is well within the allotted 25 ml maximum blood volume designated for each blood draw.

**Timing of Samples**

The initial blood sample of 25 ml will be drawn within 30 minutes of patient arrival to the Emergency Department. A second sample will be drawn at 8 hours after arrival in order to further characterize the platelet response to acute resuscitation, operative intervention, and massive transfusion protocols. Three additional 25 ml samples will be drawn at 24, 48, and 72 hours from time of arrival in order to facilitate the identification of significant trends in platelet function during the acute phase of injury and resuscitation.

**Clinical and Laboratory Data**

Demographic and descriptive data will be abstracted from the medical record. This data will be used to stratify groups according to severity of injury, degree of tissue hypoperfusion, and degree of head injury. In addition, possible modifiers of platelet function including blood pH, body temperature, and cell counts will also be collected in order to examine their influence on platelet function during trauma. Clinical data to be collected will include:

- Age, sex, height and weight.
- Mechanism of injury (e.g. blunt vs. penetrating).
- Vital signs to include blood pressure, pulse, temperature, respiratory rate, and oxygen saturation by pulse oximetry at the time of blood sampling.
- Pre-admission use of aspirin, non-steroidal anti-inflammatory medications, warfarin, heparins, or specific antiplatelet drugs.
- Major injuries as identified by physical examination, computed tomography, or during surgical intervention by anatomic distribution for calculation of the Injury Severity Score (ISS).
- Significant past medical history to include history of coronary artery disease, cerebrovascular accident, diabetes mellitus, or coagulopathy, medication history and medications used during current hospitalization.
- Volume and type of blood products transfused and crystalloid resuscitation fluids administered.
- Glasgow Coma Scale and presence/type of any significant intracranial injury.

Standard laboratory tests that are obtained on all trauma patients will also be abstracted prospectively from the available electronic medical record. VCUMC maintains an advanced integrated medical record that will greatly facilitate collection of clinical and laboratory data.
The current VCUMC laboratory testing protocol for all trauma-alerted patients upon arrival to the Emergency Department consists of:

- Basic metabolic/electrolyte profile
- Complete cell count with differential and platelet count.
- Venous blood gas analysis with base excess.
- Lactate concentration
- Prothrombin time (PT)
- International Normalized Ratio (INR)
- Activated partial thromboplastin time (APTT)
- Blood typing
- Serum alcohol level
- Serum toxicology screen for drugs of abuse.

Subsequent blood samples taken at 8, 24, 48, and 72 hours will be matched to standard laboratory test results that are abstracted from the medical record that have taken place within 2 hours of the blood draw. Clinical data to be collected at each follow-up blood draw will include:

- Vital signs with temperature.
- Medications given including type and total dose of vasopressors administered.
- Number and type of blood products transfused including activation of massive transfusion protocol.
- Total amount of intravenous crystalloid or colloid fluids administered.
- Major interventions including intubation, tube thoracostomy, laparotomy, or thoracotomy.
- Type of operative intervention to include damage-control methods.
- Dosage of any hemostatic agents administered including activated Factor VII.
- Dosage of any anticoagulants including subcutaneous heparin for deep vein thrombosis prophylaxis.
- Identified injuries by anatomic location for serial calculation and update of ISS.
- Sequential Organ Failure Assessment (SOFA) scoring

Summary

Hemostasis is arguably the highest treatment priority for the polytraumatized patient. Yet, little can currently be done to quickly recognize and treat internal hemorrhage and coagulopathy. A primary reason for the lack of viable treatment options is our lack of understanding of the cell-based clotting system during trauma. In order to address this fundamental knowledge gap, this study will provide a comprehensive characterization of the most important cellular component of the coagulation system, the platelet. What is known of the functional implications of polytrauma on platelet function offers conflicting and paradoxical results that are not readily interpretable or useful. It is clear that little progress can be made in this area without better fundamental knowledge of platelet behavior.

The comprehensive characterization of platelet function during polytrauma provided by this study will inform development of new diagnostic, therapeutic, and clinical guidelines for hemorrhage control and treatment of coagulopathy in trauma. Specifically, clearly defined relationships between platelet function and survival, transfusion, and DIC can be used to update and improve current physiologic and clinical scoring methods so to better anticipate outcomes in trauma. The information regarding changes in platelet function will also provide specific targets for further study. Preclinical model development of traumatic coagulopathy will also benefit. Our results will allow for platelet dysfunction to be incorporated into these models, thus increasing their relevance. These models will then be able to provide important platforms for further testing.
of specific diagnostics and treatments. Only then can specific therapies targeting the critical actions of platelets during polytrauma be rationally designed.