Activation of Coagulation & Inflammation in Trauma

ACIT II – Version 1.5; 20th Sept 2008

Royal London Hospital

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Synopsis

The Activation of Coagulation & Inflammation in Trauma (ACIT) study is designed to identify the clinically significant mechanisms and pathways by which the inflammatory and coagulation pathways are activated immediately following major trauma, how they lead to clinical coagulopathy and transfusion requirements, produce organ injury, and how they affect outcome in terms of organ failure and death.

Severe trauma patients will be enrolled into the study in the emergency department. Blood samples will be collected immediately and over the following three day period to characterise the nature, extent and duration of the response of the inflammation and coagulation systems to trauma. Samples will also be processed for DNA banking to assess the impact of population genetic factors to the response to trauma.

A subset of patients who are intubated, ventilated and sedated will have bronchial washing specimens analysed for markers of acute lung injury.

Clinical data on blood product transfusions, organ system failure, morbidity, ICU & hospital stay and mortality will also be collected up to day 28.

Study Aims & Hypotheses:

Aim 1: Coagulopathy and Massive Transfusion
To identify the key derangements in coagulation, fibrinolytic and endothelial cell function following trauma, determine the response to blood component therapy and identify and characterise the subsequent hypercoagulable state.

> Hypothesis ACIT: 1A
> Acute traumatic coagulopathy is caused by tissue hypoperfusion through the systemic activation of anticoagulant and fibrinolytic pathways.
>
> Hypothesis ACIT:1B
> Subsequent transfusion of red cells and blood component therapy has specific effects on the acute coagulopathy, which may be beneficial or harmful dependent on the current clinical state.
>
> Hypothesis ACIT: 1C
> Early coagulopathy leads to exhaustion of the anticoagulant system and up-regulation of antifibrinolytic systems, resulting in a hypercoagulable state.

Aim 2: Development of Organ Injury
To elucidate the effect of derangements in coagulation, fibrinolytic and endothelial cell function on the inflammatory response and the development of acute lung injury, multiple organ failure (MOF), and death.

> Hypothesis ACIT: 2A
> There is a dose-dependent effect of the severity of trauma on coagulation, fibrinolytic and endothelial cell function. These correlate with activation of a pathological systemic inflammatory response which leads to acute lung injury.

Hypothesis ACIT: 2B

There is a dose-dependent effect of the degree and duration of tissue hypoperfusion on coagulation, fibrinolytic and endothelial cell function. These correlate with activation of a pathological systemic inflammatory response which leads to acute lung injury.

Hypothesis ACIT: 2C

While tissue trauma (ACIT:2A) and cellular hypoperfusion (ACIT:2B) are different initiators, the resulting activation of the coagulation and inflammatory systems is identical and is the final common pathway in acute lung injury. Tissue trauma and cellular hypoperfusion have an additive effect on the development of ALI. The acute lung injury caused by tissue trauma and tissue hypoperfusion can be temporally separated.

Aim 3: Prediction model

To develop a prediction model for massive transfusion requirements and the development of organ injury following trauma.

Hypothesis ACIT:3A

Massive transfusion requirements can be predicted by initial physiological variables and immediate analysis of coagulation parameters. Conversely, the requirement for blood component therapy might be reduced by targeted measurement of coagulation function and biomarkers during transfusion.

Hypothesis ACIT:3B

Acute lung injury / ARDS and Acute Renal Injury can be predicted in the first hours after trauma based on trauma severity scores, tissue damage, severity and duration of tissue ischemia, with biochemical markers of coagulation or inflammation. Identify specific markers which may be clinically relevant.

Aim 4: Proteomic, analysis

To process and store samples for subsequent proteomic and genomic techniques to identify new loci for investigation, targeting drug discovery and identification of genetic susceptibility to poor outcome following trauma.

Aim 5: Trauma DNA Bank

To process and store samples for subsequent DNA typing and analysis. There appears to be a background race and genetic susceptibility to the effects of trauma. These alterations may well lie within the coagulation and inflammatory systems. Early identification of patients at risk may, in the future, allow therapy to be targeted depending on patients' racial background or even specific genetic make-up.

Hypothesis ACIT:5A

There are genetic mutations of coagulation and inflammatory genes (Factor V Leiden, Prothrombin 20210, Mannose Binding Lectin) that may protect against or increase susceptibility to the effects of tissue trauma and hypoperfusion.

Hypothesis ACIT:5B

There are Haplotype-specific (and thus race-related) variations in susceptibility and response to tissue trauma and hypoperfusion.

Background:

Over the past 100 years, advances in emergency medical systems, trauma surgery and trauma resuscitation have allowed patients who would otherwise have died on the streets to reach hospital and receive emergent treatment of their life-threatening injuries.

Patients who are bleeding may develop a disorder of the blood clotting system which leads to further bleeding, shock and potentially irreversible physiological derangements that lead to death. The nature of the clotting disorder is currently unclear, and there are no tests that have sufficiently characterised it. As such it is currently impossible to immediately assess the nature or degree of derangement of the clotting systems (coagulopathy) and no tools to guide therapy. Many patients who are bleeding get inadequate numbers of blood products. Conversely patients may be given blood products unnecessarily leading to all the complications associated with blood transfusion, including depression of the immune system, which is critical in major trauma patients.

Although they may survive this critical phase of their care, many of these patients will still die. Death, which usually occurs one to six weeks later, is due to a progressive failure of body systems – a syndrome called multiple organ failure. There is currently no specific treatment for multiple organ failure. Patients are supported on ventilators, dialysis machines and other organ support devices while the process runs its course. Patients who survive multiple organ failure may spend months in hospital, years in rehabilitation, and are usually left with some permanent disability.

Recent studies suggest that this late mortality due to multiple organ failure may be due to the body's responses to tissue damage and to blood loss that occur immediately following injury. There is a significant body of both basic science and clinical evidence that implicates the activation and dysregulation of the coagulation and inflammatory systems in the development of multiple organ failure. However, most of this data comes from research into sepsis. The mechanisms for the activation of the relevant pathways in trauma, and their relationship to clinical disease and outcomes have yet to be delineated. Identification of these key pathways will provide new directions for drug development and perhaps a specific treatment for post-traumatic multiple organ failure.

We postulate two mechanisms for the activation of these systems in trauma: tissue damage itself, and cellular hypoperfusion.

Tissue damage

Recently, two studies, the first from our group at the Royal London Hospital, have shown that trauma patients may arrive in the emergency department with severely deranged blood coagulation[1,2]. Patients with coagulopathy were three to four times more likely to die than those without. The incidence of coagulopathy was closely related to the severity of injury, and not to the volumes of fluid administered, suggesting that the injury load itself was

responsible for the activation of the coagulation systems. The mechanisms by which tissue injury activates the coagulation and inflammatory systems have not been previously studied.

Tissue hypoperfusion / hypoxia

Ischemia following hemorrhagic shock is known to lead to multiple organ failure and increased mortality. Several studies have shown that the severity of shock on admission correlates with eventual outcome. One of us (Karim Brohi) has recently finished a study examining the duration of tissue ischemia, as measured by base deficit and lactate, and found that even when sub-clinical tissue ischemia persists for over 12 hours, mortality is 38%, over twice that of patients who do not suffer a prolonged ischemic episode. Tissue hypoxia leads to endothelial injury and priming of cellular and humoral components of the inflammatory pathways.

Goals and Expected Outcomes

The entire pattern of activation of the inflammatory and coagulation systems has not been fully elucidated in trauma patients, and it remains unknown if and how this results in multiple organ failure and death. We hope that this study will allow us to fully characterise the coagulopathy of trauma and allow us to better target blood and component therapy by identifying clinically useful markers of early coagulopathy and the response to transfusion. Conversely, we expect that identifying those patients without coagulopathy might suggest that specific measures of coagulation during a massive transfusion would lead to a reduction in the total number of blood products transfused and a reduction in the number of complications.

We further hope that we will identify key junctures in the pathways that could be targeted for drug discovery and development programs. This will allow us to better understand which patients may benefit from the newer procoagulant agents such as recombinant factor VIIa. Further, it may allow us to intervene early to avoid the late complications of multiple organ failure. There has already been some headway in this area in the field of sepsis research. The anticoagulant activated Protein C (dotrecogin alpha) has recently been introduced and is the only pharmacological therapy that has shown to be effective in reducing mortality in severe sepsis. The final common pathways of sepsis induced multiple organ failure may be similar to those of tissue hypoperfusion.

Study Design

Prospective cohort multicentre observational study

The study will follow the clinical course of trauma patients on admission to the emergency department and for the next five days. Blood samples and, in some patients, bronchial washing samples will be analysed for markers of activation of the coagulation and inflammatory systems. These will be correlated with their injuries, their resulting physiological disturbances and their subsequent clinical course and outcome.

Blood sampling

Trauma patients will have blood samples drawn to measure markers of coagulation, fibrinolysis, endothelial activation and inflammation. Samples will be collected in the

emergency department at 0, 24 and between 60 to 72 hours post admission. Patients who are actively bleeding in the emergency department will have additional samples taken after administration of the 4th, 8th and 12th units of blood products, if used.

Bronchoscopy and lavage

Patients who are sedated, intubated and mechanically ventilated will have bronchoscopy and bronchial washing specimens sent for markers of acute lung injury. Samples will be collected within the first 24 hours, and subsequently at 48 and 72 hours, unless the patient is extubated before this time.

Data will also be collected on:

Patient demographics, mechanism of injury, injury type and severity, degree and duration of shock and tissue hypoperfusion, incidence and severity of organ dysfunction. These data are routinely collected in the management of injured trauma patients. No additional monitoring or interventions will be required.

Patient questionnaires

Quality of life assessment will be measured by the EuroQol EQ-5D questionnaire at hospital discharge or Day 28 and again at one year following injury. Health economic implications of massive transfusion and resource use will be collected with an additional questionnaire at the one year follow up. The in hospital questionnaire will be given to the patient and can be completed in under five minutes.

The 12-month questionnaire will be posted out to surviving patients along with a return stamped addressed envelope. The EuroQol EQ-5D questionnaire has been previously administered over the telephone, but responses can differ from those given when the patient is allowed to complete the questionnaire themselves. In addition patients will be required to answer questions on visits to outpatient clinics and GPs which is not practical in a telephone interview. Completion of the whole questionnaire will take approximately five minutes. Patients not responding within two weeks of the initial request will be telephoned as a reminder to complete the questionnaire. Confirmation with the GP and scrutiny of the hospital care record system will ensure only those patients alive at 12 months receive a questionnaire.

Outcome measures

Primary

Aim 1: Blood products transfused in the first 24 hours
Aim 2: Incidence & severity of acute lung injury & multiple organ failure

Secondary

28-day mortality, ventilator free days, ICU stay, hospital stay, blood transfusions in first 24 hours, number of infections

Procedures

Blood samples

Trauma patients will have blood samples drawn to measure activation of coagulation, fibrinolysis, endothelial injury and inflammation. Samples will be drawn at 0, 12 and between 60 to 72 hours post admission. Patients who are actively bleeding in the emergency department will have additional samples taken after administration of the 4^{th}, 8^{th} and 12^{th} units of blood products, if used. Each sample is 20mls.

Severe trauma patients not enrolled in the study would normally have blood samples drawn at minimum at 0, 12 and 24 hours and daily thereafter. Most trauma patients have blood tests drawn more frequently, especially those who have signs of hypoperfusion, coagulopathy, on-going blood loss or those that are mechanically ventilated. As most major trauma patients have an arterial or central line placed, most blood draws are not painful to the patient. Whenever possible we will coordinate our blood draws with those of clinical need, to reduce the number of needle-sticks. We will not be able to use blood that has already been collected and placed into specimen tubes as our samples will require specific handling and processing.

Coagulation markers to be tested will include: Prothrombin fragments 1+2, Protein C, Endothelial Protein C Receptor (EPCR), Thrombomodulin, Tissue factor, Plasminogen Activation Inhibitor-1 (PAI-1), tissue Plasminogen Activator, Tissue factor Activatable Fibrinolysis Inhibitor (TAFI), D-Dimers. Markers for endothelial injury will include von Willibrand Factor (vWF) and E-selectin.

Inflammation markers will include TNF-α, IL-1,IL-6,IL-8,IL-10, Complement components, heat shock proteins. Samples will also be processed and stored for DNA and proteomics analyses.

Rationale for sequential blood sampling

1. The activation pattern of coagulation following trauma is a dynamic process and the full picture will not be apparent on a single blood draw immediately following injury. Some coagulation factors are used up and the levels of others increased with increased genetic expression. Many trauma patients exhibit a hypercoagulable state in the later stages of injury [6]. How this change occurs is currently unknown, but would be identified by this protocol. This would have major implications for the treatment of coagulopathy – especially new therapies such as activated Factor VIIa.

2. A prolonged shock state has a known poor outcome[7]. This may be due to continued activation of the coagulation and inflammation systems. Further a procoagulant state has been identified late in severely injured trauma patients. Serial measurements will allow us to detect these changes and correlate them with injury and physiological factors.

3. Medical therapy in terms of fluid and blood transfusions may affect these processes – but to what degree and in which direction is currently unclear. Trauma patients receiving more than two blood transfusions are known to have a worse outcome[8]. Serial sampling will allow

us to assess the impact of fluid and blood resuscitation on the coagulation and immune systems.

Bronchoscopy & Lavage

Patients who are intubated and sedated will have bronchial washing specimens sent for markers of acute lung injury within the first 24 hours, and subsequently at 48 and 72 hours following emergency department arrival (provided they remain intubated for this time). This involves passing a fine tube through the breathing tube of a patient, injecting a small volume of saline and re-aspirating the fluid. A 50mls (three tablespoons) sample is adequate for the lavage sampling and a maximum of 150mls of saline (in 50ml aliquots) will be instilled to produce this return.

Bronchoalveolar lavage is a standard procedure on many intensive care units for the diagnosis of ventilator associated pneumonia and is similar to tracheal suctioning that is performed on all intubated patients multiple times a day. It carries minimal morbidity. The main risk of the bronchial catheter is a transient episode of mild hypoxia. We will minimise risks of the procedure by excluding patients who require high oxygen concentrations (receiving 80% oxygen or greater). Patients will be suctioned and then placed on 100% oxygen for five minutes prior to and during the procedure the procedure. If there is any significant decrease in oxygen saturation, the procedure will be discontinued (after suctioning of any remaining saline).

Experience from the Royal London intensive care unit and elsewhere[9] demonstrates that bronchoscopy and lavage can be safely performed on these trauma patients with no significant episodes of hypoxia. The alternative procedure of bronchial aspiration through a catheter has been demonstrated to be inadequate for the investigation of acute lung injury[10]. Bronchial washing will be performed by senior intensive care staff or trained members of the research team proficient in the technique.

As these patients are sedated for the purposes of mechanical ventilation, patient discomfort will be minimal. Consent for bronchial washing specimens will be in addition to the standard consent for the blood sampling, and patients' families may opt out of this segment of the study if they so wish, while still consenting to the rest of the study protocol.

Markers of Acute Lung Injury (Bronchial washing specimens) will test for the following: Cell count, differential, total protein, PC, EPCR, TF, TFPI, PAI-1, IL-6, Endothelial: vWF, Epithelial: HT156, PCP-III, SP-D, KL-6, PBEF, and samples stored for genomics/proteomics study.

Risks & Benefits

Risks

All parts of the study will be carried out to avoid patient risk and minimise discomfort at all times. At no time will patient care be compromised or delayed for the purposes of the study. The risks of blood sampling are limited to some potential bruising at the site of venepuncture, and discomforts are limited to needle puncture (where no arterial line is already in place).

Bronchoscopy is a safe procedure with a complication rate of less than 1 in 1000. The complication rate is lower in patients who have a protected airway, as in our study. Most complications are a transient episode of hypoxemia (low blood oxygen level). Rarer complications include infection and airway abrasions. Complications of bronchial lavage will be minimised in the study population as we are limiting it to sedated patients who are intubated and on controlled mechanical intervention, with full cardiorespiratory monitoring. Patients with high oxygen requirements will be excluded from the procedure, and additional oxygen will be given to all patients before and during the procedure. If there is an episode of hypoxemia the procedure will be terminated and normal ventilation continued with higher oxygen concentrations as required.

We will record all adverse events associated with the study and review them as they occur, and collectively at monthly intervals.

Participation in research may involve some degree of loss of privacy. However this risk will be minimised by our data protection methods and we are not performing any tests that might subsequently result in significant personal, financial or social risk to the research subjects. We will make every effort to ensure that our data is secured and patients' privacy is protected.

Benefits to subjects

None

Potential benefits to society

Trauma remains the leading cause of death in patients between 1 and 45 years of age, and is the fifth most frequent cause of death overall. The World Health Organization predicts that by 2020 road traffic accidents alone will be the third leading cause of death worldwide. In general, injured patients die either from major hemorrhage, traumatic brain injury or multiple organ failure. Patients who die from hemorrhage or brain injury do so within the first few days following injury. For those patients who die after the first 24 hours, 60% will die of multiple organ failure. Those patients with multiple organ failure who do not die have extensive intensive care and hospital stays and are very expensive in terms of cost, resources and personnel.

It is clear from several studies that the outcome of trauma patients is determined in the first few hours following injury[4,5]. However we currently have very little understanding of the processes at work during this early injury period. We currently have an almost total lack of understanding of the initiation and progression of activation of the coagulation and inflammation systems, and how they lead to multiple organ failure – questions this study is designed to investigate.

Trauma patients tend to be young, active members of society, often with good jobs and young families to support, who are essentially 'cut down in their prime'. We hope that this study will allow us to identify specific points in the genesis of multiple organ failure that may be

used to target interventions in the future, and hence reduce this huge burden of death and disability.

Risk/benefit analysis

Although the study carries no benefit to the subjects, the bulk of the study is observational, and interventions that are carried out are routine, carry small or minimal risk, and the study has been designed to reduce discomfort and risks to the study subjects.

Subjects

Estimated number of subjects to be enrolled: 500

The study population will include adult trauma patients admitted to the Royal London Hospital via the emergency department. Only patients who have a trauma team activation will be considered for enrolment into the study. Only sedated, intubated and mechanically ventilated patients will be considered for the collection of bronchoscopic lavage specimens.

As the study is investigating the immediate post-injury phase, patients will be recruited into the study before their full list of injuries is known (before they have X-rays, CT scans, angiograms, operations etc). From our trauma database at the Royal Hospital we know that using all major trauma activations, with the exclusion criteria listed below, will provide us with a study population with an injury severity distribution of approximately 35% severe injuries, 55% moderate injuries and 7% minor injuries.

The study will, of necessity, include trauma patients who are unable to give consent for themselves. The study may also include patients whose first language is not English. There is some evidence that patients from different racial groups have altered responses to injury, and excluding them would bias the study in favour of native English-speaking populations.

Inclusion criteria

Adult trauma patients admitted via a trauma team activation to the emergency department at the Royal London Hospital.

Exclusion criteria

- Age < 16
- Patients transferred from other hospitals
- Patients presenting more than 120 minutes after time of injury.
- Patients who have received more than 2000mls of intravenous fluids prior to emergency department arrival.
- Patients with burns > 5% of their body surface area.
- Patients taking anticoagulant medication other than aspirin (<650mg/day).
- Patients with a known bleeding diathesis.
- Patients with moderate to severe liver disease (Child's classification B or C3).

Consent

Informed Consent will be obtained by the chief investigator or co-investigators. If patients are deemed unable to consent for themselves a legally authorised representative will be

asked to give permission to enroll the patient into the study. For the first blood draw, as part of the trauma team resuscitation, this will be the Trauma Team Leader who is independent from the research protocol.

This research study focuses on the very early post-injury phase (time of injury and first few hours), and is investigating the long-term effects of injury and physiological derangements seen at this time. The majority of severely injured trauma patients are either unconscious from a traumatic brain injury or hypovolaemic shock, intubated in the prehospital phase of their care or intubated acutely in the emergency department. These patients are the core of the research study as it is patients with severe injury who will go on to develop multiple organ failure. Patients who are not unconscious have recently been through a major psychologically disturbing event, may have been a victim of violence, and are often in pain. As such they may be unable to comprehend, or it may be inappropriate to discuss, the details of a complex research trial at this time.

All trauma patients routinely have bloods drawn in the resuscitation room as part of their standard evaluation, and this study will induce no extra stress or morbidity except for the extra volume of blood drawn at this time. The patient population is unlikely to have complications related to the extra volume of blood drawn. The 20ml blood draw (equivalent to four teaspoons), is minimal compared to the total blood volume (~5 litres). Additionally, intravenous fluid and blood transfusion is commenced as soon as patients arrive in the emergency department. Blood sampling will not delay this, or any other therapy.

Where patients are awake and have relatively minor injuries, such that they would be able to comprehend the research protocol and its implications, we would consent them as soon as possible in the emergency department. However, as we are able to use blood from the initial draw for trauma management, we are still requesting a professional legally appointed representative consent to take this sample. Consenting a patient in the initial phases of trauma evaluation would be difficult and would seriously compromise patient care.

If a patient remains unidentified, the police and hospital social workers will continue to assist the investigator in identification of the patient. Daily attempts to locate family to discuss the patient's condition and study involvement will be made. Documentation of these attempts will be made in the patient's medical record.

When a personal legally authorised patient representative is found, all study procedures already performed and yet to be completed will be explained, and their consent for continued participation requested. They will also be informed that they have the right to deny continued participation. For patients who are sedated and ventilated, the legally authorised representative will also be asked to give written consent for bronchoscopy and bronchial lavage samples.

The patient will be examined regularly to determine if and when he/she is able to consent for himself/herself even if surrogate consent has already been obtained. While the duration of unconsciousness for trauma patients is very variable, the majority will regain consciousness in 2-10 days. At this time, the trial and all study procedures - performed and yet to be completed - will be explained to the patient. Again, patients will be given the option to give consent to continue participation or to withdraw from the study.

A quarterly report will be sent to the LREC regarding the Consent Process. It will include the number of subjects entered in the study, the number of subjects for who consent was obtained prior to entry, the number of subjects for whom consent was waived, the number of subjects or surrogates who later refused or agreed to continue in the study, and ongoing study results available.

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