Activation of Inflammation and Coagulation after Trauma II (ACIT II)

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2. GLOSSARY of Terms and Abbreviations

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
DMC	Data Monitoring Committee
EC	European Commission
GAfREC	Governance Arrangements for NHS Research Ethics Committees
ICF	Informed Consent Form
JRMO	Joint Research Management Office
NHS REC	National Health Service Research Ethics Committee
NHS R&D	National Health Service Research & Development
Participant	An individual who takes part in a clinical trial
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SOFA	Sequential Organ Failure Assessment
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

3. SIGNATURE PAGE

Chief Investigator Agreement

The clinical study as detailed within this research protocol **(Version 8.0 16.07.2024)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Chief Investigator Name: Dr Ross Davenport

Chief Investigator Site: Royal London Hospital, Bart's Health **Signature and Date:**

16/07/2024

Principal Investigator Agreement *(if different from Chief investigator)*

The clinical study as detailed within this research protocol **(Version 8 16.07.2024)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

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4. SUMMARY/SYNOPSIS

Short Title	*ACIT II*
Methodology	*Multicentre, prospective observational cohort study*
Research Sites	*Royal London Hospital, Bart's Health NHS Trust* *John Radcliffe Hospital, Oxford University Hospitals NHS Trust* *Royal Victoria Infirmary, Newcastle Upon Tyne Hospitals NHS Trust* *Salford Royal Hospital,* Salford Royal NHS Foundation Trust
Objectives/Aims	A prospective, observational cohort study designed to investigate the molecular mechanisms activated by traumatic injury and how these relate outcome and the effect of early therapies to treat trauma haemorrhage.
Number of Participants/Patients	*5,000 patients*

Inclusion Criteria	*All trauma patients who either:* *(1) Receive prehospital treatment by London Air Ambulance (Bart's Health) and are admitted to a study site hospital (adults only)* *(2) Admitted to a study site hospital and require a full trauma team activation e.g. conveyed by a land ambulance service or self-presentation to the emergency department (adults and children)*
Exclusion Criteria	Any patient who fulfils any of these criteria will not be considered for inclusion in the study: • Transfers from other hospitals • Burns >5% total body surface area • >120 mins have lapsed since time of injury • Deemed inappropriate for recruitment by an independent clinician (e.g. mass casualty event or futility)
Statistical Methodology & Analysis	Not applicable
Proposed Start Date	With immediate effect after ethical (REC) approval of substantial protocol amendment to version 8.0 on 16th July 2024 (ACIT II study has been active since January 2008)
Proposed End Date	Open ended
Study Duration	Ongoing

5. INTRODUCTION

5.1 Background

Trauma remains one of the world's biggest contributors to the global burden of disease, and is increasing in both incidence and severity, with over 6 million deaths worldwide as the result of injury.(1) It is the leading cause of death in those under the age of 45 years, although as the population ages it is increasingly affecting the elderly. Historically, uncontrolled haemorrhage, traumatic brain injury, and multiple organ failure have been the major causes of mortality and for the most part this remains unchanged. Of these, uncontrolled haemorrhage is the leading cause of preventable death.(2) 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. More recently there has been an explosion in our understanding of trauma sciences with significant advances made over the last decade in managing bleeding. However to date there no cure for organ failure, only supportive care. There remains much to be discovered if the trauma community is to further improve outcomes from injury and address the adverse sequela of immune dysfunction and bleeding in order to create new survivors.

Patients who are bleeding develop a disorder of the blood clotting system which leads to further bleeding, shock and potentially irreversible physiological derangements that lead to death.(3) Using early ACIT II data we have been able to characterise some elements of the global clotting disorder known as trauma-induced coagulopathy (TIC)(4-7) but many more questions remain unanswered e.g. how many different patterns of bleeding disorder exist after trauma, how they arise and what are the best diagnostic tests for rapid identification. Preliminary findings from the ACIT II have highlighted four clinically important bleeding phenotypes but currently it is impossible to fully stratify patients, assess the entire derangement in clotting or degree of dysfunction. We have shown through the introduction of viscoelastic haemostatic assays such as Rotational Thromboelastometry (ROTEM), some elements of ATC can be detected rapidly but these assays are unable to provide a complete picture of clotting with platelet function and clot breakdown pathways poorly quantified by this technology.(4, 8) As such there remains only limited tools to guide transfusion therapy. We have shown that many patients who are bleeding continue to get inadequate numbers of blood products to correct coagulopathy.(9) Conversely patients may be given blood products unnecessarily leading to all the complications associated with blood transfusion, including depression of the immune system,(10) 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 dysfunction syndrome (MODS).(11) There is currently no specific treatment for MODS. Patients are supported on ventilators, dialysis machines and other organ support devices while the process runs its course. Patients who survive MODS may spend months in hospital, years in rehabilitation, and are usually left with some permanent disability.(11)

Recent studies suggest that this late mortality due to MODS may be due to the body's responses to tissue damage and to blood loss that occur immediately following injury.(12) 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 MODS.(13) However, most of this data comes from research into sepsis which may not be directly translatable. 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 MODS, either through organ protection or organ restorative therapies.

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

Tissue damage

A number of studies including the first from our group at the Royal London Hospital,(14) have shown that trauma patients may arrive in the emergency department with severely deranged blood coagulation.(15) Significant tissue damage (in conjunction with severe blood loss) results in a reduced ability to form and maintain clots, leading to further bleeding, through an endogenous process termed acute traumatic coagulopathy (ATC).(16) Patients with this coagulopathy are three to four times more likely to die than those without.(7, 14) 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 precise mechanisms by which tissue injury activates the coagulation and inflammatory systems have yet to be fully characterised.

Tissue hypoperfusion / hypoxia

Ischemia following haemorrhagic shock is known to lead to MODS and increased mortality.(11) Several studies have shown that the severity of shock on admission correlates with eventual outcome. The duration of tissue ischemia, as measured by base deficit and lactate, and even sub-clinical tissue ischemia which persists for over 12 hours has a mortality is 38%, over twice that of patients who do not suffer a prolonged ischemic episode.(17) Tissue hypoxia leads to endothelial injury and priming of cellular and humoral components of the inflammatory pathways.(18)

It is believed that haemorrhagic shock and direct tissue injury are independently associated with the development of a hyperinflammatory state that may result in MODS. Preliminary genetic analysis of data from 70 trauma patients collected through the ACIT II platform has identified early genetic changes with associated acute immune dysfunction and the development of MODS.(12) Further, ACIT II data has identified a previously undescribed acute cardiovascular failure (19, 20) linked to injury remote from the heart, and represents an important novel area for future investigation.

Coagulopathy and injury pattern

There is increasing recognition that disorders of blood clotting after trauma may differ according to injury patterns e.g. multisystem injury with major haemorrhage versus isolated traumatic brain injury. These clotting disorders in the latter patients are poorly characterised, poorly understood and associated with worse outcomes. (21,22) Recent studies have suggested that therapies targeting coagulation may lead to improved outcomes for patients with brain injuries, offering a potential new therapy for a condition where treatments and outcomes have changed very little in the last 20 years (23-25).The specific abnormalities, mechanisms, and treatment options for blood clotting disorders in this group remain unknown.

5.2 Rationale

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 MODS and death. We hope that this study will allow us to fully characterise trauma-induced coagulopathy 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 novel procoagulant agents e.g. recombinant clotting factors, factor concentrates as well as antifibrinolytic drugs e.g. tranexamic acid.

Serial sampling from multiple tissues/organs, early after the disease onset for the first seven days, will provide a unique resource for high impact research in understanding the interplay between organ systems in the acute response to injury. Further, understanding the interplay between coagulation and acute inflammation after trauma may allow us to intervene early to avoid the late complications of MODS, thrombotic episodes and immune dysfunction and help create new survivors from major injury.

Injury is a unique model in which to investigate sterile inflammation, with a clear time point of disease onset and unrivalled potential for integration of “omics” research to study the activation of inflammatory pathways. Rapid onset and relative rapid resolution of disease (hours, days) provides the opportunity to evaluate response to therapy or prophylaxis. Characterising how the body recognises injury, and identifying early biomarkers of organ dysfunction, will provide opportunity for modulating the acute immune response to protect cells or organs. An in depth understanding of the immune response will facilitate the design of novel biologic or cellular therapeutics for precision medicine. Biomarker signature profiles will aid development of specific devices to detect and monitor organ dysfunction for earlier diagnosis or prevention of harmful long-term effects.

Research to date has predominantly focused on adult trauma patients, yet there is an expanding geriatric population who are increasingly sustaining multisystem injuries on the background of poor physiological reserve. This requires a greater understanding of the physiological responses in senescence.

Prehospital sampling

Findings from samples collected within minutes of injury, and our own preliminary data from ACIT II suggest a number of coagulation and inflammatory pathways are activated and/or deranged in the hyperacute period after trauma e.g. <60 minutes. Expanding our research capability to the patient as close to the point of injury will help characterise and understand early physiological and biochemical changes. Understanding whether these very early changes determine clinical outcome, and whether there are novel ways to modulate the response to injury are key research priorities.

Inclusion of paediatric patients

The paediatric population are grossly under-represented in human discovery trauma science with the vast majority of studies specifically excluding children. Trauma however remains a significant disease burden in this population group. Injury profiles, physiological response and management strategies for children are different compared to adults. In part this is due to their physical and physiological characteristics e.g. body size, head size, fat proportion, and reduced bone strength but increased bone pliability. These factors influence how energy from an accident is distributed and consequently children can have severe internal organ injury without bony fractures. Children are much more susceptible to hypothermia, which is known to worsen coagulopathy and organ dysfunction. In addition, clinical examination and vital signs can often be misleading as children are able to physiologically compensate for blood loss to a greater extent than adults. Classically paediatric patients may display a relatively normal physiology up to a point, before sudden and catastrophic circulatory collapse.

The vast majority of clinical evidence which guides trauma practice is translated from adult patients without dedicated paediatric evaluation. Characterisation of key pathways in coagulopathy, organ injury and MODS and therefore evidence to guide management of trauma and haemorrhagic shock in children is wholly inadequate. An important evidence gap exists in our understanding of the paediatric response to injury and blood transfusion, drivers

for organ dysfunction and whether all “adult therapies” e.g. tranexamic acid produce similar effects in the paediatric trauma population.

6. STUDY OBJECTIVES

6.1. Study Aims & Hypotheses

AIM 1: Coagulopathy and Massive Transfusion

Characterize the key derangements and describe trauma specific phenotypes in coagulation, fibrinolytic, platelet and endothelial cell function following major injury; determine the response to blood component therapy and anti-fibrinolytic medication; and further characterize the subsequent hypercoagulable state.

Hypothesis ACIT: 1A

Acute traumatic coagulopathy is caused primarily by tissue hypoperfusion which leads to systemic activation of anticoagulant and fibrinolytic pathways as well as global platelet dysfunction. Pathways of activation and dysfunction vary according to patient specific (e.g. age) and injury specific (e.g. site of injury, presence/duration of hypoperfusion) factors.

Hypothesis ACIT:1B

Subsequent transfusion of red cells, blood component therapy and antifibrinolytic drugs (e.g. tranexamic acid) have 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, up-regulation of antifibrinolytic systems and altered platelet function, resulting in a hypercoagulable state which is associated with thrombotic events and organ dysfunction.

Hypothesis ACIT 1D:

Children develop an acute traumatic coagulopathy that is distinct from that observed in adults. The coagulopathy can be identified and characterized with viscoelastic testing and coagulation biomarkers and platelet function assays.

Hypothesis ACIT 1E:

Acute traumatic coagulopathy occurs within minutes after injury and can be detected in the prehospital phase of care. Diagnostics for hyperacute identification can stratify patients for targeted treatment of precise derangements in coagulation.

Hypothesis ACIT 1F:

Patients with traumatic brain injury develop a coagulopathy that is distinct from that observed in bleeding trauma patients. The coagulopathy can be identified and characterised with viscoelastic testing, coagulation biomakrers and platelet function assays.

AIM 2: Development of Organ Injury

To elucidate the effect of derangements in coagulation, fibrinolytic, platelet and endothelial cell function on the inflammatory response and the development of acute organ injury (e.g. lung, kidney, cardiac), multiple organ dysfunctions (MODS), and death.

Hypothesis ACIT: 2A

There is a dose-dependent effect of the severity of trauma on coagulation, fibrinolytic, platelet and endothelial cell function. These correlate with activation of a pathological systemic inflammatory response that leads to acute organ injury (e.g. lung, kidney, cardiac) and MODS.

Hypothesis ACIT: 2B

There is a dose-dependent effect of the degree and duration of tissue hypoperfusion on coagulation, fibrinolytic, platelet and endothelial cell function. These correlate with activation of a pathological systemic inflammatory response that leads to acute organ injury (e.g. lung, kidney, cardiac) and MODS.

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 organ injury and MODS. Tissue trauma and cellular hypoperfusion have an additive effect on the development of organ injury and MODS.

Hypothesis ACIT:2D

Children with major trauma exhibit a specific immunological signature in response to tissue damage and blood loss that differs from adults. Particular responses are either associated with or protective for developing organ injury and MODS.

Hypothesis ACIT 2E:

Cellular pathways and biochemical signalling that produce organ injury and MODS occurs within minutes after injury and can be detected in the prehospital phase of care. Diagnostics for hyperacute identification can stratify patients for organ protective or organ restorative therapies.

AIM 3: Prediction models in major trauma

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

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 organ injury (e.g. lung, kidney, cardiac) 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: Genomic, proteomic and lipidomic analysis

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

Hypothesis ACIT:4A

There are signature transcriptomic, proteomic and lipidomic profiles associated with the risk of post-traumatic MODS and other adverse outcomes. Specific changes in circulating leukocytes and parenchymal cells occur in organs remote from the injured site and are associated with MODS.

Hypothesis ACIT: 4B

Children develop a specific transcriptomic and proteomic response to tissue trauma and hypoperfusion that is protective against post-traumatic organ injury and MODS.

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 (e.g. 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.

6.2. Primary Outcomes

For both adult and paediatric populations the outcome measures will be:

Blood products transfused in the first 24 hours

Incidence & severity of acute organ injury & MODS

6.3. Secondary Outcomes

For both adult and paediatric populations the outcome measures will be:

- 28-day mortality
- Ventilator free days
- Length of hospital and critical care stay
- Thrombotic events
- Requirement for organ support (artificial ventilation, renal replacement therapy, inotropic support)
- Infection – organ specific and each episode detailed
- Transfer destination at discharge e.g. home, other medical facility, rehabilitation unit
- Quality of Life at 28 days and 1 year (EQ5D questionnaire and extended Glasgow Outcome Scale for adults, age appropriate PedsQL for paediatric patients)

7. STUDY POPULATION

Estimated number of patients to be enrolled: 5,000

7.1. Setting

The London Air Ambulance (LAA) is the Prehospital Care service for the London Major Trauma System and provides care for injured patients at the scene of the injury. Where possible, adult (only) patients treated by LAA will be recruited under a waiver of consent to allow a

prehospital blood sample to be collected. LAA and other prehospital healthcare providers then triage appropriate Trauma patients to Major Trauma Centres (MTCs). MTCs (UK) provide advanced multi-specialty care for injured patients. The Royal London Hospital (Bart's Health NHS Trust) will be the lead site. The London Air Ambulance will be the only prehospital care provider in the study.

In addition, patients will also be recruited to at major trauma centres as partners within the International Trauma Research Network (INTRN). Such recruitment shall be according to the ACIT protocol herein and shall be conducted under the local governance of individual participating sites.

7.2. Study participants

Only patients who have received prehospital care from the London Air Ambulance and are to be conveyed to a study site ***or*** patients who arrive at a study site by other means e.g. land ambulance or self-presentation ***and*** require a trauma team activation will be considered for enrolment into the study. Only those patients undergoing surgical intervention will be considered for the collection of muscle tissue.

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 and existing ACIT study cohort 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 and by definition are in an emergency clinical situation. Patients will be assessed for eligibility to enter the trial according to the criteria set out below. If patients are eligible for entry into the study, following initial screening either in prehospital environment or upon arrival in the Emergency Department, they will be enrolled automatically under a waiver of consent. See Ethics (Section 10) for further details.

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.

Patients will be considered eligible for enrolment in this trial if they fulfil all of the inclusion criteria and none of the exclusion criteria detailed below.

7.3. Inclusion Criteria

Patients eligible for screening are all trauma patients of any age including children:

- requiring treatment by the London Air Ambulance medical team (adults only) **and/*or***
- requiring admission to the Emergency Department with a full trauma team activation (adults and children)

7.4. Exclusion Criteria

Any patient who fulfils any of these criteria will not be considered for inclusion in the study:

- Transfers from other hospitals
- Burns >5% total body surface area
- >120 mins have lapsed since time of injury
- Deemed inappropriate for recruitment by an independent clinician (e.g. mass casualty event or futility)

8. STUDY DESIGN

ACIT II is a prospective cohort multicentre observational study. It will follow the clinical course of trauma patients from injury in the prehospital phase of care and/or at admission to the emergency department and for the first 28 days. Blood samples and, in some patients, muscle biopsies will be analysed for markers of activation of the coagulation and inflammatory systems. These biomarkers will be correlated with patient injury patterns, trauma-induced physiological disturbances and subsequent clinical course and outcome.

8.1.ACIT SWIFT

ACIT-SWIFT is a sub-study of the primary ACIT study to enable a wider patient recruitment nationwide, and pool resources that are being utilized to deliver the a national trial called SWiFT which will recruit bleeding trauma patients in the prehospital phase of care. Patients enrolled into SWiFT are a specific patient subgroup (major trauma haemorrhage) of interest for the primary ACIT study and hence samples and data will be directly relevant for the general aims and hypotheses. ACIT SWIFT will specifically help to understand the treatment effect of whole blood transfusion after injury through a single blood sample taken on admission (post-dosing of the SWIFT intervention) in combination with data collected as part of the SWIFT trial – for ACIT SWIFT sub study details and paperwork please refer to appendix 4.

9.0 STUDY PROCEDURES

9.1. Informed Consent

All participants will be recruited under an emergency waiver of consent. Written informed consent or agreement to continue in the study must be obtained from the participant, personal consultee or professional consultee (independent clinician), after explanation of the aims, methods, benefits and potential hazards of the trial.

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. Most, but potentially not all, participants will be incapacitated at the time of eligibility (critical injury, mechanical ventilation, sedation), such that the Mental Capacity Act (England; 2005) provides guidance. Patients will be recruited within 2 hours of their injury, and those 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. This is also a particularly stressful time for relatives and families, an important consideration when the intervention under investigation is time sensitive. All participants will be enrolled in the study with a waiver of consent without informed participant consent due to the emergency nature of the study thus a waiver of consent will be applied for automatic study enrolment. This study requires that eligible patients are

enrolled as close to the time of injury as possible. As injury is an unexpected event, it is uncommon that relatives are present at the time of hospital admission.

Following hospital admission several approaches to obtaining consent will be used, depending on the mental capacity of the patient and availability of Personal Consultees (relative or friend). In the first instance, if a personal consultee is unavailable and the patient lacks mental capacity, a professional consultee (typically the trauma team leader who is a doctor independent of the research study) will be approached within 24 hours to provide consent for the patient to continue in the study. See Section 11 (Ethics) for a full explanation of the consenting process.

Our standard operating procedure for obtaining consent is included in **appendix 2.**

9.2. Screening and recruitment

All adult trauma patients (aged 16 and over) who are treated by London Air Ambulance, or trauma patients of any age who are admitted to the emergency department via a trauma team activation will be screened for eligibility to ACIT II. As most trauma patients will be incapacitated at the time of screening it is anticipated that a waiver of consent will be used followed by initiation of the informed consent process at a time appropriate from the participant as described in Section 9.1.

9.3 **Schedule of study interventions**

Study Activity	Prehospital	Emergency Department	HR 24	HR 48	HR 72	Day 3-7	Day 7	Day 8-288 – 28 days	Discharge/ Day 28	1-year follow up
History and Physical	Adults only	X								
Demographics	Adults only	X								
Injury characteristics	Adults only	X								
Physiological observations	Adults only	X	X	X	X	X	X	X		
Organ score (SOFA)			X	X	X	X	X	X	X	
Infections			X	X	X	X	X	X		
Thrombotic Events			X	X	X	X	X	X		
Adverse events			X	X	X	X	X	X		
Blood Sampling*	Adults only	X	X		X		X			
Muscle biopsy			(X)	(X)	(X)	(X)				
Quality of Life assessment									X	X
GOS-E									X	X

**All patients with have one blood sample taken on admission at the same as routine clinical blood tests. Bleeding patients requiring blood transfusion will have further samples taken after transfusion of the 4th, 8th and 12th unit of red blood cells in conjunction with routine clinical blood tests.* (X) Muscle biopsies will only be taken during a planned or emergency surgical procedure as part of routine clinical care

9.4. Study Intervention

9.4.1 Blood samples

Trauma patients will have blood samples drawn to measure activation of coagulation, fibrinolysis, endothelial injury and the immune system. The maximum sample volume at each time point will be 40mls (maximum 30mls during bleeding time point samples). Samples will be collected in the Emergency Department at admission (Hour 0) and then 24±2 hours, 72±12 hours and at 7 days (±24 hours) post admission. Patients who are actively bleeding will have additional samples (30ml) taken after administration of the 4th, 8th and 12th units of red blood cells, if used.

Adult patients (>16 years old) treated by the London Air Ambulance medical team will have one additional sample taken in the prehospital phase upon their arrival and as close to the time of injury as possible. Prehospital samples will be prefixed PH to indicate a sample obtained prior to Hour 0 baseline (hospital admission) to ensure consistency with previous patients recruited to the ACIT II study.

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, particularly during the first 24hours when the majority of samples will be taken, most blood draws will not be painful to the patient. When this is not the case, we will we will coordinate our blood draws with those of clinical need whenever possible, to minimise 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.

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. Characterizing these patterns of altered coagulation

would have major implications for the treatment of coagulopathy. How this change occurs is currently unknown, but would be identified by this protocol.

(2) A prolonged shock state has a known poor outcome. This may be due to continued activation of the coagulation and inflammation systems. Further a procoagulant and depressed immunological state has been identified late in severely injured trauma patients and may extend for many days after injury. Serial measurements up to Day 7 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. Serial sampling allows us to assess the impact of fluid and blood resuscitation on the coagulation and immune systems.

9.4.2 Blood sampling in paediatric patients

In view of the lower circulating blood volume in children, the volume of blood obtained will be adjusted according to age in the following manner:

- Age 0-2 years: 1ml (maximum)
- Age 3-10 years: 5-10ml (maximum)
- Age 10-15 years: 20-30ml (maximum)

Samples for standard paediatric blood testing are collected in smaller tubes not routinely carried by LAA. To avoid introducing a new blood sampling protocol in the prehospital phase of care for a patient cohort that presents additional clinical challenges, prehospital blood collection will not be performed in paediatric patients.

9.4.3 Muscle biopsies

Muscle biopsies will only be taken from adult patients undergoing surgical intervention, where the patient is anaesthetised and muscle is already exposed either by the incision or the traumatic injury itself (e.g. abdominal, chest or limb trauma). During the first seven days surgical procedures performed will be determined by clinical need and injured anatomy.

Muscle biopsies will be taken when the patient is undergoing emergency or planned surgery and serial samples will be collected from the exposed muscle during each surgical episode in the first seven days. Muscle tissues will be collected to enable extravascular tissue analysis to be performed to determine cellular responses to trauma and inflammation, in particular biomarkers for cellular death and activation of early signalling pathways within the cells which may have been initiated by the traumatic insult to the body.

A 1cm cube of muscle will be removed during the operation and immediately wrapped in saline-moistened gauze. The muscle biopsy can then be stored cool for several hours before being split to provide tissue for fixing in 4% glutaraldehyde (for visualisation/cell surveys) as well as snap frozen material (for histochemistry and biomarker analysis).

Cellular markers of inflammatory response will include T-cells, NK cells, Macrophages, B-cells and Eosinophils. Biomarkers of tissue damage and injury response will include TNF-α, IL-1 ,IL-6, IL-8, IL-10, Complement components, heat shock proteins, Syndecin-1, soluble thrombomodulin, mitochondrial DNA. Transcriptomic and proteomic studies will examine changes in cellular behaviour.

9.4.4 Additional data collection

Patient demographics, mechanism of injury, injury type and severity, transfusion of blood products and admission physiology will be recorded in real-time in the emergency department and operating theatre. Patients will then be followed up on a daily basis for organ dysfunction scoring, presence of infection, development of thrombotic events, surgical episodes and other adverse events until Day 28, discharge or death. All of this data (aside from organ dysfunction scores outside of the critical care unit) is routinely collected in the management of injured trauma patients. No additional monitoring or interventions will be required.

9.4.5 Patient questionnaires

Quality of life assessment will be measured by the use of self reported EuroQol EQ-5D questionnaire and the Extended Glasgow Outcomes Scale (GOS-E) in adults at hospital discharge or Day 28 (whichever is sooner) and again at one year following injury (see Section 9.6 for follow up data collection). In paediatric patients, quality of life will be measured using the Paediatric Quality of Life Inventory (PedsQL) questionnaire that corresponds with the age of the child at the time of injury. Assessment will be carried out at the same time points as for

adults. 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 conducted by research staff with the patient where possible, but may also be completed with the patient's personal consultee if necessary. The questionnaire can be completed in less than five minutes.

9.5 Procedure for collecting data (Case Report Forms)

Data collection for this study will be accomplished using a paper case report form (CRF) to capture data prospectively and transferred to an electronic data capture system (RedCap). CRFs are required and will be completed for each participant. It is the Investigator's responsibility to ensure the accuracy, completeness and timeliness of the data reported on the subjects CRF. CRFs will be completed in a timely fashion to support the study timelines. Future prospective data collection may be possible with validated and JRMO approved electronic data capture methods e.g. REDCap and ultimately supersede paper CRF.

9.6 Follow-up procedures

The 12-month EuroQol EQ-5D (adults) or PedsQL (paediatrics) patient questionnaire will be posted or emailed to surviving patients along with a return stamped addressed envelope. The questionnaire provides instructions for completion of the whole questionnaire, which will take approximately five minutes. Patients who have not returned the questionnaire within two weeks of the initial request will be telephoned as a reminder to complete the questionnaire and may be asked to complete it over the phone if necessary. A maximum of three recorded contact attempts will be made via phone and if these are unsuccessful, no further contact will be made and responses will be marked as not returned. Confirmation with the GP and scrutiny of the hospital care record system will ensure only those patients alive at 12 months receive a questionnaire.

9.6. Laboratory assessments

Measures of whole blood functional coagulation will be performed using equipment at the Point-of-Care and within the research laboratory. Whole blood functional testing will be performed with rotational thromboelastometry. 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 and S100A10. Markers for endothelial injury will include von Willibrand Factor (vWF) and Eselectin.

Platelet function and activation tests will include: 96-well light transmission aggregometry, Multiplate impedance aggregometry, flow cytometry and imaging studies.

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, RNA and proteomic analyses. Samples will also be available for leukocyte phenotyping with flow cytometry.

9.7. Patient withdrawal

Each participant has the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the Investigator or at the institution.

Every reasonable effort will be made to maintain protocol compliance and to retain patient participation in the study consistent with the provisions of informed consent and good clinical practice. Additionally the PI may withdraw the participant. The following are potential reasons why a patient may be withdrawn from the study:

- Withdrawal of consent: the patient, the patient's authorised representative, independent physician, or designated individual who had provided initial consent to enter the study may withdraw consent at any time throughout the duration of the trial, without prejudice to future medical care and treatment.
- Retrospective exclusion: If a patient is deemed to not meet one or more of the inclusion/exclusion criteria in retrospect they will be withdrawn from the study.
- Major protocol deviation from the study design by the participant, observed or suspected by the investigator.
- Administrative: the sponsor or monitoring committees decide to terminate or discontinue the study.

- The participant's health would be jeopardised by continued participation and is withdrawn at the discretion of the investigator.

Data collected up to the point of withdrawal will be retained. To safeguard the participants' rights, the minimum personally-identifiable information possible will be used.

Participants will be asked whether samples already collected can be retained for use in the study. Where a participant consents to storage and use of their samples, they will be asked to sign a consent form reflecting this agreement.

Any samples obtained from withdrawn participants who do not consent to storage or use of their samples will be discarded in accordance with the local NHS Trust standard procedures. Samples sent to the central laboratory at Queen Mary University London will also be discarded.

All participants withdrawn from the study will be managed in accordance with the hospital's standard procedures.

9.8. End of Study Definition

Patients are followed up when in hospital daily until day 28, death or discharge (whichever occurs sooner). If patients remain in hospital beyond day 28, their electronic records will be periodically reviewed remotely until discharge to enable collection of any outstanding endpoint data. End of study is at 12 months after completion of EQ5D (adults) or PedsQL (paediatrics) patient questionnaire.

10 STATISTICAL CONSIDERATIONS

Because of the nature of the ACIT II study and its various aims, a power calculation is not applicable.

11 ETHICS

This study will be carried out in accordance with the ethical Principals in the Research Governance Framework for Health and Social Care, Second Edition, 2005 and its subsequent amendments as applicable and applicable legal and regulatory requirements. The study has

been reviewed and approved by the Research Ethics Committee: London – City and East (07/Q0603/29).

11.1 Informed Consent

Our standard operating procedure for obtaining consent is included in Appendix 2. All participants will be enrolled in the study with a waiver of consent due to the emergency nature of the study. Several approaches to obtaining consent will then be used following hospital admission, depending on the mental capacity of the patient and availability of Personal Consultees (relative or friend).

[1] If a patient is considered to have mental capacity they will be approached directly, and their informed consent requested.

[2] For patients who lack capacity and where it is not possible to take agreement from a Personal Consultee we will use approaches consistent with the Mental Capacity (England) Act (2005), as below:

[a] If the patient has capacity, they will be approached.

[b] If the patient lacks capacity and a personal consultee not available (which is anticipated to be the case in the majority of instances) an independent clinician acting as a professional consultee will be approached within 24hrs of study enrolment to provide consent for the patient to continue in the study. This will usually be a substantive consultant in emergency medicine acting as trauma team leader. The clinician must not be a named local PI or local individual directly involved in the research project and must be of consultant or senior emergency medicine registrar status. In all cases they must have received prior information about the research to enable them to provide advice regarding inclusion in the emergency situation and be included on a log of professional consultees. It is anticipated that this individual will be best placed to advise as they will be aware of the patient's condition and will be the clinician in charge of the immediate clinical care of the patient. Where the trauma team leader is connected with the research project, or unavailable e.g. due to other clinical demands, it is acceptable to ask the opinion of another emergency or trauma physician who is not connected with the research.

[c] When entry to the study is based on the consent of a doctor who is not connected with the research project (professional consultee), the personal consultee will be approached at the earliest opportunity for agreement to remain in the trial. If the Personal Consultee does not agree to the patient remaining in the trial for further blood tests and data collection to day 28, the research team will clarify whether samples already collected can be retained and where applicable will ask the personal consultee to sign a consent form. All data collected up to the point of withdrawal will be retained. In cases where a personal consultee is not available, we will use the consent signed by the professional consultee until the patient regains mental capacity and they will be approached directly for obtaining personal consent (see point [d]). In cases where a personal consultee is available but all our attempts (2 telephone calls and 2 letters) to contact him/her are unsuccessful, the patient will be kept in the trial based on the consent signed by the Trauma Team Leader (professional consultee). All attempts at contact will be recorded.

[d] All patients who lacked mental capacity at the time of recruitment will be approached for consent to remain in the trial at the earliest opportunity once they regain capacity. It will be made clear during the consent process that any intervention (blood and/or tissue sample collection) has already taken place and that we are asking for their consent for the collection of further samples and data collection. In cases where patient has regained mental capacity but we didn't manage to obtain a personal consent (because of an early hospital discharge) we will make and document all attempts to contact the patient up to a maximum of 2 calls and 2 letters to the registered address. If these attempts are unsuccessful in obtaining written consent, the patient will be kept in the trial based on the consent signed by the professional or personal consultee. Verbal agreement from the patient regarding their agreement to partake in the study will also be documented in medical record.

[e] If the patient regains capacity but decides they do NOT wish to remain in the trial we will ask the patient to clarify one of the following: [i] they wish to be removed from the trial, decline any further tissue sampling or data collection and wish for all tissue samples already collected to be destroyed in accordance with local hospital policy. A withdrawal form will be completed. [ii] they do not consent to further tissue sampling but consent to the use of tissue samples that have already been collected. Agreement to tissue storage and use for the study will be documented on the consent form. All data collected up to the point of withdrawal will be retained.

[f] Patients who die during the follow up period or do not regain mental capacity, will be included in the study based on the advice provided by the personal consultee and/or professional consultee. In cases where the subject dies before our attempt to obtain consent from the patient or the personal consultee, the patient will remain in the trial based on the consent obtained by the professional consultee and we will not attempt future telephone contact with relatives/personal consultee in order to minimise stress and anxiety associated with the unexpected and traumatic death of their relative / next of kin.

[g] In general, the same procedures and principles will apply to the recruitment of paediatric patients with parents or legal guardians providing consent for children aged under 16 years who lack capacity. Paediatric patients who are Gillick competent will give consent for their own involvement. Wherever appropriate children will be involved in the discussion (with parents or legal guardians) regarding consent to use their samples and data. Discretion will be applied by the recruiting member of the research team regarding approaching a parent or legal guardian at all times, particularly during initial screening and recruitment in order to avoid causing undue stress and anxiety. Advice will be taken from the independent clinician in cases of uncertainty. Procedures for paediatric consent are detailed fully in appendix 2.

11.2 Safety Considerations

All parts of the study will be carried out to avoid patient risk and minimize discomfort at all times. At no time will patient care be compromised or delayed for the purposes of the study.

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 minimized 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, in accordance with our local information governance guidelines.

11.3 Specific Risks

11.3.1 Blood sampling

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).

11.3.2 Muscle Biopsy

The risks associated with performing a muscle biopsy under general anaesthesia are minimal as it will only be taken during a procedure where muscle is already exposed and surgery is being performed on the same anatomical location. There may be a very small amount of bleeding at the site of the biopsy but will be controlled at the time of surgery with conventional surgical practice (e.g. diathermy).

11.4 Benefits to study participants

None

11.5 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 (1). In general, injured patients die either from major haemorrhage, traumatic brain injury or multiple organ failure (2). Patients who die from haemorrhage 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 (11).

It is clear from several studies that the outcome of trauma patients is determined in the first few hours following injury. 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'. However, the growing elderly population now represents a larger and important demographic in trauma since the combination of multisystem injury and age-related comorbidities or low energy and poor physiological reserve produce poor outcomes. 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.

11.6 Risk/benefit analysis

Although the study carries no direct 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.

12 PUBLIC INVOLVEMENT

The Centre for Trauma Sciences PPI group - Patient & Public Advisors in Injury Research (PAIR) was set up after the ACIT study first started in 2007. However the group are integral to all protocol amendments and revision of patient consent forms and information sheets. All documentation aimed at children recruited to ACIT have also been reviewed by a RLH youth PPI group – the Youth Empowerment Group. Key research findings generated from the ACIT II study are presented at the PAIR meetings with input on how best to disseminate to the lay public.

13 DATA HANDLING AND RECORD KEEPING:

13.1 Data management

The investigator/institutions will keep records of all participating participants (sufficient information to link records e.g., CRFs, hospital records and samples), all original signed informed consent forms and copies of the CRF pages. CRF data will be uploaded to a secure database. The database has no interaction with other systems. Documented training will be provided to investigators entering data onto the CRF and electronic database.

It is the responsibility of the Chief Investigator (CI) to maintain adequate records for the study including completed CRFs, signed Informed consent documents and all correspondence with the

REC and the sponsor. Original documents will be retained as part of the audit trail. Any amendments or corrections to original documents will be signed and dated.

All paper records including the CRF and consent forms will be stored in a secure restricted access location at the recruiting site. Only de-identified information, as required by the study protocol and captured on the CRF, will be uploaded onto the study database.

13.2 Confidentiality

All investigators and trial site staff must comply with the requirements of the General Data Protection Regulation (GDPR) 2018 and Data Protection Act (2018) with regards to the collection, storage, processing and disclosure of personal information and will uphold the regulation's core principles.

The Principal Investigator has a responsibility to ensure that participant confidentiality is protected and maintained. They must also ensure that their identities are protected from any unauthorised parties. Information with regards to study participants will be kept confidential and managed in accordance with the Data Protection Act, the General Data Protection Regulation (GDPR), NHS Caldecott Principles, the UK Policy Framework for Health and Social Care Research, and the conditions of Research Ethics Committee favourable opinion.

The Chief Investigator and the study team will adhere to these parameters to ensure that the Participant's identity is protected at every stage of their participation within the study.

Subject identifiable information (name, date of birth, hospital number) will be recorded for the purposes of consent (on the consent log). Access to all identifiable information will be limited to the study investigators.

13.3 Data Custodian Details

Queen Mary University of London is the sponsor for the study based in the United Kingdom. They will be using information from participants and their medical records in order to undertake the study and will act as the data controller for the study. This means that Queen Mary University of London are responsible for looking after the information and using it properly.

Participant's rights to access change or move their information is limited, as it needs to be managed in specific ways in order for the research to be reliable and accurate.

13.4 Record Retention and Archiving

During the course of research, all central and site records are the responsibility of the Chief Investigator and must be kept in secure conditions. When the research trial is complete, it is a requirement of the UK policy framework for health and social care research and Trust Policy that the records are kept for a further 25 years.

Site files from other sites must be archived for 25 years at the originating site and cannot be stored within Queen Mary University of London.

Destruction of essential documents will require authorisation from the Sponsor.

14 LABORATORIES

14.1 Central Laboratories

The research laboratory on Ward 12D, The Royal London Hospital, Bart's Health NHS Trust will be the primary site for processing and preparation of blood samples for future use. The Blizard Institute Queen Mary University of London will perform the biomarker analyses for locally collected samples and those sent from external sites when collaborative cohort studies are conducted. Other recruiting sites may also conduct biomarker analysis within their local laboratories according to their own specific local laboratory protocols and procedures. All procedures will be documented in the laboratory manual.

14.1 Local Laboratories

Local NHS UKAS accredited Haematology and Clinical Biochemistry laboratories at each participating centre will perform the analysis of routine clinical blood samples taken.

14.3. Sample collection and preparation

Blood taken for future use will be drawn from either a central, arterial or large bore peripheral line sited for purposes of patient care or, from femoral vein or antecubital fossa. Muscle biopsies will only be taken from the wound edge at surgical sites from tissue that would otherwise be discarded.

Biological samples will be collected, processed and stored at the research centre where the participant is enrolled. All samples will be allocated the unique study identifier (pseudoanonymised) given to the participant at enrolment and stored in teams' local laboratories at -80^{o}C for future use. Samples should be clearly labelled with the participants unique study identifier, sample type and sample time point. Samples may be stored in their original vacutainer or processed and aliquoted into suitable freezer proof sealable eppendorfs. Guidelines for sample preparation and storage can be found in Appendix 1.

14.4 Laboratory procedures

All plasma samples will be measured by a commercially available ELISA kits or via automated assay.

14.6 Sample storage and transfer

Biological samples processed and stored at the research centre where the participant is enrolled. Samples may be shared with selected International collaborators to maximise the findings of the study. These samples will be transferred and used in a secure and ethical manner that ensures protection participants fundamental rights and privacy under an appropriate Material Transfer Agreement.

15 FINANCE AND FUNDING

Departmental funding from the Centre for Trauma Sciences pays for all consumable costs for patients recruited at Bart's Health with a long-standing agreement from Werfen Ltd who provide research support costs for ROTEM equipment and consumables. Assays are funded through individual grants. Additional study sites are self-funded.

16 INDEMNITY

The insurance that Queen Mary University of London has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm.

17 DISSEMINATION OF RESEARCH FINDINGS:

Data will be written up, presented and disseminated in a timely manner. The main outputs will be research papers for general journals and trauma specialty journals, research abstracts for presentation to national and international meetings and a final report summarising the overall findings.

Study collaborators will be named as authors on any publication or report. Findings will be disseminated via the centre for trauma sciences website, and via online seminars for MTC participation. Public dissemination will be achieved through our various educational outreach events and at regular PAIR meetings.

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19 APPENDICIES

Appendix 1: Blood Processing SOP

Appendix 2: Consent SOP

Appendix 3 – The ACIT SWIFT Study

Appendix 4- ACIT SWIFT paperwork

4a Participant Information Sheet ACIT SWIFT V1 09.08.2022

4b Personal Consultee Information Sheet ACIT SWIFT v 1 09.08.2022

4c ACIT SWIFT Consent Form Participant V 1 09.08.2022

4d ACIT SWIFT Consent Form Personal Consultee V1 09.08.2022

4e ACIT SWIFT Professional Consultee Consent Form V2.0 02.06.2023

Appendix 4 – The ACIT SWIFT protocol

Summary:

Short Title	*ACIT SWIFT Sub Study*
Methodology	*Multi-centre, prospective observational cohort study*
Research Sites	*A maximum of 10 UK MTC's with confirmed capacity and capability*
Objectives/Aims	A prospective, observational cohort study designed to investigate the molecular mechanisms activated by traumatic injury, to understand how these relate outcomes and the effect of early therapies to treat trauma haemorrhage.
Number of Participants/Patients	386 patients
Inclusion Criteria	Trauma patients who have been enrolled into the prehospital Study of Whole Blood In Frontline (SWiFT) randomized controlled trial and are transported to a Major Trauma Centre in England, Wales or North Ireland
Exclusion Criteria	Any patient who fulfils any of these criteria will not be considered for inclusion in the study: • Transfers from other hospitals • Burns >5% total body surface area • >120 mins have lapsed since time of injury • Deemed inappropriate for recruitment by an independent clinician (e.g. mass casualty event or futility)

Proposed Start Date	June 2022 to coincide with start date of the SWIFT trial
Proposed End Date	June 2024
Study Duration	2 years

ACIT SWIFT is a sub-study of the primary ACIT study to enable a wider patient recruitment nationwide, and pool resources that are being utilized to deliver the national SWiFT trial which will recruit bleeding trauma patients in the prehospital phase of care. Patients enrolled into SWiFT are a specific patient subgroup (major trauma haemorrhage) of interest for the primary ACIT study and hence samples and data will be directly relevant for the general aims and hypotheses. ACIT SWIFT will specifically help to understand the treatment effect of whole blood transfusion after injury through a single blood sample taken on admission (post-dosing of the SWIFT intervention) in combination with data collected as part of the SWIFT trial (reference Appendix 1 SWIFT Trial Summary).

SWiFT is a multi-centre, prehospital randomized control trial (RCT) to evaluate whether leukocyte-depleted Whole Blood (WB) stored at 2 – 6^{o}C is superior to standard of care (red cells and any form of plasma) in bleeding trauma patients. A total of 848 patients (424 in each arm) will be recruited over a 2-year period, with recruitment planned to start in June 2022. The trial is sponsored by NHS Blood and transplant (NHSBT) and funded jointly by NHSBT, nine air ambulance charities and UK Defence Medical Services. The primary objective of the trial is to determine whether WB transfusion (platelet rich) is better than standard care (blood component transfusion and/or lyosophilized plasma) in reducing 24 hr mortality or massive transfusion (defined as >10 units of any blood component) in patients with life-threatening haemorrhage.

International guidelines consider platelet transfusions integral to a massive haemorrhage protocol to treat trauma-induced coagulopathy although the mechanism by which platelets support coagulation and improve outcome are largely unknown. In severe

trauma patients, both thrombocytopenia and platelet dysfunction, as measured by decreased aggregation in response to agonists, have been shown to be directly associated with increased mortality. Even in the presence of a normal platelet count upon admission, dysfunction occurs in around half of critically injured patients and is an independent predictor of in-hospital mortality.

In the last two decades, the resuscitation of bleeding trauma patients has evolved from purely haemodynamic resuscitation (restoration of tissue perfusion/oxygenation) to haemostatic or balanced resuscitation to preserve or improve clot function. In a sub-analysis of the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial early platelet transfusions were found to increase survival and rates of haemostasis in major trauma haemorrhage (https://pubmed.ncbi.nlm.nih.gov/25647203/). Platelets are the principle effectors of haemostasis to both create a "plug" at the site of vessel injury for primary haemostasis, and provide a phospholipid membrane necessary for large scale thrombin generation to initiate coagulation factor assembly, activation and fibrinogen cleavage to create a cross-linked fibrin clot. Following activation platelets in addition release several procoagulant and antifibrinolytic mediators contained within alpha-granules that further support coagulation and inhibit fibrinolysis e.g. plasminogen activator inhibitor-1 (PAI-1). In bleeding trauma patients who received platelet transfusions we have shown higher PAI-1 levels in conjunction with reduced markers of fibrinolytic activation compared to those who did not receive platelets.

Platelets components until recently were universally stored at room-temperature to fulfil logistical requirements of blood banks. Cold stored platelet (CSP) components kept at 4°C have increased haemostatic competence compared to RTP, in addition to reduced rates of storage lesions (receptor downregulation and mitochondrial dysfunction). While the use of RTP is nowadays a mainstay of haemostatic resuscitation, there is a growing body of evidence from in vitro studies showing that CSP have improved aggregation, increased degranulation and microvesiculation, and lead to a faster and stronger clot formation with diminished fibrinolysis thus in theory may provide a more efficacious product. A recently published small randomized control trial showed a non-statistically significant decrease in post-operative bleeding with a signal for reduced transfusion of plasma components in patient who received CSP during cardiothoracic surgery, when compared to RTP.

Platelet transfusions cannot be administered in the prehospital setting and even in hospital are frequently administered late during the major haemorrhage protocol. Whole blood contains all elements necessary to support coagulation including platelets. The use of whole blood, which can be stored at 4°C, is undergoing global re-evaluation in trauma resuscitation following a resurgence of its use in the military with emerging evidence of benefit in small civilian studies.

Rationale

SWiFT is the first large scale RCT that will determine the effectiveness of whole blood on reducing mortality in bleeding trauma patients. Understanding how whole blood transfusion corrects clotting abnormalities and how this may alter clinical outcomes will require both (i) randomized clinical outcome data from SWiFT trial and (ii) detailed biochemical and functional analysis of coagulation which is not collected as part of the pragmatic clinical trial but is a key objective of the primary ACIT study.

Patients will be recruited into SWiFT before arrival at the Major Trauma Centre and will have trial data collected during the bleeding episode and hospital admission. To optimize the limited research resource across the national trauma system patients will be co-enrolled into ACIT SWIFT with a single blood draw to coincidence with clinical admission samples in ED. Given the constraints in emergency research capability at the majority of Major Trauma Centres we have significantly reduced the ACIT study activities. We have removed all serial blood sampling and in hospital data collection in ACIT SWIFT to evaluate key coagulation and immune proteins in patients randomized to the SWiFT intervention. Samples will be banked in accordance with the wider aims of the primary ACIT study for proteomic and genomic analysis. A post-dose blood draw will enable an understanding of how whole blood transfusion vs component therapy modulates the acute changes we have observed in coagulation and the immune system after injury, that have been characterized from earlier ACIT samples.

In our lab we have performed ex vivo pilot work to evaluate the effect of whole blood and platelet concentrates on coagulopathic blood sampled from bleeding trauma patients in ACIT soon after arrival in the Emergency Department. Platelet concentrates and whole blood appear to primarily prevent dilutional effects and further deterioration in platelet dysfunction, clot strength and clot formation times typically observed during current MHP transfusion. This preliminary analysis using rotational elastometry (ROTEM) demonstrates a signal for improved thrombin generation profiles of coagulopathic blood mixed with whole blood compared to red cell and plasma transfusion. Data from this work have been used for the sample size calculations for the primary outcome (thrombin generation) and allows for 25% dropouts and

STUDY POPULATION

Estimated number of patients to be enrolled: 386

STUDY AIMS and OBJECTIVES

AIM 1: Characterize the therapeutic mechanism and efficacy of whole blood transfusion in trauma major haemorrhage

Determine the key derangements and describe trauma specific phenotypes in coagulation, fibrinolytic, platelet and endothelial cell function following major injury; determine the response to whole blood therapy and anti-fibrinolytic medication.

Hypothesis ACIT-ED

Whole Blood transfusion is better than standard care (component transfusion and/or lyosophilized plasma) in correcting acute traumatic coagulopathy and supporting haemostasis during major trauma haemorrhage.

AIM 2: Coagulopathy and Massive Transfusion

Characterize the key derangements and describe trauma specific phenotypes in coagulation, fibrinolytic, platelet and endothelial cell function following major injury; determine the response to blood component therapy and anti-fibrinolytic medication; and further characterize the subsequent hypercoagulable state.

AIM 3: Genomic, proteomic and lipidomic analysis

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

AIM 4: 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.

Primary Outcomes

Primary outcome is endogenous thrombin generation assessed via 10ml research blood sample collected within 60 minutes of arrival in the Emergency Department.

Secondary Outcomes

Secondary outcomes will include incidence of acute traumatic coagulopathy on arrival (defined by platelet count, prothrombin time, fibrinogen, fibrinolysis); admission blood lactate; admission clotting factor levels and markers of fibrinolytic activation/inhibition; quantitative analysis and phenotyping of microparticles; relative contributions of microparticle subtypes to thrombin generation.

STUDY POPULATION

Estimated number of patients to be enrolled: 386

Setting

Participating Major Trauma Centres (MTCs) receive trauma patients from the ten Air Ambulance Services that are participating the SWIFT trial. MTCs provide advanced multi-specialty care for injured patients.

Study participants

Adult patients (>16 years) who been enrolled in the SWIFT trial and are admitted to the Emergency Department of a MTC will be considered for enrolment into the study. As the study is co-enrolling patients who have been recruited to SWiFT the ACIT SWIFT patients by definition will all have sustained life-threatening haemorrhage after major injury.

The study will, of necessity, include trauma patients who are unable to give consent for themselves and by definition are in an emergency clinical situation. Patients will be assessed for eligibility to enter study according to the criteria set out below. If patients are eligible for entry into the study, upon arrival in the Emergency Department, they will be enrolled automatically under a waiver of consent. See Ethics (Section 10) for further details.

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.

Patients will be considered eligible for enrolment in ACIT SWIFT if they fulfil all of the inclusion criteria and none of the exclusion criteria detailed below.

Inclusion Criteria

Patients eligible for screening are adult (>16 years) trauma patients enrolled into the SWIFT prehospital randomized controlled trial and are transported to a Major Trauma Centre in England, Wales or North Ireland

Exclusion Criteria

Any patient who fulfils any of these criteria will not be considered for inclusion in the study:

- Transfers from other hospitals
- Burns >5% total body surface area
- >120 mins have lapsed since time of injury
- Deemed inappropriate for recruitment by an independent clinician (e.g. mass casualty event or futility)

STUDY DESIGN

ACIT SWIFT is a prospective cohort multi-centre observational sub-study of ACIT II that will co-enroll patients who have been recruited into SWiFT. Through a data sharing agreement between SWiFT and ACIT SWIFT it will enable capture of patient data from injury in the prehospital phase of care (when dosing for SWiFT occurs) with a ACIT SWIFT blood sample on admission to the emergency department. Data for SWiFT will be collected for the first 28 days or hospital discharge. Biomarkers from the blood sample will be compared between treatment arms in SWiFT and correlated with patient injury patterns, trauma-induced physiological disturbances and subsequent clinical course and outcome.

STUDY PROCEDURES

Informed Consent (same as primary ACIT study)

The standard operating procedure for obtaining consent is included in appendix 2.

Primary Outcomes

Primary outcome is endogenous thrombin generation assessed via 10ml research blood sample collected within 60 minutes of arrival in the Emergency Department.

Secondary Outcomes

Secondary outcomes will include incidence of acute traumatic coagulopathy on arrival (defined by platelet count, prothrombin time, fibrinogen, fibrinolysis); admission blood lactate; admission clotting factor levels and markers of fibrinolytic activation/inhibition; quantitative analysis and phenotyping of microparticles; relative contributions of microparticle subtypes to thrombin generation.

Screening and recruitment

All SWiFT trial adult patients (aged 16 and over) who are admitted to the emergency department at an MTC via a trauma team activation will be screened for eligibility to ACIT-ED. As most SWiFT patients will be incapacitated at the time of screening it is anticipated that a waiver of consent will be used followed by initiation of the informed consent process at a time appropriate from the participant as described in Section 9.1.

Schedule of study interventions

Study Activity	**Emergency Department**	**Discharge/ Day 28**
History and Physical	SWIFT data	
Demographics	SWIFT data	
Injury characteristics	SWIFT data	
Physiological observations	SWIFT data	
Adverse events	ACIT-ED	ACIT-ED
Blood Sampling*	ACIT-ED	
Clinical outcomes		SWIFT

**All patients have one blood sample taken on admission at the same as routine clinical blood tests*

Study Intervention

Blood samples

SWiFT trial patients co-enrolled into ACIT SWIFT will have a blood sample drawn to measure activation of coagulation, fibrinolysis, endothelial injury and the immune system. The maximum sample volume for the single blood draw on admission will be 20ml. Severe trauma patients not enrolled into either SWiFT or ACIT would normally have blood samples drawn at minimum at 0, 12 and 24 hours and daily thereafter.

As most major trauma patients have an arterial or central line placed, particularly during the first 24hours when the ACIT SWIFT sample may be taken it will not be painful to the patient. When this is not the case, we will coordinate our blood draw with those of clinical need whenever possible, to minimise 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.

There will be no serial blood sampling during the bleeding episode and at 24hr, 72hrs and Day 7 as research resource will not enable robust data collection 24/7 when SWiFT patients will be arriving at the MTC. The initial post-dosing sample after the SWiFT intervention has been delivered is the most important sample. As the patients will be randomized we can determine how whole blood transfusion improves hemostasis through comparison between trial arms.

Additional data collection

There will be no additional data collected for ACIT SWIFT(outside of the SWiFT trial which will be responsibility of the SWiFT team).

Patient questionnaires

There will be no quality of life assessment collected as part of ACIT-ED. The UK Trauma & Audit Research Network (TARN) now routinely collects patient reported outcome measures on Day 28 and at 6 months which are available to MTCs locally and will be shared with the SWIFT trial team.

Procedure for collecting data

Data collection for this study will be limited to record of blood sample being taken and noted in the SWiFT trial to enable samples to be matched to the SWiFT study ID. It is the Investigator's responsibility to ensure the accuracy, completeness and timeliness of the record to ensure matching can take place.

Follow-up procedures

Follow up will be limited to SWiFT study procedures only.

Patient withdrawal

Each participant has the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the Investigator or at the institution.

Every reasonable effort will be made to maintain protocol compliance and to retain patient participation in the study consistent with the provisions of informed consent and good clinical practice. Additionally the PI may withdraw the participant. The following are potential reasons why a patient may be withdrawn from the study:

- Withdrawal of consent: the patient, the patient's authorised representative, independent physician, or designated individual who had provided initial consent to enter the study may withdraw consent at any time throughout the duration of the trial, without prejudice to future medical care and treatment.
- Retrospective exclusion: If a patient is deemed to not meet one or more of the inclusion/exclusion criteria in retrospect they will be withdrawn from the study.
- Major protocol deviation from the study design by the participant, observed or suspected by the investigator.
- Administrative: the sponsor or monitoring committees decide to terminate or discontinue the study.
- The participant's health would be jeopardised by continued participation and is withdrawn at the discretion of the investigator

Data collected up to the point of withdrawal will be retained. To safeguard the participants' rights, the minimum personally-identifiable information possible will be used.

Any samples obtained from withdrawn participants will be discarded in accordance with the local NHS Trust standard procedures. Samples sent to the central laboratory at the MTC will also be discarded.

The study withdrawal form will be completed for these patients and a reason for withdrawal captured where possible. All participants withdrawn from the study will be managed in accordance with the hospital's standard procedures.

End of Study Definition

Patients are followed up when in hospital in accordance with the SWiFT protocol which is daily until day 28, death or discharge (whichever occurs sooner). If patients remain in hospital beyond day 28, their electronic records will be periodically reviewed remotely until discharge to enable collection of any outstanding endpoint data.

STATISTICAL CONSIDERATIONS

Ex vivo work at the Centre for Trauma Sciences in which patients with ATC had blood samples spiked with either whole blood, or red cells & plasma, has demonstrated a 12% increase in the maximum velocity of thrombin generation in those samples spiked with whole blood. ACIT-ED will use 90% power to detect an increase in maximum velocity of thrombin generation of 10% at 5% significance level and a two tailed test. The required sample size to meet specified power requirements is 438 patients in total (219 in each arm). We have increased this by 20% to allow for drop outs, unbalanced recruitment to ACIT-ED across arms of the SWiFT trial and sampling errors leading to a total of 526 participants.

ETHICS

This study as a sub study of ACIT II will be carried out in accordance with the ethical Principals in the Research Governance Framework for Health and Social Care, Second Edition, 2005 and its subsequent amendments as applicable and applicable legal and regulatory requirements. The ACIT II study has been reviewed and approved by the Research Ethics Committee: London – City and East (07/Q0603/29).

11 Safety Considerations

All parts of the study will be carried out to avoid patient risk and minimize discomfort at all times. At no time will patient care be compromised or delayed for the purposes of the study.

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 minimized 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, in accordance with our local information governance guidelines.

Specific Risks

Blood sampling

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).

Risk/benefit analysis

Although the study carries no direct 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.

PUBLIC INVOLVEMENT

The Centre for Trauma Sciences PPI group - Patient & Public Advisors in Injury Research (PAIR) was set up after the ACIT study first started in 2007. However the group are integral to all protocol amendments and revision of patient consent forms and information sheets. All documentation aimed at children recruited to ACIT have also been reviewed by a RLH youth PPI group – the Youth Empowerment Group. Key research findings generated from the ACIT II study are presented at the PAIR meetings with input on how best to disseminate to the lay public.

DATA HANDLING AND RECORD KEEPING:

Data management

The investigator/institutions will keep records of all participating participants (sufficient information to link records e.g., CRFs, hospital records and samples) and all original signed informed consent forms. A data sharing agreement will be drawn up between NHS Blood & Transplant and QMUL to enable ACIT-ED and SWiFT data to be linked and analysed together.

It is the responsibility of the Chief Investigator (CI) to maintain adequate records for the study including completed CRFs, signed Informed consent documents and all correspondence with the REC and the sponsor. Original documents will be retained as part of the audit trail. Any

amendments or corrections to original documents will be signed and dated.

All paper records including the CRF and consent forms will be stored in a secure restricted access location at the recruiting site. Only de-identified information, as required by the study protocol and captured on the CRF, will be uploaded onto the study database.

Confidentiality

All investigators and trial site staff must comply with the requirements of the General Data Protection Regulation (GDPR) 2018 and Data Protection Act (2018) with regards to the collection, storage, processing and disclosure of personal information and will uphold the regulation's core principles.

The Principal Investigator has a responsibility to ensure that participant confidentiality is protected and maintained. They must also ensure that their identities are protected from any unauthorised parties. Information with regards to study participants will be kept confidential and managed in accordance with the Data Protection Act, the General Data Protection Regulation (GDPR), NHS Caldecott Principles, the UK Policy Framework for Health and Social Care Research, and the conditions of Research Ethics Committee favourable opinion.

The Chief Investigator and the study team will adhere to these parameters to ensure that the Participant's identity is protected at every stage of their participation within the study.

Subject identifiable information (name, date of birth, hospital number) will be recorded for the purposes of consent (on the consent log). Access to all identifiable information will be limited to the study investigators.

Data Custodian Details

Queen Mary University London is the sponsor for the study based in the United Kingdom. They will be using information from participants and their medical records in order to undertake the study and will act as the data controller for the study. This means that Queen Mary University London are responsible for looking after the information and using it properly.

Participant's rights to access change or move their information is limited, as it needs to be managed in specific ways in order for the research to be reliable and accurate.

Record Retention and Archiving

During the course of research, all central and site records are the responsibility of the Chief Investigator and must be kept in secure conditions. When the research trial is complete, it is a requirement of the UK policy framework for health and social care research and Trust Policy that the records are kept for a further 25 years.

Site files from other sites must be archived for 25 years at the originating site and cannot be stored within Queen Mary University of London.

Destruction of essential documents will require authorisation from the Sponsor.

LABORATORIES

Central Laboratories

The research laboratory on Ward 12D, The Royal London Hospital, Bart's Health NHS Trust will be the primary site for processing and preparation of blood samples for future use. The Blizard Institute Queen Mary University London will perform the biomarker analyses for locally collected samples and those sent from external sites when collaborative cohort studies are conducted. Other recruiting sites may also conduct biomarker analysis within their local laboratories according to their own specific local laboratory protocols and procedures. All procedures will be documented in the laboratory manual.

Local Laboratories

Local NHS UKAS accredited Haematology and Clinical Biochemistry laboratories at each participating centre will perform the analysis of routine clinical blood samples taken.

Sample collection and preparation

Blood taken for future use will be drawn from either a central, arterial or large bore peripheral line sited for purposes of patient care or, from femoral vein or antecubital fossa. Muscle biopsies will only be taken from the wound edge at surgical sites from tissue that would otherwise be discarded.

Biological samples will be collected, processed and stored at the research centre where the

participant is enrolled. All samples will be allocated the unique study identifier (pseudoanonymised) given to the participant at enrolment and stored in teams' local laboratories at -80oC for future use. Samples should be clearly labelled with the participants unique study identifier, sample type and sample time point. Samples may be stored in their original vacutainer, or processed and aliquoted into suitable freeze proof sealable eppendorfs. Guidelines for sample preparation and storage can be found in Appendix 1.

Laboratory procedures

All plasma samples will be measured by a commercially available ELISA kits or via automated assay.

Sample storage and transfer

Biological samples processed and stored at the research center where the participant is enrolled. Upon completion of the ACIT-ED recruitment some samples will be sent to the Centre for Trauma Sciences for analysis.

FINANCE AND FUNDING

Departmental funding from the Centre for Trauma Sciences pays for all consumable costs for patients recruited at Bart's Health with a long-standing agreement from Werfen Ltd who provide research support costs for ROTEM equipment and consumables. Assays and consumable costs as well as research time for consenting, sample processing etc at each MTC will be funded through a charitable grant or a major grant body.

INDEMNITY

The insurance that Queen Mary University of London has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm.

DISSEMINATION OF RESEARCH FINDINGS:

Data will be written up, presented and disseminated in a timely manner. The main outputs will be research papers for general journals and trauma specialty journals, research abstracts for presentation to national and international meetings and a final report summarising the overall findings.

Study collaborators will be named as authors on any publication or report. Findings will be disseminated via the centre for trauma sciences website, and via online seminars for MTC participation. Public dissemination will be achieved through our various educational outreach events and at regular PAIR meetings.

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