1. TITLE

THE ORiF **P**rocedure m**E**chanisms of **R**ib fix**A**tion (OPERA) STUDY

2. SUMMARY OF RESEARCH (ABSTRACT)

Research question

Do the anticipated inflammatory pathway effects account for the mechanisms of action of rib fixation in patients with multiple traumatic rib fractures. Specifically, do they influence recovery and clinical outcomes, and do patient characteristics modulate these processes, enabling more stratified and improved clinical care?

Background

Injuries from trauma are a leading cause of death in the UK, and the biggest killer of under 35 year olds worldwide. Judicious surgical intervention in the traumatised patient is known to improve outcomes; it is thought to limit the damage caused by the injury, and restore function. Many patients who sustain severe traumatic injuries require admission to the intensive care unit following injury and may require a combination of lifesaving resuscitative surgery, injury limiting surgery and longer-term reconstructive surgery. Earlier surgery can limit the physiological stress from the untreated injury, however, it also exposes the patient to a surgical insult which leads to a further stress response (1) at a time of physiological instability. It is now understood that the timing of surgical intervention can be important and that the surgical reconstructive strategy should be tailored to a patient's individual physiological response characteristics (2, 3). There is substantial evidence that the response to the same traumatic and physiological insult varies between patients (e.g. by age (4) and gender (5), and by the time frame (3)) throughout the healing phase. Specifically, this variation is linked to multiple factors including the inflammatory response to trauma, the role of body composition (6, 7), the influence of the gut microbiome (8-10), the timing of surgical intervention (2) and key patient characteristics; all of which are believed to potentially alter the recovery pathway and clinical outcomes. We hypothesise that rib fixation results in reduced generalised hypoxia/hypoxemia and localised hypoxemia by lowering the risk of atelectasis and decreasing, via lower pain levels, the production of catecholamines. Both of these pathways could explain the mechanisms whereby there is less localised inflammatory responses after receiving rib fixation surgery. These pathways would in turn be modulated by the intrinsic patient characteristics described above. To date no mechanistic studies have been carried out to establish the impact of rib fixation on inflammatory pathways or its interaction with key individual patient characteristics and their impact on outcomes in the traumatic rib fracture population.

In this mechanisms of action study (OPERA), we will use the ORiF (The Operative Rib Fixation) study, an NIHR HTA funded randomised controlled trial (RCT), as a vehicle to assess these important questions surrounding the optimisation of the physiological response to trauma, the mechanism by which surgery interacts with the determinants of recovery and how these are reflected at a molecular, cellular and patient level. We hypothesise that the common inflammatory pathway suffered by patients during major injury and surgery drives all complications via a common pathway injury including death, multiple organ failure, sepsis, infection and thromboembolic disease. The main outcome of interest in the OPERA study is complication free recovery (i.e. no medical complications surgical complications or prolonged recovery).

The common pathway for complications following trauma and surgery is inflammation. We have identified the two most likely drivers of inflammation that would be moderated by surgery for rib fractures (pain and hypoxia) and the mechanisms through which this modulation is likely to happen and relevant modulators (development of SIRS modulated by gut microbiome, body composition and muscle strength, healing measured with resolvins and bone healing markers) The mechanistic findings from traumatic rib fracture patients and the role of rib fixation in this setting will serve as a model for surgical intervention in the wider setting of major traumatic injury.

Aims and objectives

Aim To inform patient care by assessing whether the patient pro-inflammatory responsiveness to injury and patient characteristics, impact upon the proposed inflammatory pathway mechanisms of action of rib fixation for severely injured patients with traumatic rib fractures.

We will thus understand the mechanisms underlying fib fixation and how they influence clinical outcomes, and the related clinical decisions surrounding patient care.

Objectives These are:

1. To assess if the efficacy of rib fixation surgery is through modulation of patient pro-inflammatory responses by reducing pain mediated catecholamine responses, and by reducing hypoxia through improved ventilation thereby resulting in a lower risk of atelectasis. These effects will be measured by:
   a. Assessing if the patient's inflammatory response is altered by rib fixation by;
   b. Quantifying the effect of rib fixation on the patient's gut bacterial microbiome and on secondary sepsis and SIRS;
   c. Establishing if higher diaphragmatic and costovertebral muscle strength contributes to improved ventilation, and increased sarcopenic decline or lower baseline muscle strength results in higher breathlessness, hypoxemia/hypoxia and inflammation.
2. To assess treatment pathway mechanisms by
   assessing if the proposed patient stratification and treatment pathway modifiers affect the efficacy of rib fixation;
3. To identify biological targets, responsible for the mechanisms of action of rib fixation surgery, for potential ancillary treatment to optimise the efficacy of rib fixation surgery.
4. To explore the prognostic value of the findings by undertaking prediction modelling of recovery using both treatment pathway and biological factors to quantify how predictive the hypothesised mechanisms involved in the efficacy of rib fixation are.

Study design

The ORiF Procedure mEchanisms of Rib fixAtion (OPERA) will utilise data from 3 sources: a) The ORiF trial cohort (n=532 including body composition); b) Additional blood and faecal samples in a subset of ORiF participants from both arms (n= 212) to assess inflammatory and muscle strength biomarkers. Planned statistical analyses for the different objectives include mixed modelling, mediation analysis, propensity score weighted analysis and prognostic modelling.

Patient and public involvement

We designed the study with the help of a panel of 15 patients who had surgery after a serious injury or broken ribs. They all thought the design was acceptable to patients and that the research question was important. They were keen that doctors looked at the patient as a whole. They felt the study may allow future patients to have more personalised information, which they don't currently receive.

Timelines for delivery

Some study set up will begin prior to the start of the grant. This is possible due to the infrastructure of Royal College of Surgeons (RCS) Surgical Intervention Trials Unit (SITU), ORiF trial and the availability of key personnel. Ethical approval (via substantial amendment) will be in place by the grant start date. Recruitment to the sample collection ORiF sub-study component will be run from months 1-14, follow-up and sample transfer completed by month 18. Sample laboratory analyses and processing will run from months 15-24. Months 25-29 will focus predominantly on laboratory and cellular mechanistic analyses and month 25-30 will be write up of publications and the report. The associated statistical work will be ongoing from months 13 utilising interim data (ORiF baseline data) to set up models, with the biological data analyses conducted once recruitment has been completed.

Anticipated impact and dissemination

This study will have impact at various levels. First, it will influence the care of patients with traumatic rib fractures and the role of surgical fixation. Whatever the findings of ORiF trial, the exploration of the generalisability of the result, and the mechanisms of action underlying surgery will be important. Second, it will also assess potential areas to improve current supportive care informing the development of interventions to benefit all traumatic rib fracture patients. Thirdly, it will serve to inform potential future mechanistic research related to patients with traumatic injuries in general, an underdeveloped but potentially impactful, clinically relevant and rich research area. The generalisability of the results from this mechanistic study, therefore, will potentially have significant impact for the treatment of traumatic injuries, not just rib fractures.

3. BACKGROUND AND RATIONALE

How the OPERA study fits into the EME remit and the mechanistic study call

The proposed study takes advantage of an NIHR funded HTA programme RCT, ORiF, to understand the mechanisms of action of rib fixation surgery and associated non-operative treatments in chest wall trauma (flail chest and fractured ribs) and recovery. The proposed research explores the mechanisms of treatment efficacy, which in turn lead to improvements in health and patient care. It also specifically falls within the current injuries, accidents and urgent and emergency care themed call which the EME programme is participating in.

TABLE 1 - ORiF TRIAL SYNOPSIS

Title	The Operative Rib Fixation (ORiF) Study	
Study Design	RCT with registry embedded data collection	
Study Participants	Adult patients with multiple rib fractures suitable for surgical fixation.	
Planned Sample Size	532	
	Objectives	**Outcome Measures**
Primary	● To assess differences in all-cause mortality between the rib fixation with plates and screws in addition to supportive management versus supportive management alone at 12 months; ● To assess differences between rib fixation with plates and screws in addition to supportive management versus supportive management alone groups in quality of life over 12 months following injury.	● All-cause mortality data ● EQ-5D-5L index with direct trial collection of primary outcome data

We propose the OPERA project developed in response to prior work to address hypotheses on the mechanisms underlying the development of major complications of multiple rib fractures, and the role of surgical treatment. Specifically, the study aims to confirm suggested mechanisms which would allow identification of individuals at higher risk of serious complications, such as systemic inflammatory response syndrome (SIRS) (11, 12), multiple organ dysfunction (MOD) (13, 14) and sepsis (8, 11, 15), which impede recovery and can lead to mortality. It will also assess which patients benefit from surgical treatment and when. The mechanism of action in terms of clinical outcomes particularly complications and mortality using a composite outcome measure of complication free recovery will be explored. This research will inform whether and how to intervene early in these severely ill individuals, and to target their treatment (both medical and surgical) accordingly to address the drivers of complications, increased hospitalisation and mortality. Collectively this research will drive forwards future mechanistic work in traumatic injuries, and it will clarify the most valuable direction for further hypothesis driven studies.

Overview of the ORiF Trial

The opportunity presented by the NIHR HTA programme funded ORiF trial (16/61/10) will be exploited to assess mechanisms of action related to the injury response and treatment (particularly

surgical fixation) of severely injured patients. The ORiF study proposal was originally submitted to EME and incorporated a mechanism of action embedded study. However, it was transferred to HTA programme partly due to their being a similar proposal to that programme. We were successful in securing funding for the ORiF study (see Table 1 for a synopsis of the ORiF trial). It is progressing well and is at an early stage of recruitment (156 of the target 532 as of the 8th Jan 2021). Submission of a mechanisms of action study was specifically invited at the time of transfer to HTA programme.

Why this research is now needed

Trauma continues to be a national and global significant health care burden. In 2016, 80,000 patients presented with major injuries in England, of whom 13,000 had a chest injury due to a moderate or severe traumatic injury. In addition to being the biggest killer of under 35s, the morbidity associated with survivors of trauma is significant (14). Despite increases in road safety, severe chest injury due to road traffic accidents is a common cause; alongside assault, and in the older person, frailty related falling chest injuries are increasing as the population ages. Surgical intervention can improve both survival and quality of life; the ORIF study is designed to assess both outcomes for patients with traumatic rib fractures. Following injury patients suffer a major metabolic response and outcomes including death and disability are thought to be driven by inflammatory cascades. Judicious surgery is known to be able to improve outcomes, however the added burden of surgical insult can worsen prognosis. The underlying mechanisms of recovery following traumatic rib facture injury, and the treatment of it need evaluating.

The NHS has recognised urgent and emergency care, and specifically trauma and injuries as a major healthcare challenge in its 'Long Term Plan (2019)' the NHS recognised the importance of '*improving and developing new ways to look after patients with the most serious illness and injury*', with major trauma and associated care pathways identified as a major challenge and an area that can be developed to reduce morbidity and mortality. This is reflected in the NIHR current priority area and has commissioned a pan-NIHR call "Injuries, accidents and urgent and emergency care themed call". This commissioned call recognises the need to evaluate delivery of interventions in trauma within the secondary care setting. The EME programme is participating in this themed call, and this proposal seeks to increase the understanding of traumatic rib fractures and the use of surgery to treat a major injury in the emergency care setting. Furthermore, this work is submitted under the Mechanism of Action of Health Interventions Call (19/119). In line with the EME programme's stated objectives for the call this application examines in a hypothesis driven manner the underlying possible efficacy. This proposal is timely as there is currently a limited window of opportunity to carry out additional data sample collections on a subset of ORiF participants to address key mechanisms. Furthermore, it will provide funding for vital, but time-consuming and relatively complex statistical analyses to i) assess how surgical rib fixation influences inflammatory mechanisms linked to body composition, gut microbiome, and responses to rib fracture injuries ii) evaluate generalisability of findings and whether purported risk factors function in the hypothesised manner in terms of patient selection and intervention timing, iii) explore the predictive value of these factors biological and demographic can be explored in terms of adverse outcomes, and iv) identify targets for new (medical) interventions.

Chest injuries are common in young patients suffering from high energy trauma (such as road traffic accidents) and in the elderly, where they are the second most common fragility fracture. Both populations carry a high morbidity and mortality (16) despite developments in supportive management. Poorer outcomes are seen in patients requiring ventilatory or cardiovascular support on ICU. Patients managed on ICU require specialist nursing, cardiovascular and often ventilatory support. Rehabilitation is prolonged and results in lifelong exertional restriction and secondary disability for those who survive (17). Qualitative work from our own group (18) suggests a significant long term burden with prevalence of chronic pain. Care of patients with chest wall injuries (including both traumatic fractures and fragility rib fractures) represents a major financial and social burden to the NHS. Chest trauma was the primary cause of death in 25% of polytrauma patients (19). The most common type of blunt chest wall injury is rib fractures, which are associated with considerable morbidity and mortality due to respiratory complications resulting

from pain and impaired respiratory and ventilatory function (20). Our previous work (systematic review and meta-analysis (21)) showed that pneumonia is a common complication following rib fractures, and one that seems to reduce following fixation, within the limits of the available data (the ORiF trial will provide more definitive evidence). In those traumatised patients with multiple rib fractures, up to 31% of patients will experience nosocomial pneumonia, prolonged respiratory failure, prolonged hospitalisation or death (22) (23). This finding underpins our hypothesis that the key mechanism of action for surgical rib fixation efficacy is related to improved ventilatory function.

The second major serious complication seen in rib fracture patients is systemic inflammatory response syndrome (SIRS) which is defined by a set of criteria that indicate a hyperinflammatory state representing a complex pathophysiologic response to tissue damage following an insult such as infection, trauma, burns or a variety of other insults. Majorly injured patients suffer from SIRS and this over-activation of the inflammatory cascade (11, 15). The development of SIRS during major injury is not fully understood and whilst judicious surgery can reduce the insult, it is well known that surgery can also increase the risk of developing SIRS, complications and death. SIRS is a hyper-inflammatory state common to all severely unwell patients that is driven by a cytokine storm and culminates in migration of neutrophils into inflamed tissue to release free radicals. In the respiratory system SIRS is characterised by capillary congestion, leukocyte and macrophage infiltration into alveolar spaces, in the gastrointestinal system it is characterised by loss of the endothelial barrier and translocation of gut bacteria and toxins across the gut endothelium, both are harmful and major drivers of complications. Whilst SIRS is well studied, little is known about the drivers for the pathway or which patients are most likely to experience SIRS following trauma or how surgery might modulate this response. The molecular pathways that drive SIRS are identical irrespective of aetiology with common inflammatory markers such as TNF (tumour necrosis factor)-α and interleukins being seen in both sepsis/non-sepsis SIRS (24).

SIRS accompanying hospital-acquired pneumonia in intensive care unit (ICU) patients with acute respiratory failure more than 48h after intubation is defined as ventilator associated pneumonia (VAP) (25); and affects around 30% of the most vulnerable patients (26), increasing morbidity, mortality, length of stay (LOS), and costs (27). Sepsis, SIRS caused by an infection, is categorised as severe if complicated by the presence of multi-organ dysfunction (MODS) (28). Development of SIRS is one of the key predictors of morbidity, ICU time, and mortality in rib fracture patients (29). Patients are not suitable for surgery once SIRS is established due to high mortality, and failure of supportive measures, however there is evidence that early rib fixation surgery my reduce ventilator days and development of respiratory complications including SIRS (21).

Fixation of the ribs in an unstable (flail) chest wall injury (Abbreviated Injury Scale (AIS)(30) 3-5) can improve ventilatory mechanics which reduces ICU stay and complications such as pneumonia and death. Published studies evaluating surgical fixation are limited to three small randomised studies (31) (32) (33) and a number of mostly very small observational studies (34) (35) (22) (21). The most recent approach to rib fracture fixation using plates and screws is becoming more widely used though currently only for the more severely injured patients, but not those who are peri-morbid. Existing evidence, despite its limitations, suggests benefits of fixation including the possibility of a substantial practice shifting mortality benefit. There is substantial uncertainty regarding for whom, and when, rib fixation should be carried out for patients with traumatic rib fractures. The ORiF trial is assessing the efficacy of the surgical intervention in addition to supportive medical management for multiple rib fracture patients. However, existing work suggests that a number of factors influence the clinical outcomes of surgery.

The timing of surgery has been suggested to affect the outcome of treatment in previous, mostly relatively small scale and crude evaluations (2)(36)(37)(38). Patients need to be sufficiently stable to undergo surgery which represents a significant psychological and physiological stress so the immediate negative impact of the surgical procedure needs to occur at the appropriate time post injury; thereby reducing the risks of worsening the stress response and SIRS. The ORiF trial (see above for more details on this study) which is currently recruiting, will assess rib fixation within 72 hours of traumatic injury; however, this time window reflects partly limitations in the existing

evidence to be more specific, current clinical practice guidance (BOAST-15(17)) informed to a large extent by expert opinion, and also constraints within the current NHS emergency care system. Assessing the timing of rib fixation surgery will inform optimal care. There is evidence that variation, even within 72 hours, may affect the benefit received from fixation (2) (36) (37) (38). Outside of the ORiF trial, timing of rib fixation surgery will vary between sites and patients.

A number of key patient characteristics are believed to affect the outcome of traumatic rib fracture patients, influence the disease progression and the benefit of rib fixation surgery. Specifically, the hypothesised impact of injury severity (routinely measured as the AIS score) with fixation surgery increasingly in use as AIS score, and severity, reduces), polytrauma status, age at injury (two distinct subpopulations existing of typically young road traffic accident injuries and frailty-related injuries in the elderly who it has been proposed have differing injury responses and tolerance for both the injury and rib fixation (39), and gender (evidence suggests for a given age men respond worse than women to an equivalent traumatic event). However, there is little evidence on this applied to the trauma patients, and even less to the care of traumatic rib fracture patients. To date assessments have been relatively crude, unadjusted and/or small scale studies (22) (34) (35).

In this OPERA project, the ORIF study will be used as a vehicle to assess these important questions surrounding the anticipated physiological response to trauma, the mechanism of action of surgical fixation and the determinants of outcomes and how these are reflected at a molecular and cellular level through to patient population level. This will be carried out as a sub-study of the ORIF trial using the same rib fracture trauma population. All participants in ORIF will be approach to participate in the OPERA additional patient collection (until the sample size of 212 is reached). The findings from the traumatic rib fracture population and the role of rib fixation in this will serve as a model for surgical intervention in the wider major injury setting.

The aim of surgical fixation is to restore compromised respiratory function in patients with multiple rib fractures. Rib fractures reduce local ventilation which inhibits gaseous exchange, resulting in end alveolar hypoxia and resultant inflammation(40) as has been widely verified in COVID- 19 patients (41) In addition, reduced ventilation generates pulmonary oedema, localised acidosis and pneumonia. Improved ventilation improves gas exchange, reduces hypoxia thus reducing local tissue damage and inflammation thereby facilitating recovery and preventing further complications. During surgery the patient goes under general anaesthesia and receives ventilation support during the surgery usually including PEEP (positive end expiratory pressure) which serves to increase ventilation in the end alveolar further reducing localised hypoxia and inflammatory related ischaemia. In the unoperated patient intervention includes pain relief starting with mild analgesics, progressing to weak opioids, morphine and eventually anaesthesia. During the first few days following injury this exposes the unoperated patient not just to hypoxia due to lack of ventilation but to increased pain mediated production of catecholamines (42, 43) which in turn will increase both oxygen demands and inflammatory response (44, 45). Like all operative interventions, rib fixation surgery itself is traumatic and localised tissue damage results in activation of local and systemic inflammatory cascades. Individuals vary in how they are able to tolerate surgery following injury. Other factors such as the severity and nature (i.e. polytraumatic or not) of the injury, their age, gender (5, 22, 39) and comorbidities also have a role. Additionally, it is known that the immune response to the initial traumatic injury varies over the early period after the injury. There is therefore a strong rationale, and specific hypotheses as outlined below, relating to each of these factors that suggest a stratified approach to surgical fixation in the area as they affect and mediate the mechanisms of action.

The knowledge gap this research will address

The ORiF study is designed to assess the efficacy of rib fracture fixation in the setting of blunt chest wall injury. The OPERA study is designed to assess the underlying mechanisms for efficacy via a clear hypothesis driven approach. We will assess cellular, molecular, patient demographic, treatment and injury characteristics at a cellular, patient, and population level to answer the hypothesis and draw inferences to guide the treatment of future patients. The ORiF study includes both isolated chest injury and polytraumatised patients. We will further explore the relevance of

our findings in the polytrauma setting, to assess the impact of the surgical insult, and appraise treatment strategies for multiple injuries where rib fracture surgery treats only one of the injuries. OPERA has been designed to sample the population of both operative and non-operative patients recording complications and markers of healing (including resolvins). In combination with body composition data and serum cytokines we will be able to establish which mechanisms drive complications and delays to recovery. OPERA will also assess the contribution of the patients gut microbiome during injury and recovery on outcomes. It will establish if patients' gut microbiome composition and levels of gut permeability are implicated in outcomes following injury, surgery and development of complications in the same way as it is in other acute and hyperacute settings.

We hypothesise that rib fixation in operated patients will result in reduced generalised hypoxia and localised hypoxemia by improving end alveolar ventilation. It will also decrease, via lower pain levels, the production of catecholamines. These two mechanisms will result in lower localised inflammatory responses in those receiving rib fixation surgery which will in turn be modulated by intrinsic patient characteristics. Accordingly, we hypothesise that:

I. Higher resolvin:leukotriene ratio at baseline will be linked to a higher probability of a complication free recovery following rib fixation surgery. Increases over time in this ratio will correlate with better clinical outcomes, with greater muscle loss and higher pro-inflammatory cytokine levels at follow-up.

II. Higher gut microbiome alpha diversity will correlate with a higher probability of a complication free recovery post-rib fixation and with lower pro-inflammatory cytokine levels in response to surgical insult;

III. Higher gut permeability (measured by lipopolysaccharide (LPS) and zonulin in serum) will correlate with higher risk of complicated recovery & infection following rib fixation surgery;

Unoperated patients' higher diaphragmatic and costovertebral muscle strength are crucial to contribute to improved ventilation, and increased sarcopenic decline or lower baseline muscle strength will result in higher breathlessness, hypoxemia/hypoxia and inflammation. Therefore we hypothesise that:

IV. Higher muscle mass from CT scan body composition, lower levels of muscle loss biomarkers, higher levels of muscle anabolism, will correlate with lower inflammatory cytokines at baseline and a higher probability of a complication free recovery among the unoperated patients but will be less important than in those receiving rib fixation.

The place of rib fixation within the treatment pathway can be refined in light of the ORiF trial result whatever they be given as rib fixation is the current treatment of choice for the most severely injured. We hypothesise that:

V. The timing of rib fixation surgery is important and early surgery is associated with better clinical outcomes given the presumed mechanism of action;

VI. The clinical outcome following rib fixation surgery is determined by a number of key patient characteristics as proposed in the literature: reduced severity of injury is associated with better clinical outcome, polytrauma status has poorer clinical outcome, that outcome varies by age (younger individuals have better clinical outcome) and gender (women have better clinical outcome for like injury) reflecting the presumed mechanisms of action of the surgery. These will be explored as able within the constraints of the ORiF trial and OPERA additional data.

Molecular pathways involved in the development of SIRS

Early proinflammation via innate immune system activation may cause early organ dysfunction, while anti-inflammation, via inhibition of the adaptive immune system and apoptosis, may induce immunoparalysis, impaired healing, and multiple organ failure (13). A recent pathway analysis of a murine model of sepsis identified pathways involved in SIRS (46). The major gene set involved was "signaling in immune system" including innate immunity, Toll receptor cascade, and inflammatory signalling pathways (e.g. TNF signaling). The authors confirmed differential upregulation of several key members of these pathways (*Tlr2, Tlr4, TNF-a*) across organs during using qPCR in an independent set of animal experiments and concluded that, given the size and functional profile of this gene sets, it is likely a dominant player in multi-organ response during SIRS. Based on this and clinical evidence (see below) we have focused on three specific narrow set of markers as mediators of the SIRS response in the context of rib fractures and flail chest.

Specialized pro-resolving mediators (SPMs also known as resolvins)
Resolvins, or soluble pro-resolving mediators (SPMs) have been proposed for almost a decade now as possible analgesics for inflammatory pain. Their name signifies 'resolution phase interaction products', They enable inflamed tissues to return to homeostasis once the need for inflammatory response is over they assist in the resolution of inflammation and act in nanomolar and picomolar dose ranges in vitro and in vivo (47). Resolvins are not immunosuppressive and, in fact, counter-regulate a variety of inflammatory mediators. They limit further recruitment of peripheral mononuclear neutrophils, and counter regulate the initial regulators produced in the inflammatory response. Resolution of inflammation is an active process with different mediators and control mechanisms (47), (48). Whilst resolvins inhibit prostaglandins and leukotrienes, they promote wound healing and have immunoresolvent effect (49). Recent evidence demonstrates that situations of excessive inflammation, correspond to a low expression of resolvins and a high abundance of inflammatory mediators such as cytokines, prostaglandins and leukotrienes (48). Antimicrobial functions are particularly compromised in individuals with low levels of these substances. Investigations in trauma patients (n=96) have recently shown that those with uncomplicated recoveries had higher resolvin pathway gene expression and lower gene expression ratios of leukotriene:resolvin pathways (49). Based on the above and on extensive evidence from animal studies (50) (51) we expect a strong predictive relationship between resolvin levels on incidence of SIRS in our clinical trial and complications.

Gut microbiome composition, wound healing and SIRS/sepsis
The gut microbiome or intestinal microbiota is now known to be an important regulator of inflammation (52), and part of its effect on inflammation is known to be via Toll-like receptor signalling (53), one of the key pathways identified in murine models of SIRS (46). This may account for some of the strong effects of the gut microbiome that have been seen linked to recovery after traumatic injury (54). Dramatic changes in gut microbiome composition have been seen in patients after a major trauma (55) with significant drops in microbiome diversity and the production of anti-inflammatory short chain fatty acids by gut microbes. Importantly gut dysfunction promotes distant organ injury. During the injury and treatment pathways physiological insult in the form of nosocomial and iatrogenic 'hits' exaggerate the immune response, contributing to MODS. This is suggestive that infectious and non-infectious causes of inflammation may trigger, heighten, and perpetuate an inflammatory response culminating in MODS and death. Emerging evidence suggests posttraumatic injury mechanisms, such as intestinal mucosal disruption and shifting of the gut microbiome into a pathological state (54). In murine models of injury gut dysbiosis impairs recovery (10) and can be reversed by the addition of healthy gut microbes via faecal matter transplantation or probiotic supplementation.

We have described a straight-forward measure of gut dysbiosis (low gut microbiome alpha-diversity) (56) which indicates high inflammation and low production of anti-inflammatory short chain fatty acids. Importantly, a recent study in the US has described how both alpha and beta microbiome diversity measures are significantly correlated with risk of mortality, LOS, ICU LOS, number of days on the ventilator, infections, and ARDS (Acute Respiratory Distress Syndrome) sustaining severe injury admitted to a Trauma Centre (9). We propose to compute these measures in a subset of ORIF participants. In addition to the crucial role of the gut microbiome in modulating innate immune responses and controlling inflammation, gut dysbiosis, via gut permeability, also contributes to sepsis (8) (57). in critically ill patients and is often underdiagnosed. It is therefore also necessary to account for the extent to which the gut has become leaky and to do so we propose to measure levels of zonulin and lipopolysaccharide in serum.

Muscle loss and clinical outcomes
Sarcopenia (muscle loss) has been increasingly reported as a prognostic factor for outcome in emergency surgery. The quantity of skeletal muscle calculated from computed tomography (CT) images has been shown to correlate with ventilation-free days, patients' length of stay in the intensive care unit, and 28-day mortality.(6) It has also been linked to poor functional outcomes at discharge from hospital in patients following trauma (7) as well as a resultant depending living

status on hospital discharge (4). Sarcopenia is prevalent in geriatric trauma ICU patients and is an independent predictor of poor functional outcomes (7). Indeed, assessing for sarcopenia has great potential as a prognostic tool in older trauma patients (7). However, a recent study suggests this predictive power may be confounded by the presence of chronic renal insufficiency and cancer. (58) We therefore intend to assess the role of muscle loss on uncomplicated recovery and then to adjust for inflammatory status (measured by cytokine profiles and two specific measures of gut dysbiosis) and healing status (measured by the resolvins and bone formation markers). We will thus determine whether muscle loss is a causal pathway of worse outcomes for trauma patients or simply a reflection of other ongoing pathologic processes. If the former, addressing muscle loss will become imperative as part of the clinical strategy to treat patients at risk of SIRS.

4. AIMS AND OBJECTIVES

Aim The study's aim is to inform patient care by assessing whether the proposed patient pro-inflammatory responsiveness to injury and treatment pathway characteristics, impact upon the recovery and efficacy of surgery for severely injured patients with traumatic rib fractures.

We have identified two key factors or 'pre-conditions' related to an individual patient in the form of sarcopenia and inflammation that interact with trauma as well as rib fixation surgery to determine the nature and magnitude of innate immune response resulting in clinical outcomes. We will thus understand the mechanisms underlying the influence of these determinants and the clinical decisions surrounding patient care.

Objectives These are:

1. To assess if the efficacy of rib fixation surgery is through modulation of patient pro-inflammatory responses by reducing pain mediated catecholamine responses, and by reducing hypoxia through improved ventilation thereby resulting in a lower risk of atelectasis. These effects will be measured by:
    a. Assessing if the patient's inflammatory response is altered by rib fixation;
    b. Quantifying the effect of rib fixation on the patient's gut bacterial microbiome and on secondary sepsis and SIRS;
    c. Establishing if higher diaphragmatic and costovertebral muscle strength contributes to improved ventilation, and increased sarcopenic decline or lower baseline muscle strength result in higher breathlessness, hypoxemia/hypoxia and inflammation.
2. To assess treatment pathway mechanisms by assessing if the proposed patient stratification and treatment pathway modifiers affect the efficacy of rib fixation status.
3. To identify biological targets, responsible for the mechanisms of action of fib fixation surgery, for potential ancillary treatment to optimise the efficacy of rib fixation surgery.
4. To explore the prognostic value of the findings by undertaking prediction modelling of recovery using both treatment pathway and biological factors to quantify how predictive the hypothesised mechanisms involved in the efficacy of rib fixation are.

Deliverables from the project

This project will produce a number of deliverables:

1. Collection of blood and faecal samples for 212 individuals taking part in this ORiF mechanism study over the 3 months follow-up period;
2. Assessment of hypothesised impact of body composition biomarker impact upon clinical outcomes following rib fixation;
3. Assessment of hypothesised impact of gut microbiome, resolvins and muscle loss upon clinical outcomes following rib fixation;
4. Assessment of hypothesised surgical fixation effect mediation and modifiers upon clinical outcomes;
5. We will explore if treatment and exposure effects seen in surgery for isolated rib fractures are seen replicated in rib fracture surgery in the polytraumatised cohort; within the constraints of the ORIF trial and OPERA additional sample data.
6. Publications of main findings of the study (three high impact papers are anticipated)
7. Production of report to the funder.

5. RESEARCH PLAN / METHOD

Research design

This proposed research study seeks to the utilise the unique opportunity present by the ongoing ORiF trial to carry out hypothesis driven mechanism evaluations of the impact of severe chest trauma injury upon patients and to assess the corresponding role of rib fixation surgical treatment. It will utilise the data collected within the ORiF trial (59), from the national registry within which it is embedded on the ORiF cohort, TARN (trauma Audit and Research) (60) registry (see below for further details), along with specific recruitment of patients to additional data collection for this study (OPERA). The study overview is shown in **Figure 1**.

Study population

The target population is patients with multiple rib fractures suitable for surgical fixation. The ORiF trial patient population reflects current accepted national standards and practice. Patients with either isolated chest injuries or polytrauma including chest injury will be eligible. Analysis of the TARN data extract (2) demonstrates over 95% of rib fracture fixations occur in patients with a severe (AIS 3+) thoracic injury. The ORiF trial inclusion/exclusion criteria is as follows:

Inclusion/exclusion criteria

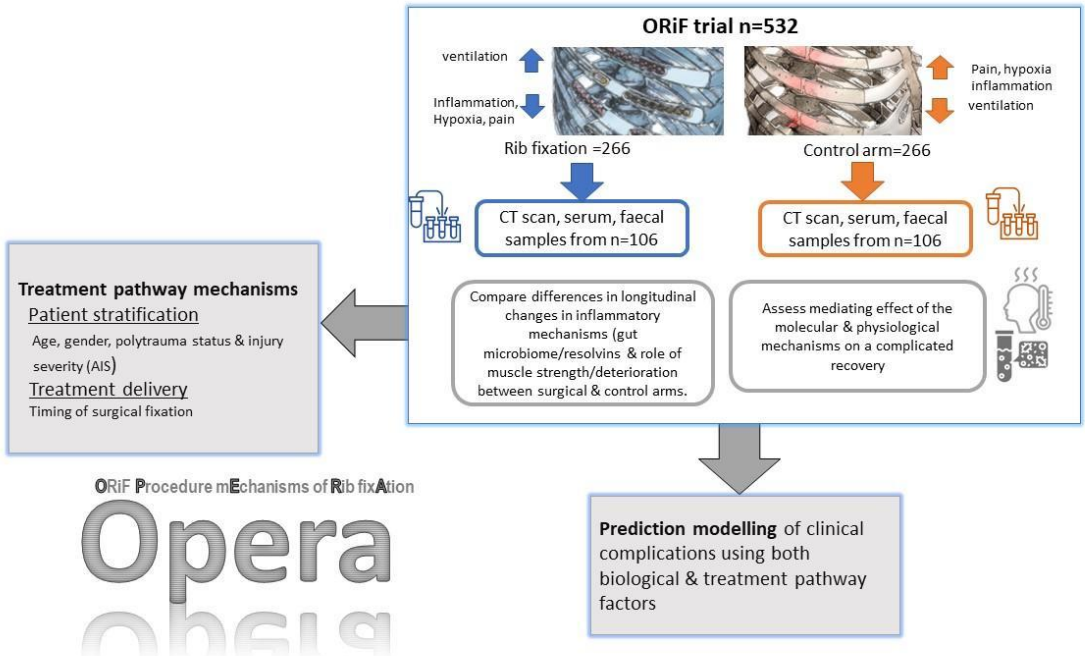
Inclusion criteria is adult patients (16 years and above) who will be suitable for inclusion in the study if they:

- Present with multiple (3+) rib fractures suitable for surgical repair
- Have, as per BOAST-15 Standard 8, indications for fixation as: Clinical flail chest, respiratory difficulty requiring respiratory support or uncontrollable pain using standard modalities
- Are a surgical candidate

Patients will be excluded if:

- They have a head or thoracic injury requiring emergent intervention.
- Cannot be operated on within 72 hours as unfit for surgery
- Significant thoracic injury requiring surgery where conservative management would be inappropriate.

Figure 1 Overview of the ORiF Mechanism of action study



Sample/data from interventional studies

Overview of data utilised in the OPERA study

The ORiF trial cohort will be utilised in various ways to collect the required data:

1. Patient characteristics and data relating to the delivery of rib fixation including its timing is already being collected within the *ORiF trial (See section 3)*.
2. *The* ORiF trial is embedded within *a population cohort* (*TARN –Trauma Audit & Research Network*) increasing clinical data acquisition for ORiF participants .
3. *Additional data collection* The ORiF trial is described in detail in section 3 along with a description of the relevant data collection collected on a subset of the ORiF trial participants (see below for details). .

TARN registry data

The TARN registry is a national registry to which data submission is mandated by NHS England and therefore has excellent capture of patients. It is linked to both Hospital Episode Statistics (HES) and the Office for National Statistics (ONS). Data completeness of TARN data is of a high standard. Importantly inclusion is not dependent upon receipt of particular treatment and therefore is a suitable infrastructure within which to nest a RCT. Remuneration in the form of best practice tariff for treatment of trauma patients is dependent on the corresponding TARN submission, and hospitals are benchmarked on their data completeness. The TARN national registry is be used to collect core clinical data, process measures including admission details and patient demographics and short-term secondary outcomes as part of the ORiF trial. The TARN dataset is queried monthly to ensure data completeness and the trial team will raise data queries with centres for missing clinical data and contact patients directly for data missing from patient reported questionnaires. The TARN population registry will also be utilised to collect details about other injuries and pathway procedures such as admission details. The ORiF trial primary outcomes (mortality and EQ-5D-5L questionnaire) data is collected via robust and proven mechanisms (NHS Spine (61) and CRF completed in hospital or by post). The use of HES and ONS data linkage in TARN, combined with best practice tariff incentive and national dash boarding, results in accurate records for hospital admissions for all patients. The TARN registry conducts continuous data quality monitoring using HES linked data to establish completeness of patient entries within TARN ("case ascertainment") by Major Trauma Centres and also data accreditation purposes. It includes a check of at least 25 fields for each patient. This includes accreditation for intervention operative data (operation performed, surgeon, grade, speciality, anaesthetist), detailed injury descriptions and CT scan reports. This is national benchmarked data and publicly available at www.tarn.ac.uk. The case completeness and data accreditation (respectively) for 2016 for example at ORiF centres Nottingham (100%, 95%) Oxford (100%, 96%), Royal London (96-100%, 92%), Newcastle (100%, 94%), Swansea (100%, 96%) and Southampton (100%, 96%) reflecting exceptional coverage of cases and data accreditation. Overall there is reason to be confident about the data quality of the core items provided by TARN. The TARN registry represents the best and most complete dataset of trauma patients within the UK. It is currently used as the "host" for a number of randomised trials including the UK-REBOA (HTA funded, 14/199/09) and the Cryostat-2 (NIHR PGfAR funded, ORiF trial co-applicant with Brohi as lead-applicant) trials. The hosting of the ORiF trial with TARN enable efficient data collection (62).

Additional data collection

For a subset of participants randomised to the ORiF trial (n=212) we will collect additional samples which will allow exploration of three molecular and cellular level mechanisms; for each mechanism of interest relevant biomarkers will be collected (wound healing markers using the resolvin to leukotriene ratio, circulating markers of muscle loss and measures of gut dysbiosis, specifically gut microbiome alpha and beta diversity and circulating lipopolysaccharide and zonulin levels) and assessed as predictors of a complication free recovery i.e. no medical complications (e.g SIRS, pneumonia, etc.), surgical complications (e.g. infection, return to theatre, etc.) or prolonged recovery. We will measure in addition the inflammatory status (cytokine panels, c-reactive protein) and bone healing status for these individuals. To achieve this, a subset of ORiF trial patients will have blood and faecal samples taken over 3 months follow-sup. They will also be phenotyped

including body composition measures using a validated CT-based technique. The schedule of the additional samples is given in Table 2 below.

Table 2 Additional sample collection schedule

	Baseline	**Day 1 post op (when applicable)**	**once patient is home**	**6 weeks**	**90 days**
serum sample	**y**	**y**	**n**	y	**n**
Resolvins (healing response)	**y**	**y**	n	**y**	n
inflammatory cytokines	**y**	*n*	n	y	n
muscle loss markers	**y**	*n*	n	y	n
faecal sample	n	n	**y**	y	y
gut microbiome composition (diversity and permeability)	n	n	**y**	y	y

Hypothesized predictive markers derived from blood and faecal samples
We hypothesize that the following markers, based on published human and animal data will be linked to patient recovery:
1) Resolvin/leukotriene ratio. This has been shown to be predictive of a complication free recovery(49) using a gene expression approach. We will measure the actual lipid levels in serum using the Mass Spectrometry facilities at the University of Nottingham as we have done in previous publications (63, 64). Briefly, the liquid chromatography mass spectrometry (LC-MS/MS) method used for eicosanoid analysis in human serum samples, based on the method previously developed (63, 64). will be performed using fully extracted calibration standards for each of the analytes using for HPLC a Shimadzu series 10AD VP LC system and an Applied Biosystem MDS SCIEX 4000 Q-Trap hybrid triple-quadrupole-linear ion trap mass spectrometer. The resolvin to leukotriene ratio will be computed by adding the concentrations resolvin D types 1, 2 and 3 and dividing by the concentration of leukotriene B4;
2) Gut permeability. We will measure by ELISA (Enzyme-linked immunosorbent assay) levels in serum of lipopolysaccharide-binding protein (LBP), a surrogate marker of endotoxemia and gut leakiness, and zonulin, a marker of gut permeability;
3) Muscle loss. N-terminal peptide of procollagen type III (P3NP) and C-terminal agrin fragment (CAF) provide minimally invasive and clinically informative measures of skeletal muscle status (65) they will be measured by ELISA.

Laboratory assays
Serum aliquots will be used to measure inflammatory and disease status markers – these molecules will be used as potential confounders and to confirm clinical status, they will not be used to predict outcomes as they are part of the outcome.
The following assays will be carried out to measure the above markers:
1) A panel of inflammatory cytokines as markers of the inflammatory state of the patient i.e. SIRS. These assays will be performed by ELISA and will measure levels of TNFα, IL-1, IL-6, IL-8, and IL-10 which are the main ones altered during SIRS cytokine storms (12)
2) A key marker of bone formation (N-terminal propeptide of type I procollagen (PINP) (66)) which will indicate if the fractures are healing. This will also be measured by radioimmunassay.

Faecal samples will be stored at –80C and shipped to ErasmusMC where DNA will be extracted and the V4 region of the 16s ribosomal RNA gene amplified and sequenced, as described in (67). Alpha (α) diversity, or the intra-population diversity (microbial diversity within individual patients at each time point), will be estimated by calculating the number of observed OTUs (richness), and the Shannon Diversity Index. α-diversity measures of gut dysbiosis, with lower values corresponding to high dysbiosis and will be calculated by rarefying the OTU table down to 7000 sequences per sample 50 times and taking the average. Beta (β) diversity, or the inter-population diversity (the microbial diversity between patients at each time point), will be estimated by constructing principal coordinate analysis plots for both weighted and unweighted UniFrac distances. These analyses will be carried out in QIIME 2 (v2018.11).

Clinical outcomes
The main clinical outcome of interest in the OPERA study is occurrence of complication free recovery (i.e. no medical complications, surgical complications or prolonged recovery) over a 90 day follow-up period. Most analyses involving clinical complications will focus upon the composite of any of these events given the relatively small OPERA biological and ORiF trial cohorts.

Proposed sample size
The sample size of 532 for the ORiF trial cohort was previously calculated in order to assess the co-primary outcomes of mortality and quality of life and will be sufficient for use of the corresponding data within this mechanism study.

The additional data sample collection for a sub-set of ORiF patients is anticipated to be a minimum of 212 individuals. Allowing for 5% missing data this leads to 201 analysable observations. Based upon the combined clinical complication free recovery outcome of 67% (prevalence of 33% for any complication) this will allow an effect size of 0.55SD to be detected with 90% statistical power, for 201 individuals (groups of 60 and 141). An effect size of 0.45 would be detected with 80% power keeping the other assumptions constant. If the level of missing data was markedly higher at 25%, an effect size of 0.6SD would still be detectable with 90% power. Based on the resolvin study published on trauma (49) this seems perfectly reasonable for this mechanism and is larger than the sample size (n=67) of a recent study that found significant differences in mortality and LOS in trauma patients based on gut microbiome alpha and beta diversity (9). Specifically, the microbiome analyses identified that survivors of trauma carried on average 546 types of different bacteria (operational taxonomic units) compared to 480 in those who died, corresponding to an effect size of 1.9 (standard deviation units). This sample size will be more than sufficient to quantify variability of biomarkers and correlations between pro-inflammatory biomarkers and clinical outcomes. With regards to quantifying correlations, a sample of 212 would produce a 95% confidence interval (CI) for Pearson's correlation of width of 0.2 or smaller if the correlation is 0.6 or higher (even after allowing for 25% missing data) (68). For a variance, a sample of around 212 (again allowing for up to 25% missing) has a CI width of approximately 22% of the variance value. Therefore quantification of correlations and variances related to the continuous biomarkers can also readily be achieved with reasonable levels of precision with the OPERA patient samples (69).

Statistical analysis
General approach
The statistical analyses will be agreed by the OPERA study group in advance in an addendum to the ORiF trial Statistical Analysis Plan (SAP). Separate statistical analyses are intended for the different types of hypotheses, which reflect the nature of hypothesis as it relates to the mechanism, current thinking, and data available. All statistical analyses will be carried out in Stata (currently version 15.0)(70) or R (currently version 3.6.1)(71) software. No imputation of missing data is planned though appropriate sensitivity analyses will be considered.

Analysis of pro-inflammatory (Inflammation, body composition and gut microbiome) factors
The three key hypotheses to be assessed are:

1. A resolvin to leukotriene ratio which has been shown to correlate with uncomplicated recovery from general trauma (49)
2. Gut microbiome = alpha diversity measuring gut dysbiosis, beta diversity assessing and zonulin/LPS levels measuring gut permeability
3. Muscle loss markers = a combination of muscle markers and body composition

The key outcome of these mechanistic analyses will be a composite measure of complication free recovery (see above for the definition) over a 90 day follow-up. It is expected, based on a large previous study, to have a prevalence of complications for multiple rib fractures (22) of ~ 70% of participants in the OPERA sample. A key aspect of the analysis plan will be the ability to quantify and integrate diverse influences (patient's demographics and inflammatory responsiveness, and treatment pathway), thereby being able to quantify the relevance of the various mechanisms for

predicting a complicated recovery. Although the analysis of these mechanisms is limited to the subset of ORiF with biospecimen collection (n=212 see **Figure 1**), this would, to our knowledge, be the largest study to date integrating biological mechanisms with treatment and trauma clinical outcomes.

The statistical analysis will assess the anticipated relationships (observed associations) according to the respective hypothesised mechanisms using the processed data from the ORiF mechanistic study CT-based body composition, blood and faecal samples. Anticipated bivariate relationships will be explored first using Pearson and Spearman pairwise correlations with corresponding 95% confidence intervals also generated. Descriptive analyses of clinical and process outcome data will also be carried out using appropriate summary measures (e.g. number of events and percentage for binary measures). Hypothesised relationships will be assessed using generalised linear models with corresponding assessment of goodness of fit (univariate and multivariate for the full set of factors) will be carried out and association between the three key mechanisms and uncomplicated recovery adjusting for potential confounders (age, sex, BMI), treatment arm (surgery vs control) baseline cytokine profile and change in bone markers will be assessed. Transformation of the dependent variables will be explored as necessary along with use of bootstrapping of 95% confidence intervals to address non-normality for continuous dependent variables. Measures will be quantified, and as appropriate, an associated 95% confidence interval calculated (e.g. using the Wilson score interval method (or an equivalent) for binary measures).

Analysis of treatment pathway factors
To assess the impact of the hypothesised mechanisms on the key outcomes of interest (complication free recovery and all-cause mortality), process outcome (absence of mechanical ventilation and length of stay, and select key complications (i.e. pneumonia) will be assessed.

The hypothesis related to the timing of surgical intervention (patients who are operated upon within 72 hours have a better outcome) will be assessed using various approaches to allow assessment of the reliability and some generalisability of findings. The total observed effect of rib fixation on mortality will be estimated and decomposed into the direct and indirect effects using a causal mediation modelling approach (72), (73). In this regard the timing of surgery can be viewed as a mediator of the offer of rib fixation. (72) Causal modelling approaches are an active area of research (72), (73), (74) (76). However, their use in randomised trials data is relatively limited to date(77). Such models require multiple assumptions though they do allow adjustment for measured potential confounders of the mediator outcome relationship(75), (72). The analysis will be conducted using only the subset of patient who took part in both the ORiF trial and the OPERA study. These causal “mediation” analyses will be done using the paramed command in Stata which will enable attribution of effect to timing of surgery versus receipt of surgery per se. In this sense timing of surgery can be viewed as a mediator of the offer of surgery. This decomposition of effect is of interest irrespective of the overall findings of the ORiF trial, as the optimal timing of rib fixation is of dispute in the literature (2) (36-38). Surgery causes a further inflammatory response which could lead to organ and respiratory dysfunction. Appropriate timing may reduce such risk with operating within some evidence suggesting operating on trauma patients within 24 hours is optimal (2). In the case of a no overall benefit for surgery it could elucidate that the timing of surgery was sub-optimal (in ORiF an arbitrary, though practical 72 hours window for delivering rib fixation is used)(59, 78). It requires, amongst other things no unmeasured confounders of mediation and outcome (72,73). The sensitivity of the findings to violation of the assumptions will be explored (79).

Assessment of prognostic value
In a complementary analysis, the prognostic value of the various measures will be assessed using an elastic net logistic model (simplifying as appropriate) to predict absence of complication free recovery over 90 days (80). This model will include the measures of the aforementioned (patient pro-inflammatory and treatment pathway) mechanisms with their inclusion in the model informed by the findings on the underlying mechanisms. Such an approach seeks to assess overall prognostic value not the causal relationships of the individuals measures thus is complementary

to the above statistical analyses. The elastic net method allows for restriction of the complexity of the overall model and also compensation for the anticipated "collinearity" between candidate predictors (80). Furthermore a cross-validation approach will be incorporated into the estimation to reduce potential optimism bias. The combined discriminatory value will be estimated by calculating the area under the curve (C-statistic) with a corresponding 95% confidence interval.

6. DISSEMINATION, OUTPUTS AND ANTICIPATED IMPACT

What we intend to produce from the OPERA project

The study will be registered on a publicly accessible online database such as the International Standard Randomised Controlled Trial Number (ISRCTN) and the entry will be kept up to date. The study protocol will be available on the NIHR website and will also be published in a peer reviewed journal (anticipated to be BMC *Trials* journal). The trial management team and other collaborators will prepare the study report following study completion and this will be submitted for publication in the NIHR EME journal produced according to the programme's and journal's requirements. Furthermore, individual research papers will be sent for publication to high impact factor journals and will be made available by open access so that a high visibility of the work will be maintained. It is expected that this will result in 4 high impact results publications, i) detailing the joint predictive value of molecular and clinical factors to outcomes of multiply injured patients, ii) detailing the mechanistic trajectories of molecular pathways during healing or during SIRS, iii) integrating the molecular data with the effects of ORiF surgery, and iv) the other relating to the and treatment pathway stratification, and generalisability of the ORiF trial result, regarding the value of rib fracture fixation. This will allow for the results to be disseminated across the orthopaedic and rehabilitation communities, the wider medical community and NICE. In addition, the findings of the study will be presented at the annual meeting of the British Orthopaedic Trauma Society. The findings will inform evidence-based guidance to inform the management of patients with rib fractures and also further research in this field on improving patient outcome and potential medical interventions. The results generated from this body of research will be presented at local, national and international conferences on surgery, anaesthesia, metabolism and perioperative care (e.g. Association of Surgeons of Great Britain and Ireland, American College of Surgeons, Society for Academic and Research Surgery). Research papers will be made available by open access so that a high visibility of the work will be maintained.

How we will inform and engage patients, NHS and the wider population

We will make the study information available on the NIHR, Universities (Nottingham and Oxford), and study website, including progress and results of the study. The OPERA PPI group will lead on the dissemination of the study results to patients and the wider public. Patients and carers will also be made aware of the findings through patient associations and special interest/focus groups. Lay summaries written in conjunction with the PPI group and the patient representative on the ORiF Trial Steering Committee (TSC) in conjunction with scientific abstracts and publications will be published on the study website. Video presentations and podcasts will be uploaded on the Universities website so that interested parties may access the work in bite-sized quantities.

How the outputs of the research will influence health and care system

The work will also be presented at the collaborating institutes and the results will be incorporated in future national and international guidelines and framework documents, including NICE guidelines. Local, national and international workshops and training programmes will be held to help implement the findings so that they become part of routine clinical care and help encourage further research in the field. Clinical collaborators in the ORiF trial include the National Clinical Directors for Trauma, and Intensive Care Networks, and the Chair of the National Injuries and Emergencies Specialty Group of the NIHR Clinical Research Network. This will ensure highest visibility and impact of positive or negative findings on a national and international stage. It will be presented at the NIHR Musculoskeletal Trauma Trials Days held twice a year to allow dissemination to a wide multi-professional audience of trialists, clinicians and allied health professionals. We will use the press offices of both Universities, the NIHR, and the MRC to publicise the research. The University of Oxford and Nottingham and both hospital trusts have communications officers who will work with the NIHR to widely and effectively disseminate the

research findings. We will also use and scientific dissemination articles such as those in http://theconversation.com/uk for which one of the co-applicants is a regular contributor (e.g. https://theconversation.com/profiles/ana-valdes-345983/articles). The proposed project is the first to our knowledge embedded and designed mechanistic study in a surgical trial in the UK. This will constitute a stepping stone for building capacity and infrastructure and future applications.

Further funding or support required if this research is successful
The results from this mechanistic study will feed directly into the University of Nottingham BRC's MSK and GI themes. Having identified significant predictors of surgical outcome for the multiply injured we will pursue experimental medicine studies aimed at understanding how to implement early detection in an emergency setting and how to modify these factors. Patient biological characteristics that show large effects in the OPERA study will form the basis of future stratified medicine approaches in trauma trials and we intend to apply for funds to carry out such studies.

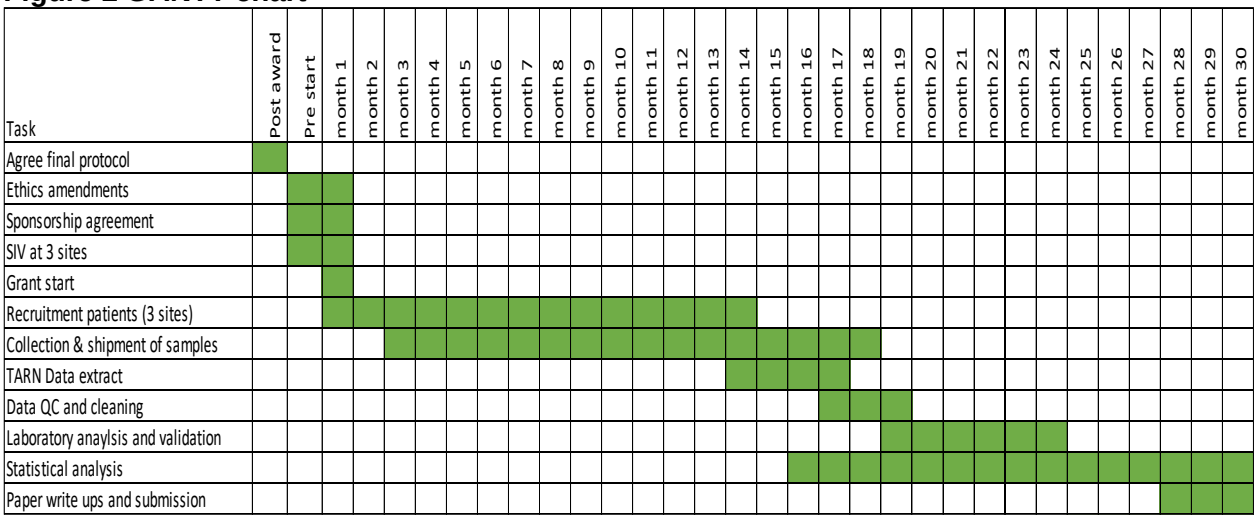
Possible barriers for further research, development, adoption and implementation
Potential barriers to adoption relate to constraints inherent in the NHS emergency care system, along with possible inertia from the clinical community. First, demonstrating optimal care, as well as assessing in a robust manner the value of personalisation of treatment is the first step in improving care for all patients. We will engage with the various communities, and clinical guidance bodies to encourage implementation of findings as well as exploitation of findings for future research to further address patients' needs.

The impact of the OPERA study and who will be influenced
First, this work will explore the generalisability of the trial finding across the wider potential population and also where specific subgroups of participants benefit more. Second, it will assess mechanisms which can lead to development of treatment to support patients with both traumatic rib fracture injuries, polytraumatised and needing surgery during the acute phase of their traumatic injury. Third, it will highlight area for further research to target future interventions. Fourth, it will provide a model for future studies of traumatic injuries and related mechanism along with the role of surgical intervention. The project will train a postdoctoral fellow to analyse body composition data and to integrate molecular data to outcomes of the multiply injured patient building capacity.

7. PROJECT / RESEARCH TIMETABLE

The project will run over 30 months (See Figure 2 for the GANTT chart). Months 1-14 will be recruitment to collect the additional samples outlined above. Months 15-29 will be the laboratory analyses, and substantial statistical analyses (months 16-30) involved to address the outlined objectives. Months 25-30 will be write up of the findings and the beginning of dissemination of findings to relevant stakeholder groups.

Figure 2 GANTT chart



8. PROJECT MANAGEMENT

The study will be managed by an established team within the RCS Surgical Trials Unit (SITU) with the support of the Clinical Trials unit (CTU), the Oxford Clinical Trials Research Unit (OCTRU). The project co-ordinator (SITU) will oversee all aspects of daily study management including the running of the OPERA project management group. The core Nottingham and Oxford teams (Applicants and co-ordinating team) will teleconference on a monthly basis, when setting up the sample collection study, monitor recruitment progress to the mechanism study and ensure project milestones are met. The group will reviewprogress on the various components of the study (e.g. sample collection, body composition measurements, laboratory assays, data transfer and statistical analysis). The CTU will provide further trials management, statistical and programming support, and study database and co-lead Cook will act as the local link. OPERA as with ORiF will be run in strict accordance with the SOP's and operational policies of OCTRU (the accredited CTU) and this sub-study will also follow these where applicable. The project group will report to the ORiF study trial management group and TSC. It will also be subject to monitor by the study sponsor, and funding body (e.g. via progress reports).

9. ETHICS / REGULATORY APPROVALS

We do not anticipate any ethical concerns with this study. Most of the data and permission required are covered by the original ORiF trial ethical approval (REC reference: 18/SC/0666). The general approach to potential participants will follow-up the ORiF trial ethical process (59). A substantive amendment will be submitted via the IRAS system for REC/HRA approval to enable to the additional consent process and approvals required for the collection of blood and faecal samples and the related uses of this personal data. The sponsor of the ORiF study have confirmed they will sponsor the OPERA study. The local R&D committee of participating hospitals will approve local involvement in the sub-study. Study documentation preparation prior to start of grant will have appropriate PPI involvement. The OPERA study as ORiF does, will be run in strict accordance with the SOP's and operational policies of OCTRU (the accredited CTU). These all adhere to UKCRC regulatory requirements.

10. PATIENT AND PUBLIC INVOLVEMENT

In addition to the extensive PPI work undertaken to develop the ORIF trial additional specific PPI work has been undertaken for the development of this study. Two focus groups were conducted to discuss the design of the study. The groups consisted of a total of 15 participants. All participants in the groups had recently had surgery following major orthopaedic trauma, some participants had sustained rib fractures as part of their injury and 2 participants who had been involved in a trial as part of their treatment. The two focus groups were led by a trained PPI facilitator and considered three areas:

1. Trial Design The design of the trial was entirely acceptable to patients. Patients did not consider the addition of blood and faecal samples to the existing study protocol to be intrusive. Patients rationalised this by noting that the experience of having major trauma required intensive and intimate nursing care and thus providing further blood/faecal samples would be relatively routine to them. Individuals were keen to avoid additional clinic appointments, but that an additional appointment would not have prevented them participating. Participants had no objection to having existing scans reviewed be a research fellow to glean further information for research purposes, some mentioned that it may be useful to explain the process for handling incidental findings on the study paperwork as this was a concern for some.

2. Consent Participants were ambivalent regarding whether inclusion into this embedded study should be a separate consent event or whether it could be included as part of the study consent. Participants who had experience of being in a study, leaned towards having a separate consent form and information sheet for this study as they were concerned about information overload and felt they may find it easier to process if they were presented as two separate studies. Some however felt that the procedures were so minor they could be included as an optional section on the bottom of the existing consent form for brevity.

3. Importance Participants agreed that the research question was important, although admitted the concepts were difficult to understand. Participants were grateful that attention was being paid to the more holistic aspects of the management – such as nutrition, as they felt this could confer better outcomes. Other patients felt that this study may allow future patients to have better access

to more personalised prognostic information which many felt they had lacked. The more scientific concepts were hard to explain to patients, although all individuals could understand that their samples would be in laboratory based experiments and that these in-vitro experiments could be used to make inferences about the mechanisms of trauma physiology. Participants were impressed that bench research was happening in trauma as they felt this sort of research was limited to other areas such as cancer.

11. PROJECT / RESEARCH EXPERTISE

A multi-disciplinary team is required to support the study with joint co-leads Benjamin Ollivere and Jonathan Cook. *Benjamin Ollivere* is Associate Professor of Trauma Surgery and Chief Investigator (CI) of the ORIF trial. He has expertise as in orthopaedic trauma RCTs (CI of EMADE, WRIST, Innovate UK SilverNails; Co-I of UKSTAR and IRMINE) and holds grants from NIHR HTA, InnovateUK, Versus Arthritis and AOUK. He has particular expertise in rib fracture fixation having led a number of studies in this area (including a systematic review of treatments and an observational study). *Jonathan Cook* is Associate Professor and Deputy Director of SITU. He is a statistician and methodologist with expertise in design, conduct, analysis and reporting of surgical and RCTs in general (H-index of 51). He has been involved in a large number of NIHR funded projects including 14 ongoing studies (including the TOPKAT, ACL SNNAP, NEON, ProCuRE, and FUTURE-GB trials). He has led 6 projects as CI and has over 150 peer reviewed publications including 28 and 24 as first and last author. He will oversee the statistical analysis and all the investigators will contribute to the write-up of publications. *Ana Valdes* is Associate Professor and Reader in Musculoskeletal Genetics at the University of Nottingham and Honorary Senior Lecturer at the School of Medicine King's College London. Trained as a genetic epidemiologist, she has expertise in molecular epidemiology and mechanistic studies, with and H-index of 76 and over 220 peer reviewed publications she has co-authored high impact research on the molecular mechanisms of complex diseases and ageing. She has coordinated or participated in the past in several large EU programmes (TREAT-OA, EurHEALTHAgeing, HEALs) and is currently Research Area Lead within the University of Nottingham BRC MSK theme. She is the CI for an RCT assessing a digital intervention for knee OA measuring inflammatory marker changes. She has pioneered microbiome studies in Nottingham and Bangalore and is co-I of GCRF funded lifestyle interventions in Kerala India and has extensive links with the biotech industry. *Katie Rollins* is Assistant Professor at the University of Nottingham. She is a co-applicant on the ORIF study. She has specific expertise in body composition analysis as well as methodological issues pertaining to CT-based body composition analysis. She was awarded a European Society of Metabolism and Clinical Nutrition (ESPEN) research fellowship grant for research on body composition measures in pancreatic cancer and elected to ESPEN's Early Career Faculty in 2019. *Simon Craxford* is a Research Fellow at the University of Nottingham. He has experience in qualitative methodology, PPI, patient reported outcomes and measures (PROM) and clinimetrics. He is a PI for the ongoing Outcomes after Chest Trauma Score" study (OCTS) recruiting in open 20 sites with 193 rib fracture patients recruited. OCTS involves extensive trauma and rib fracture patient qualitative research in developing and validating a new PROM instrument for rib fracture.

12. SUCCESS CRITERIA AND BARRIERS TO PROPOSED WORK

The main risk in a clinical study is lack of recruitment. However, that is not considered a substantive risk to the OPERA project given that recruitment to ORiF is ongoing and that the figures proposed are conservative relative to the number of patients being currently recruited into this trial. A second risk to consider would be the lack of any effects from the proposed mechanisms. We consider this to be extremely unlikely as all hypothesized mechanism have already strong evidence in animal models and humans and therefore the major risk would be if these factors proved not to be predictive enough to generate clinically useful evidence to guide patient care. Even in such an unlikely scenario, the data generated will still be valuable to clinicians and scientists regarding providing robust evidence on the limitations in our current understanding and would stimulate new research to why this has occurred. The specific deliverables that will indicate success of the project are complete collection of samples and clinical data necessary to carry out the assays and analyses, the completion of the statistical analyses including clinical and molecular data and the write-up of the results deriving from these.

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