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Neutrophil oxidative burst capacity for peri-operative immune monitoring in trauma patients

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ABSTRACT

Background: Post injury immune dysfunction can result in serious complications. Measurement of biomarkers may guide the optimal timing of surgery in clinically borderline patients and therefore prevent complications. Aim: peri-operative measurement of neutrophil oxidative burst capacity as an indicator of the immune response to major orthopaedic surgical procedures.
Methods: Prospective cohort study of trauma patients aged ≥16 yrs with pelvic, acetabular, femoral shaft or tibial shaft fractures requiring surgical intervention. Blood samples were taken immediately pre-op and at 30 min, 7, 24 and 72–96 h post-operatively. Neutrophil oxidative burst capacity was measured both with and without stimulation by formyl-methionyl-leucyl-phenylalanine (fMLP, a chemotactic factor). Clinical outcomes measured were mortality, length of stay, MOF, pneumonia, acute respiratory distress syndrome (ARDS) and sepsis.
Results: 100 consecutive orthopaedic trauma patients were enrolled over a 16 month period. 78% were male, with a mean age of 42 ± 18 years and an average ISS of 19 ± 13. Neutrophil oxidative burst capacity was significantly elevated at 7 h ($p = 0.006$) and 24 h ($p = 0.022$) post operatively. Patients who developed infective complications (pneumonia and sepsis) had higher levels of oxidative burst capacity pre-operatively (pneumonia: 1.52 ± 0.93 v 0.99 ± 0.66 $p = 0.032$, sepsis: 1.39 ± 0.86 v 0.97 ± 0.56 $p = 0.024$) and at 24 h post op (pneumonia: 2.72 ± 2.38 v 1.12 ± 0.63 $p = <0.001$, sepsis: 2.16 ± 2.09 v 1.10 ± 0.54 $p = <0.001$). When analysed by operation type, no statistical difference was seen between major and minor operations. No correlation was found between length of stay, length of ICU stay, ISS or age and neutrophil oxidative burst capacity at any time point.
Conclusions: Neutrophil oxidative burst capacity response to orthopaedic trauma surgery is associated with the infective post injury complications. There was no correlation between magnitude of injury or operation and oxidative burst capacity. These results are promising for the development of tools for prediction of post-operative complications and guidance for optimal timing for surgical intervention.

Introduction

Post injury immune dysfunction can result in serious complications. Acute respiratory distress syndrome, multiple organ failure, post-operative sepsis and pneumonia, continue to be serious causes of morbidity and mortality in the trauma population [1–4]. The predictors of these complications include (1) patient factors, (age, gender, genetic predisposition, co-morbidities, BMI), (2) injury factors (severity, blunt vs penetrating, contamination, ischaemia–reperfusion injury, shock), and (3) treatment factors (duration of shock, blood transfusion (quantity and age of cells), crystalloid administration, sepsis, abdominal compartment syndrome, surgical intervention) [5–7].

Neutrophil leukocytes play a critical role in the host immune defence system. They respond to various stimuli and combat foreign pathogens through the release of reactive oxygen species (ROS) and extracellular proteases. Inappropriate or excessive stimulation of this defence system may result in unintended host tissue damage. Second-hit theory postulates that neutrophils are 'primed' by pro-inflammatory mediators such as TNF-a [4,8–10] released during the initial tissue injury. Priming upregulates neutrophil fMLP-receptors which enhances the neutrophils oxidative burst capacity [4,11,12]. There is a temporal window

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The MOF daily score is the addition of the worst values for the day for each organ system. MOF is defined as a score >3.

Dysfunction	Grade0	Grade1	Grade2	Grade3
PulmonaryPaO2/FiO2ratio	<250	250-200	200-100	<100
Renalcreatinine(umol/l)	<159	160-210	211-420	>420
Hepatic total bilirubin (umol/l)	<34	34-68	68-137	>137
Cardiac	No inotropes	Only 1 inotrope at small dose	Any inotrope at moderate dose or >1 inotrope at any dose	Any inotrope at large dose or >2 inotropes at moderate doses

Fig. 1. Denver postinjury multiple organ failure score.

post-injury [12] during which, subsequent, or 'second-hits', can result in activation of these potentiated neutrophils and an excessive production of ROS. This hyperstimulation, and subsequent unintended host tissue damage, forms the basis for post-operative complication such as ARDS and MOF in trauma patients [13–16]. Thus the timing of surgery is a potential modifiable risk factor in the development of post-operative complications.

Orthopaedic trauma patients frequently require non-lifesaving operative intervention during periods of physiological imbalance following injury, when the stress of resuscitation and surgical intervention can provoke a heightened inflammatory response. Timing of this intervention is the subject of ongoing debate [17,18]. Whilst, biological mechanisms for the development of MOF and prediction and management of complications have been well explored in the literature [5,16,19–21], there remains a lack of good indicators for the timing of non-lifesaving surgery.

This paper aims to measure the oxidative ability of host neutrophils during the peri-operative timeframe of major orthopaedic trauma operations. We hypothesise that neutrophil oxidative burst capacity would correlate with post-operative complications in orthopaedic trauma patients.

Methods

A prospective cohort study was performed. All trauma patients aged >16, undergoing orthopaedic operative management admitted to our level one trauma centre between October 2011 and December 2012, were enrolled. Orthopaedic interventions were chosen for study as they are a frequent presentation with a standard set of operative management options.

Patients recruited underwent surgery for femoral shaft, tibial shaft, acetabular and/or pelvic fractures. Surgery involved supra-acetabular external fixation, percutaneous insertion of sacroiliac screws, symphyseal plating, and Kocher-Langenbeck and/or ilio-inquinal approaches for the acetabulum and formal open approaches to the anterior and posterior pelvic ring. Tibial and femoral shaft fractures were managed with external fixation, intramedullary nailing, percutaneous plating, or open reduction and internal fixation.

Demographics including gender, age, and ISS were collected. Other injuries were recorded. Volume of blood and crystalloid transfused, admission base deficit and blood pressure were also collected. Blood results were only included following their index operation. Subsequent operations were not included.

The clinical outcomes were defined as mortality, length of stay, length of ICU stay, multiple organ failure [7] acute respiratory distress syndrome [13], pneumonia [22] and sepsis (Fig. 4) [23]. Multiple organ failure was defined using the Denver postinjury MOF score, with a score >3 for more than 3 days considered positive (Fig. 1). ARDS followed the Berlin consensus definition (Fig. 2). Pneumonia was a clinical diagnosis with empirical use of antibiotics with the presence of key criteria (Fig. 3). Sepsis was deemed present in a patient who was positive for SIRS and had a positive blood culture result (Fig. 4).

One six millilitre sodium heparin (NaHep) collection tube of venous whole blood was collected from each patient: pre-operatively and at several post-operative intervals; 30 min post-op, 7 h, 24 h and 72–96 h post-op. Two aliquots of 100 μl of blood were stained with dihydrorhodamine 123 (DHR 123—an uncharged ROS indicator measurable by flow cytometry when oxidised to cationic rhodamine 123). One aliquot was then stimulated with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP—a commonly used general purpose laboratory agent that induces cell activation of granulocytes). Both aliquots were then incubated at 37 degrees. The samples were then washed and analysed on a flow cytometer. 20,000 neutrophils were counted and the mean fluorescence intensity (MFI) of both the stimulated and unstimulated tubes was measured.

The results were then analysed using Graphpad Prism 6 statistical analysis software [24]. A paired Student's *t*-test was used for statistical analysis comparing means with a significance level of $p \leq 0.05$. Correlations were expressed using Pearson's correlation coefficients. Unless otherwise stated, means are expressed as value ± standard deviation.

This study received ethics approval from the Hunter New England Human Research Ethics Committee. Informed written consent was obtained from all participants.

Results

100 consecutive orthopaedic trauma patients were enrolled over a 16 month period. Average age was 42 ± 18 years, 78% were male and average ISS was 19 ± 13. 105 fractures were fixed in 100 initial procedures. 27 femoral nails, 24 tibial nails, 22 pelvic ORIF, 16

- Lung injury of acute onset, within 1 week of an apparent clinical insult and with progression of respiratory symptoms
- bilateral opacities on chest imaging not explained by other pulmonary pathology (e.g. pleural effusion, pneumothorax or nodules)
- respiratory failure not explained by heart failure or volume overload
- decreased arterial PaO2/FiO2 ratio
    - Mild ARDS: ratio is 201 - 300 mmHg (≤ 39.9 kPa)
    - Moderate ARDS: 101 - 200 mmHg (≤ 26.6 kPa)
    - Severe ARDS: ≤ 100 mmHg (≤ 13.3 kPa)

Fig. 2. Berlin definition of acute respiratory distress syndrome.