Investigating Interleukin – 8 and TGF Beta as Early Biomarkers Predicting Poor Clinical Outcome in Major Trauma

LUHAIB ABBOOD

MPhil/ MASTER OF PHILOSOPHY

Science, Engineering and Environment School

University of Salford

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TABLE OF CONTENTS

List of Figures

List of Tables:

Dedication

This research degree is dedicated to the memory of my grandfather, Mohamed Al Janabi, who passed away last year. He always believed in my ability to be successful in the academic field and in my talents. You are gone, but your belief in me has made this journey possible.

Acknowledgements

I would like to express my special appreciation and thanks to my supervisor Professor Niroshini Nirmalan. You have been my guidance and support in my research and general life. I would like to thank you for encouraging me in my challenging times and for giving me the motivation to achieve my MPhil degree. Your knowledge and kindness have made me grateful to have you as an advisor and role model.

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Finally, my special thanks to my talented daughter Rand for her continuous love, help and support.I am grateful to her for always being there for me.

Abbreviations

AECC - American-European Consensus Conference

APACHE-Acute Physiology and Chronic Health Evaluation

ARDS - Acute Respiratory Distress Syndrome

CARS - Compensatory Anti-Inflammatory Response Syndrome

CRP - C-Reactive Protein

DAMPS - Damage Associated Molecular

PatternseGFR - Estimated Glomerular Filtration

Rate FiO2 - Fraction of Inspired Oxygen

HMGB1 - High Mobility Group Box 1

HNP - Human Neutrophil Peptide

ICAM- Intercellular Adhesion Molecule

IL-8 - Interleukin-8

ISS - Injury Severity Score

LRR - Leucine Rich Repeats

MAC-1 - Macrophage-1 Antigen

MAP - Mean Arterial Pressure

MAPK - Mitogen-Activated Protein Kinase

MCP-1 - Monocyte Chemotactic Protein 1

MODS - Multi-Organ Dysfunction Syndrome

MOF - Multiple Organ Failure

NF-κB - Nuclear Factor Kappa-light-chain-enhancer of activated B cells

NISS - New Injury Severity Score

PAMPs - Pathogen Associated Molecular Patterns

PCWP - Pulmonary Capillary Wedge Pressure.

PECAM - Platelet/endothelial Cell Adhesion Molecule 1

PRR - Pathogen Recognition Receptor

qSOFA- Quick Sequential Organ Failure Assessment

sIL-6R - Soluble Interleukin-6 Receptor

SIRS - Systemic Inflammatory Response Syndrome

SOFA - Sequential Organ Failure Assessment

Th-1 - Class 1 T Helper Cells

Th-2 - Class 2 T Helper Cells

TIC - Trauma-Induced Coagulopathy

TIR - Toll/Interleukin-1 Receptor

TIRAP - Toll-Interleukin 1 Receptor (TIR) domain containing Adaptor Protein

TLR - Toll Like Receptor

TNFα - Tumour Necrosis Factor Alpha

TRAF - TNF Receptor-Associated Factor

TRAM - TRIF-Related Adaptor Molecule

Treg - Regulatory T cells

TRIF - TIR-Domain-Containing Adapter-Inducing Interferon-β

Abstract

Trauma is a leading cause of death and disability. Globally, traumatic injury is the main cause of death under the age of 45. Recent studies from the UK report a demographic change in major trauma. The most common mechanism of traumatic injury is falling from less than 2 meters, with the primary demographic being elderly males. The imbalance between pro and anti-inflammatory immune states following trauma is postulated to cause an exaggerated compensatory anti-inflammatory response. This leads to an immuno-suppression and increased susceptibility to sepsis and multi-organ failure, thus contributing to the late phase deaths due to major trauma.

Here where test the hypothesis that early cytokine changes defining these immune imbalances could be exploited as biomarkers to predict poor clinical outcomes and second phase deaths in major trauma. IL-8 as a pro-inflammatory cytokine and TGF-β as a pleiotropic cytokine with a potent regulatory activity were investigated for their potential as early biomarkers. The availability of such biomarkers will aid inpatient stratification, thus enabling prioritisation of such patients for early, focused clinical and therapeutic interventions. The results will add to a larger multi-centric study looking at cytokine,cellular immune and metabolomic biomarkers as early predictors of poor clinical outcome in major trauma.

Blood samples, clinical, biochemical and demographic data were collected for major trauma patients admitted to Central Manchester and Salford Royal Foundation Trusts on Day 1 and Day 5 post trauma (NIHR Portfolio study BIT 19377). IL-8 and TGF-β trends monitored using BD Biosciences cytometric bead arrays showed a steady, statistically significant decline from days 1-5 post-trauma in the whole trauma cohort (IL-8 P=0.013; TGF-β P= 0.000). The high levels on Day 1 of both cytokines suggest the potential use of these cytokines as early biomarkers if they related to poor clinical outcome.

In order to do this, the patient cohort (IL-8. n = 53; TGF-β, n = 38) was analysed based on calculated Sequential Organ Failure assessment SOFA scores on Day 1 and 5. A SOFA score of ≥3 was deemed as a cut off to denote early organ failure. IL-8 and TGF-β levels on Day 1 were compared in patients clustered based on SOFA scores (≥3 cut offs) on Day 5. While higher levels were observed for IL-8 and TGF-β on Day 1 for the poor outcome cluster (SOFA ≥3) than when compared to the good outcome control (SOFA <3) the values were not statistically significant (IL-8 p = 0.218 ; TGF-β p = 0.624). When the SOFA cut offs were increased to cluster on the basis of more severe organ

failure (SOFA ≥ 6), the p values obtained were as follow; significant (IL -8 p = 0.894; TGF-β p = 0.075). While this is still not statistically significant, increase in the significance for TGF-β, when clustered with patients with more adverse outcomes is noteworthy and needs to be further evaluated in a larger cohort patient. The pleiotropic nature of TGF-β makes it harder to hypothesise whether a pro or anti-inflammatory mode of action is adopted in its response. The multifaceted role of this cytokine needs a better understanding of the function in combination with other immune modulatory factors by investigating the pattern of cytokine level along the acute period of trauma and during the recovery.

CHAPTER 1

1. INTRODUCTION

1.1 Incidence of trauma

The World Health Organisation reported that trauma is a leading cause of death and disability. Ten percent of people die, and approximately 16% are disabled due to trauma (WHO, 2014). Traumatic injury is the main cause of death under the age of 45 in the worldwide, particularly in developed countries (Sakran et al., 2012). In 2012, traumatic injuries caused 11,000 deaths in the UK (Kellezi et al., 2016). The trauma–related world death toll is higher than the numbers reported as results of other virulent diseases such as HIV, malaria and tuberculosis. In addition, trauma is the major cause of long-term complications; for each fatal incidence of trauma, there are survivors with long-term injuries and disabilities. According to National Audit Office (NAO), the economic cost borne by the National Health Service (NHS) for treating major trauma in England ranges between £300 and £400 million per annum (NHS confederation, 2010). Complex trauma results in patients staying in hospitals, ICU units which results in a significant financial impact on health services in the UK. Each patient gets admitted to the hospital costs £50,000 which leads to £1.6 million a year, approximately 7 per cent of the National Health Services (National Institute for Health and Care Excellence, 2015). Road traffic accidents are anticipated to be the seventh leading cause of death (World Health Organisation, 2018) and one fifth of deaths are anticipated because of trauma (WHO, 2008). Head injuriesare the major cause of death among traumatic injuries (Gunst et al., 2010). A recent large study reported a demographic change in major traumatic injuries in the UK over a 23year period. The study found a slight increase in the mean age from 1990 until 2013, while a rapid change was observed in 2006 reaching to 58 years in 2013. The study reported major trauma is a main cause of death in elderly people. Kehoe et al. reported that the mechanism of major trauma had also changed, the most common mechanism of traumatic injury was falling from < 2 m, and 63.3% were male. (Kehoe et al., 2015, Trauma audit & research network, 2017).). Ischemia-reperfusion is the tissue, and systemic damage that are caused by the lack of oxygen supply following the restoration of blood to a tissue or an organ (e.g. after major trauma, stroke and vascular surgery) this can initiate a complex inflammatory response and drop the function of the organ (Dorweiler et al., 2009). Trauma-haemorrhage stimulates a systemicinflammatory reaction by the production of pro and anti-inflammatory mediators (Gebhard, 2000;

Keel, 2005). This inflammation is known as Systemic Inflammatory Response Syndrome SIRS (Bone,1996).

1.2 The pathophysiology of trauma

Major traumatic injuries stimulate systemic responses that could lead to acute and nonspecific immunity. In many cases of severe traumatic injuries this can cause sepsis and multiple organs damage leading to multiorgan failure and death. The management of haemorrhage following major trauma has been improved, and that has led to more trauma survivors (Lord et al., 2014).

1.2.1 Damage and Pathogen associated molecular patterns (PAMP & DAMP)

Abnormal physical conditions can cause a tissue injury which leads to promoting damage-associated molecular pattern (DAMP) molecules. Most DAMPs are proteins with intracellular function. Microorganisms enhance the inflammation response by promoting pathogen-associated molecular patterns (PAMP) molecules. DAMP and PAMP both contribute to the initiation of inflammation by promoting certain receptors production including Tall-like receptors (TLRs), the node-like receptors (TNRs) and Rig-l-like receptors (RLRs) as shown in figure 1. They have many shared receptors, some of them are membrane bound, e.g. TLR4, and some are intracellular, e.g. TLR3, all NLR5 and RLR5 (Rubartelli & Lotze, 2007).

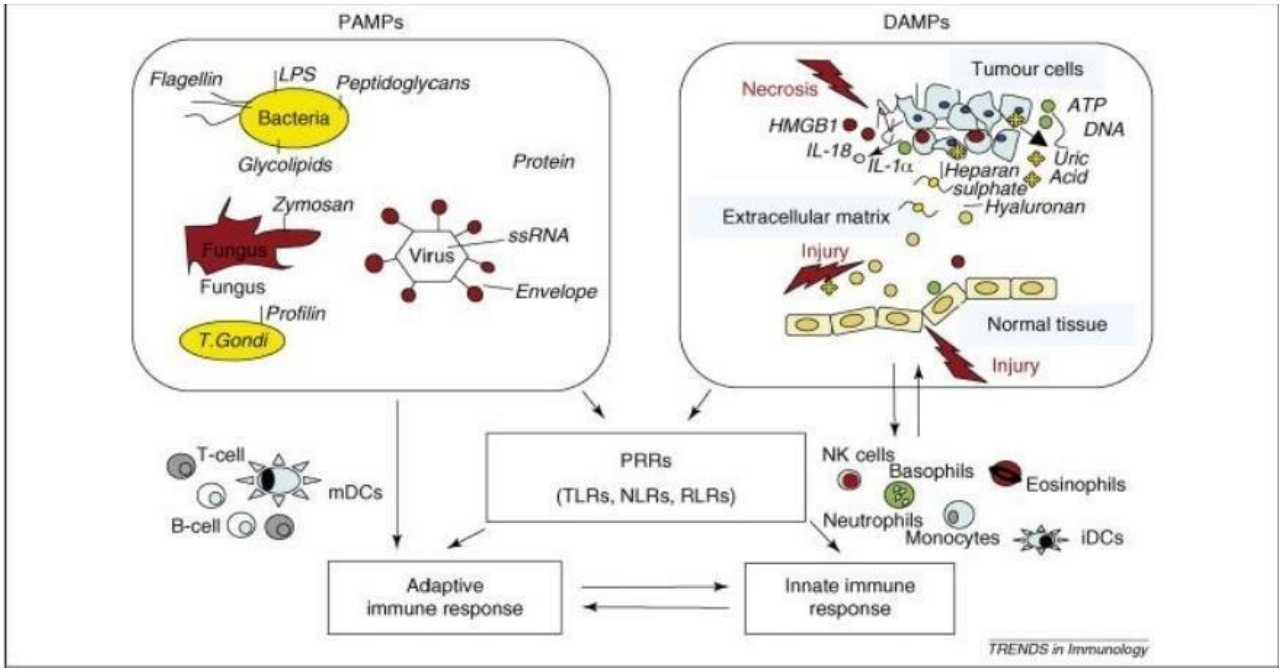

Figure 1: Molecules that initiate an adaptive and innate immune response, including DAMP and PAMP events. (Rubartelli & Lotze, 2007).

Cells undergoing stress can secrete endogenous molecules. This can be sensed as a dangerous signal (Galucci, 2016). Following trauma, DAMPs are released to the bloodstream by damaged tissues into the extracellular milieu. DAMPs are recognised by adaptive and innate immune cells. Innate immunitycells are, mainly antigen-presenting cells (APC) such as dendritic cells (DCs) and neutrophils (PMNs) recognize DAMPs. Activation of PMNs stimulates the release of cytokines (e.g. tumour necrosis factor (TNF-α and interferon –γ(INF-γ)) and chemokines for local tissue repair. The abnormal regulation of cytokines, and in response to DAMPs release, initiate a systemic inflammatory response syndrome (SIRS) that is responsible for physiological alterations such as increased heart rate, hyper or hypothermia and increased/decreased leukocyte counts (Bone et al., 1992). SIRS can also increase the risk of pneumonia (Bochicchio et al., 2002) and organ failure (Roquilly et al., 2011).

Following trauma or sepsis, secondary infections may occur and contribute to immunosuppression (IS). Uncontrolled IS makes patients more prone to develop secondary infections (bacterial, fungal and viral) (Roquilly et al., 2011). DAMPs are originated from SIRS and IS, thus they can be promising biomarkers for traumatic patients.

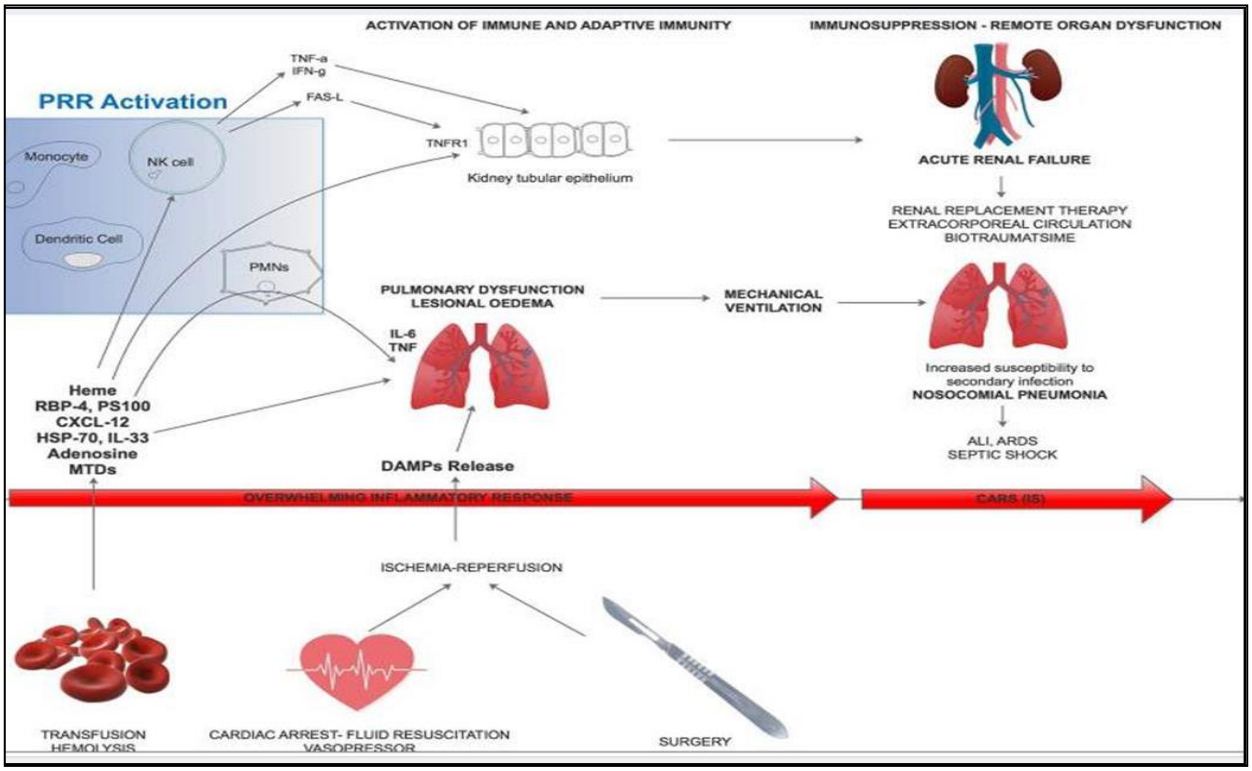

Figure 2: The effect of DAMP on remote organ dysfunction following trauma. (Roquilly et al., 2011).

After severe trauma, DAMPsare released and caused oedema or kidney tubular epithelium activation. Also, subsequent transfusion, fluid resuscitation and surgery lead to remote acute organ failure. ALI: acute lung injury, ARDS: acute respiratory distress syndrome; CARS: compensatory anti-inflammatory response, PMN: polymorphonuclear leukocytes, MTD: mitochondrial damage-associated molecular patterns, RBP4: retinol-binding protein-4, HSP: heat-shock protein, TNFR1: Type 1-TNF receptor; TNF-α: tumour necrosis factor-α. Adapted from (Vourc et al., 2018).

1.2.1.1 High Mobility Group Box 1 (HMGB1)

HMGB is a histone chromosome binding protein which is in mammalian cell nucleotides. HMGB1 has two domains (A, B) and an acidic C-terminus (Bustin,1996 & Ferraris, 1994). The promoting of transcription of a few genes by different mechanisms is the main function of HMGBs, as shown in figure 3 (Marco, 2005). The B-domain plays an important role of being pro-inflammatory and promotes cytokines, while the binding sites on A-domain promote the reduction of inflammatory cascade cytokines (Li J 2003; Mess Mer, 2004; and Yony H, 2004). HMGB1 is produced actively by certain immune cells such as monocytes, dendritic cells and macrophages (Wang, et al., 1999; Lotze, 2005; Abraham et al., 2000). However, almost all nucleated cells are releasing HMGB1 passively after getting signals by neighbouring cells during the cells damage (Scaffidi, 2002).

HMGB1 can be released by stimulating of different receptors to promote the pro-inflammatory cascade. Rohiainen and his team in 2007 have reported that the cell signalling cascade can be promoted through the interaction of HMGB1 with Tall-like receptor 2 and 4 to produce pro-inflammatory cytokines.

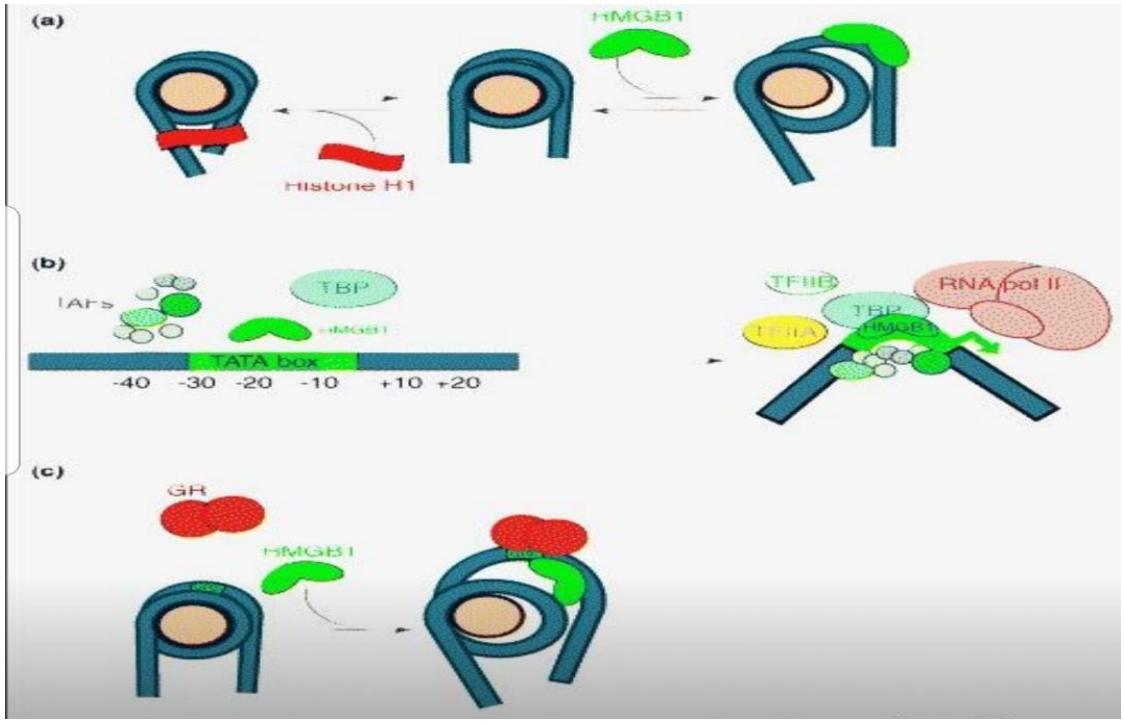

Figure 3: Mechanisms of gene expression control by HMGB proteins. (a) HMGBs interact with nucleosomes: linker DNA is the binding site for both HMGB1 and histone H1. Their binding exerts opposite effects: H1 blocks nucleosome sliding and induces chromatin compaction. By contrast, HMGB1 behaves as a chromatin fluidizer, facilitating nucleosome-remodeling. (b) HMGB1 bends promoter DNA, thus increasing TBP affinity for the TATA box. The recruitment of TFIIB, TFIIA and RNA pol II follows with increased efficiency.
(c) HMGB1 can bend the DNA and can make the DNA sequence accessible to transcription factors, stabilize transcription factors on their targets, promote the recruitment of further interacting proteins, or a combination of any of these features(Bianchi & Agresti, 2005).

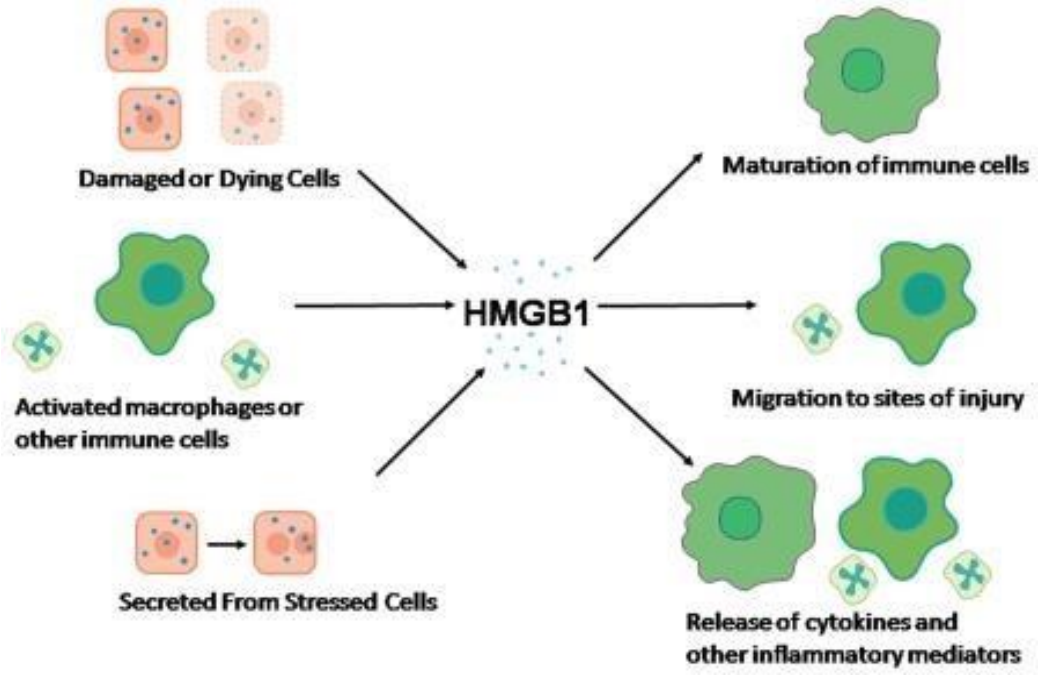

Figure 4: HMGB1 is an endogenous nuclear protein that is released due to a variety of stimuli to activate proinflammatory responses in multiple cell types (Klune et al., 2008).

1.2.1.2 S100 Proteins

S100 Proteins are cytosolic calcium-binding proteins which play a role in stimulating inflammatory responses in various immune cells such as lymphocytes, macrophages, endothelial cells and vascular smooth muscle cells. (Sorci G et al., 2010; Sorci G et al., 2013). B100 Proteins can stimulate multiple intracellular functions such as migration, cell growth, calcium homeostasis and cell cycle regulation (Donato, 2001; Heizman, 2002; Bertheloot & Latz, 2017). Whereas the extracellular functions of S100 proteins have been investigated in few studies for certain S100 Proteins which are known by S100A8, S100A9 and S100A12 (Roth, 2003; Roth, 2001; Foell, 2007) S100 A8 and S100 A9 exist in monocytes, neutrophils and myeloid progenitors and they can be stimulated during the inflammation (Foell et al., 2007) while S100A12 is presented only in neutrophils (Yang, 2001; Fuellen, 2003). Preventing of tumour metastasis can be enhanced by S100A8 and S100A9. (Kaplanet al., 2006).

1.2.1.3 Heat Shock Proteins

Heat shock proteins are recognised as conserved proteins produced by all cells. Increasing of HSPs expression has been found under stressful conditions cells from aggregation and denaturation in addition to easing of protein transport through the cell membrane (Demaio,1999; De Meester et al., 2001; Grundtman et al., 2011).

Despite HSPs being distinguished as intracellular proteins that can be produced by necrotic cells through a passive mechanism (Calder Wood et al., 2007).they are currently recognised as a production of non-necrotic cells by an active mechanism through releasing of HSPs as free proteins and within highly immunogenic properties (Asea, 2008).

The most known family of HSPs is HSPA1A (Hsp70) which have been recognised in wide studies related to inflammation. (Kampinga et al., 2009). HSPA1A has potential roles in the immune response: it activates the preparation of antigens for presentation to T-lymphocytes, it has a property of immune reactivity endogenous HSPs in addition to its role in the activation of the classical complement pathway (Pockley et al., 2008). It has been found that HSP levels can be increased in patients with severe traumatic injuries and acute conditions such as infections (Pittit et al., 2006; Kolinsk, 2018). HSPs have been considered as potential biomarkers of immunosuppression in the first hours following traumatic injuries (Guisasola et al., 2018).

A significant reduction has been found in HSPA1A values at 24 and 28 hours in comparison to the values at the first 12 hours following polytraumatic injury, as shown in figure 5 (Guisasola et al., 2015).

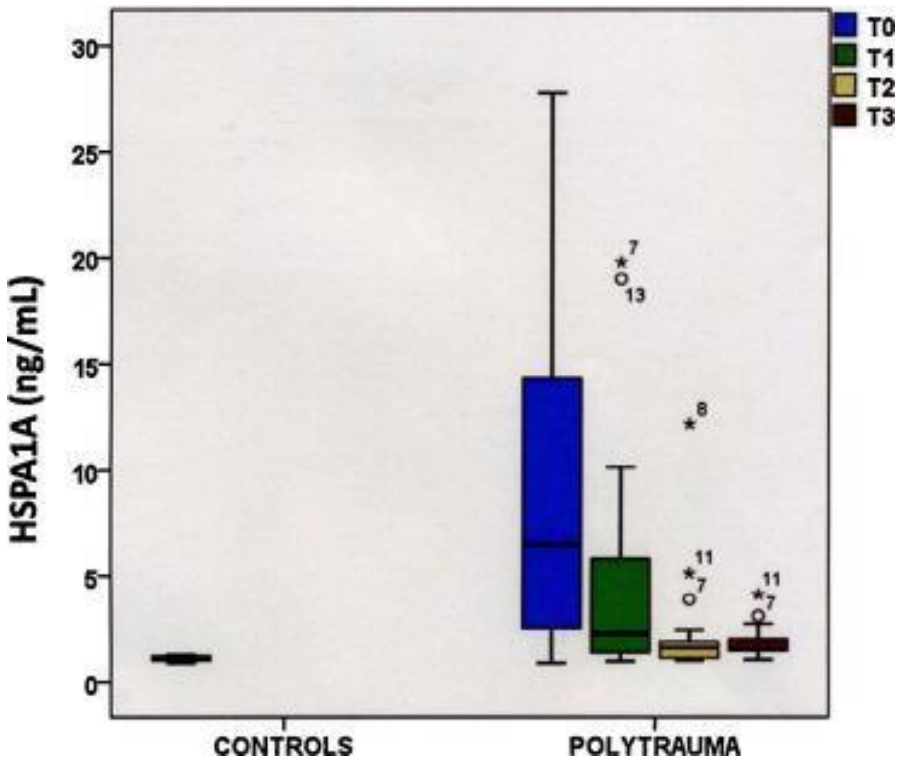

Figure 5: Serum HSPA1A concentrations in polytrauma patients compared to healthy control subjects, up to 10 times higher after injury and permanently elevated until 48 h after the accident.(Guisasola et al., 2015).

1.2.2 Toll-like Receptors TLR

TLRs are a family of proteins that have an important role in the innate immune response. This family includes TLR1-TLR13. Most TLRs are found in humans apart from TLR11, TLR12 and TLR13 (Mahla, 2013). TLRs are integrated proteins that are characterised by an extracellular domain and a cytoplasmic tail. A luminal ligand-binding domain is an extracellular domain that is formed by leucine = rich repeat (LRR) motifs, while the cytoplasmic tail containing a signalling region interleukin-1 (IL-1 receptor), (TIR domain) (Nishiya & De Franco, 2004).

TLR engaged by ligands of PAMP or DAMP to promote different signalling pathways**.** Kawai & Akira, 2010; Lim & Staudt, 2013). The signalling pathways can be either dependent on My D88 or independent. This calcification is based on using adaptor My D88 (O'Neill & Bowie, 2007).The My D88 pathway promotes the activation of all TLRs except TLR3 (Akira, 2006). The My D88 can enhance inflammatory gene expression by TLR2,4 and 5, while TLR7 and TLR9 stimulate the production of type 1 IFN. The My D88-independent pathway uses TRIF adaptor of TLR3 and TLR4. (Yamamoto, 2003).

TLRs play an important role in the initiation of innate immunity and promoting the progress of the adaptive immune response. Releasing of TRLs by macrophages and neutrophils rapidly has been recognised following trauma (Binkowska, 2015).

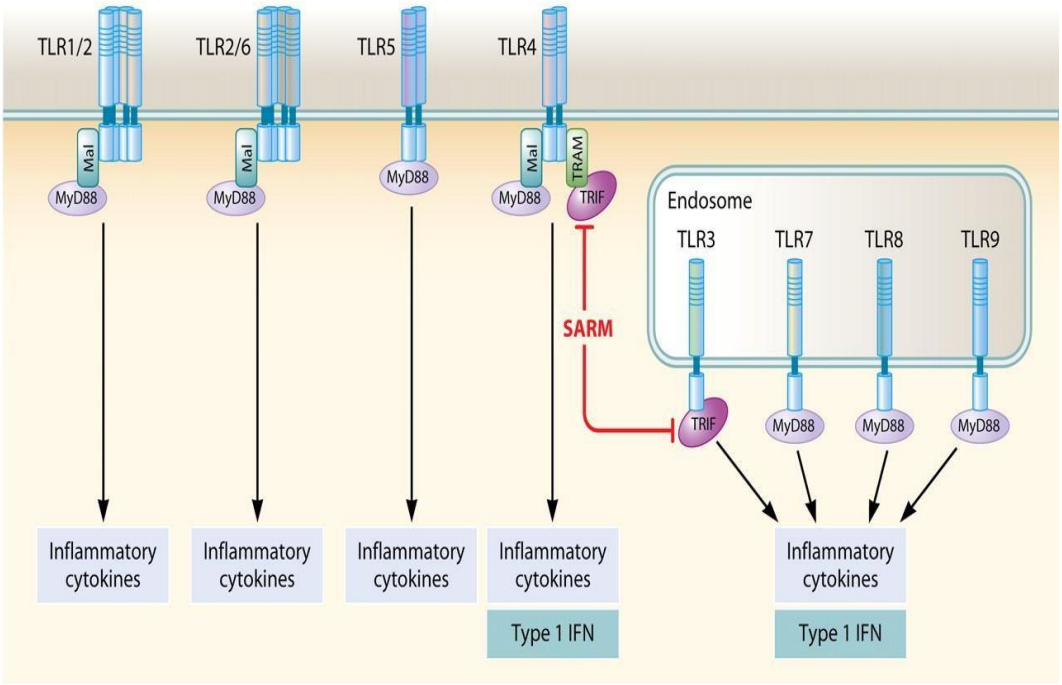

Figure 6: TLRs and TIR domain-containing adaptor molecules. TLR1/2 and TLR2/6 utilise MyD88 and Mal as adaptors. TLR3 is dependent on TRIF for signalling. In the case of TLR4, four different adaptors, i.e., MyD88, Mal, TRIF, and TRAM, are involved, whereas TLR5, -7, -8, and -9, utilise only MyD88. The fifth adaptor, SARM, negatively regulates TRIF-dependent signalling. Overall, MyD88-dependent signalling induces proinflammatory cytokine production, whereas TRIF-dependent signalling stimulates a type I IFN response. In pDCs, stimulation of TLR7 or TLR9 induces type I IFN production by a mechanism dependent on MyD88 (Mogensen, 2009).

1.2.3 The coagulation response during major traumatic injury

After trauma, coagulation/ anti-coagulation balance and fibrinolysis are altered, leading to impaired hemostasis. The alterations after trauma can be summarised in three phases (Martini, 2016).

1.2.3.1 Acute post-trauma phase (within hours after trauma)

This phase is also called acute traumatic coagulopathy (ATC). It is characterised by prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT) immediately after trauma (Maegele et al., 2007; MacLeod et al., 2003). This complication is associated with an increased requirement for blood transfusion and might lead to mortality. This early recognition of coagulopathy before blood transfusion could help for a clear understanding of trauma-related coagulopathy. During this phase, the increase of DAMPs production leads to an imbalance of the generation of coagulant and anti-coagulant factors. For example, the concentration of high-mobility group box 1 (HMGB1) that is released from activated, suppressed immune cells, non-immune cells and DNA-histone complex trigger intravascular coagulation DIC (Ito, 2014). For instance, N-formyl peptides (F-MIT), DNA and RNA stimulate coagulation cascades whereas histones promote platelets aggregation (Wenceslau et al., 2015), thrombin production and clot formation (Wenceslau et al., 2015). Similarly, HMGB1 activates coagulation via enhancing the expression of tissue factor on neutrophils and monocytes outside the tissues. In contrast, HMGB1 and histones inhibit coagulation and impair the activation of Protein C. Inhibition of protein C activation is contributed to hypo coagulation and hyperfibrinolysis (Bouillon et al., 2010).

1.2.3.2 Resuscitation phase (1-2 days) post-trauma

This phase is associated with the development of metabolic acidosis (Meng et al., 2003) and hypothermia as well as to haemodilution. Due to severe blood loss, metabolic acidosis develops, causing clotting enzyme dysfunction. This, in turn, contributes to prolong the PT and aPTT and reduces the levels of VII/ VIIa by 90% (Martini et al., 2005).

1.2.3.3 Later phase (prothrombotic phase) - few days after trauma injury.

In severe trauma, there is an overproduction of pro-inflammatory cytokines and thrombin. The endothelial cell function converts from antithrombotic to thrombotic. Thrombomodulin and fibrinolysis are down-regulated due to endothelial cell activation. During this phase, it is essential for traumatic patients to be prescribed anti-coagulant drugs (e.g. heparin).

1.2.4 The inflammatory response to trauma

Inflammation is an immune reaction following traumatic injuries. The initial immune response is

pro-inflammatory, which is mediated by the cells of the innate immune system. The adaptive immunity, including anti-inflammatory reaction or immunosuppression, is involved in the inflammation process following traumatic injuries (Choilean, 2006).

1.2.4.1 Cellular and vascular changes in inflammation

Inflammatory response induces cellular and vascular changes to facilitate the leukocyte migration process through blood vessels to the inflamed sites. Some changes occur to the shape of leukocytes such as neutrophils, monocytes and T-cells and cells adhesiveness to ease leukocytes motility. Stimulated endothelial cells and some perivascular cells, i.e mast cells and macrophages can control the migration of leukocytes which involve four main steps: rolling, crawlling, adhesion and transmigration as shown in figure 7 (Noursharg & Alon, 2014).

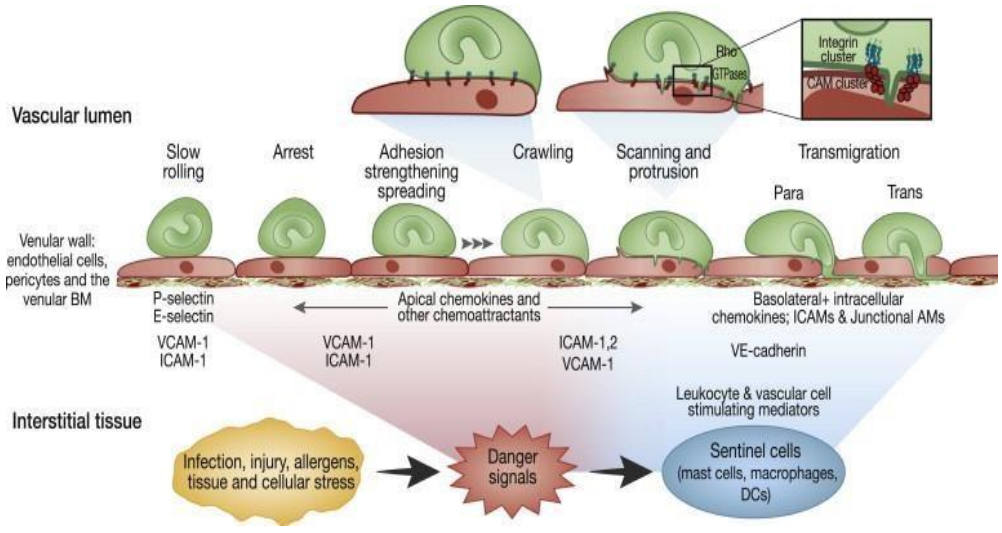

Figure 7: The main steps of leukocytes migration : rolling, crawlling, adhesion and transmigration. Mast cells and macrophages play an important role in controlling the migration. (Noursharg & Alon, 2014).

Leukocyte rolling is defined as the rotational movement of leukocytes inside the vascular. This movement is formed by the force of blood flow to create an interaction between leukocytes and the vascular endothelium. (Mozow, 2015).

Firm interactions are developed by the binding of leukocyte integrins and endothelial ligands (Alon and Duslin, 2007). Free-flowing leukocytes and endothelium interactions require leukocyte glycoprotein mediators: PSGL-1 and selectin-p and E, while free flowing leukocytes can interact with attached leukocytes by L-selectin-PSGL-1 binding (Walcheck et al., 1996). Leukocyte rolling is controlled by microvilli through the interactions of chemokines receptors and integrins with their endothelial ligands (Chen & Springer, 1999). The interactions between leukocytes and endothelial cells increase firmly by the binding of chemokines to chemokine receptors (Herter & Zarbok, 2013). This binding is followed by the interaction of leukocytes such as LFA-1 and MAC-1 with ICAM-1, ICAM-2 and VACM-1 molecules on the endothelial surface as shown in figure 8. This interaction can promote the migration of leukocytes into the inflamed- tissue (Harris et al., 2000).

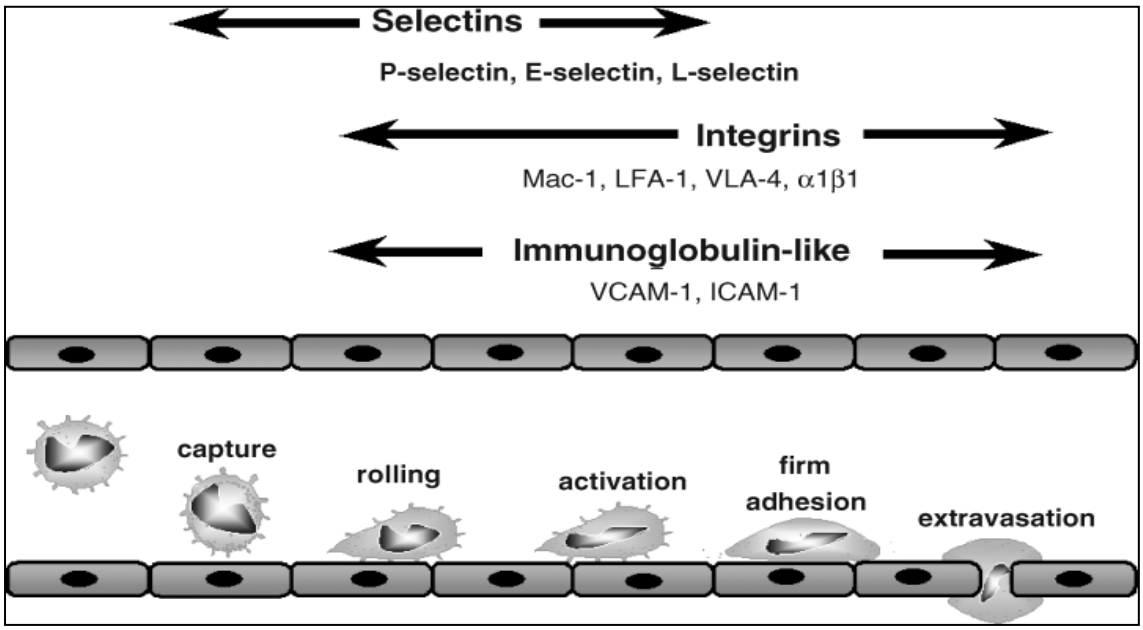

Figure 8: Cellular and vascular response to injury in response to trauma causes damage to the intact endothelial layer causing upregulation of CAMs including P-selectin, E-selectin and L-selectin. VCAM-1 and ICAM-1 and integrins such as Mac-1, LFA-1, LFA-4 and α1β1 are expressed on smooth muscle cells and endothelial cells which all contribute to the firm adhesion and extravasation of the leukocytes through the activated endothelium. VCAM-1 (vascular cell adhesion molecule-1), ICAM-1 (intracellular cell adhesion molecule-1), LFA-1 (leukocyte function-associated antigen-1 (LFA-1), LFA-4 (leukocyte function-associated antigen-4 (LFA-4) and very late antigen-4 (VLA-4). Adapted from (Jung et al., 1998).

Transmigration (TEM) can be represented by the lateral border recycling component (LBRC), which includes molecules such as platelet/endothelial cell adhesion molecules, poliovirus receptor (CDAY 1SS), and (CD99). The targeted LBRC prevents monocytes, lymphocytes and neutrophils transmigrating by their movement within the membrane. This movement needs kinesin to be stimulated (Muller, 2014). A high percentage of leukocyte TEM occurs on the endothelial cell border. However, leukocyte can migrate towards endothelial cell cytoplasm (Camm CV et al., 2007; Milian, 2006). This occurs due to specific conditions such as certain chemokine activators or other components on the surface of cytokine–activated endothelium. The LBRC regulates leukocyte transmigrates by its movement from the endothelial border to leukocytes (Mamdouh et al., 2009).

1.2.4.2 The acute phase response to major trauma

The body's early defence mechanism is represented by innate immunity to prevent infection and promote inflammation. The innate immunity includes physical barriers, toll-like receptors, phagocytes and complements (Janeway, 2001).

The acute phase response (APR) is a systemic reaction that occurs following infection, trauma, tissue injury and immunological disorders. APR is caused by the releasing of pro-inflammatory cytokines and chemokines which are produced by monocytes, fibroblasts, macrophages and T cells that leads to the stimulation of protein production by the liver (Gruys et al., 2005; Janeway, 2001). These proteins are playing a powerful role in inflammation and tissue repairing. (Sander etal., 2010). Complement proteins such as C3a, C3b, C4b and C5a are involved in different inflammatory responses (Sharma & Ward, 2011; Markicwski &Lambris, 2007). C3b and C4b can enhance phagocytosis by the binding of these protiens with pathogens and antibody-antigen complexes to stimulate pathogen recognition by phagocytes (Sharma & Ward, 2011). C3a and C5a play a role in the inflammatory cascade and stimulate the acute phase response (Markicwski& Lambris, 2007).
In traumatic injury, activation of complement is strongly linked with coagulation cascade activation. Thrombin produced in the coagulation cascade has been found to be an activator for C5a in the complement system (Huber-Lang et al., 2006). In the site of injury, monocytes and endothelium release pro-inflammatory cytokines including, interleukin-1β (IL-1β), TNF-α, IL-6, IL-

8 and IFN-γ (Svoboda et al., 1994). IL-1 and IL-8 are the first cytokines released and responsible for triggering the production of other pro-inflammatory cytokines such as IL-6 and IL-8 and anti-inflammatory cytokine IL-10 (Lyons et al., 1997; Kola Czkwska and Kubes,2013). IL-6 is the earlier marker that can be detected inthe plasma of traumatic patients within 1 hour. It stimulates the release of C-reactive protein (CRP). The level of secreted IL-6 is strongly correlated with the severity of trauma and can indicatethe risk of post-surgical operation (Nast-Kolb et al., 1997; Hengst, 2003). α2 macroglobulin is a plasma protein that plays an important role in the inhibition of thrombin which leads to the decreasing of fibrin production and blood clot formation. Additionally, it can act as a transporter to transport various cytokines and growth factors (Rehman, Ahsan & khan, 2013).

1.3 The role of cytokines in inflammation

1.3.1 Pro and anti-inflammatory response

The pro-inflammatory response is the initial inflammatory response and includes the release of pro-inflammatory cytokines (TNF-α, IL-1β and IL-16 and IL-8) which are released by activated macrophages, endothelium, and neutrophils in the site of injury. The release of pro-inflammatory cytokines is accompanied by changes in the release of chemokines and the complement system. The pro-inflammatory response after traumatic injury commonly leads to systemic inflammatory response syndrome (SIRS), which is followed by the initiation of an anti-inflammatory response (Zhang et al., 2010). The anti-inflammatory response is called the compensatory anti-inflammatory response syndrome (CARS) which includes the release of anti-inflammatory cytokines including IL-1Ra, IL-10, IL-11 and IL-13, to suppress the pro-inflammatory response. The balance between inflammatory and anti-inflammatory responses is vital to achieving homeostasis. The overactivity of CARS could lead to infection and sepsis (Tsukamoto et al., 2010).

1.3.2 Interleukin - 8 (IL-8)

Interleukin-8 is a pro-inflammatory cytokine initially released by white blood cells early after injury. It mainly attracts neutrophils and can be identified by the release of enzymes that cause connective tissues degradation. Lysosomal enzymes share with other cytokines, DNA sequence properties that orchestrate common regulatory pathways. (French et al., 2017).

IL-8 can play a role in stimulating monocytes, T- cells and basophils. (Aloisi et al., 1992). IL -8 (CXCL8) is one of chemokine family members which shows two potential receptors, CXCR1 and CXCR2. These proteins can stimulate certain cells as an immune response due to inflammatory and homeostasis (Remo et al., 2014). Chemokine proteins are divided into two subclasses, C-X-C (motif chemokine ligase 8) and C-C (motif) ligand. The C-X-C chemokines are chemotactic against neutrophils and T-cells but not against monocytes, whereas C-C chemokines only exhibit chemotactic activities against monocytes (French et al., 2017). IL-8 increases the expression of Mac-I receptor (CDAY 11b/CDAY 18) and complement receptor (CR-I) (Harada et al., 1994; Bickel, 1993), and enhances the adherence to non-activated endothelial cells (Harada et al., 1994). IL-8 belongs to the IL-8 supergene family that includes chemotactic polypeptide consisting of 72 amino acids. IL-8 production has been found produced by vascular endothelial cells, dermal fibroblasts, keratinocytes (Larsen et al., 1989), hepatocytes (Wigmore et al., 1997) and human melanoma cells (Luca et al., 1997). The production of IL-8 occurs in the presence of an inflammatory-induced inflammatory response such as lipopolysaccharide (LPS), IL-1 and TNF-α which all regulate cell survival, apoptosis and metabolic activities as shown in figure 9 (Campbell et al., 2013).

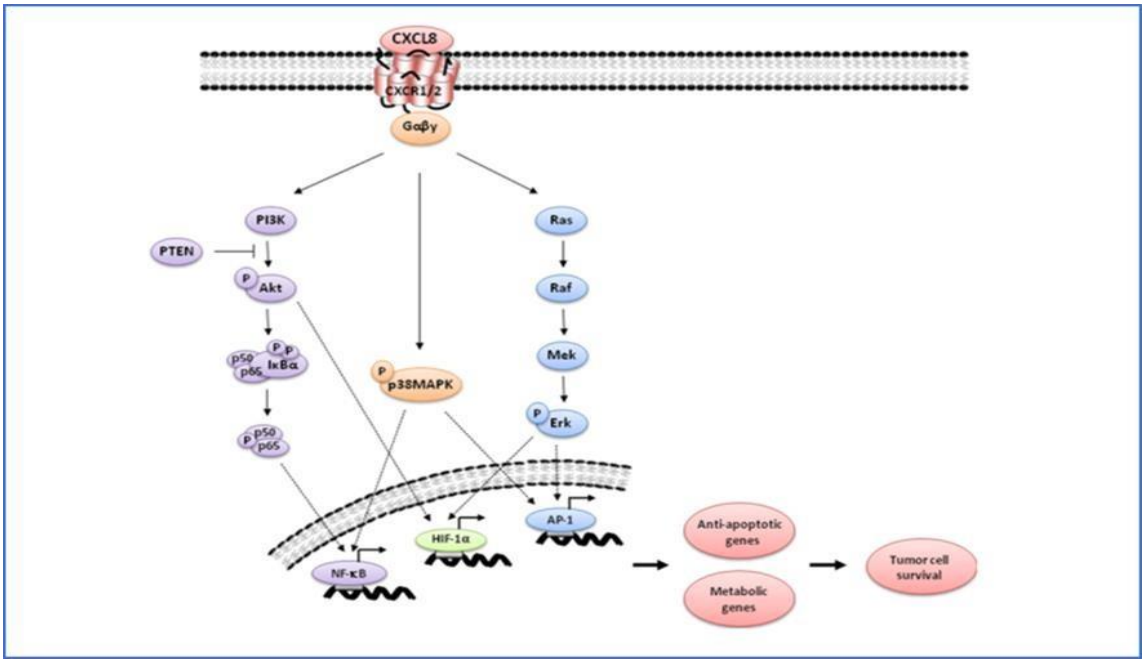

Figure 9: The principal signalling pathways of IL-8: IL-8 pathways are activated through Akt, MAPK and Erk by IL-8 and their relevance to cell survival. IL-8 induces the promotion of transcription factors (NF-κB, HIF-1 or AP-1) and increases the expression of apoptosis and metabolism genes, allowing survival and adaptation to stressful environments. Adapted from (Campbell et al., 2013).

A recent study demonstrated that IL-8 induced neutrophil chemotaxis through a non-receptor tyrosine kinase called Janus Kinase 3 (JAK3) (Henkels et al., 2011). Hence, IL-8 is a potential marker for the inflammatory response thatcan be detected early within 6h of injury and stays elevated for 24-48 hours in inflammatory diseases(Volpin et al., 2014). Elevated levels of IL-8 can potentially help to diagnose traumatic death (Mimasaka et al., 2007). IL-8 can be a main factor in causing secondary brain damage. A correlationhas been found between rising mortality rates and the increasing level of IL-8 in cerebrospinal fluid CSF following a major head injury (Morgani, 1997). Ziebell JM and Morgani MC in 2010 reported the high concentration of CSF IL-8 can cause blood-brain barrier dysfunction. A recent study investigated the level of some pro- inflammatory cytokines, including IL-8 in the severe andmoderate groups of traumatic patients. The study found a significant increase in IL-8 levels in injured patients compared to controls. The increase of IL-8, IL-6, TGF-β and the decrease of IL-4 were indicated as potential biomarkers following trauma. The high level of pro-inflammatory cytokines and reduction of anti-inflammatory cytokines during the acute phase of trauma can be a cause of Systemic Inflammatory Response Syndrome (SIRS). This can lead to some serious health issues such as Multi Organ Failure (MOF) and Acute Respiratory Distress Syndrome (ARDS) (Volpin G et al., 2014).

1.3.3 Transforming growth factor-β (TGF – β)

Transforming growth factor-beta (TGF-beta) is a potential cytokine that exists in the extracellular matrix. TGF-β is a family of 33 members that can play an important role in maintaining the regulation of lymphocyte proliferation, differentiation, and survival. TFG-β has two receptors, transforming-growth factor receptor-I (TGF-β IR) and transforming-growth factor receptor-II (TGF-β IIR) (Shi Minlong et al., 2011; Cazac and Roes, 2000). TGF-β has an important role in regulating the differentiation and function of immune system cells after tissue injury. It could act as a leukocyte stimulator or suppresser depending on the cytokine conditions, as shown in Fig 10 (Dobaczews et al., 2011). The normal serum level of TGF-β1 is in range (1-33 ng/ml), median (16 ng/ml) (Maria-Christina et al.,1998; Czarkowska-Paczek et al., 2006).

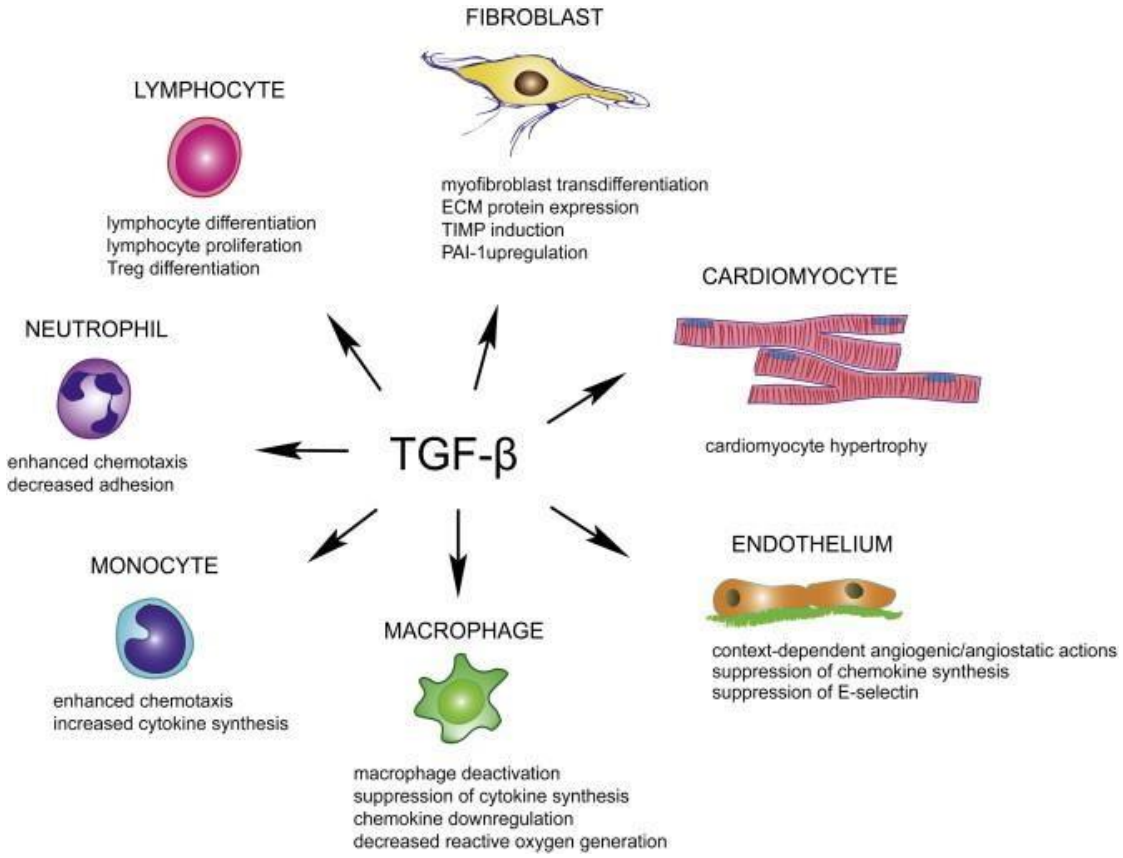

Figure 10: The cellular effects of TGF-β. TGF-β applies multiple effects on all cell types involved in cardiac injury, repair and remodelling (Dobaczews, 2011).

TGF-β has two recognised pathways, SMAD dependent and SMAD independent pathways. The binding of TGF- β with TBR II receptors on the cell surface stimulates the SMADs pathway by the formation of a complex which can phosphorylate TBR I receptor (ALK5 or ALK1). This phosphorylation leads to promoting downstream signals involving certain SMADs proteins. Phosphorylation of ALK5 leads to the formation of SMAD2/ SMAD3-SMAD4 complex, while ALK1 enhances the formation of the SMAD/ SMADAY 5-SMAD4 complex. This results in stimulating one of the two processes, gene transcription or suppression according to accumulating of either co-activators or co-inhibitors. SMAD6 and SMAD7 act as inhibitors and downregulate TGF-β in addition to the SMADs independent pathways such as Erk, JNK, p38 MAPK and PP2A, as shown in figure 11 (Bujak M et al., 2007).

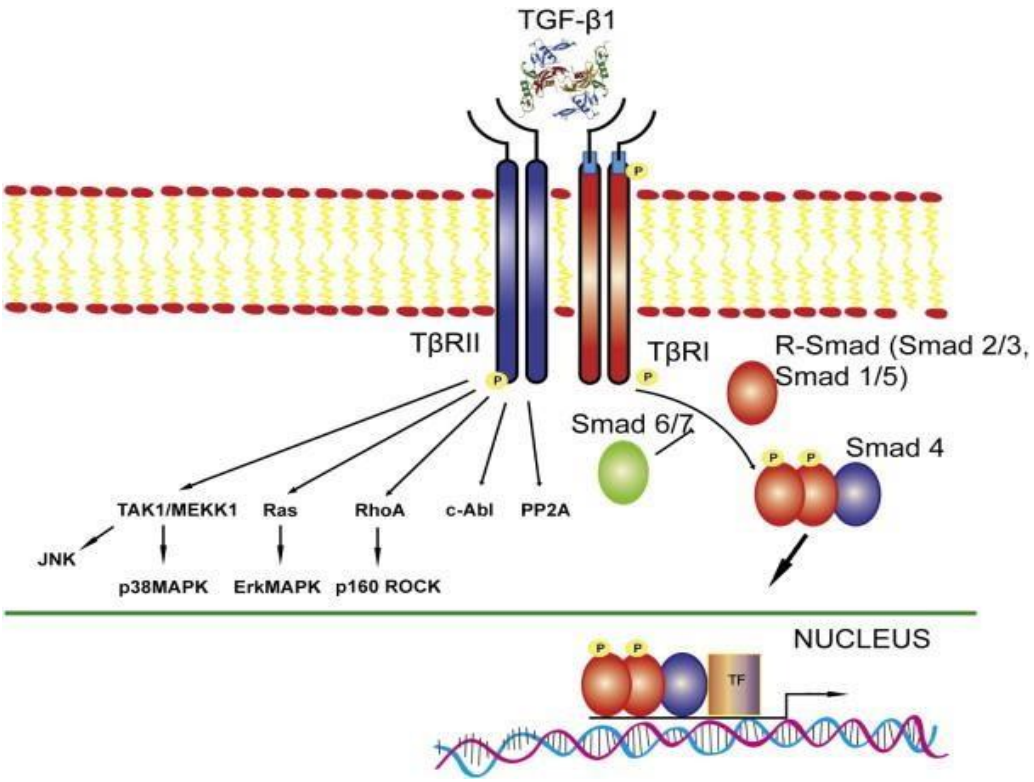

Figure 11: SMAD-dependent and SMAD-independent pathways in TGF-β signaling. Phosphorylation of ALK5 leads to the formation of SMAD2/ SMAD3-SMAD4 complex, while ALK1 enhances the formation of the SMAD/ SMADAY 5-SMAD4 complex. This results in promoting one of the two processes, activation or suppression according to accumulating of either co- activators or co-inhibitors. (Bujak M et al., 2007).

TGF-β can regulate the initiation and resolution of inflammatory responses through the regulation of chemotaxis, activation, and survival of lymphocytes, natural killer cells, dendritic cells, macrophages, mast cells, and granulocytes (Wrzesinski et al., 2007). On the other hand, TGF-β inhibits T-cells function through IL-2 suppression (Kehrl et al., 1986). Alternatively, TGF-B suppresses T cells by up-regulating the expression of cycle regulators in T cells such as cyclin-dependent kinase inhibitors, including p15, p21, and p27 and down-regulating cell cycle–promoting factors, including c-myc, cyclin D2, cyclin-dependent kinase 2 and cyclin E (Datto et al., 1995;Wolfraim et al., 2004).

Witsch and his team reported that TGF-β inhibits autoimmune diseases progression without affecting normal immune cell function to pathogens and acts as a tumour suppressor. However, elevated levels of TGF-β are associated with cancer progression and metastasis (Witsch et al.,

2010). Yang L et al. reported the stimulating apoptosis in marine RAW 264.7 macrophages after the application of pristine graphene through the associated signalling pathways including mitochondrial, MAPKs and TGF-β pathways (Yang L et al., 2011). Gershon V et al. reported a significant increase in TGF-β, IL-6 and IL-8 concentration and the decrease in IL-4 level in traumatically injured patients. This change of cytokines level; the high increase of pro-inflammatory cytokine concentrations and the reduction of anti-inflammatory cytokines level may cause SIRS, MOF and ARDS (Gershon V, 2014).

1.3.4 Cytokines detection methods

There are different methods to detect the measurements of proteins in different biological samples. These techniques can support the scientific research. The two main assays used to measure proteins include Enzyme-linked Immurement Assay (ELISA) and Cytometric Bead Array (CBA).

1.3.4.1 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a traditional quantification technique to detect certain antigens. This technique is used to measure the soluble mediators including chemokines, cytokines and other secretory proteins in plasma. The principle of this technique is based on antigen-antibody reaction. There are different types of ELISA, direct, indirect and sandwich, as shown in figure 12.

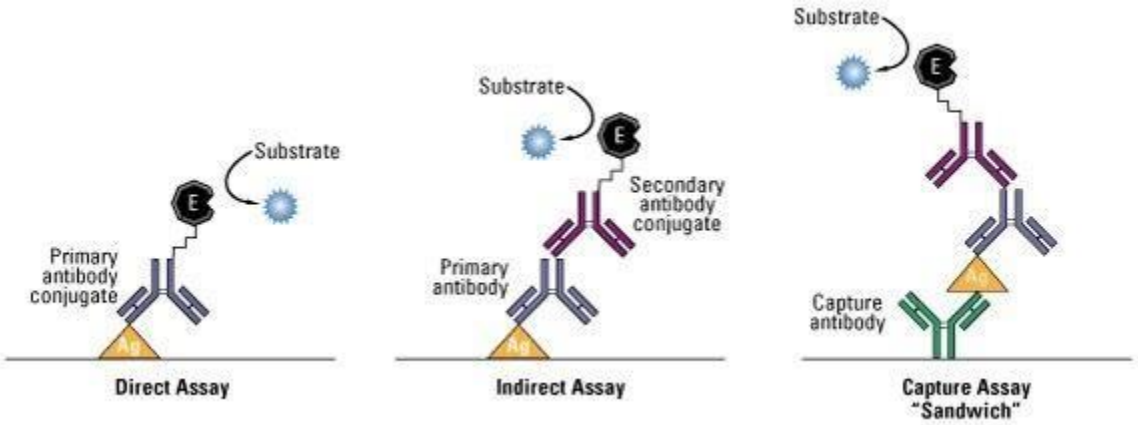

Figure 12: In the assay, the antigen of interest is immobilised by direct adsorption to the assay plate or by first attaching a capture antibody to the plate surface. Detection of the antigen can then be performed using an enzyme-conjugated primary antibody (direct detection) or a matched set of unlabeled primary and conjugated secondary antibodies (indirect detection). Image adapted from thermo fisher scientific site.

The direct method, a specific antigen attached on the well binds to the primary antibody conjugated to an enzyme. primary antibody conjugated to an enzyme. In the indirect technique, two antibodies are involved: primary and secondary conjugate. The secondary antibody is conjugated to a specific enzyme to detect the primary antibody Figure 12 (Gan & Patel, 2013). Sandwich ELISA Figure 12 is the standard method in which two antibodies are used primary and secondary in addition to the capture antibody. The antigen attached on the capture antibody. The primary antibody attaches to the other end of the antigen for detection and amplification. ELISA detects a single analyte and provides quantitative and reproducible results. Therefore, It is widely used in laboratories and medical research (Leng et al., 2008

1.3.4.2. Cytometric Bead Array (CBA)

CBA (Figure.14) is recently developed from a traditional ELISA. It is a multiplexed assay that detects more than one analyte (30>) simultaneously in one reaction in one small volume of sample. CBA has several advantages, including high efficiency (potential time saving), less volume of sample required, reproducibility, repeatability and accurate results (Leng et al., 2008). The cells pass through a light source that causes a light scattering in two directions, sideways and forward. The changes of scattering and fluorescence are recorded by detectors to produce data analysis on the computer. CBA is a method that can detect the cellular components that interact with antibodies by using fluorescent dyes to stain them. This can be used for quantification of internal cellular components (Givan, 2013). CBA uses beads coated with antibody and a wide range of fluorescence to detect the analyte concentration in different samples. A flow cytometeris used to capture the selected analyte, which is differentiated by different light scatters (Morganet al., 2004).

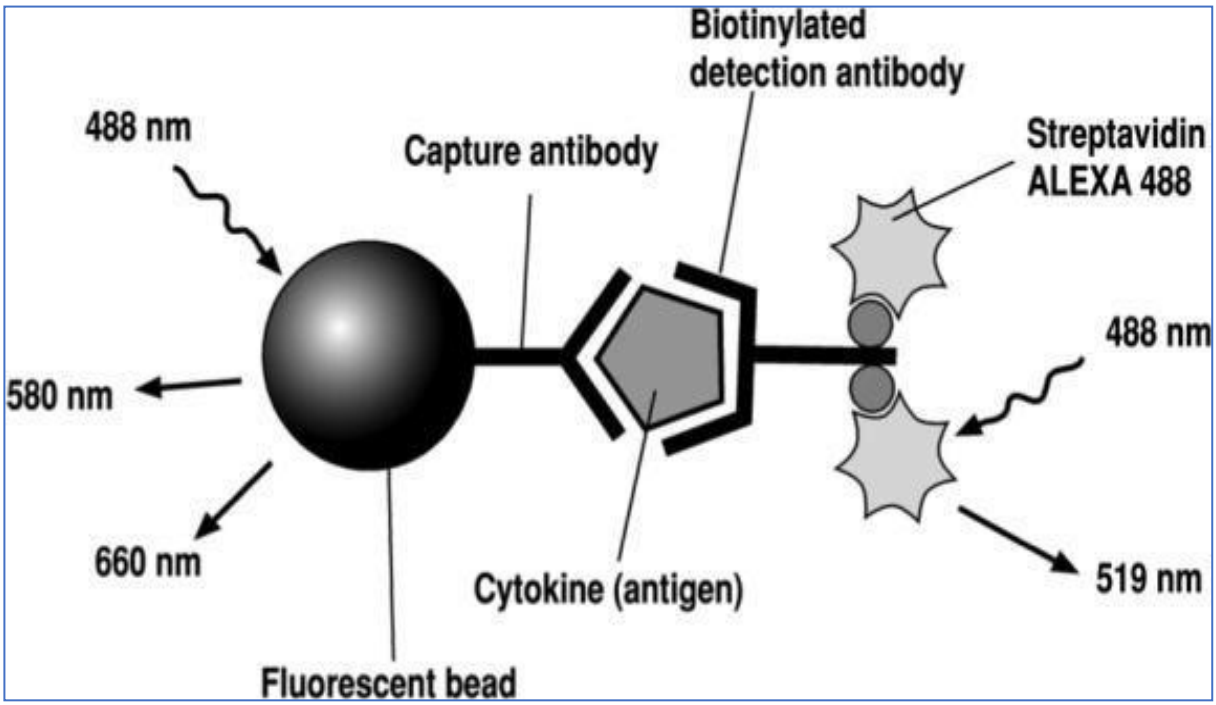

Figure 13: A schematic representation of bead-based multiplex assay. The beads are labelled internally With two fluorescent dyes, one labelling the capture antibody and the second labelling the detection antibody, same as ELISA but with more additional differential detection power (Givan, 2013).

1.4 Clinical response to traumatic Injury

The clinical response to traumatic injury initiates with a primary inflammatory response which can be aggravated to form a “second hit “model. This may lead to multi-organ dysfunction (MODS) to increase the mortality in traumatic patients (Butt and Shrestha, 2008). The first hit is represented by the first traumatic event while the second hit is caused by surgical intervention or nosocomial infections. That can lead to enhancing a Systemic Inflammatory Response Syndrome (SIRS) which can develop into MOF or MODS. As a response to severe traumatic injury, different pro-inflammatory cytokines will be involved such as TNF α, IL-1 and IL-8. To achieve homeostasis, the level of anti-inflammatory mediators increases.

However, the unbalanced increase of anti-inflammatory mediators can cause compensatory anti-inflammatory response syndrome (CARS) (Morris et al., 2015).

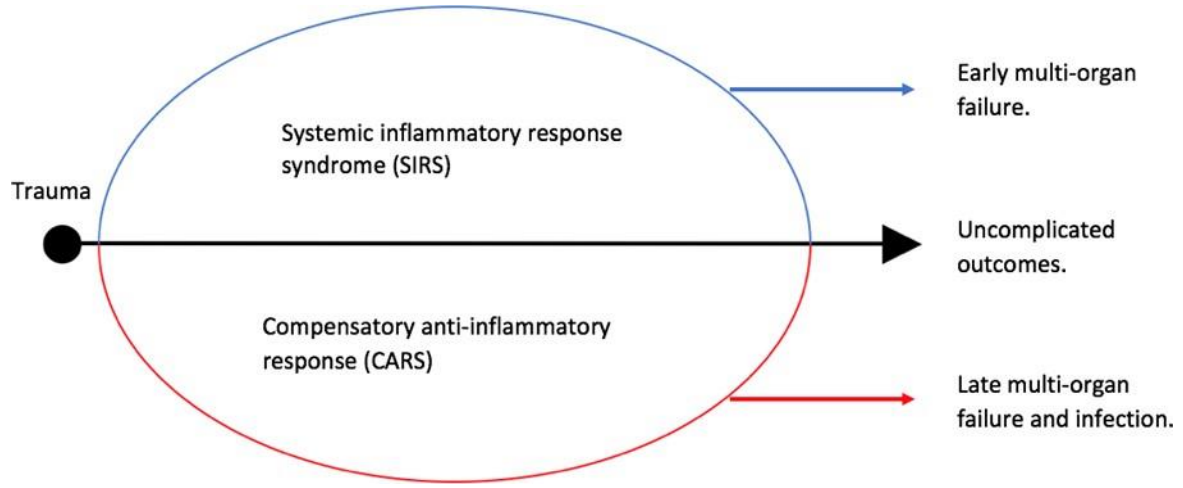

Figure 14: Unbalanced SIRS and CARS can lead to complications (Rosenthal & Moore, 2016).

1.4.1 Systemic Inflammatory Response Syndrome (SIRS)

Scientists define SIRS as sepsis when it is caused by any infection. However, SIRS can be related to sterile factors such as traumatic injuries, major surgeries and burns (Bone et al., 1992). The exhibition of two or more of the criteria mentioned in (table 1) can be considered as evidence of developing SIRS. The American college of chest physicians and the society of critical care medicine have created a range of terms for different levels of sepsis (table 2). (Roberts O & Cooper Smith, 2006). SIRS represents the host response following infection or trauma, that can be involved in different pathophysiology mechanisms in both innate and adaptive immune systems. The innate immune response is an immediate general line that includes multiple cell types, e.g., macrophages and neutrophils. These cells can recognise PAMPs and promote cytokine production and involve TLRs and pathogen binding. (Beuler et al., 2004). TLRs can stimulate by invading gram-negative bacteria after recognition of the lipopolysaccharide. However, TLRs may be involved in non-infectious SIRS that be presented in certain endogenous mechanisms rather than exogenous pathways. (Jonson et al., 2004). The immune response can be upgraded to an adaptive immune response which is more specific and involves T and B lymphocytes. CD4T – helper cells take a part in the production of pro and anti-inflammatory cytokines, and Th1 cells can shift to Th2 cells in SIRS. Non-survivor septic patients showed a reduction in cytokine levels that were produced by Th1 cells with no conversion to Th2 cells (Heidocke D,1999).

PARAMETERS	LEVEL
Heart Rate	>90 beats/min
Temperature	>38^{o}C or 36^{o}C
White Blood Cell Count	>12K or <4K or >10 immature forms.
Respiratory Rate	>20 breaths/min

Table 1: Criteria used in the determination of SIRS (Robertson & Coopersmith, 2006).

TERM	DEFINITION
Sepsis	SIRS caused by infection
Severe Sepsis	Sepsis with at least one organ dysfunction or hypoperfusion
Septic Shok	Severe sepsis associated with hypotension that is resistant to adequate fluid resuscitation
Bacteremia	The presence of viable bacteria in the bloodstream
Multiple Organ Dysfunction Syndrome (MODS)	Impairment of two or more organ systems in an acutely ill patient where homeostasis cannot be maintained without therapeutic intervention

Table 2: Consensus definitions of a spectrum of clinical entities that result in organ failure (Robertson& Coopersmith, 2006).

A major study in a murine burn model has shown an interaction between the innate and adaptive immune systems as a type of sterile SIRS (Murphy et al., 2005). A 25% body surface burn includes the increase of pro-inflammatory cytokines production, TLR2 and TLR4 can play role as mediators in this mechanism. This mechanism can be overstated in Rag mice with lack of T–lymphocytes (Roberton & Coopersmith, 2006).

The systemic response represented by the production of different pro-inflammatory cytokines can develop a cytokine storm which can lead to organ damage or failure such as the blockage of the airways caused by an accumulation of immune cells and fluids (Ti Soncik et al., 2013).

1.4.2 Compensatory Anti-Inflammatory Response Syndrome (CARS)

CARS stands for Compensatory Anti-inflammatory Response Syndrome, which was represented by maintaining the homeostasis in sepsis through the deactivation of systemic immunity mediated by certain cytokines and cellular responses (Ward NS et al., 2008). It is important to know that inflammation can be stimulated by two ways, any pathogenic infection or accumulation of tissue destruction. The innate immune system has cells with receptors that can recognize certain pathogenic cells, and this pathogen-receptor binding can lead to lymphocyte activation, that promotes monocyte production. The increasing monocyte level stimulates the production of pro-inflammatory cytokines such as IL-1 and TNF. CARS can inhibit many of these mechanisms (Oberholzer et al., 2001), including the decrease of lymphocytes and monocytes and the increase of certain cytokine levels such as IL-10 which can inhibit TFN production (Ward et al., 2008). Lymphocytes are involved in both innate and adaptive immune responses and play a powerful role in coordinating the inflammatory response (Mahawy et a.l, 1985). An increase of inhibitory co-receptors such as PD-1, CD47 and TLA4 on lymphocytes while a decrease of co-activator receptors such as CD28 on lymphocytes have been reported following traumatic injury (Bandyopadhay et al., 2007).

This mechanism can enhance the reduction of certain mediators that play an important role in immunity and develop sepsis which increases the mortality in traumatic injury patients (Mahony et al., 1985 and Stephan, 1987). It was found that T-lymphocytes can lose the ability of proliferation in addition to failing to produce IL-2 or IL-12 cytokines in patients who have sepsis

(Heideck et al., 1999 and Rodrick et al., 1986). Activated T-lymphocytes are divided into two sub-set; Th1 and Th2. The function of each T-lymphocyte can be dependent on the type of lymphokines secretion. Th1 has exhibited secretions of pro-inflammatory cytokines (TNF-αand IL – 2) while Th2 secretes anti-inflammatory cytokines, e.g. IL-4 and IL- 10. The level of Th1 cytokines was decreased, and the Th2 cytokines level was increased in patients with sepsis or burns (O Sullivan et al., and Rodrick et al., 1986).

Monocytes play an important role in killing pathogens and stimulating the pro-inflammatory response by the expression of HLA receptors and producing pro-inflammatory cytokines (Krakaver & Oppenhein, 1993). It has been shown that the reduction of HLA production leads to a decrease of TNF – α and IL – 1 in septic patients (Stiz et al., 1996).The high level of lymphocyte apoptosis was investigated in patients with septic shock (Le Tulzo et al., 2002).

1.5 Measuring of traumatic injury severity

A number of scoring systems have been used to predict morbidity and mortality in the Intensive Care Unit (ICU). The purposes of scoring systems are to perform matching in clinical trials, assess the quality of care in IC, assess its performance and to quantify the severity of illness (Rao et al., 2008). The common scoring systems used for trauma are as follows:

1.5.1 Acute Physiology and Chronic Health Evaluation score (APACHE)

The APACHE score depends on physiological parameters and clinical data (Norris et al., 1995). Indeed, APACHE was less specific compared to other scoring systems in assessing the severity of disease and predicting the morbidity and mortality. In 1985, it was developed to APACHE II, which is composed of the acute physiology score, points of age and chronic health. There are 13 parameters, and each parameter has a score range from 0-4. Likewise, chronic health and age points are scored from 0-5 for the former and 0-6 for the latter. Based on the total score, mortality is predicted (Chhangani et al., 2015). The total of the scores predicts mortality. APACHE II has been shown to be accurate, specific and most used as an international severity scoring system worldwide for mechanical trauma.

1.5.2 Sequential-related Organ Failure Assessment (SOFA)

SOFA is a tool to predict mortality by quantitatively assessing multiple organ dysfunction failures after traumatic injury. SOFA was developed in 1994 by the European Society of Intensive Care Medicine. The sofa scores 6 organ systems (respiratory, renal, hepatic, cardiac, coagulation and Central Nervous System (CNS) from 0-4 according to the degree of dysfunction as shown in table3 Llompart-Pou et al., 2014). The latter is described as the changes of ≥2 organs (with a score three or more (≥3) (Durham et al., 2003). The maximum score obtained by SOFA is strongly correlated with mortality. Patients who totally score more than 15 are at high mortality likelihoodof over 90%. The sofa score is highly balanced between sensitivity and specificity of 99% (Bache, 2016).

Organs	Variables	0	1	2	3	4
Respiratory	PaO_2/FiO_2		< 400	< 300	< 200 AND respiratory support	< 100 AND respiratory support
Renal	Creatinine (µmol/L)		110-170	171-299	300-440	> 440
Hepatic	Bilirubin (µmol/L)		20-32	33-101	102-204	> 204
Cardiac	Inotropes (µg/kg/min)		Mean arterial pressure < 70mmHg	Dopamine ≤ 5 OR Dobutamine any dose	Dopamine > 5 OR Epinephrine ≤ 0.1 OR Norepinephrine ≤ 0.1	Dopamine > 15 OR Epinephrine > 0.1 OR Norepinephrine > 0.1
Coagulation	Platelets ($x10^3/mm^3$)		< 150	< 100	< 50	< 20
CNS	Glasgow Coma Score (GCS)		13-14	10-12	6-9	< 6

Table 3: SOFA scoring system, the criteria used to detect the overall SOFA score for trauma patients based on the function of six organ systems. (Llompart-Pou et al., 2014).

1.5.3 Injury Severity Score (ISS)

ISS is an anatomical scoring system and an important predictor for the severity of traumatic injuries. ISS scoring can range from 0-75. High scoring of ISS is associated with an increased incidence of sepsis and trauma (Brattström et al., 2010; Kisat et al., 2013). ISS represents the sum of the squares of the highest Abbreviated Injury Scale (AIS) for the most severely injured regions in the body. The body is divided into six areas: head and neck, face, thorax, abdomen including pelvic viscera, limbs including the bony pelvis and body surface. The level of injury severity starts from 0 for no injury to 6 for afatal injury. Table 4 (Kingston & Oflanga, 2000).

SCORE	DESCRIPTION
0	No injury
1	Minor injury
2	Moderate injury
3	Serious injury.
4	Severe injury.
5	Critical injury.
6	Fatal injury

Table 4: The ISS is a comprehensive coding system for injuries of all types in every part of the body, with a description of the characteristics defining each grade of severity from 0 (no injury) to 6 (fatal injury). (Kingston & Oflanga, 2000).

1.5.4 Glasgow Coma Score (GCS)

GCS assesses the level of clinical consciousness and predicts sepsis (Kisat et al., 2013). The GCS was first introduced by Teasdale and Jennett in 1974 (Jennett and Teasdale, 1977). It is a physiological scoring system that represents the sum of 3 components. It evaluates impairment of a patient's motor response, verbal response and eye-opening to stimuli. GCS ranges from 3 to 15 (Middleton, 2012). The method of scoring depends of the level of response. It is scored from '1' for no response to '4' for eye opening normal response, '5' for verbal normal response and '6'for motor normal response (Balestreiet et al., 2004).

Eye opening response- score		Verbal response- score		Motor response- score	
Spontaneous	4	Oriented	5	Obey commands	6
To speech	3	Confused conversation	4	Localise pain	5
To pain	2	Words (inappropriate)	3	Withdraws from pain	4
None	1	Sounds (Incomprehensible)	2	Flexion to pain	3
		None	1	Extension to pain	2
				None	1

Table 5: GCS scoring system, It is scored from 1 to 6 (Balestreiet et al., 2004).

1.5.5 Lactate level

Changes in lactate level can be used as a marker for sepsis after (12-24) hrs. It is an indicator of inadequate oxygen supply, i.e. hypoxia. Increased serum levels of lactate in traumatic patients are a predictor of higher mortality (Jin et al., 2014). It was suggested that a lactate level over 4 in traumatic patients is linked to inflammatory response and thus requires treatment in an intensive care unit. Other studies found that pre-hospital lactate is a predictor of the requirement for surgical treatment (Guyette et al., 2011).

1.6 Complications of traumatic injury

1.6.1 Infections and sepsis

The most common cause of death in trauma patients is sepsis. Trauma patients are at a high risk of developing infections and sepsis because of an inadequate nutritional status, transfusion and depressed immune function. It was found that after trauma, patients have lower lymphocyte counts after receiving 20 units of blood. Patients with trauma are more prone to developing pneumonia compared to non-traumatic individuals. All these factors cause abnormality in host defence that consequently leads to sepsis and infection (Coccolini et al., 2017; Morgan, 1992). The septic process involves complex events including inflammation, circulation, disruption and hormonal and cellular responses (Hotchkiss, 2003; Gullo , 2006).

The severity of sepsis is dependent on different signs and symptoms. The diagnosis of sepsis in the early stage can be useful in guiding treatment (Kumar, 2006; Zanbon, 2008).

Biomarkers play important roles in identifying the cause of the infection, antibiotic therapy and differentiation of multiple types of bacteria as a sepsis cause and the progress of organ failure (Dellinger et al., 2008)

1.6.2 Multiple Organ Failure (MOF)

MOF is the main cause of mortality in 50-60% of all dead patients after weeks of trauma (Bochicchio et al., 2002). When MOF is associated with SIRS (hospital-acquired infections), the mortality can reach 50-80% (Durham et al., 2003). Post-traumatic MOF patients usually have longer stays in the intensive care unit at hospitals. Because of the imbalance in the immune system, MOF is followed by ischemia-reperfusion after hemorrhagic shock (Dewar et al., 2009).

Respiratory failure plays an important role in early phase of MOF which reaches 99% of all cases and usually leads to heart failure within a few hours (early death) and liver and kidney dysfunction following 5 days (late stage death) due to sepsis and MFO (Gunst et al., 2010; McGwin et al., 2009).

Mediators and effectors that potentially cooperate for post-trauma MOF development are summarised in figure 15.

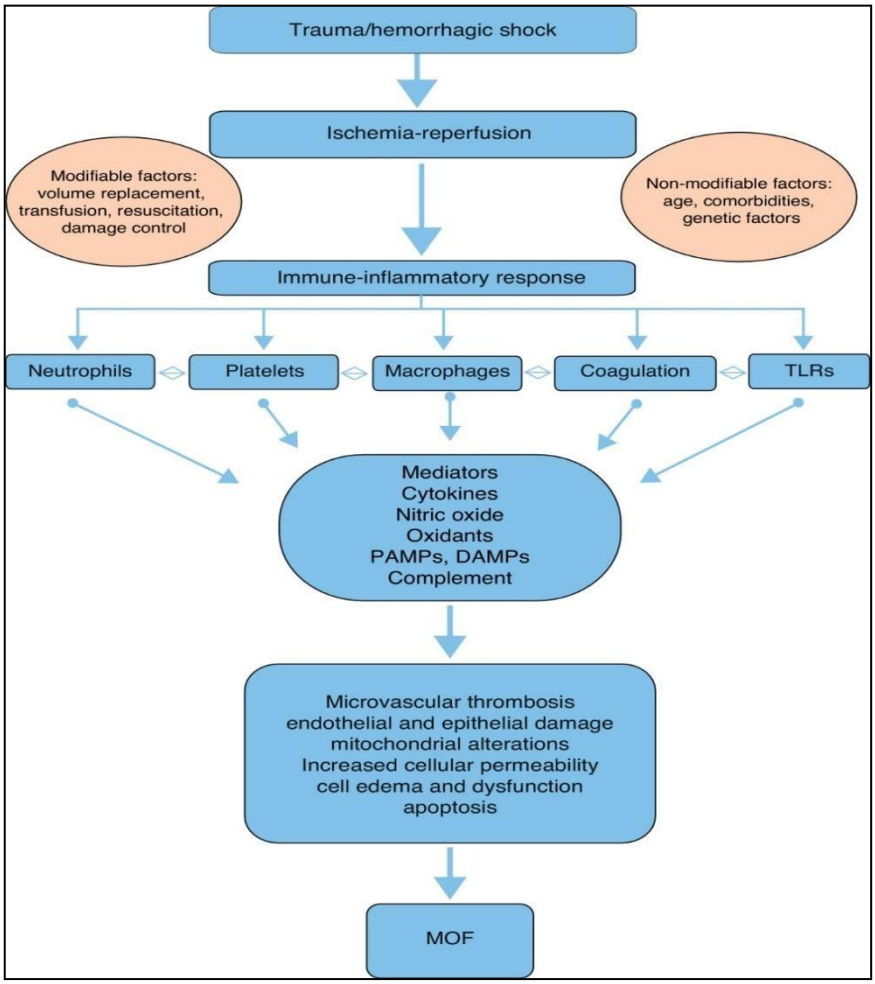

Figure 15: The pathophysiological development of multiple organ failure (MFO) following severe trauma. Adapted from (Llompart-Pou et al., 2014).

1.6.3 Acute Respiratory Distress Syndrome (ARDS)

ARDS is initiated following MOF in traumatic patients. In these patients, the mortality rate can reach 50-80% (Durham et al., 2003). ARDS patients have longer stays in hospital leading to higher costs and lower quality of long-term-related quality of life in traumatic individuals. Prediction models have been identified to reduce the risk of more complications including subject age, Acute Physiology and Chronic Health Evaluation (APACHE) II Score, Injury Severity Score (ISS), and the existence of blunt traumatic injury, lung contusion, massive transfusion, and flail chest injury. This preventive model could predict the development of ARDS in the future (Watkins et al., 2012.) ARDS can be developed directly by causing damage to the lung epithelial tissue or by an external factor that could stimulate inflammations involving some cytokines. The pathophysiological process of ARDS can progress in three stages, acute phase, proliferative phase and fibrotic phase.The first phase lasts for a week following the injury which can include the progress of hypoxaemia,

pulmonary infiltrates such as pus, blood or proteins that can be observed by chest radiograph and reduction of the ability of lung muscles contractions. These symptoms are combined with some clinical changes in the alveoli, haemorrhage, increase the diffusion of neutrophilic alveolar infiltrate to cause tissue injury (Mackay & Al haddad, 2009). The inflammatory response is mediated by releasing cytokines including; IL-1, IL-6 and TNF α. The pro- inflammatory response can lead to a rapid increase of the neutrophilic response and aggregation of leukocytes and erythrocytes (Bhatia & Moochhla, 2004). The proliferation stage can be started from day five onwards. The main symptoms of this stage is a reduction of the oxygen level in the blood resulting in prolonged hypoxaemia, decreasing of the ability of lung muscle contraction, rapid increasing of type 2 alveolar cells, fibrosis and promoting of microvascular thrombosis to prevent tissue damage. These symptoms can cause capillary function disruption. This stage could be followed by healing and recovery, or it can develop to a fibrotic stage in other cases. Patients in the fibrotic phase exhibit lung disability, resulting in decreasing of carbon dioxide excretion (Mackay & Al haddad , 2009). This can make the patient in need of external mechanical ventilation (Herridge et al., 2011)

1.6.4 Coagulation disorders

Haemorrhage is the most common coagulation disorder, and in traumatic patients accounts for 40% of trauma-related death (Kauvar et al., 2006). It is followed by coagulopathy that is generated from fluid resuscitation, hypothermia and acidosis which all lead to coagulopathy and death. Acidosis is a key factor to develop hypoperfusion and increased fibrinolysis. It was suggested that coagulopathy in trauma patients is associated with protein C activation, impaired thrombin formation, inactivation of Plasminogen Activator Inhibitor-1 (PAI)-1 and increased fibrinolysis (Gando et al., 1992). However, the relation between fibrinolysis and coagulopathy is still not fully determined in trauma. Acidosis can lead to blocking of the binding of clot formation enzymes complexes and the membrane lipids. (Tieu et al., 2007). Increasing some processes following trauma, including blocking of anticoagulant mechanisms, the lack of fibrinogen and excessive fibrinolysis mechanisms can lead to Trauma Induced Coagulopathy (TIC) (White, 2013).

1.7 Aim of study

The main aim of this MPhil is characterising TGF-beta and interleukin-8 in the context of major trauma. The ultimate aim of the project was to identify if both or either of these cytokines can be used as potential biomarkers for predicting poor clinical outcome in patients following major trauma. TGF beta and IL-8 levels are studied in 38 and 53 major trauma patients respectively, recruited as part of the NIHR Portfolio study (BIT 19377) conducted at the Salford Royal Foundation Trust (SRFT) and Central Manchester Foundation Trust (CMFT).

CHAPTER 2:

2. Materials and Methods

2.1 Study Design:

This study is designed to identify the association between the immune system and clinical outcomes in patients following major trauma. This study was granted UKCRN-NIHR Portfolio status (BIT 19377), which supported research nurse funding for clinical activities in Salford Royal Foundation Trust (SRFT) and Central Manchester Foundation Trust (CMFT).

The overall study is set to recruit 200 patients with major trauma injuries from both Foundation Trusts. This MPhil research analysed 38 samples for TGF- beta and 53 samples for IL-8 from both hospitals.

2.1.1 Patients recruitment

Three major criteria were identified in recruiting suitable samples from SRFT and CMFT as shown in table 6. Before collecting the samples from the suitable patient, consent must be taken.

PATIENTS RECRUITMENT	Yes/No
Age >16 and <90	Yes
Male and Female	Yes
ISS >15	Yes
Immediate surgical intervention	No
ICU admission	Yes

Table 6: Recruitment criteria.

The suitable patients will have collected 20ml of blood in three different days. The blood samples were taken on Day 1 (within 24 hours after injury), day 3 (third day after injury) and Day 5 (fifth day after injury) as shown in table 7.

	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8
Blood sample	X		X		X			
Data collection	X		X		X			X

Table 7: The time points of blood samples and data collection.

In addition to the blood samples collected, clinical data was taken on Day 1, Day 3, Day 5 as well as Day 8. The data collection sheet is shown in table 8.

Day			Date		
HR		BP	___/__	Temp	____°C
Hb		WCC		PLT	
eGFR		Creatinine		Bilirubin	
PT		Intubated	Y / N	NIV/CPAP	Y/N
FiO2	____%	P/F Ratio	____kPA	Lactate	___mmol/L
Noradrenaline	___µg/kg/min	CRP	____mg/L	CVVH/IHD	Y/N
Sedated		GCS			
Treated with antibiotics	Y / N	Source of sepsis		____empirical/unknown	

Table 8: the sheet of clinical data collection.

Patients SOFA scores were calculated by using the clinical data. TGF- beta and IL-8 concentrations were compared with SOFA scores to detect the association between patient outcomes with TGF-beta and IL-8 concentrations.

2.1.2 Detection of patient outcome

For this MPhil study, blood and data were collected in addition to TGF-beta and IL-8 being examined to identify patient outcomes. SOFA scores were calculated for Day 1 and Day 5 against concentrations of TGF-beta and IL-8 for the respective days. The method of measuring a good or poor outcome is using the SOFA score. Organ failure will be distinguished if the SOFA score ≥3 for this organ. In contrast, a good patient outcome will be identified if SOFA score <3.

2.2 Ethical approval

The project was designed to find correlations/associations between certain cytokines as biomarkers and patients clinical outcome following traumatic injury. The research has gained ethical approval from the Local Ethics Commit Manchester, NHS/HSC Research and Development offices (**IRAS ID 172620**) and the ethical committee at the University of Salford, under ethics code **ST1617-17**. This grant was supported by research nurse funding for clinical activities in Central Manchester Foundation Trust (CMFT) and Salford Royal Foundation Trust (SRFT).

2.3 Sample preparation

2.3.1 Blood sample collection

Anonymised and coded blood samples were collected from CMFT and SRFT and sent to the University of Salford within 3 hours to examine cytokines after serum separation.

2.3.2 Serum separation

At the Cockroft Laboratories of the University of Salford, 5 ml of blood was placed in two 15 ml centrifuge tubes and centrifuged for 5 minutes at 2000 rpm (644 xg). After centrifugation, the top layer of serum is separated, and 300ul of aliquots is collected in 8 labelled cryovials to store in the blood biobank at – 80 c°.

2.4 Sample quantification – Cytometric Bead Array (CBA)

2.4.1 Principle OF CBA

The main principle of CBA is represented by using flow cytometry to detect soluble analytes with beads and fluorescence. In this MPhil study, BD CBA Human Soluble Protein Flex Set and BD CBA Human Soluble Protein Master Buffer kits are used for blood serum, bead-based immunoassay. The main advantage of this assay is detecting very low concentrations of soluble analytes (10 to 2500 pg/ml). The CBA assay employs two main reagents, the first reagent is capture bead which has distinct fluorescence and is coated with capture antibody. The other reagent is the detection reagent which is containing phycoerythrin (PE) and conjugated antibodies. After incubation of the samples with reagents, sandwich complexes will be formed (capture bead + analyte + detection reagent). These complexes can be detected by flow cytometry to distinguish the fluorescent particles. The study used a FACSVerse flow cytometer which uses dual lasers (488 nm and 640nm lasers).

2.4.2 Cytometric Bead Array for standard of IL-8 and TGF- β

2.4.2.1 Determination of IL-8 standard concentrations

To creating a standard curve for IL-8 a lyophilised standard sphere from BD, a CBA Flex Set was used. A standard sphere was added into a 15 ml tube which contains 4ml of assay diluent to be reconstituted. This tube is labelled as a "Top Standard" which was the highest concentration (2500 pg/ml), the tube was left for 15 minutes to be equilibrated. A serial dilution was performed by labelling 9 tubes, as shown in (table 9), and 500 ul of assay diluent was added to each tube. Starting from Top Standard tube, 500 ul of the solution was transferred to the following tube to create serial dilution in addition to the negative control tube, which contained only assay diluent.

Tube number	Standard dilution	Concentration/pg/ml
1 (Top Standard)	1:1	2500
2	1:2	1250
3	1:4	625
4	1:8	312
5	1:16	156
6	1:32	80
7	1:64	40
8	1:128	20
9	1:256	10
10 (Negative control)	No Dilution	0

Table 9: Serial dilution of standard concentrations of IL-8.

The next step is the preparation of capture beads. Each test requires 1ul of capture bead stock to be added. To resuspend the capture bead stock, it needs to be vortexed for 15 seconds. The required volume was added to a tube and washed with 500ul of washing buffer then centrifuged at 200 X G for 5 minutes. After the washing, the supernatant was discarded, and the pellet was resuspended in 50 ul of capture beads diluent per test. After mixing the calculated volume of capture beads diluent with the pellet vortex the tube is done and the tube then was incubated for 15 minutes at room temperature.

Preparation of PE detection reagent was done by adding 1 ul of PE detection reagent to 49 ul of PE detection reagent diluent per test and reaching a final 1:50 dilution. PE detection reagent was kept at 4Ċ to be protected from light. The required volume of PE detection reagent was added to 490 ul of PE detection reagent diluent to obtain a final volume of 500 ul. A new set of Eppendorf

tubes was prepared and labelled to correspond to the first set of standard concentrations. Each tube contained 50 ul of mixed capture beads added to 50 ul of standard concentration. The tubes were incubated in room temperature for one hour. After incubation, a 50 ul of mixed PE detection reagent was added to each tube and left at room temperature for two hours in the dark. Following the second incubation, each tube was washed with 1 ml of washing buffer and centrifuged at 200 X G for 5 minutes. The supernatant was discarded, and the pellet was resuspended with 300 ul of washing buffer to be ready for flow cytometry

2.4.2.2 Determination of TGF-β standard concentrations

A standard sphere for TGF-β was provided by a BD CBA flex set to create a standard curve into a 15 ml test tube, and a standard sphere was added and mixed with 1 ml assay diluent. The tube was left for 15 minutes to equilibrate. A serial dilution was performed by preparing 9 labelled tubes as shown in table (10). 500 ul of assay diluent was added to each tube. To create a serial dilution, 500 ul of the solution was transferred from each tube to the following one. The Top Standard" tube is the most concentrated which is 10000 pg/ml, while the lowest concentrated tube is 40 pg/ml. A negative control was prepared, which was containing just assay diluent.

Tube number	Standard dilution	Concentration/pg/ml
1 (Top Standard)	1:1	10000
2	1:2	5000
3	1:4	2500
4	1:8	1250
5	1:16	625
6	1:32	312
7	1:64	156
8	1:128	80
9	1:256	40
10 (Negative control)	No Dilution	0

Table 10: serial dilution for standard concentrations of TGF-β.

A mixed capture bead was prepared by adding 1 ul of capture beads to 50 ul of capture bead diluent for each test. A new set of Eppendorf tubes was prepared and labelled as corresponding to the firs set of standard concentrations. 50 ul of standard concentration was added to each tube then mixed with 50 ul of mixed capture beads for 2 hours at room temperature.

PE detection reagent was prepared by mixing 1 ul of PE detection reagent and 49ul of PE detection reagent diluent per test to reach a final 1:50 dilution. PE detection reagent was kept in 4 C̊ to be protected from light. After the first incubation, the tubes were washed with 1 ml of wash buffer and centrifugation for 5 minutes at 200 X G. The supernatant was discarded, and 100 ul of it has left in each tube. 50 ul of mixed PE detection reagent was added and mixed gently to be incubated for 2 hours in the dark at room temperature. A second washing was done following the incubation with 1 ml of wash buffer per tube and centrifugation at 200 X G for 5 minutes. The supernatant was discarded, and the pellet was resuspended with 300 ul of wash buffer and flow cytometry detection was performed.

2.4.3 Cytometric Bead Array Analysis In Serum Samples

2.4.3.1 Cytometric Bead Array Analysis For IL-8 In Serum Samples

Triplicate Eppendorf tubes were prepared. Patient serum samples were removed from the serum biobank in -80 C freezer then they were defrosted at room temperature. The first step is the preparation of 50 ul of mixed capture beads (as mentioned in standard concentration determination). 50 ul of patient serum was prepared in each Eppendorf tube then mixed with 50 ul of mixed capture beads to be incubated for an hour at room temperature. Following the first incubation, 50 ul of PE detection reagent (prepared as mentioned in standard concentrations determination) was added to each tube then was incubated at room temperature for 2 hours. After the incubation, 1 ml of wash buffer was added to each tube then centrifuged for 5 minutes at 200 xg. The supernatant was discarded, and the pellet was resuspended by 300 ul of wash buffer to be ready for flow cytometry analysis.

2.4.3.2 Cytometric Bead Array Analysis for TGFβ in serum samples

2.4.3.2.1 Patients serum activation

A samples activator was prepared by forming two different solutions. Acetic acid/ urea solution was prepared by adding 50 ul of 2.5 N of acetic acid to 8 M urea. NaOH/HEPES solution was created by mixing 50 ul of 2.7 N of NaOH with 1M HEPES. To activate serum samples. 50 ul of serum was

mixed well with 50 ul of Acetic acid/Urea in an Eppendorf tube and left for 10 minutes at room temperature. After the incubation sample neutralization was done by transferring 50 ul of (sample + acetic acid/urea) to 50 ul of NaOH/HEPES. Serum sample was then diluted 10-fold with assay diluent. The calculated concentration for each patient sample was multiplied by a dilution factor of 30 then converted into ng/ml.

2.4.3.2.2 CBA procedure for TGF-β in serum samples

Following the serum sample activation, a mixed capture bead was prepared, as mentioned in the TGF-β standard concentrations method. 50 ul of activated serum sample for Day 1 and Day 5 was added separately to 50 ul of mixed capture beads in triplicate and was mixed gently to be incubated for 2 hours at room temperature. The tubes were washed in I ml of wash buffer per tube and centrifuged at 200 X G for 5 minutes. The pellet was discarded, and the pellet kept with 100 ul of the supernatant.

A mixed PE detection was prepared as mentioned in the TGF-β standard concentrations method previously. 50 ul of mixed PE detection reagent was added to each tube then mixed gently and incubated in the dark for 2 hours in room temperature. Following the second incubation, the second washing was done by adding 1 ml of wash buffer to each tube and centrifuging at 200 X G for 5 minutes. 100 ul of supernatant was left in each tube and the pellet resuspended at 300 ul of wash buffer. The flow cytometry analysis was done.

2.4.4 Flow cytometry analysis for IL-8 and TGF-β

The flow cytometry was done by using the FACS machine, and daily QC (quality control) is performed to test the efficiency of the cytometer. Different capture beads for IL-8 and TGF-β were identified from the general bead population. Moreover, PE fluorescence intensity (MFI) was measured for each standard or patient sample in this analysis.

The differences between data taken for the samples at Day 1 and Day 5, and the correlation between some clinical parameters and cytokines concentrations were determined. The main clinical parameter used in this analysis SOFA score and the data were represented by mean, standard deviation and standard error of the mean.

2.5 Statistical analysis

In this study, mean, standard deviation and standard error of the mean were measured. Statistical analysis performed by making comparisons between cytokine concentrations at different points and clinical parameters such as SOFA score. Lactate concentration comparison between DAY 1 and DAY 5 was performed. The statistical analysis used Minitab statistical software (version19) for testing the normality, and Mann-Whitney U-test was used for non- parametric distribution to find the statistical significance at $P < 0.05$ level.

CHAPTER 3

3. RESULTS

3.1 Optimization of cytometric bead array

In this study, experiments for IL-8 and TGF-β were done to create standard curves for each cytokine. The experiment followed the manufacturer's protocols. The standard curves were generated to find the corresponding concentrations for each of IL-8 and TGF-β by applying median fluorescence intensity (MFI) of PE on the standard curve formula. The serum samples were collected, and the cytokine levels were calculated by using the standardised method.

The experiments for IL-8 and TGF-β were carried out individually. The population of capture beads were first gated as P1 then the beads were detected and gated as IL-8 or TGF- β as shown in figure (17 and 18) respectively.

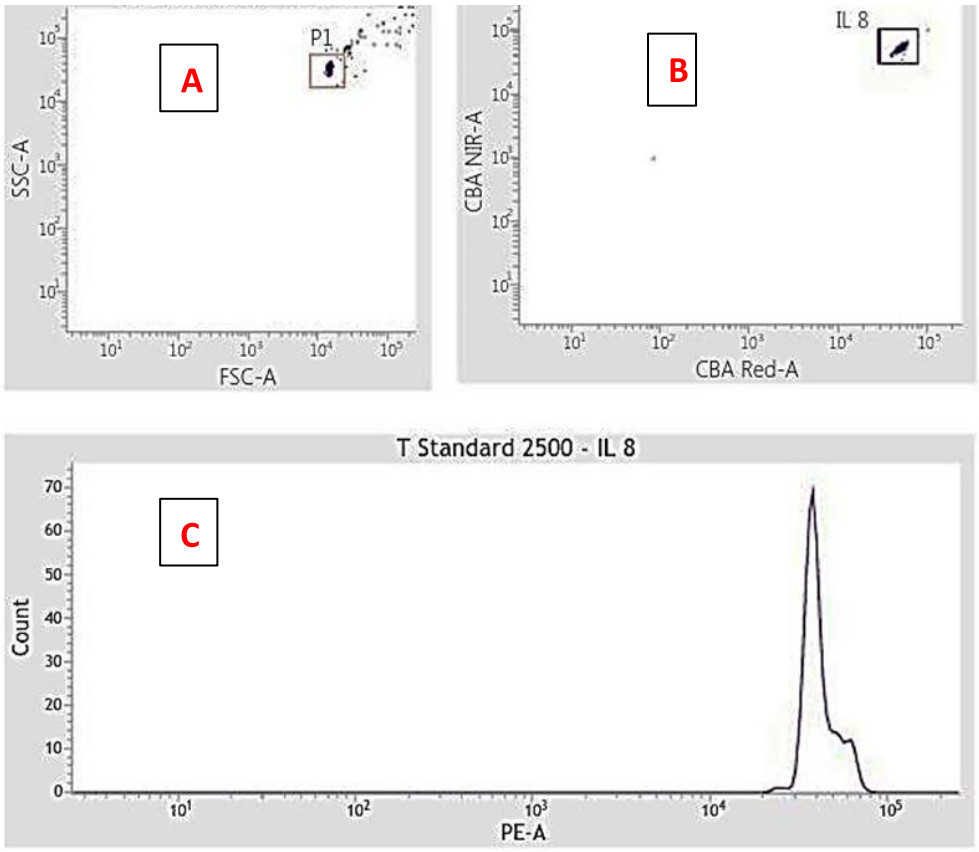

Figure 17: A. populations gate in P1, B. IL-8 cytokine position, and C. MFI of PE for IL -8

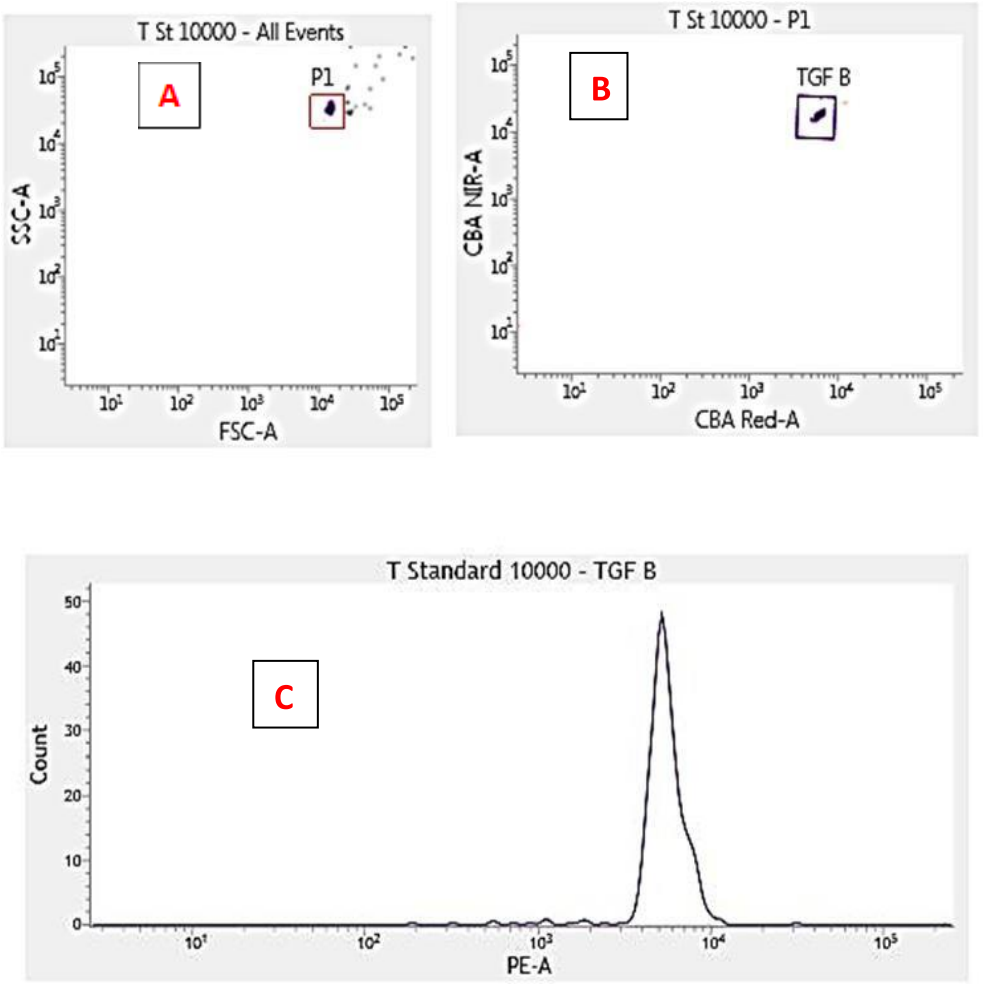

Figure 18: A. populations gate in P1, B. TGF-β position, and C. MFI of PE for TGF-β.

The standard curve was generated by a range of concentrations in pg/ml represented by the X axis, while the Y axis represented the PE – Median Fluorescence Intensity (MFI) as shown in figures 19A and 19B for IL-8 and figures 20A and 20B for TGF – β.

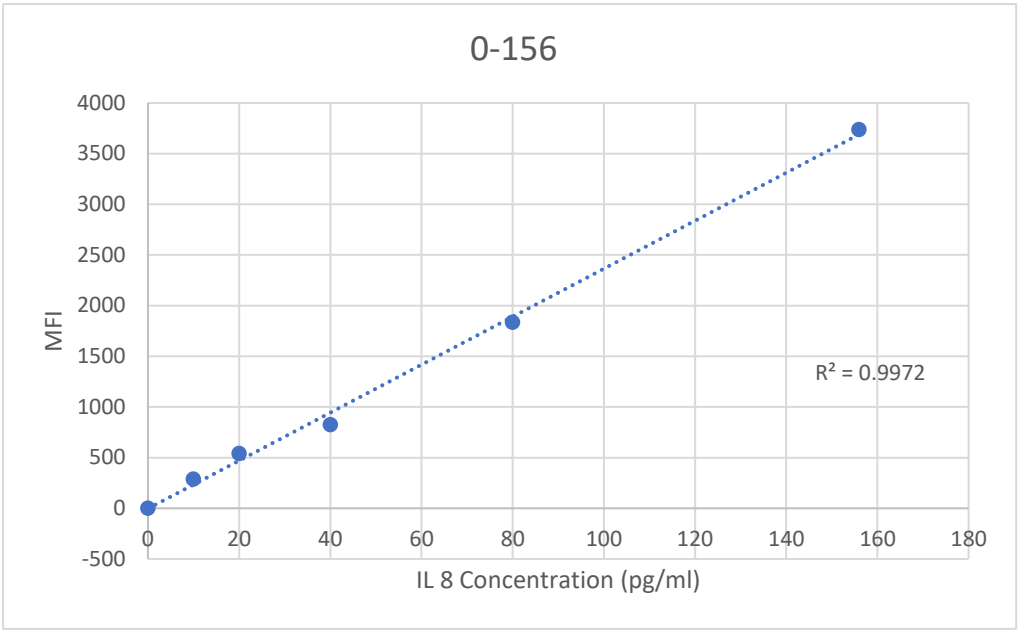

Figure 19 A: Standard curve for IL-8. Concentrations range 0-156.

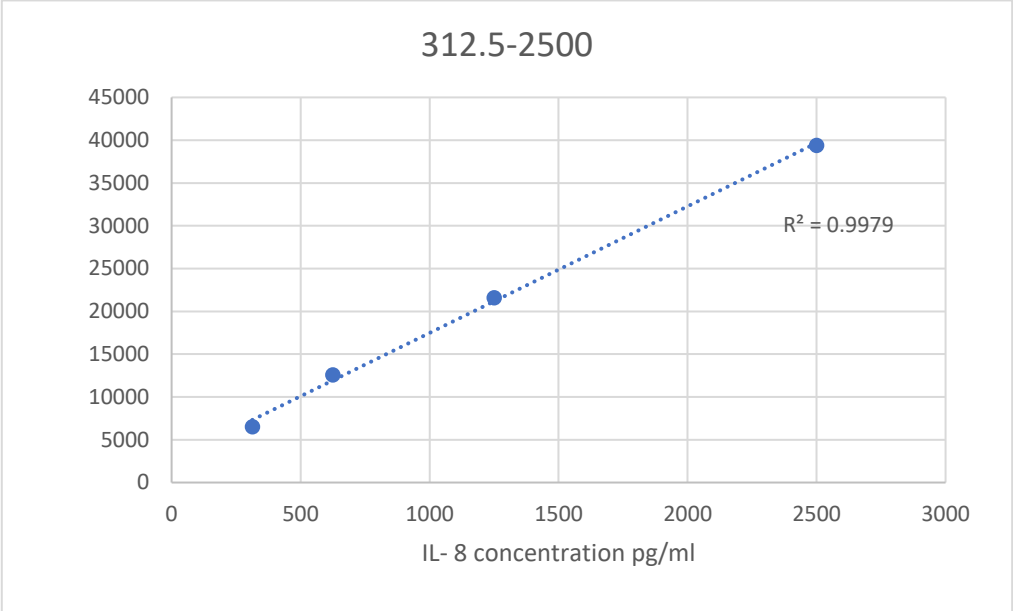

Figure 19 B: Standard curve for IL-8. Concentrations range 312.5-2500.

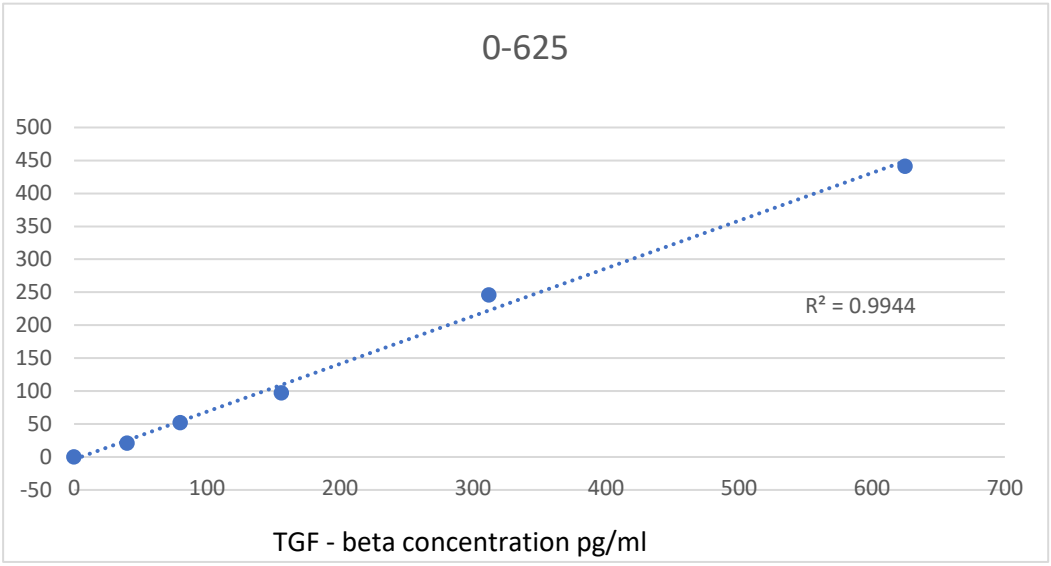

Figure20 A: Standard curve for TGF-β. Concentrations range 0-625.

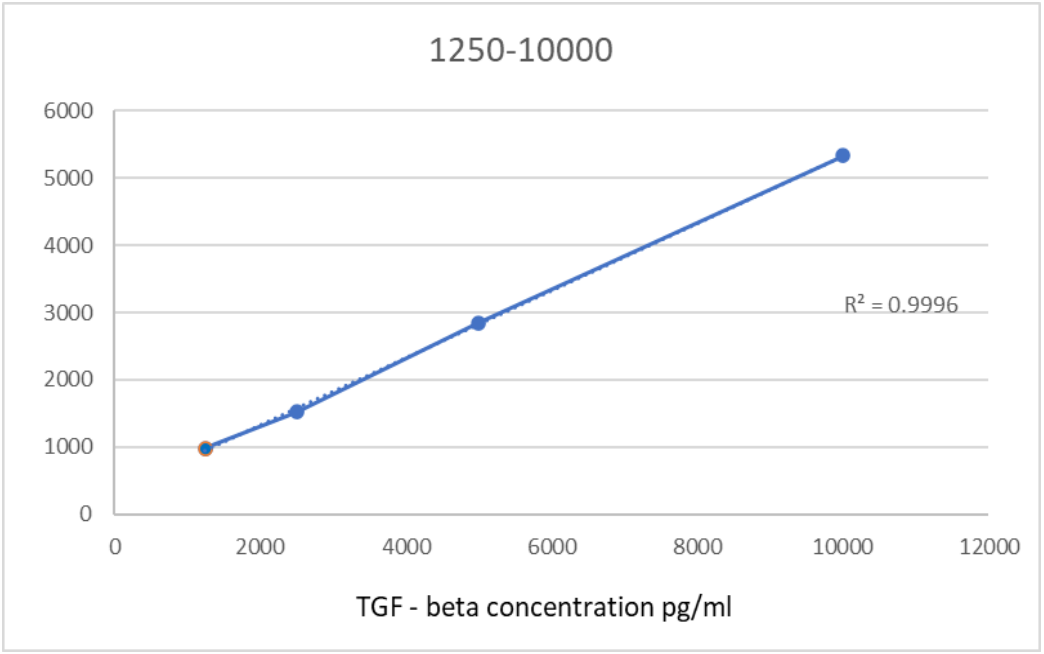

Figure 20 B: Standard curve for TGF-β. Concentration range 1250 - 10000.

3.2 Prediction of trauma patient outcomes by IL-8 concentration.

3.2.1 Detection of cytokine IL-8 concentration in the serum of traumatic patients.

The cytokine IL-8 concentration was measured in triplicates in the serum of 53 patients on Day 1 and Day 5 following traumatic injuries, as shown in figure 21.

IL-8 concentration was varied in Day 1 and Day 5 (appendix6). The sample concentrations in Day 1 ranged from 5.205330 pg/ml to 94.304483 pg/ml with a median of 18.46882656 pg/ml and a mean value of 21.2795421 pg/ml. The sample concentrations in Day 5 ranged from 5.2757314pg/ml to 36.0690067 pg/ml with a median value of 13.96318042 pg/ml and a mean value of 15.36003699 pg/ml. The average concentration was decreased from 21.2795421pg/ml in Day 1 to 15.36003699 pg/ml in Day 5. A significant statistical decrease in Day 5 compared to Day 1 at p = 0.013 using the Mann- Whitney U- test.

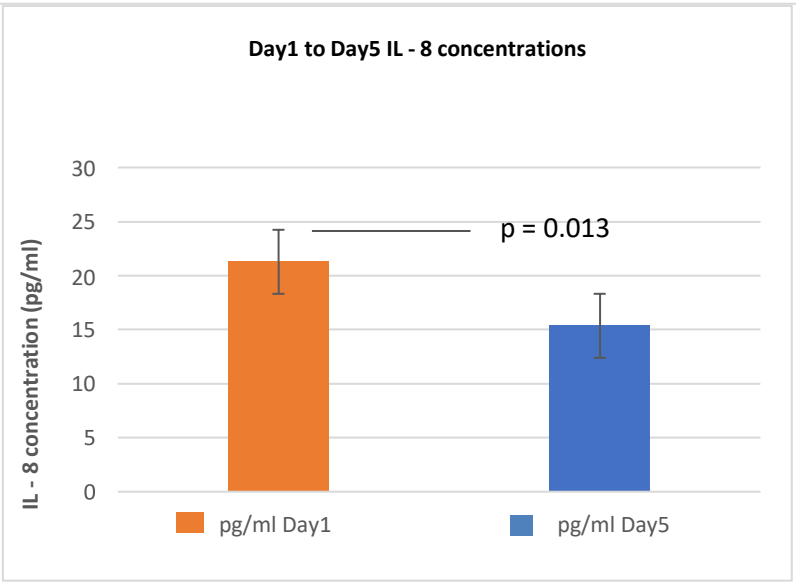

Figure 21: A comparison between Day 1 and Day 5 IL-8 average concentration for each patient. Day 1 IL – 8 average concentration represented by ■, Day 5 IL – 8 average concentration represented by ■. A significant statistical decrease in Day 5 compared to Day 1 at p value 0.013.

3.2.2 A comparison between IL-8 concentration and SOFA score

SOFA score was calculated for each patient on Day 1 and Day 5 as shown in figure 22 (appendix 6). The mean value of Day 1 SOFA was 7.35 compared to 7.58 in Day 5.

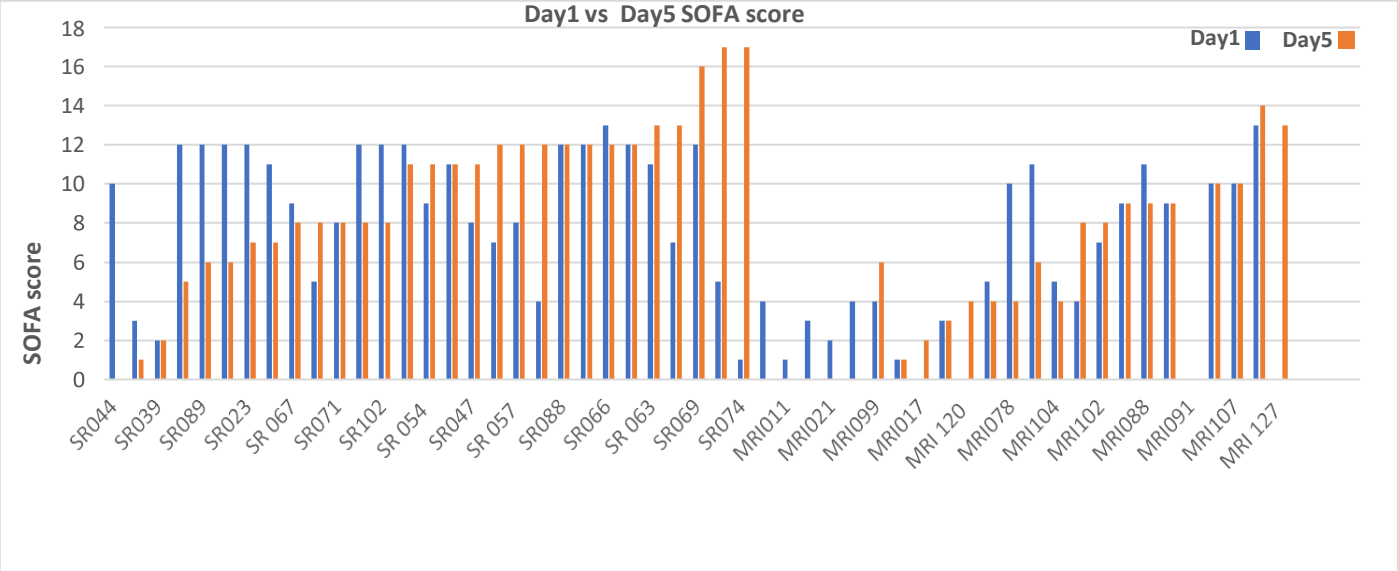

Figure 22: A comparison between Day 1 and Day 5 SOFA score. SOFA Day 1 represented by ■, SOFA Day5 represented by ■.

3.2.2.1 A comparison between Day 1 IL-8 concentrations (pg/ml) and Day5 SOFA scores.

A comparison between Day 1 IL -8 concentration and Day 5 SOFA score was done in order to infer the relationship between early IL-8 levels (Day 1) and the clinical outcome of the patient on Day 5, as defined by the calculated patient SOFA score. The DAY 1 IL-8 concentration ranged between 5.205330 – 94.30448312 pg/ml with corresponding DAY 5 SOFA score between 17–6. The average of DAY 1 IL-8 concentration was 21.2795421 pg/ml, and the DAY 5 SOFA score average was 7.5. Patients were clustered of on the basis of poor and good outcomes at Day 5 (either using a SOFA cut off <3 and ≥3, or <6 and ≥6) two enable stratification for two ranges of severity.

3.2.2.2 A comparison between Day 1 IL-8 and Day 5 SOFA scores at the thresholds <3 and ≥3.

The Day 1 IL-8 concentration for the patients was grouped according to Day 5 SOFA score (<3 and ≥3) into two groups (figure 24). The patients with SOFA scores <3 represent patients with good outcomes, while patients with SOFA scores ≥3 represent patients with poor outcomes (Durham et al., 2003).

The mean value of IL-8 concentration for the patient with DAY 5 SOFA ≥3 was 23.19741 pg/ml. Whereas, the mean value of IL -8 concentration for the patients with DAY 5 SOFA <3 was 20.71822 pg/ml. The difference however was not statistically significant (p=0.218, Mann-Whitney U-test was used to compare the two groups) as shown in figure 23 A.

3.2.2.3 A comparison between Day 1 IL-8 and Day 5 SOFA scores at the threshold <6 and ≥6.

The Day 1 IL-8 concentration for the patients were grouped according to Day 5 SOFA score into two groups (SOFA <6) and (SOFA ≥6) . To predict the correlation between IL- 8 level andmulti - organ failure, the concentration of IL- 8 was measured for the patients of SOFA score ≥6 on day 5 (Ferreira et al., 2001).

This study reported that the mean value of IL-8 concentration for the patients with SOFA ≥ 6 is 20.36716 pg/ml. Whereas, the mean value of IL-8 concentration for the patients with SOFA < 6 was 19.63627 pg/ml. Figure 23 B shows a moderate decrease of the cytokine level for the patients of SOFA score ≥ 6 compared to the patients with SOFA score < 6 but it is not a statistically significant difference, P = 0.894 (Mann-Whitney U-test was used to compare the two groups) as shown in figure 23 B.

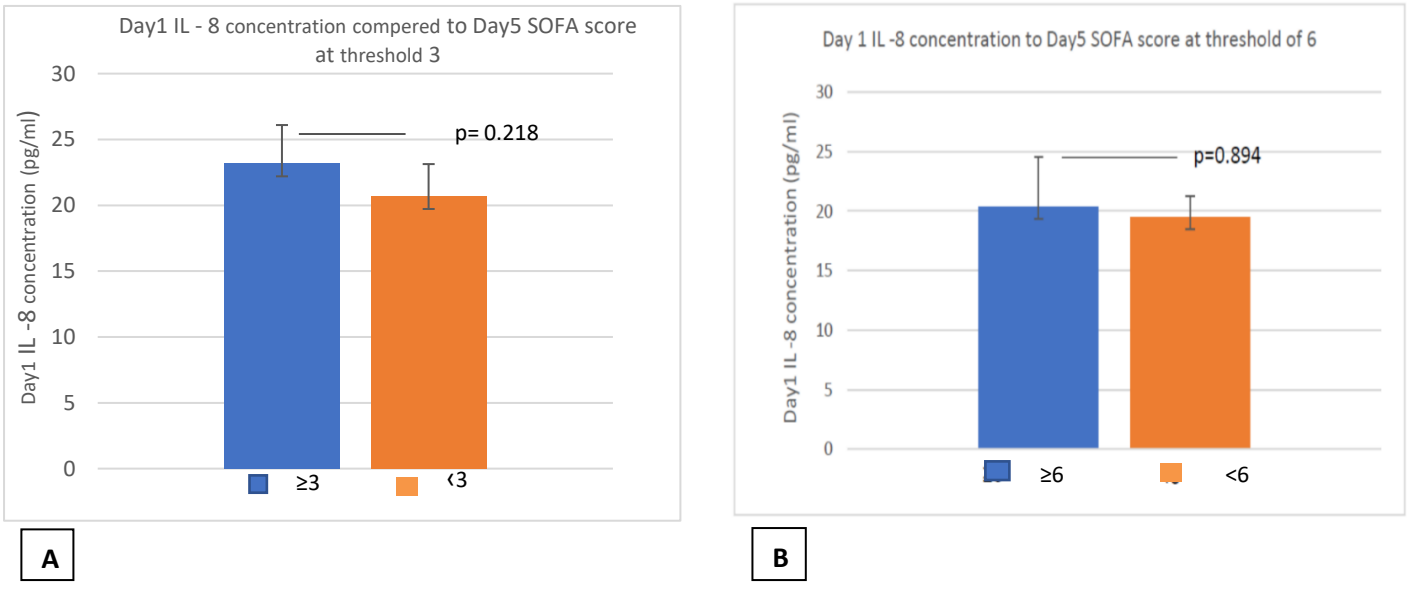

Figure 23: **A**. A comparison between Day 1 IL-8 concentration and Day 5 SOFAs score at threshold of 3, **B**. A comparison between Day 1 IL-8 concentration and Day 5 SOFAs score at threshold of 6. Patients with SOFA score ≥3, ≥6 represented by ■, patients with SOFA score <3 and <6 represented by ■.

3.2.2.4 A comparison between good and poor patient Outcomes at SOFA thresholds of ≥3 or ≥6

Patients were considered into two groups; good outcome (SOFA<3) and poor outcome (SOFA ≥3) groups (Durham et al., 2003). The average of Day 1 IL-8 concentration in the poor outcome group was 23.19741 pg/ml compared to 20.71822 pg/ml in the good outcome group. Whereas, the difference between the good outcome and poor outcome patients increased in Day 5 as the average concentration for good outcome patients was 13.60444pg/ml compared to 18.18888 pg/ml for the poor outcome group. Both good and poor outcomes decreased in Day 5 as shown in Figure 24.

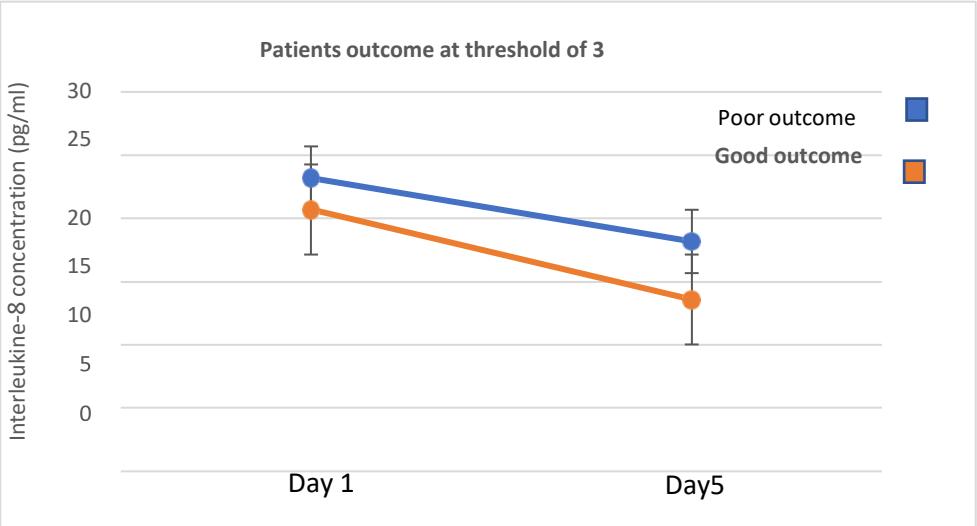

Figure 24: A comparison between the IL-8 concentrations (pg/ml) of good and poor outcome patientsat a SOFA threshold of ≥3.

Patients were also considered into two groups; good outcome (SOFA<6) and poor outcome (SOFA ≥6) groups (Ferreira et al., 2001).The average of Day 1 IL-8 concentration in the good outcome group was 19.63627 pg/ml compared to 20.36716 pg/ml in the poor outcome group. Whereas, the average concentration for good outcome patients was 14.49988pg/ml compared to 16.34973 pg/ml for the poor outcome group. Both good and poor outcomes decreased in day 5 as shown in figure 25.

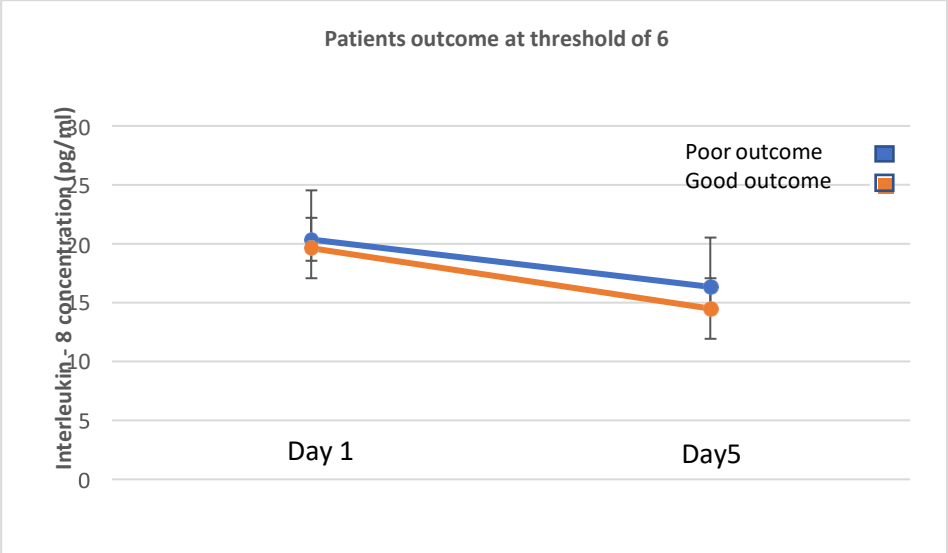

Figure 25: A comparison between the IL-8 concentrations (pg/ml) of good and poor outcome patients at a Day 1 and Day 5 SOFA threshold of ≥6.

3.3 Prediction of trauma patient outcomes by TGF-β concentration.

3.3.1 Detection of cytokine TGF-β concentration in the serum of trauma patients.

The cytokine TGF-β concentration was measured in ng/ml in triplicates for the serum of 38 patients on Day 1 and Day 5 following traumatic injuries, as shown in figure 26.

TGF-β concentration was varied on Day 1 and Day 5 (appendix7). The sample concentrations on Day 1 ranged from 2.593823ng/ml to 15.32153 ng/ml with a median of 5.227017 ng/ml and a mean value of 7.05612742 ng/ml. The samples concentrations in Day 5 ranged from 0.111573 ng/ml to 9.707431 ng/ml with a median value of 3.734753 ng/ml and a mean value of 4.391352421 ng/ml (figure 26). A statistically significant decrease on Day 5 compared to Day 1 at $p = 0.000$ was seen using the Mann-Whitney U-test.

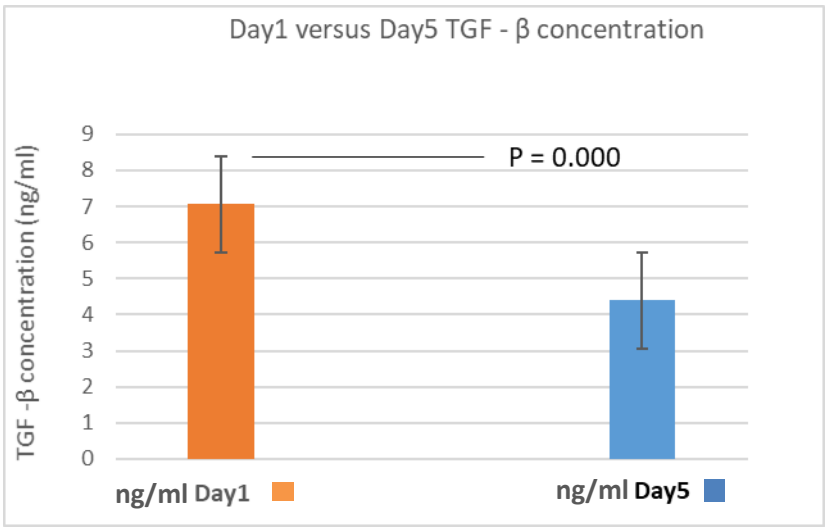

Figure 26: A comparison between Day 1 and Day 5 TGF-β average concentration Day 1 TGF-β average concentration represented by , Day 5 TGF-βaverage concentration represented by . A significant statistical decrease in Day 5 compared to Day 1 at p value 0.000.

3.3.2 A comparison between TGF-β concentration and SOFA score

SOFA scores were calculated for each patient on Day 1 and Day 5, as shown in figure 30 (appendix 7). The mean value of the Day 1 SOFA was 8.236. Whereas the mean value of the Day 5 SOFA was 7.473

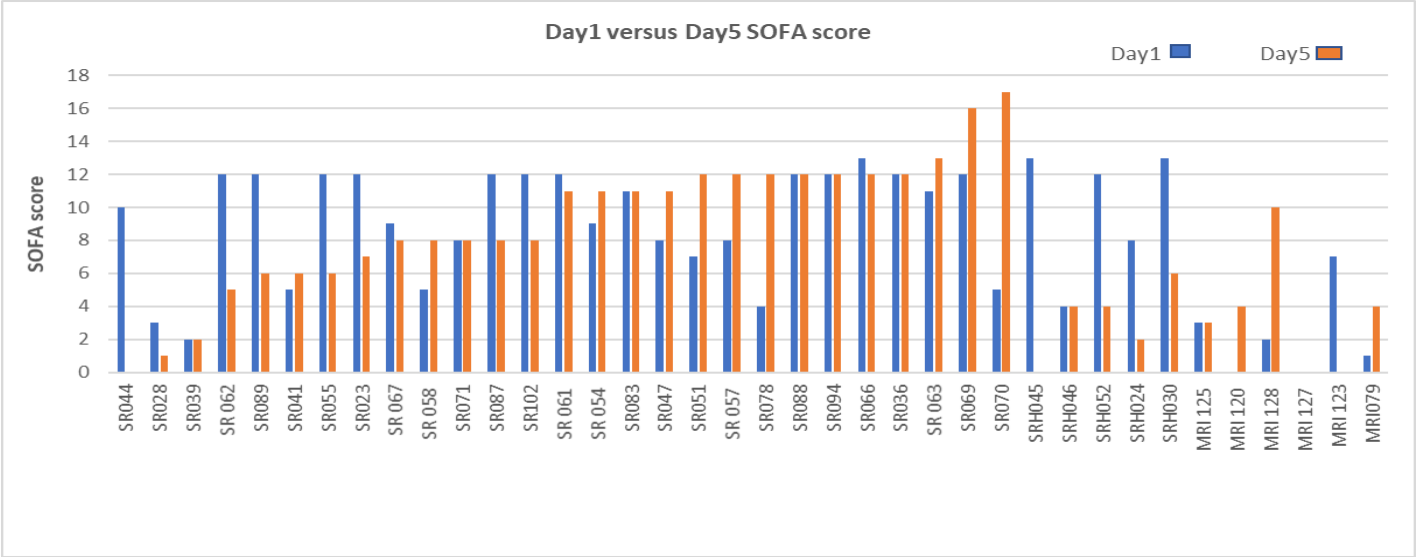

Figure 27: A comparison between Day 1 and Day 5 SOFA score. SOFA Day 1 represented by , SOFA Day5 represented by .

3.3.2.1 A comparison between Day 1 TGF-β concentrations and Day 5 SOFA score.

A comparison between Day 1 TGF-β concentrations and Day 5 SOFA scores was made. The DAY 1 TGF-β concentrations ranged between 2.593823ng/ml to 15.32153 ng/ml with corresponding DAY 5 SOFA scores between 6 and 4. The average DAY 1 TGF-β concentration was 7.056127429 ng/ml, and the DAY 5 SOFA score average was 7.4.

3.3.2.2 A comparison between Day 1 TGF-β concentration and Day 5 SOFA scores at the threshold of ≥3

The Day 1 TGF-β concentration for the patients was grouped according to Day 5 SOFA scores (<3 and ≥3) into two groups (figure 30). The patients with SOFA scores <3 represent patients with good outcomes, while the patients with SOFA scores ≥3 represent patients with poor outcomes (Durham et al., 2003).

The mean value of TGF-β concentration for the patients with DAY 5 SOFA ≥3 was 8.242936 ng/ml. Whereas, the mean value of TGF-β concentration for patients with DAY 5 SOFA <3 was 6.833601 ng/ml. Figure 31 shows a difference between the two groups (SOFA < 3 and SOFA ≥ 3) but it is not a statistically significant difference. (Mann-Whitney U-test was used to compare the two groups.

3.3.2.3 A comparison between Day 1 TGF-β concentrations and Day 5 SOFA scores at the threshold of ≥6

The Day 1 TGF-β concentration for patients was grouped according to Day 5 SOFA scores into two groups (SOFA <6) and (SOFA ≥6) (figure 33). The patients with SOFA scores ≥ 6 represented patients with organ dysfunction and multi-organ failure (Ferreira et al., 2001)

The mean value of the TGF-β concentration for the patients with DAY 5 SOFA ≥ 6 was 8.81642 ng/ml. Whereas, the mean value of TGF-β concentration for the patients with DAY 5 SOFA < 6 was 5.788112 ng/ml. Figure 32 shows a difference between the two groups (SOFA < 6 and SOFA ≥ 6), but it is not a statistically significant difference. (p=0.075, Mann-Whitney U-test was used to compare the two groups).

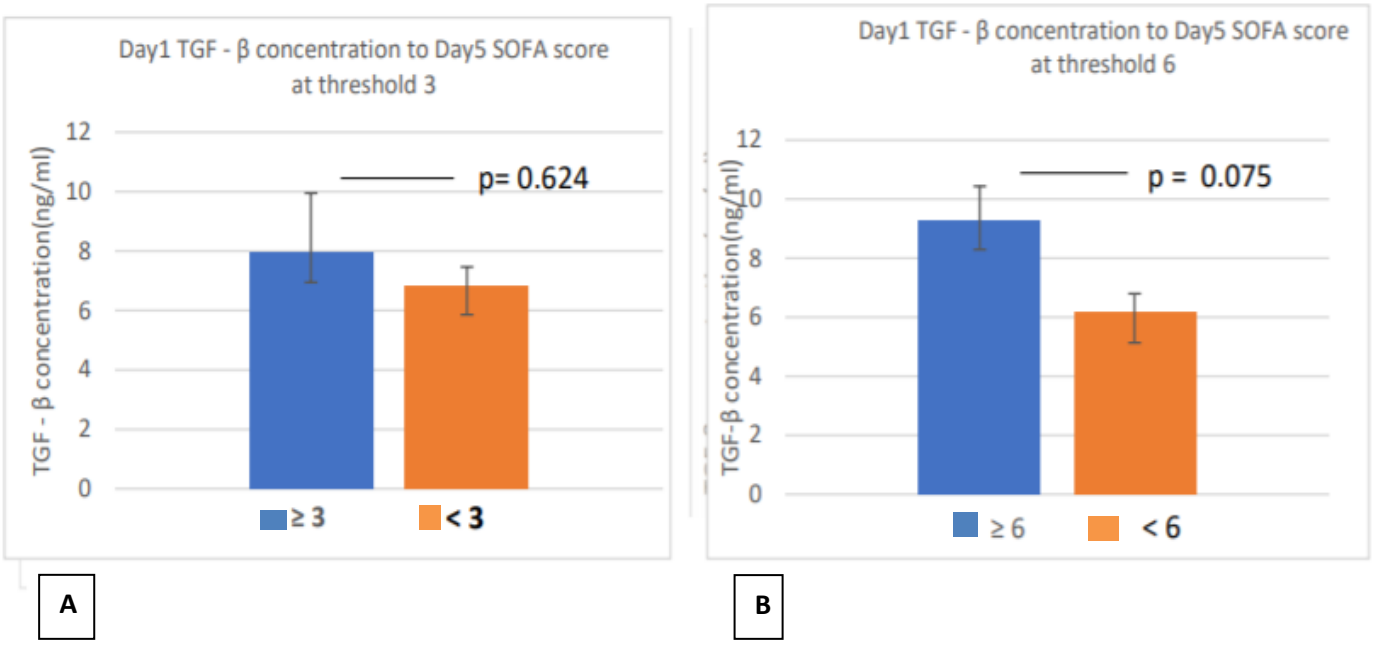

Figure 28: **A**. A comparison between Day 1 TGF-β concentration and Day 5 SOFAs score at threshold of 3, **B**. A comparison between Day 1 TGF-β concentration and Day 5 SOFAs score at threshold of 6. Patients with SOFA score ≥3, ≥6 represented by ■, patients with SOFA score <3 and <6 represented by ■.

3.3.2.4 A comparison between good and poor patient outcomes at SOFA thresholds of ≥3 or ≥6

Patients were considered into two groups; Good Outcome (SOFA<3) and Poor Outcome (SOFA ≥3) (Durham et al., 2003). The average of Day 1 TGF-β concentration in poor outcome patients was 8.24293 ng/ml compared to 6.833601 ng/ml in good patient outcome. The difference between good outcome and poor outcome patients decreased on Day 5 as the average concentration for poor outcome patients was 4.703897 ng/ml compared to 4.320778 ng/ml for the good outcome group (Figure 29).

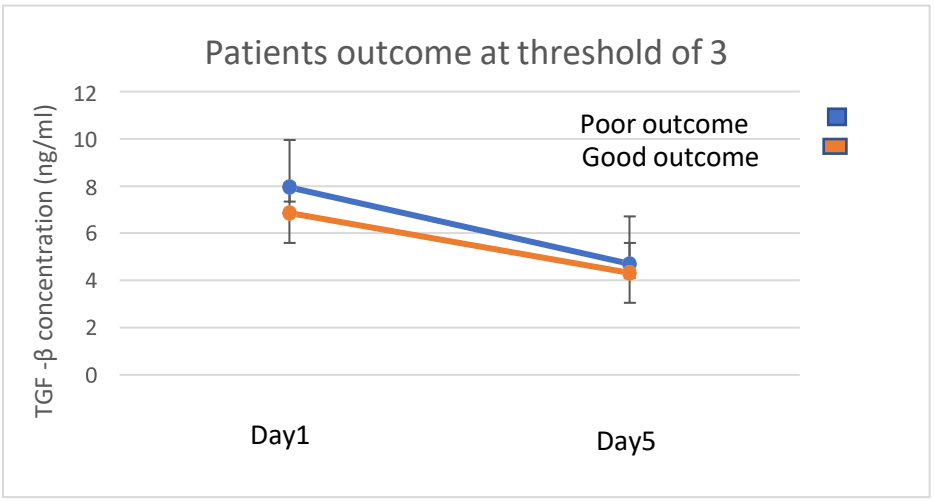

Figure 29: A comparison of good and poor patient outcomes at a SOFA threshold of ≥3

Patients were also considered into two groups; good outcome (SOFA<6) and poor outcome (SOFA ≥6) groups (Ferreira et al., 2001). The average of Day 1 TGF - β concentration in the good outcome group was 5.788112 ng/ml compared to 8.81642 ng/ml in the poor outcome group. Whereas, the good outcome average measured as 3.909817 ng/ml compared to 5.43678 ng/ml for the poor outcome average in Day 5. Both good and poor outcomes decreased on Day 5 (Figure 30).

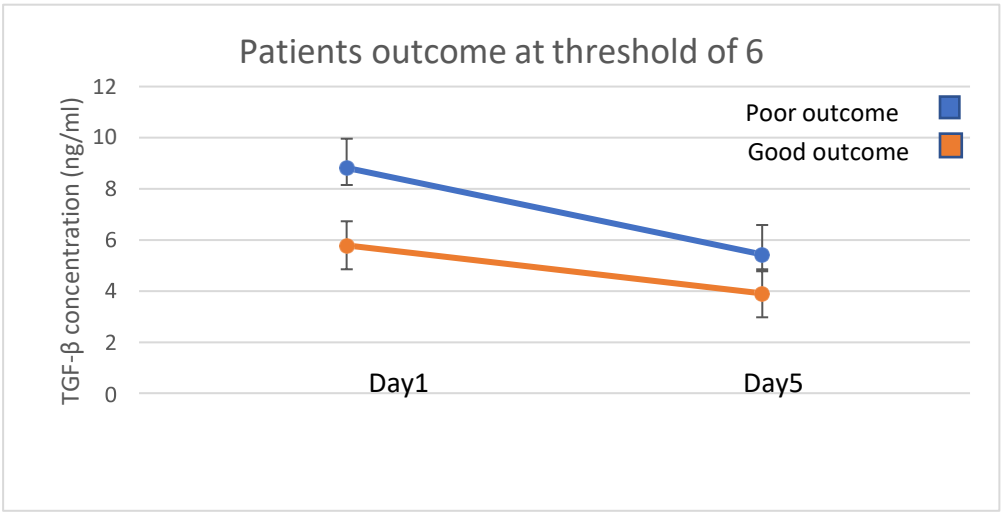

Figure 30: A comparison of good and poor patient outcomes at a SOFA threshold of ≥6

3.4 Using Lactate concentration as a biomarker

The lactate concentration was used as a marker for inflammation and organ dysfunction (Nguyen et al., 2010) for 23 traumatic patients. The Lactate concentration on Day 1 ranged from 0.8 m M/L to 6 m M/L. Whereas, the concentration on Day 5 ranged between 0.8 m M/L and 1.9 m M/L. The average lactate concentration for the patients decreased from 3.043 m M/L on Day 1 compared to 1.185 m M/L on Day 5, as shown in figure 35. A statistically significant decrease was measured in DAY 5 lactate concentration compared to DAY 1 at p = 0.000 level using the Mann-Whitney U-test.

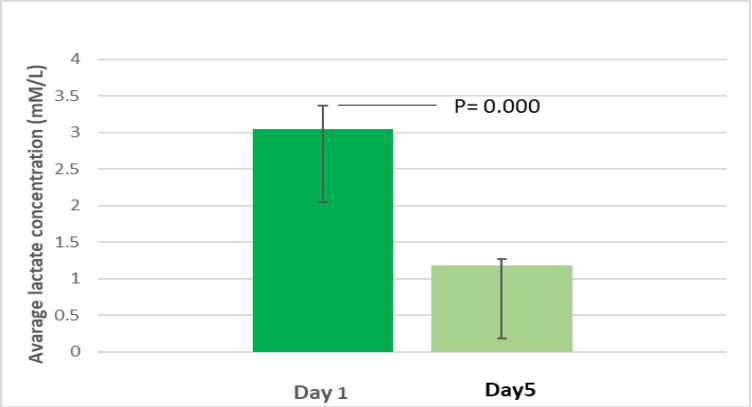

Figure 31: The average concentration of lactate for all samples, at Day 1 and Day 5 following traumatic injury.

CHAPTER 4

Discussion & Conclusion

The inflammatory response to major trauma is rapid and complex. The early systemic pro-inflammatory response is followed by a counter-balancing anti-inflammatory response in a bid to achieve homeostasis. The working hypothesis for this study is that an imbalance of the two arms, be it due to over-activity or suppression, could result in poor clinical outcomes and increased mortality. The data presented in this dissertation contributes to a larger study on this patient cohort, exploring cellular immune, cytokine and metabolic responses post-trauma ((NIHR Portfolio study BIT 19377). The final object of the study is to define early biomarkers (cellular, cytokine or metabolic) which could predict poor clinical outcomes post-trauma, thus identifying/stratifying this subset of 'high-risk' patients early, so as to enable focused and aggressive management regimes (e.g. Antibiotics, surgical stabilisation, biological response modification etc.) to prevent poor outcomes. The availability of such markers is currently very limited in clinical practice. The study has increased relevance to the United Kingdom. The TraumaAudit Research Network (TARN) report identified changing trends in the population admitted with major trauma in the UK (Kehoe et al., 2015). As opposed to patterns observed in the developing countries where younger populations and high speed RTAs were the main causal factors, a changing trend in the UK identified the dominant cohort as elderly people (> 65 years) with the leading mechanism of trauma attributed to falls from a height of>2m. With an expanding elderlypopulation, the economic burden on the NHS for treating major trauma in the UK is set to increasesignificantly. The existence of multiple co-morbidities in this cohort often mask clinical diagnosticparameters for sepsis, SIRS and MODS, and the availability of early predictive biomarkers will be a valuable resource.

This project aims to determine if the serum concentrations of IL-8 and TGF-β can be used as biomarkers in predicting poor patient outcomes in major trauma. The cytokine concentrations in a cohort of major trauma patients were defined on Day 1 and Day 5 post-trauma for correlative analysis against clinical outcomes, as measured through patient SOFA scores. This study intends to define trends for the expression for cytokines IL-8 and TGF-β in the days following the traumatic insult and to determine if these cytokines can be used as early biomarkers predicting late onset complications in major trauma patients. Clinical data collected for each patient was used to

calculate Sequential organ failure assessment scores (SOFA) on Days 1 and 5, to serve as a proxy for the onset of MODS. SOFA score cut-offs of ≥3 or ≥6 were used in the analyses as two varying thresholds of poor outcome. The high level of pro-inflammatory cytokines and declined level of anti-inflammatory cytokines can develop SIRS and this can lead to ARDS and MOF. (G Volpin,2014)

IL-8 is a chemokine that is involved in pro-inflammatory responses and is produced by macrophages, epithelial cells, airway smooth muscle cells (Hedges et al., 2000) and endothelial cells (Wollf et al., 1995). A cohort of 53 major trauma patients were recruited for this study from the Central Manchester Foundation Trust and Salford Royal Foundation Trust and patient clinical, biochemical data collated on Day 1 and Day 5 as stipulated in the methods section of this dissertation (Appendices 2,3,4,5). Serum samples collected on Days 1 and 5 were analysed for IL-8following validation of the processes as per manufacturer's instructions.

Comparison of average cytokine concentrations for the whole cohort on Days 1 and 5, showed increased levels in response to trauma and a decrease on progression to Day 5. This trauma-induced increased pro-inflammatory response in an early stage has been reported in a previous study (Schinkel et al., 2014). The early increase of IL-8 levels also corroborated published data for other pro-inflammatory cytokines in previous studies (Sears et al., 2009; Giannodis et al., 2004; Volpin et al., 2014). IL-8 concentration were derived as averages from triplicate flow cytometer reads. To assess the cytokine as a potential early biomarker for poor clinical outcome, IL- 8 concentrations on Day 1, were measured against SOFA scores on Day 5 at SOFA clustering thresholds of 3 and 6. No statistically relevant correlations were observed with either cut off.

IL-8 can be a neutrophil chemotactic factor as it stimulates neutrophils to migrate to the site of infection or damage and promotes phagocytosis. The binding between CXCI 8 and their ligand CXCR 1/2 receptors can stimulates the upregulation of integrin expression by neutrophils and this can promote the chemotaxis process (Dixit N et al,2012).Neutrophils play a major role in inflammation as they are the first leukocytes to be recruited to the damaged site.(Kola czkowska,2013). A significant increase of pro-inflammatory cytokines (IL-8, IL-6) and the regulatory cytokines TGF-β was reported in a previous study as an early immune response following trauma injury. This can stimulate the activation of neutrophils. (Volpin,2014)

The similar study design was applied to investigate TGF-β as a potential marker of poor clinical outcome following major trauma (n=38). TGF-β is a pleiotropic regulatory cytokine which is

involved in proliferation, differentiation, apoptosis and wound healing. TGF-β plays a key role in immune response and inflammatory activity. It can be a suppressor of T- cells and acts as an anti-inflammatory mediator to maintain homeostasis. TGF-β works effectively in controlling the innate immune cells, leading to this cytokine having been used as a therapeutic target and a treatment for different conditions such as autoimmune diseases, cancers and Alzheimer's disease.

The immune response of TGF-β is varied and depends on cellular and environmental conditions. It can stimulate Th17 differentiation that leads to producing a set of cytokines including IL-22 (Shomysehet al., 2009). Its increased expression in response to traumatic injury when compared to normal controls was reported by Volpin et al., 2014, with attribution to its pro-inflammatory influence and its role in fracture healing following injury.

To investigate if TGF-β can be used as a biomarker to predict patient outcomes following traumatic injury, a comparison between TGF-β concentration in Day 1 and Day 5 was done for the trauma patient cohort. Averaged TGF-β levels were increased as an early response to trauma (Day 1) and the levels subsequently declined significantly with progression to Day 5 ($p = 0.000$). The early upregulation of the cytokine corroborates with data published by Volpin et al., 2014. In order to evaluate its potential as a biomarker for poor clinical outcome, TGF-β concentrations on Day 1 was compared against SOFA scores on Day 5 at thresholds ≥ 3 and ≥ 6. Day 1 TGF-β comparisons based on patients clusters from D5 SOFA thresholds of < 3 and ≥ 3, showed a higher averaged TGF-β expression in the poor outcome cluster (SOFA ≥3). At the more stringent cut off for severity of outcome SOFA < 6 and SOFA ≥ 6 a similar pattern was observed (SOFA ≥ 6 was higher than SOFA < 6. While both cut offs failed to show statistical significancein the assessment of the cytokine as a predictive biomarker, the increase in significance of the p value in the higher severity clustering is noteworthy. A previous study was reported the increase of TGF-β following trauma can demodulate the immune response from pro-inflammatory to anti-inflammatory (Sagiv, JY,2015). The pleiotropic nature of TGF-β makes it harder to hypothesise whether a pro or anti-inflammatory mode of action is adopted in this response. However, the results warrant re-evaluation of TGF-β in a larger cohort.

A high level of lactate can be correlated to organ dysfunction (Frink et al. 2009) and associated with infectious complications, and patients needing prolonged ICU and hospital stays. The early management of this parameter can reduce the complications and mortality through early surgery and antibiotic therapy (Billeter et al., 2009). The lactate concentrations were analysed in this study (n=23) where the data was available.

The mean lactate concentrations for the trauma patient cohort showed a statistically significant decrease from D1 to D5 ($p = 0.000$), a result corroborating previous studies (Frink et al., 2009; Billeter et al., 2009). However, due to the smaller cohort numbers and an insufficient range in the SOFA profile, the correlation to outcome was not carried out.

In conclusion, this study has shown that both cytokines play an important role in inflammation after traumatic injury, with significant upregulation as an early response to traumatic injury. While higher levels of the cytokines were associated with patients clustered on the basis of higher SOFA scores and therefore poorer outcomes, the correlations were not statically significant. The high level of IL-8 can indicate the potential role in neutrophils activation and the pro-inflammatory function. Further study is needed on a larger size cohort for a significant result. The improved significance of the TGF-β data following clustering based on higher SOFA cut offs (more severe outcomes) may yield statistical significance of the cohort size in expanded and is therefore worthy of follow up. The pleiotropic nature of TGF-β makes it harder to hypothesise whether a pro or anti-inflammatory mode of action is adopted in its response. A further research is needed to study TGF- β mode by investigating the pattern of cytokine level along the acute period of trauma and during the recovery in combination with other immune modulatory factors.

CHAPTER 5

References

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Chapter 6 - Appendices

Appendix 1 - Patient recruitment consent form

Central Manchester University Hospitals NHS
NHS Foundation Trust

Investigator: Prof Kevin Mackway-Jones and Dr Richard Body

Patient Information Sheet

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish.
This sheet tells you the purpose of this study, what will happen to you if you take part and provides more detailed information about how the study will be carried out. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?
We are investigating a condition called major trauma. This is a process whereby a person becomes severely injured. It is known that, after a major injury, the body activates inflammatory mechanisms that are designed to promote healing. This inflammatory process and other mechanisms that reverse this process can become exaggerated after a significant injury. This can result in the person developing further illness after the initial injury.

We are investigating the levels of inflammation proteins called cytokines and cells in the immune system called T-regulatory cells. It is hoped that these markers in the blood can be used to predict whether someone will survive after a major trauma and be of use in targeting treatments in the future for patients.

Why have I been chosen?
You have been asked to take part in this study as you have suffered a major injury requiring hospitalisation. We are planning to study 200 patients in total, admitted to Manchester Royal Infirmary.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part in the study, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part at all, will not affect the standard of care you receive.

What will happen to me if I take part?
You will be asked to provide a 20ml blood sample on the day of your injury and on the third and fifth days afterwards. The blood samples will be sent to a laboratory to estimate the levels of inflammatory cytokines and T regulatory cells. We request that blood samples are treated as a gift and are able to be stored at the University of Salford after the completion of this study in order to perform further analysis at a later date.
All samples will be coded and not contain any personal identifying information. These samples will initially be stored at this hospital and then be sent to the University of Salford for storage and analysis. Samples will be stored beyond the end of this study in accordance with the Human Tissue Act.

What do I have to do?
You will not have to do anything different if you decide to take part. The medical and nursing staff will take the blood samples while in the emergency department and on the ward. We will continue to collect daily clinical information from your medical notes relating to your condition throughout your stay in hospital.
With your consent, we will share your name, postcode and date of birth with the Health and Social Care Information Centre. This will enable the Health and Social Care Information Centre and other central UK NHS bodies to provide us with information about your health status after hospital discharge for up to 6 months.
If you do not wish to be part of this study, no further information will be collected about you for the trial and the doctors will continue to provide you with whatever medical treatment is needed.

Will this affect the way I am treated in hospital?
No. Inclusion in the study will not change the care that you receive and the doctors and nurses caring for you will not be aware of the results of the tests in the study.

What are the possible benefits of taking part?
This study will improve our understanding of why some people survive major trauma and others do not and hopefully improve our care for people with major trauma in the future. However, this study will not have any direct benefits to your health.

What are the possible disadvantages and risks of taking part?
Blood samples will need to be collected. This will usually be done from existing lines, but it might be necessary to collect a sample from a new needle, which might result in some minor discomfort during collection and possibly a small bruise.

Will information from this study be kept confidential?
All information, including personal information, which is collected about you during the course of the research will be kept password protected and strictly confidential. Any information about you which leaves the hospital will have your name, hospital number and address removed and will be identified only by your Trial subject number, date of birth and initials, so that you cannot be recognised from it. This is with the exception of information obtained from The Health and Social Care Information Centre as described earlier. Only theresearchers and representatives of regulatory authorities and research ethics committees may have direct access to it. Other doctors in this hospital treating you will be told of your participation in this study.

What will happen to the results of the research study?
The results of this study will be presented at medical meetings and published in scientific journals. Only group information and no personal information will be presented. If you are interested in the results you will be able to contact the investigators for further information.

Who is organising and funding the research?
This study is being organised by doctors and scientists at Manchester Royal Infirmary andthe University of Salford. It is funded by the University of Salford.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people called a ResearchEthics Committee.

Who can I contact for independent research information?
If you have any questions about being in a research study, you can contact the Trust's Patient Advice Liaison Service (PALS). They will give you advice about who you can talk to forindependent advice.

Further information
Thank you for considering participation in this study. If you have any questions about thisresearch, the local study staff will be more than happy to answer them. Their contact details are:

Study Investigators Contact details:

Study Investigator	Prof Kevin Mackway-Jones and Dr Richard Body
Study Nurse	Richard Clark
Day time Telephone	0161 276 6777
Emergency Telephone	0161 276 4712

CONSENT FORM FOR PATIENTS ABLE TO GIVE CONSENT

Patient #		Site #	
Name of Research Doctor			

Please initial each box if you agree with the following:

☐ I *(forename and surname*) …… .....freely agree to take part in the study.

☐ I confirm that I have read and understood the patient information sheet dated January 2015 Version 1.0 for the above study and have been able to ask questions which have been answered fully.

☐ I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐ I understand my identity will never be disclosed and any information collected will remain confidential.

☐ I agree that my medical records and other personal data generated during the study may be examined by the research team and by representatives of Regulatory authorities, where it is relevant to my taking part in this research.

☐ I agree that I will not seek to restrict the use to which the results of the study may be put.

☐ I agree to gift my samples to a tissue bank for future scientific study.

☐ I understand that information held and managed by The Health and Social Care Information Centre and other central UK NHS bodies may be used in order to provide information about my health status. To do this, I understand that my name, postcode and date of birth will be shared with The Health and Social Care Information Centre.

Appendix 2 - Day 1 - clinical data for the patients recruited in Central Manchester Foundation Trust.

BIT	Day 1	Sys BP	Dia BP	MAP	Temp	Hb	WCC	PLT	eGFR	Creat	Bili	PT	Intubated	NIV / CPA	FiO2	P/F	Lactate	Norad	CRP
BIT001	115	110	60	76.66667	37.8	134	31.6	356	42	132	34	15.6	N	N	0.21	N	1.2	0	19
BIT002	125	122	84	96.66667	38	122	18.6	142	50	134	28	16.6	N	N	0.28	38.2	3.9	0	63
BIT003	94	70	45	53.33333	36	123	11.5	180	55	116	6	15.4	N	N	0.3	N	N	0	2
BIT004	129	101	61	74.33333	36.5	112	16.2	213	76	85	7	14.4	N	N	0.21	63.1	0.9	0	102
BIT005	82	135	85	101.6667	37.9	110	8.4	199	90	70	7	15.9	N	N	0.28	N	0.4	0	32
BIT006	84	111	71	84.33333	36.2	79	9.8	472	81	74	3	15.4	N	N	0.21	N	N	0	15
BIT007	110	126	66	86	37.5	140	19	298	90	76	12	14.7	N	N	0.35	48.3	0.9	0	60
BIT008	110	85	60	68.33333	37.7	99	5.9	152	65	69	13	20.8	Y	N	0.21	58	2	0	23
BIT009	90	102	55	70.66667	37.9	108	22	232	81	101	15	16.3	N	N	0.28	N	N	0	6
BIT010	116	99	35	56.33333	37.8	130	9.9	286	80	68	7	13.7	N	N	0.3	21.6	1.3	0	100
BIT011	90	103	71	81.66667	36.4	114	15.4	318	27	158	8	19.7	N	N	0.4	N	N	0	117
BIT014	112	102	56	71.33333	36.2	78	16.6	129	78	88		16.3	Y	N	0.4	29.2	3.4	0	8
BIT016	117	72	48	56	36.3	115	26.6	150	50	126	9	15.7	N	N	0.35	34.7	3.5	0	5
BIT017	127	94	63	73.33333	36.2	120	10.9	222	72	83	6	13.7	Y	N	0.6	71.9	1.7	0	6
BIT018	109	175	90	118.3333	35.4	146	16.8	260	90	49	6	11.3	N	N	1	N	0.7	0	89
BIT021	146	90	70	76.66667	35.2	109	33.5	235	50	146	10	14.2	Y	N	0.3	50	3.4	0	1
BIT022	97	117	75	89	35.6	128	27.1	408	90	79	5	10.8	N	N	0.28	N	N	0	3
BIT023	98	88	65	72.66667	35.7	112	22.1	156	83	91	18	12.3	N	N	0.32	43.6	1.1	0	17
BIT024	115	74	40	51.33333	38.2	83	16.4	155	90	56	8	11.2	Y	N	0.6	62.3	3.8	0	170
BIT025	122	85	45	58.33333	33.9	97	16.1	130	31	241	20	11.9	N	Y	0.35	9.34	6.3	0.07	345
BIT027	129	163	65	97.66667	38	100	28.8	203		111	61	10.7	Y	N	1	27.6	1.4	0	30
BIT028	130	89	50	63	35	87	16	191	43	166	4	11.2	N	N	0.4	N	N	0	38
BIT029	94	159	84	109	35.5	119	6.8	132	90	74	28	11.2	N	N	1	N	N	0	226
BIT034	75	88	55	66	37.8	129	9.7	235	26	229	10	11.4	N	N	0.6	24.2	1.4	0	N
BIT035	89	168	93	118	36.2	139	14	295	67	83	15	11.2	N	N	0.85	N	N	0	43
BIT040	110	109	88	95	36.1	131	33.2	226	80	99	11	11.4	N	N	0.6	61.6	1.5	0	10
BIT042	99	135	67	89.66667	38.4	118	19.5	213	N	93	5	11.9	N	N	21	N	N	0	1
BIT043	109	79	30	46.33333	37.7	117	19	194	81	101	9	12	N	N	35	33.4	3.3	0	
BIT044	126	135	81	99	35.9	136	14	237	59	128	5	11.4	N	N	21	N	2.2	0	1
BIT046	92	80	50	60	35.5	73	29.6	78	59	81	16	13.4	N	N	85	31.4	1.8	0	6
BIT047	160	77	56	63	35	147	28.7	248	67	114	12	12.4	Y	N	70	41.9	4.8	1.01	75
BIT048				0		145	27.1	287	81	91	12	12.1	y	n	N				71
BIT049	123	177	77		35.1	121	16.4	129	89	102	27	12	n	n	32	39.2	7.7	0	1
BIT050	97	123	59		35/6	149	8.7	359	64	116	10	11.5	y	n	28	0	2	0	20
BIT052	112	151	76		36.5	94	13.3	161	66	88	12	13	y	n	35	40.3	4.1	0	2
BIT053	85	103	52	69	34.6	117	8.1	154	62	77	7	11.5	n	n	85	30.6	1.7	0	32
BIT055	88	165	75	105	38.2	105	8.9	146	82	81	18	12.3	n	n	28	49.8	1.6	0	38
BIT060	130	70	40	50	35.7	80	13.9	101	45	146	25	12.7	Y	N	95	27.7	5.4	0.19	
BIT061	116	180	105	130	38.1	128	14.4	220	89	63	10.9	11	y	n	80	19.9	2	0	18
BIT064	95	148	93	111.3333	35.6	134	19.4	154	90	69	6	10.9	n	n	85	n	2.8	0	1
BIT065	100	106	61	76	35.6	123	29.1	338	90	47	19	11.2	N	N	24	44	0.7	0	41
BIT066	22?	162	85	110.6667	35	138	13.2	178	81	83	8	11.9	n	n	28	n	n	0	3
BIT067	82	146	81	102.6667	36.9	124	12.5	166	99	84	15	11.4	n	n	35	n		0	13
BIT068	109	109	55	73	35.8	114	21.4	230	90	88	4	12.1	y	n	50	61.2	1.3	0	1
BIT069	112	95	55	68.33333	35.5	104	19.7	257	79	104	15	11.9	y	n	85			0	4
BIT070	110	178	117	137.3333	35.8	143	26.9	339	89	88	11	11.8	y	n	N			0	n
BIT071	36.5	86	42	56.66667	35.5	88	16	199	90	61	3	11.7	y	n	30	n	0.6	0	1
BIT072	53	138	72	94	35.6	130	6.8	242	n	76	20	12.6	n	n	21	n	n	0	1
BIT073	53	138	72	94	35.6	129	16	210	56	87	32	11.7	n	n	70	9.79	2	0	116
BIT074	90	130	90	103.3333	36.2	110	11.3	238	90	65	13	11.4	n	n	21			0	29
BIT076	112	87	52	63.66667	35.7	105	17.3	381	46	93	<3	11.1	y	n	30	30.2	2.8	0.11	20
BIT030	71	70	48	55.33333	36.2	113	6	146	90	67	5	10	y	n	85	n	0.8	0	4
BIT078	88	102	51	68	35.7	118	16.4	174	83	92	11	11.2	y	y	85	32.4	2.6	0.11	9
BIT079	144	219	136	163.6667	38.3	155	16	240	70	102	25	12.2	y	n	35	n	7.6	0	272
BIT080	94	140		46.66667	35.1	132	5.4	253	59	97	5	12.3	y	n	85	n	n	n	<1
BIT084	78	108	59	75.33333	36.1	80	11	168	70	102	11	12.5	y	n	50	n/k	n/k	n	40
BIT085	140	196	129	151.3333	35	181	16.3	350	79	76	3	10.8	y	n	65	25	3	0	18
BIT087	50	140	87	104.6667	35.9	138	17.7	199	90	82	9	11.7	N	N	21	n/a	n/d	0	1
BIT088	125	94	55	68	36.9	135	38.5	409	76	111	30	10.9	N	N	85	n/a	2.8	0	12
BIT091	49	185	81	115.6667	35.2	81	9.1	235	30	152	10	10.9	y	N	40	39.5	1	0.09	8
BIT092				0		136	143	274	90	89	12								61
BIT093				0		84	21.2	259	38	165	18								
BIT094		60	25	36.66667	36.2	123	4.2	307	40	137		11.8							
BIT095	103	147	22	63.66667	36.6	119	28	200	28	230	7	11.6	N				0.7		
BIT096				0		146	20.7	141	90	74	13	10.6							
BIT098	107	100	66	0	36.7	152	11	301				10.8	N				2.9		
BIT099	95	130	90	103.3333		144	5	187	90	50			N						
BIT100				0		98	3.9	289	90	86		11.4	N	N			10.3		
BIT102	82	110	60	76.66667	37	165	12.8	233	46	136	11	11.2	Y	N	40		0.8		
BIT103	121	84	53	63.33333	33.1	85	3.2	142	61	120	3	10.4	Y	N	50	49	15		3
BIT104	90	100	50	66.66667	37.4	89	11.6	195	90	43	6	10.3	N	N	40		0.7		15
BIT105	118	93	52	65.66667	37	102	11.6	171	90	99			N	N	60		2		5
BIT107	99	77	44	55	38	113	4.8	134	65	101	30	11	Y	N	70	11.25	12.6	0.1	7
BIT109	100	94	54	67.33333	35.8	107	6.6	57	90	50	16	12.8	N	N	21		2		
BIT111	64	94	44	60.666	34.7	83	17.4	216	62	82	11	11.8	N	Y	34	28	4.3	NA	111
BIT113	133	160	108	125.3333	37.9	102	19.1	195	36	193	51	11.2	Y	N	40	31	3.5	NA	58
BIT115	129	164	94	117.3333	35.2	153	14.8	267	69	100	4	10.9	N	Y	34	23	3.6	n/a	1
BIT117	110	90	60	70	38.2	107	15.9	181	71	115	11	12.3	Y	N	50	19	5.3	0.23	63
BIT118	129	97	53	67.66667	37.6	105	13.3	17.1	85	58	7	12	N	N	24	56	2.3	NA	6
BIT119	124	146	73	97.33333	37.1	88	11.1	173	>90	63	12	11	N	N	60	38	3.1	NA	24
BIT120	98	158	98	118	37.5	126	12.7	199	>90	69	15	10.7	N	N	35	n/a	2.4	n/a	15
BIT121	54	109	75	86.33333	36.8	104	11.2	135	90	64	NA	10	N	N	50	NA	0.7	NA	32
BIT123	135	61	44	49.66667	37	113	22.9	137	>90	86	16	10.9	Y	N	70	17.9	1.9	3	7
BIT124	109	177	76	109.6667	35.5	110	13.3	211	69	70	7	9.8	N	N	35	31.4	1.1	N	37
BIT125	59	94	37	56	35.5	98	11.1	446	83	79	11	11	n	N	60	16	2.4	NA	N
BIT126	113	168	83	111.3333	38.1	125	23	147	74	68	16	10.8	N	N	60	n/a	2.7	n/a	80
BIT127	91	118	69	85.33333	36.8	101	10.4	197	77	90	NA	11	N	N	40	NA	NA	NA	28
BIT128	108	140	90	106.6667	35.4	121	22.1	198	61	108	6	n/a	N	Y	60	27	3.3	n/a	39
BIT129	59	93	88	89.66667	37.2	119	9.3	200	>90	58	15	12	N	N	32	34	2.9	0	37

BIT	CVVH/HD	Sedated	GCS	Antibiotic	Septic source	MAP SOFA	PLT SOFA	CREAT SOFA	BILI SOFA	RESP SOFA	GCS SOFA	SOFA DAY 1
BIT001	N	N	15	Y		0	0	1	2	0	0	3
BIT002	N	N	15	Y		0	1	1	1	2	0	5
BIT003	N	N	15	Y		1	0	1	0	0	0	2
BIT004	N	N	15	N		0	0	0	0	0	0	0
BIT005	N	N	15	N		0	0	0	0	0	0	0
BIT006	N	N	15			0	0	0	0	0	0	0
BIT007	N	N	15	Y		0	0	0	0	1	0	1
BIT008	N	Y	N	Y	empirical	1	0	0	0	0	0	1
BIT009	N	N	14	Y		0	0	0	0	0	1	1
BIT010	N	N	15	N		1	0	0	0	3	0	4
BIT011	N	N	15	N		0	0	1	0	0	0	1
BIT014	N	Y	N	Y	empirical	0	1	0	0	2	0	3
BIT016	N	N	15	Y		1	1	1	0	2	0	5
BIT017	N	Y	N	Y	empirical	0	0	0	0	0	0	0
BIT018	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT021	N	N	15	Y	empirical	0	0	1	0	1	0	2
BIT022	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT023	N	N	14	Y	empirical	0	0	0	0	1	1	2
BIT024	N	Y	N	Y		1	0	0	0	0	0	1
BIT025	N	N	15	Y	empirical	3	1	2	1	4	0	11
BIT027	N	Y	N	Y	empirical	0	0	1	2	2	0	5
BIT028	N	N	15	N		1	0	1	0	0	0	2
BIT029	N	N	15	Y	empirical	0	1	0	1	0	0	2
BIT035	N	N	15	N		0	0	0	0	0	0	0
BIT040	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT042	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT043	N	N	15	N		1	0	0	0	2	0	3
BIT044	N	N	15	Y	empirical	0	0	1	0	0	0	1
BIT046	N	N	15	Y	empirical	1	2	0	0	2	0	5
BIT047	N	Y	N	Y	empirical	4	0	1	0	1	0	6
BIT048	n	y		y	e	1	0	0	0	4	4	9
BIT049	n	n	15	y	empirical	1	1	0	1	2	0	5
BIT050	n	y		y	empirical	1	0	1	0	4	4	10
BIT052	n	y		y	empirical	1	0	0	0	1	4	6
BIT053	n	n	15	y	empirical	1	0	0	0	2	0	3
BIT055	n	n	15	y	empirical	0	1	0	0	1	0	2
BIT060	N	Y	N	Y	empirical	4	1	1	1	2	0	9
BIT061	n	y		y	empirical	0	0	0	0	3	4	7
BIT064	n	n	15	y	empirical	0	0	0	0	0	0	0
BIT065	N	N	13	N		0	0	0	0	1	1	2
BIT066	n	n	15	y	empirical	0	0	0	0	0	0	0
BIT067	n	n	15	y	empirical	0	0	0	0	0	0	0
BIT068	n	n		y	empirical	0	0	0	0	0	4	4
BIT069	n	y		y	empirical	1	0	0	0	4	4	9
BIT070	n	y		y	empirical	0	0	0	0	4	4	8
BIT071	n	y		y	empirical	1	0	0	0	0	4	5
BIT072	n	n	14	y	eye	0	0	0	1	0	1	2
BIT073		n	15	y	empirical		0	0	1	4	0	5
BIT074	n	n	15	y	empirical	0	0	0	0	4	0	4
BIT076	n	y	3	y	empirical	4	0	0	4	2	4	14
BIT030	n	y		y	e	1	1	0	0	0	4	6
BIT078	n	y	3	y	emp	4	0	0	0	2	4	10
BIT079	n	y	15	y	emp	0	0	0	1	0	0	1
BIT080	n	y	3	y	emp	4	0	0	0	0	4	8
BIT084	n	y	15	y	emp	4	0	0	0	0	0	4
BIT085	n	y	n/a	y	emp	0	0	0	0	3	0	3
BIT087	n	n	15	N		0	0	0	0	0	0	0
BIT088	n	n	15	Y	empirical	1	0	1	1	0	0	3
BIT091	n	y	n/a	Y	empirical	3	0	1	0	1	0	5
BIT092	N					1	0	0	0	4	4	9
BIT093					empirical	1	0	1	0	4	4	10
BIT094	N		15	Y	unknown	1	0	1	0	4	0	6
BIT095			15	Y	empirical	1	0	2	0	4	0	7
BIT096						1	1	0	0	4	4	10
BIT098			15			1	0	0	0	4	0	5
BIT099			15			0	0	0	0	4	0	4
BIT100				Y	Empirical	1	0	0	0	4	4	9
BIT102	N		10			0	0	1	0	4	2	7
BIT103	N				Empirical							
BIT104	N		15		Empirical	1	0	0	0	4	0	5
BIT107	N		3		Empirical	3	1	0	1	4	4	13
BIT109	N				Empirical	1	2	0	0	4	4	11
BIT111	N	N	15/15	N	NA	1	0	0	0	2	0	3
BIT113	Y	Y	14	Y	Empirical	0	0	0	2	2	1	5
BIT115	N	N	14	Y	Empirical	0	0	0	0	2	1	3
BIT117	N	Y	14/15	Y	Empirical	4	0	1	0	3	1	9
BIT118	N	N	15/15	N	NA	1	0	0	0	0	0	1
BIT119	N	N	15/15	Y	Empirical	0	0	0	0	2	0	2
BIT120	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT121	N	N	3	Y	Emiprical	0	1	0	NA	0	3	4
BIT123	n	Y	15	n		3	1	0	0	3	0	7
BIT124	N	N	15	Y	Emiprical	0	0	0	0	2	0	2
BIT125	N	N	15/15	N	n/a	1	0	0	0	2	0	3
BIT126	N	N	15/15	N	n/a	0	0	0	0	0	0	0
BIT127	N	N	15/15	N	n/a	0	0	0	0	0	0	0
BIT128	N	N	15/15	N	n/a	0	0	0	0	2	0	2
BIT129	N	N	15/15	Y	empirical	0	0	0	0	2	0	2

Appendix 3 - Day 5 - clinical data for the patients recruited in Central Manchester Foundation Trust

BIT	HR	Sys BP	Dia BP	MAP	Temp	Hb	WCC	PLT	eGFR	Creat	Bili	PT	Intubated	NIV / CPA	FiO2	P/F	Lactate	Norad	CRP
BIT001	94	111	78	89	37.2	102	10.1	356	90	84	N	N	N	N	0.21	N	N	0	18
BIT002	107	131	79	96.33333	36.6	91	7.5	191	90	65	18	16.9	N	N	0.28	45.2	0.8	0	244
BIT003	110	85	65	71.66667	36.6	83	6.3	211	86	78	13	14	N	N	0.21	N	N	0	113
BIT004	117	92	76	81.33333	37.3	102	12	221	90	45	17	15	N	N	0.21	53.6	0.5	0	329
BIT005	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N
BIT006	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N
BIT007	85	115	85	95	36.5	122	7.7	217	90	61	10	13.1	N	N	0.21	N	N	0	55
BIT008	108	102	62	75.33333	35.7	85	20.3	199	90	48	22	12.5	Y	N	0.28		1.3	0	210
BIT009	80	115	47	69.66667	37.1	83	7.6	233	90	91	15	14.7	N	N	0.21	N	N	0	95
BIT010	124	126	94	104.6667	36.2	114	6.7	270	90	38	7	13.6	N	N	0.4	N	N	0	181
BIT011	98	115	60	78.33333	36.9	N	N	N	N	N	N	N	N	N	0.32	N	N	0	
BIT014	103	130	64	86	35.9	91	10.6	357	90	78	N	N	N	N	N	N	N	0	194
BIT016	100	113	70	84.33333	36.4	83	10	125	58	111	11	14.1	N	N	0.4	29.8	1.4	0	174
BIT017	112	140	65	90	37.7	82	6.2	85	90	60	8	13.6	N	N	N	N	N	0	124
BIT018	113	176	96	122.6667	37.6	111	8.5	228	90	53	13	10.7	Y	N	0.4	25.8	1.2	0	92
BIT021	98	133	78	96.33333	36	109	9.7	339	90	70	9	10.4	N	N	0.21	N	N	0	26
BIT022	54	130	75	93.33333	36.1	N	N	N	N	N	N	N	N	N	N	N	N	0	
BIT023	80	111	58	75.66667	37.1	N	N	N	N	N	N	N	N	N	0.21	N	N	0	
BIT024	95	105	50	68.33333	36.7	82	9	198	90	52	6	9.9	Y	N	0.3	45.4	0.6	0	
BIT025	75	96	46	62.66667	34.7	92	14.9	166	83	79	16	11.1	N	N	0.6	22.1	1.4	0	
BIT027	66	116	80	92	36.4	118	11.9	350	90	75	N	N	N	N	0.21	N	N	0	33
BIT028	60	132	65	87.33333	37.7	96	9	346	90	70	11		N	N	0.21	N	N	0	105
BIT029	86	107	69	81.66667	35.4	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N
BIT034	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N
BIT035	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N
BIT040	117	134	82	99.33333	38.5	103	12.2	409	90	86	21	N	N	N	0.24	N	N	0	309
BIT042	106	127	73	91	37.4	101	8.6	239	N	64	8	11.3	N	N	0.21	N	N	0	150
BIT043	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N
BIT044	N	N	N		N	N	N	N	N	N	N	N	N	N	N	N	N	Norad	N
BIT046	110	86	37	53.33333	35.8	80	48.7	84	83	60	24	13.4	N	N	0.5	23.5	0.9	0	181
BIT047	112	139	65	89.66667	38.2	86	7.1	179	90	76	22	11.4	N	N	0.28	43.9	0.8	0	227
BIT048				0		74	12.7	245	19	321	12	11	y	n					350
BIT049	97	147	81		37.2	123	8.2	248	90	71	8	n	n	n	0.21	n	n	0	3
BIT050	98	115	74	87.66667	36.1	119	7.2	339	90	69	11	n	n	n	0.28	n	n	0	267
BIT052	98	132	87	102	35.9	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n
BIT053	105	155	72		37.5	102	6.2	207	90	48	n	11	n	n	0.32	n	n	0	123
BIT055	90	98	63	74.66667	37.5	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n
BIT060	125	105	50	68.33333	37.7	77	6.5	165	75	94	15	10.8	Y	N	0.6	11.3	1.2	0	
BIT061	97	140	55	83.33333	35.8	82	8.8	213	90	38	14	11.1	n	n	0.35	27.4	0.9	0	152
BIT065	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N
BIT066	87	145	72		35.7	129	9.9	261	90	62	13	n	n	n	0.21	n	n	0	53
BIT069	110	149	88	108.3333	37.1	125	8.4	242	90	67	7	11.1	n	n	0.28	63.7	1.1	0	n
BIT070	56	145	63	90.33333	36	123	10.6	349	78	98	n	n	n	n	0.21	n	n	0	n
BIT072	104	136	71	92.66667	35.7	n	n	n	n	n	n	n	n	n	n	n	n	0	n
BIT073	83	131	83		36.9	127	5.6	254	76	67	7	n	n	n	0.28	n	n	0	22
BIT074	89	137	77	97	37.8	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n
BIT076	121	112	70	84	37.8	89	7.8	282	>90	48	4	n/d	n	n	0.24	n	n	n	n
BIT030	74	91	52	65	35.9	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n
BIT078	105	141	70	93.66667	37.9	101	6.2	217	>90	80	n	n	n	n	0.21	n	n	n	127
BIT079	n/k	n/k	n/k		n/k	96	7.5	239	86	85	9	10.7	n	n	n/k	n	n/k	n	151
BIT080				0															
BIT082				0															
BIT084	n/k	n/k	n/k		n/k	78	20.7	404	>90	57	9	n/d	n	n	n/k	n/k	n/k	0	243
BIT085				0															
BIT088				0		85	15.5	293	90	86	19	10.5							
BIT091				0		79	10	262	17	128	7								
BIT092				0		100	11.6	233	90	71	7								
BIT093				0		94	12.6	313	88	79	26								103
BIT094				0		108	4.8	45	90	77									
BIT095	DISCHARGED			0															
BIT096				0															
BIT098	97	140	86	104	36.9	124	16.9	247	90	59	15	11.1	N	N					
BIT099				0		132	8.8	182	90	53	31		N	N					178
BIT100	105	88	64	72	37.3	84	1.6	155	84	98	8		N	N					
BIT102	91	100	70	80	36.6	126	11.1	185	67	98	15	10.9	Y	N	0.4		0.8		251
BIT103	109	115	63	80.33333	36.8	79	12.4	158	90	65	13	10.3	N	N	35		0.9		350
BIT104	80	102	56	71.33333	39.2	62	8.6	302	90	49	8	11	N	N	21				110
BIT107	113	94	50	64.66667	38	75	9.1	125	64	102	21	11	Y	N	60		1	0.4	372
BIT109	101	140	79	99.33333	36.4	73	3.4	88	90	35	31		N	Y	0.21		1.5		
BIT111				0		79	6.4	207	90	47	10		N	N					
BIT120	60	142	82	102	36.2	127	5.1	266	>90	65	12	10.5	N	N	0.35	n/a	n/a	n/a	n/a
BIT121	55	112	43	66	36.3	104	NA	185	90	60	N	9.4	N	N	0.21	N	NA	N	NA
BIT123	111	141	84	103	36.2	101	9.1	292	>90	54	16	NA	N	N	0.28	N	NA	NA	110
BIT124	87	171	70	104	35.7	122	7.2	190	90	47	10	9.9	N	N	0.35	N	NA	N	107
BIT125	66	91	34	53	36.8	84	10.9	256	87	76	14	10	N	N	21	27	1.7	N	N
BIT127	78	103	64	77	37.1	95	5.3	217	90	79	N	NA	N	N	28	N	NA	0	186
BIT128	136	137	72	93.66667	37.9	71	31.1	391	41	152	56	10	Y	N	80	16	1		289
BIT129	64	116	68	84	37.2	123	7.6	217	90	58	N	NA	N	N	0.21	N	N		NA

BIT	CVVH/HD	Sedated	GCS	Antibiotic	Septic sour	Steroids	CAM +ve	MAP SOFA	PLT SOFA	CREAT SOFA	BILI SOFA	RESP SOFA	GCS SOFA	SOFA DAY 5
BIT001	N	N	15	Y		N	N	0	0	0	0	0	0	0
BIT002	N	N	15	Y		N	N	0	0	0	0	1	0	1
BIT003	N	N	15	Y		N	N	0	0	0	0	0	0	0
BIT004	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT005	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT006	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT007	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT008	N	Y	N	Y	Abdomen	Y	N	0	0	0	1	4	0	5
BIT009	N	N	15	Y		N	N	1	0	0	0	0	0	1
BIT010	N	N	15	Y	Chest	N	N	0	0	0	0	0	0	0
BIT011	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT014	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT016	N	N	15	Y		N	N	0	1	1	0	2	0	4
BIT017	N	N	15	N		N	N	0	2	0	0	0	0	2
BIT018	N	Y	N	Y	empirical	N	N	0	0	0	0	3	0	3
BIT021	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT022	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT023	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT024	N	Y	N	Y		N	Y	1	0	0	0	1	0	2
BIT025	N	N	15	N		N	N	1	0	0	0	3	0	4
BIT027	N	N	15	Y	POST OP	N	N	0	0	0	0	0	0	0
BIT028	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT029	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT034	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT035	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT040	N	N	15	Y	empirical	N	N	0	0	0	1	0	0	1
BIT042	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT043	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT044	N	N	N	N	N	N	N	N	N	N	N	N	N	N
BIT046	N	N	13	N	empirical	N	N	1	2	0	1	3	1	8
BIT047	N	N	15	N		N	N	0	0	0	1	1	0	2
BIT048	n	y		y	empirical	n	n	1	0	3	0	4	4	12
BIT049	n	n	15	n		n	n	1	0	0	0	0	0	1
BIT050	n	n	15	n		n	n	0	0	0	0	0	0	0
BIT051								1	4	0	0	4	4	13
BIT052	n	n	15	n		n	n	0	0	0	0	0	0	0
BIT053	n	n	15	n		n	n	1	0	0	0	0	0	1
BIT054								1	4	0	0	4	4	13
BIT055	n	n	15	n		n	n	0	0	0	0	0	0	0
BIT056								1	4	0	0	4	4	13
BIT057								1	4	0	0	4	4	13
BIT058								1	4	0	0	4	4	13
BIT059								1	4	0	0	4	4	13
BIT060	N	Y	N	Y	empirical	Y	Y	1	0	0	0	4	0	5
BIT061	n	n	15	y	empirical	n	n	0	0	0	0	2	0	2
BIT065	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT066	n	n	n	n		n	n	1	0	0	0	0	0	1
BIT067								1	4	0	0	4	4	13
BIT068								1	4	0	0	4	4	13
BIT069	n	n	15	n		n	n	0	0	0	0	0	0	0
BIT070	n	n	15	y	empirical	n	n	0	0	0	0	0	0	0
BIT071								1	4	0	0	4	4	13
BIT072	n	n	15	y	eye	y	n	0	0	0	0	0	0	0
BIT073	n	y	15	y	empirical	y	n	1	0	0	0	0	0	1
BIT074	n	n	15	y	empirical	n	n	0	0	0	0	0	0	0
BIT076	n	n	15	y	emp	n	n	4	0	0	0	0	0	4
BIT030	n	n	15	n		n	n	1	0	0	0	0	0	1
BIT078	n	n	15	y	emp	n	n	4	0	0	0	0	0	4
BIT079	y	n	15	y	emp	n	n	4	0	0	0	0	0	4
BIT080								1	4	0	0	4	4	13
BIT082								1	4	0	0	4	4	13
BIT084	n	n	15	y	abdo	n	n		0	0	0	0	0	0
BIT085								1	4	0	0	4	4	13
BIT087								1	4	0	0	4	4	13
BIT088								1	0	0	0	4	4	9
BIT091								1	0	1	0	4	4	10
BIT092								1	0	0	0	4	4	9
BIT093				Y	spleen			1	0	0	1	4	4	10
BIT094	N		15	Y	CHEST			1	3	0	0	4	0	8
BIT095								1	4	0	0	4	4	13
BIT096								1	4	0	0	4	4	13
BIT098				Y	empirical			0	0	0	0	4	4	8
BIT099	N	N	15					0	1	0	1		0	6
BIT100								0		0	0			
BIT102	N	N		Y	empirical	N		0	0	0	0	1		8
BIT103	N	N						0	4	0	0	2		
BIT104	N	N	15					1	4	0	0	2	0	7
BIT107	N	Y	3					4	2		1	0	4	14
BIT109	N	N	15					0	3	0	1	2	0	6
BIT111	N	N	15					0	0	0	0		0	
BIT120	N	N	15	N	n/a	N	N	4	0	0	0	0	0	4
BIT121	N	N	15	Y	EMPIRICAL	N	N	0	0	0	0	0	0	0
BIT123	N	N	15	N	N	N	N	0	0	0	0	0	0	0
BIT124	N	N	15	Y	Wound	N	N	0	0	0	0	0	0	0
BIT125	N	N	15	N	N	N	N	1	0	0	0	2	0	3
BIT127	N	N	15	N	N	N	N	0	0	0	0	0	0	0
BIT128	N	Y	3	Y	empirical	N	N	0	0	1	2	3	4	10
BIT129	N	N	15	Y	Empirical	N	N	0	0	0	0	0	0	0

Appendix 4 - Day 1 - clinical data for the patients recruited in Salford Royal Foundation Trust

BIT	HR	Sys BP	Dia BP	Noradren	MAP	MAP SOFA	PLT	PLT SOFA	Creat(µmol/l)	Creat SOFA	Bili (µmol/l)	Bili SOFA	P/F	P/F (mm Hg)	RESP SOFA	GCS	GCS SOFA	SOFA DAY 1
SR001	115	110	60	0	76.66667	0	356	0	132	1	34	2	38.2	290.32	2	15	0	5
SR002	84	149	59	5	167	4	161	0	47	0	7	0		0	4	3	4	12
SR003	126	138	80	30	206	4	186	0	127	1	12	0	0.22	1.672	4	N	0	9
SR007	110	158	60	0	172	0	205	0	58	0	13	0	11.32	86.032	4	11	2	6
SR008	132	119	80	0	93	0	169	0	53	0	11	0	n	N	0	13	1	1
SR009	108	209	139	0	162	0	307	0	84	0	13	0	n	N	0	6	3	3
SR010	101	147	78	0	101	0	250	0	79	0	10	0	n	N	4	3	4	8
SR011	84	125	50	5	75	4	193	0	84	0	3	0	n	N	4	N	0	8
SR012	97	180	101	0	127	0	124	1	75	0	14	0	N	N	4	3	4	9
SR013	95	105	74	0	84	0	235	0	59	0	6	0		N	4	3	4	8
SR014	58	158	61		93	0	246	0	54	0	8	0	375.05	2850.38	0	N	0	0
SR015	70	120	80	0	93.3	0	175	0	69	0	7	0	N	N	0	15	0	0
SR018	92	104	97	0	99.3	0	169	0	76	0	7	0	n	N	0	15	0	0
SR019	101	126	59	12	81.3	4	251	0	85	0	9	0	N	N	4	3	4	12
SR020	76	132	66	5.5	88	4	237	0	75	0	22	1	N	N	4	3	4	13
SR021	69	110	60	6	76.7	4	87	2	49	0	23	1	N	N	0	14	1	8
SR022	101	121	65	0	83.7	0	121	1	56	0	15	0	N	N	4	N	0	5
SR023	53	110	52	6	71.3	4	165	0	42	0	5	0	N	N	4	3	4	12
SR024	103	110	50	0	70	0	246	0	57	0	7	0	32.9	250.04	4	3	4	8
SR025	50	111	41	0	64.3	1	308	0	53	0	12	0	21.3	161.88	3	N	0	4
SR026	101	102	71	0	81.3	0	348	0	130	1	8	0	172	1307.2	0	3	4	5
SR027	86	130	68	0	88.7	0	78	2	67	0	28	1	N	N	4	6	3	10
SR028	58	91	49	0	63	1	242	0	79	0	21	1	N	N	0	14	1	3
SR029	100	114	70	0	84.67	0	310	0	51	0	9	0	N	N	0	14	1	1
SR030	117	113	64	2	93.67	4	276	0	120	1	11	0	N	N	4	3	4	13
SR031	80	123	53	6	76.33	4	162	0	47	0	5	0	N	N	4	3	4	12
BSR032	95	115	60	0	78.33	0	208	0	62	0	21	1	N	N	0	15	0	1
SR033	90	136	62	0	86.67	0	185	0	102	0	12	0	N	N	0	15	0	0
SR034	104	121	73	4	89	4	109	1	148	1	15	0	N	N	4	3	4	14
SR035	60	97	48	0	64.33	1	198	0	68	0	13	0	N	N	0	14	1	2
SR036	60	136	72	7	93.33	4	202	0	61	0	5	0	N	N	4	3	4	12
SR037	54	118	95	0	102.67	0	212	0	66	0	12	0	N	N	4	3	4	8
SR039	63	114	58	0	76.67	0	82	2	97	0	16	0	N	N	0	15	0	2
SR041	126	110	80	0	90	0	207	0	82	0	21	1	N	N	4	N	0	5
SR044	106	60	98	2	75.33	4	116	1	97	0	28	1	0.5	3.8	4	15	0	10
SR045	66	98	50	0.1	65	3	193	0	192	2	10	0	N	N	4	3	4	13
SR046	102	161	69		99.7	0	248	0	71	0	12	0	2	15.2	4	15	0	4
SR047	95	150	72	N	94	4	214	0	81	0	19	0	0.23	1.748	4	15	0	8
SR051	85	123	55	0.03	78	3	270	0	67	0	14	0	0.56	4.256	4	ND	0	7
SR052	72	123	58	N	75	4	200	0	80	0	14	0	0.4	3.04	4	3	4	12
SR053	100	128	99	N	108.67	4	274	0	95	0	7	0	N	N	0	15	0	4
SR054	79	138	50	N	68	4	327	0	57	0	5	0	0.26	1.976	4	14	1	9
SR055	94	130	70	N	90	4	146	1	51	0	7	0	0.36	2.736	4	8	3	12
SR056	99	143	82	N	102	4	328	0	102	0	7	0	ND	N	0	15	0	4
SR057	58	165	88	N	115	4	177	0	45	0	11	0	0.51	3.876	4	15	0	8
SR058	94	135	82	N	99.67	4	236	0	77	0	13	0	ND	N	0	14	1	5
SR060	100	130	67	N	88	4	307	0	86	0	17	0	ND	N	0	15	0	4
SR061	73	120	39	0.26	64	4	211	0	57	0	12	0	ND	N	4	3	4	12
SR062	106	102	56	N	72	4	174	0	62	0	29	1	ND	N	4	6	3	12
SR063	70	105	58	N	73.67	4	156	0	56	0	6	0	0.22	1.672	4	7	3	11
SR064	55	120	60	0.03	80	3	196	0	68	0	16	0	0.25	1.9	4	15	0	7
SR066	86	122	58	0.33	78	4	129	1	70	0	12	0	0.33	2.508	4	3	4	13
SR069	46	130	58	10	76	4	171	0	89	0	7	0	0.42	3.192	4	3	4	12
SR070	76	126	74		91.33	0	213	0	57	0	14	0	0.51	3.876	4	13	1	5
SR071	44	135	67		89.67	0	160	0	61	0	19	0	0.22	1.672	4	3	4	8
SR073	66	166	77	0.18	107	4	185	0	67	0	12	0	0.68	5.168	4	14	1	9
SR074	95	116	60		78.67	0	205	0	56	0	4	0	ND	N	0	13	1	1
SR076	72	178	60	0.133	94	4	235	0	66	0	24	1	0.57	4.332	4	3	4	13
SR077	72	129	76		93	0	233	0	75	0	4	0	0.58	4.408	4	5	4	8
SR078	85	135	57		83	0	269	0	63	0	6	0	0.31	2.356	4	15	0	4
SR 079	94	140	70	0	93.33333	0	171	0	101	0	8	0	0.34	2.584	4	15	0	4
SR 080	72	150	90	0	110	0	220	0	72	0	23	1	0.4	3.04	4	13	1	6
SR 081	55	168	58	0.098	94.66667	3	229	0	62	0	10	0	0.405	3.078	4	3	4	11
SR 082	100	110	52	0.288	71.33333	4	267	0	101	0	18	0	0.446	3.3896	4	6	3	11
SR 083	114	107	54	0.062	71.66667	3	267	0	101	0	18	0	0.432	3.2832	4	3	4	11
SR 084	88	125	68	0	87	0	259	0	68	0	16	0	0.39	2.964	4	15	0	4
SR 085	90	182	94	0	123.3333	0	327	0	78	0	4	0	0.23	1.748	4	14	1	5
SR 086	84	105	55	No Weigh	71.66667	4	188	0	67	0	30	1	0.62	4.712	4	15	0	9
SR 087	75	120	77	0.17	91.33333	4	260	0	84	0	15	0	0.51	3.876	4	3	4	12
SR 088	69	130	60	0.369	83.33333	4	309	0	82	0	17	0	0.63	4.788	4	3	4	12
SR 089	75	126	65	0.122	85.33333	4	183	0	120	1	N	0	0.502	3.8152	4	6	3	12
SR 092	95	130	70	0	90	0	272	0	87	0	29	1	0.5	3.8	4	15	0	5
SR 093	95	125	70	0	88.33333	0	231	0	90	0	7	0	0.49	3.724	4	15	0	4
SR 094	58	130	55	0.09	80	3	199	0	65	0	24	1	0.555	4.218	4	3	4	12
SR 097	84	110	58	N	75.33333	4	277	0	72		7		N	N	0	15	0	4
SR 098	90	110	58	0	75.3333	0	253	0	95	0	6	0	0.6	4.56	4	7	3	7
SR 099	72	120	60	0	80	0	213	0	66	0	15	0	0.69	5.244	4	10	2	6
SR 100																		
SR 101	90	110	57	0.031	74.6667	3	291	0	66	0	20	1	0.581	4.4156	4	15	0	8
SR 102	137	115	50	0.524	71.667	4	349	0	59	0	4	0	0.447	3.3972	4	3	4	12
SR 103	74	118	65	0.4075	82.6667	4	214	0	66	0	6	0	0.4	3.0932	4	6	3	11

BIT	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	FiO2	Lactate	CRP	CVVH/HD	Sedated	Antibiotics	Septic source
SR001	37.8	134	31.6	42	15.6	N	N	0.21	1.2	19	N	N	Y	empirical/unk
SR002	38.7	108	10.9	N	11.7	Y	N	0.98	3.6	N	N	Y	N	N
SR003	39.1	136	28.5	62	12.1	Y	N	0.8	2.3	N	N	Y	N	N
SR007	36.7	132	7	>90	12.8	N	N	0.28	0.9	N	N	N	N	N
SR008	35	104	8.8	>90	11.9	N	N	0.21	1	N	N	N	N	N
SR009	37.1	97	14.4	60	9.9	N	N	1	4.3	N	N	N	N	N
SR010	37.5	134	18.3	>90	12.4	Y	N	0.25	7.1	N	N	Y	N	N
SR011	38.2	127.9	15.4	>90	10.7	Y	N	0.25	2.9	N	N	Y	Y	N
SR012	37	134	13.7	85	12.4	Y	N	0.21	1	N	N	Y	N	N
SR013	36.1	85	8.5	85	11.2	Y	N	0.4	1.8	N	N	Y	N	N
SR014	37.7	145	15.6	>90	14.1	Y	N	0.21	2.1	N	N	Y	Y	N
SR015	36.8	115	11.4	73	11.7	N	N	0.3	N	4.5	N	N	N	N
SR018	36.8	110	7	>90	13.3	N	N	0.0021	2	N	N	N	Y	5
SR019	37.6	89	15	87	13.1	Y	N	0.21	1.6	N	N	Y	Y	6
SR020	36.9	134	17.1	N	13.9	Y	N	0.21	2.2	N	N	Y	N	N
SR021	36.6	104	9.5	N	13.6	N	N	0.21	2	N	N	N	Y	6
SR022	38	104	10	>90	13.5	Y	N	0.21	1.8	N	N	Y	Y	6
SR023	36.7	130	15.2	>90	11.5	Y	N	0.3	1.5	N	N	Y	Y	1
SR024	36.7	135	10.2	>90	13.1	Y	N	0.4	1.7	N		Y	Y	6
SR025	33.1	95	6.7	>90	16.6	Y	N	0.4	2.7	N	N	Y	N	N
SR026	36.4	155	30.8	N	12.4	Y	N	0.21	1.7	N	N	Y	Y	
SR027	37.9	103	9.1	>90	13.7	Y	N	0.21	3.1	N	N	N	N	N
SR028	37.8	134	16.6	89	11.6	N	N	0.21	1.2	N	N	N	Y	6
SR029	37	127	16.5	>90	11.9	N	N	0.21	3.9	N	N	N	Y	6
SR030	36.7	124.1	14.6	53	11.7	Y	N	0.3	6.6	N	N	Y	N	N
SR031	37.9	125.8	9.6	N	12.7	Y	N	0.21	3.9	N	N	Y	N	N
SR032	36.4	118	6.1	>90	11.4	N	N	0.21	0.8	N	N	N	Y	6
SR033	37.5	106	11.4	73	11.6	N	N	0.21	1.7	N	N	N	Y	6
SR034	38.7	94	16.8	32	12.6	Y	N	0.3	4.8	N	N	Y	Y	6
SR035	38.6	135	16.3	>90	11.6		Y	0.4	1.3	N	N	N	Y	6
SR036	36.6	132.9	8.4	>90	12.8	Y	N	0.4	2.6	N	N	Y	Y	6
SR037	37	116	9.2	89	12.2	Y	N	0.25	0.25	N	N	Y	Y	1
SR039	37.4	117	11.5	64	14.2	N	N	0.4	2.6	N	N	N	N	N
SR041	39.2	130.1	15.9	81	15.4	Y	N	0.35	1.5	N	N	Y	Y	6
SR044	36.5	85	10	N	11.6	N	N	0.21	3	58	N	N	Y	6
SR045	37	121	10.4	24	14.4	Y	N	0.4	1.4	N	N	Y	Y	1
SR046	38.2	157	12.9	>90	11.1	N	N	0.5	3.4	N	N	N	N	N
SR047	37.5	134	15.2	87	11.6	N	N	0.4	1.6	N	N	N	N	N
SR051	37.2	121	11.6	79	12	Y	N	0.21	2.4	N	N	Y	N	N
SR052	38	118	8.9	>90	13.4	Y	N	0.21	0.9	ND	N	Y	Y	6
SR053	36.8	160	16.6	73	12	N	N	0.21	1.9	ND	N	Y	Y	6
SR054	37.7	113	10	ND	11.5	Y	N	0.21	4.6	ND	N	Y	N	N
SR055	36.4	135	14.3	>90	14.1	Y	N	0.3	1.1	ND	N	Y	N	N
SR056	37.2	145	12.9	ND	12.4	N	N	0.21	ND	ND	N	N	Y	6
SR057	36.3	150	12.7	ND	11.1	N	N	0.21	0.9	ND	N	N	Y	6
SR058	37.1	128	14.6	>90	ND	N	N	0.25	1.1	ND	N	N	Y	6
SR060	36	128	12.7	76	11.4	N	N	0.21	1.5	ND	N	N	Y	1
SR061	36.9	104	17.8	89	ND	Y	N	0.28	5	ND	N	Y	N	N
SR062	36	96	18.8	>90	10.8	Y	N	0.3	0.4	ND	N	Y	Y	6
SR063	37.2	124	14.8	>90	12.2	Y	N	0.25	2.77	ND	N	Y	N	N
SR064	38	146	8.1	>90	11.4	N	N	0.21	1.1	ND	N	N	N	N
SR066	36.7	82	19	>90	12.8	Y	N	0.35	3.8	ND	N	Y	Y	6
SR069	37.8	134	13.1	78	171	Y	N	0.3	4.07	ND	N	Y	Y	6
SR070	37	121	9.4	>90	12.7	N	N	0.21	1	ND	N	N	N	N
SR071	35.5	134	19.4	>90	12.4	Y	N	0.5	1.2	ND	N	Y	Y	N
SR073	36.6	104	14.4	>90	11.5	N	N	0.28	1.5	ND	N	N	N	N
SR074	35.9	113	7.7	>90	10.8	N	N	0.21	1.5	ND	N	N	Y	6
SR076	36.4	139	18.2	>90	13	Y	Y	0.21	0.9	ND	N	Y	Y	6
SR077	36.9	137	8.6	>90	10.2	Y	N	0.25	1.32	ND	N	N	N	N
SR078	37.8	101	6.5	82	12.4	N	N	0.35	0.8	ND	N	N	Y	1
SR 079	36.9	99	10.9	46	10.9	N	N	0.21	1.7	N	N	N	Y	6
SR 080	36.3	138	20.4	90	13.4	N	N	0.25	N	N	N	N	N	N
SR 081	37	138	16	>90	12	Y	N	0.3	N	32	N	Y	N	N
SR 082	36.8	136	20	N	12.3	Y	N	0.5	4.1	N	N	Y	Y	6
SR 083	38.3	136	20	N	12.3	Y	N	0.25	2.8	N	N	Y	N	N
SR 084	37.8	149	13.7	>90	12.4	N	N	0.24	0.83	N	N	N	N	N
SR 085	38.6	139	11.6	61	30	N	N	0.3	1.9	36	N	N	N	N
SR 086	35.9	121	20.7	N	17	N	N	0.21	3.9	N	N	N	Y	6
SR 087	39.2	141	25.5	87	12.7	Y	N	0.21	6	N	N	Y	N	N
SR 088	36.4	115	16.4	N	20	Y	N	0.21	5.6	N	N	Y	Y	1
SR 089	37.1	123	11	54	12	Y	N	0.5	5.7	N	N	Y	Y	5
SR 092	37.3	114	13.5	77	11.7	N	N	0.21	1	N	N	N	N	N
SR 093	36.3	120	13.4	58	13	N	N	0.21	2.8	N	N	N	Y	5
SR 094	35.8	135	16.1	N	14.3	N	N	0.4	1.8	N	N	Y	N	N
SR 097	37.3	111	7.2	>90	11.2	N	N	0.21	N	34	N	N	Y	6
SR 098	37.3	137	11.3	72	13.9	Y	N	0.3	2.18	N	N	Y	Y	6
SR 099	37.2	134	16	>90	12.6	Y	N	0.25	2.7	N	N	Y	N	N
SR 101	37	128	11.5	>90	13	N	N	0.21	1	N	N	N	N	N
SR102	38.6	128	23.4	>90	12.4	Y	N	0.3	3.9	N	N	Y	N	N
SR103	36.6	138	8.3	>90	11.1	Y	N	0.4	1.6	N	N	Y	N	N

Appendix 5 - Day 5 - clinical data for the patients recruited in Salford Royal Foundation Trust

	Hb	WCC	eGFR	PT	Intubated	IV / CPA	FiO2	Lactate	CRP	CVVH HD	Sedated	Antibiotics	Septic source	Steroids	CAM +ve	HR	Sys BP	Dia BP	Norad	MAP	MAP SOFA	PLT	PLT SOFA	Creat	Creat SOFA	Bili	Bili SOFA	P/F	P/F	ESP SOFA	GCS	GCS SOFA	SOFA DAY 5
SR001	77	18.1	90	16.7	N	N	0.21	2	145	N	N	Y	Clinical Junior	N	N	94	111	78	0	89	0	356	0	84	0	N	0	40	304	1	15	0	1
SR002	116	11.3	>90	N	Y	N	30%	0.7	N	N	Y	N	N	N	N	100	169	81	N		4	195	0	57	0	9	0	N	N	4	5	4	12
SR003	87	14.2	85	14.5	Y	N	30%	0.9	N	N	Y	N	N	N	N	134	172	82	9	112	4	176	0	85	0	10	0	0.34	2.584	4	N	0	8
SR004																																	
SR005																																	
SR006																																	
SR007	115	6.1	>90		13.6	N	N	N	N	N	N	N	N	N	N	67	130	72	0	187	0	309	0	60	0	15	0	N	N	0	14	1	1
SR008	93	10.6	>90		13.2	N		21	1.1	N	N	N	Y		1	N	74	112	50	0	70	0	207	0	207	2	N	0	N	N	0	15	0	2
SR009	74	16.9	28	9.9	Y	N		21	3.3	N	N	N	N	N	Y	N	123	162	52	0	88	0	188	0	110	1		3	0	N	N	4	N	0	5
SR010	126	20	>90		12.9	Y	N	45	1.9	N	N	Y	N	N	N	N	96	150	70	10	96	4	194	0	59	0	5	0	N	N	4	N	0	8
SR011	120	8.8	>90		12.2	Y	N	21	N	N	N	Y	Y	N	N	N	85	125	61	0	82	0	174	0	46	0	10	0	N	N	0	15	0	0
SR012	88	14.5	>90		14.4	N	N	21	0.7	N	N	N	N	N	N	N	122	137	58	N	84	4	192	0	76	0	6	0	N	N	0	13	1	5
SR013																RIP																	RIP
SR014	128	11.7	N		12.8	Y	N	30	0.9	N	N	Y	N	N	N	N	83	150	50	7	83.3	4	328	0	51	0	7	0	295.03	224	2.228	0	N	0	4
SR015	131	6.4	>90		12.3	N	N	21	N	N	N	N	N	N	N	N	68	120	80	0	93.3	0	163	0	58	0	9	0	N	N	0	15	0	0
SR016																																	
SR017																																	
SR018	95	8.1	N		15.1	N	N	21	2	184	N	N	Y		5	N	N	71	124	64	0	84	0	N	0	N	0	N	0	N	N	0	15	0	0
SR019	73.9	9.9	>90		11.6	Y	N	21	0.7	N	N	Y	Y		6	N	90	171	64	0	99.7	0	225	0	53	0	10	0	N	N	4	3	4	8
SR020	11.1	12	N		14.5	Y	N	21	0.6	N	N	Y	Y		6	N	N	76	152	59	4	90	4	228	0	48	0	6	0	N	N	4	N	0	8
SR021	75	12.3	N		10.8	N	N	21	0.9	N	N	N	Y		6	Y	N	67	111	43	0	65.7	1	104	1	35	0	19	0	N	N	0	14	1	3
SR022	102	10.3	N	N	N	N	N	21	0.7	N	N	N	Y		6	N	N	90	188	80	0	116	0	N	0	N	0	N	0	N	N	0	14	1	1
SR023	105	9.3	N		12	N	N	21	1.2	N	N	N	Y		1	N	N	86	119	60	9	88	4	224	0	44	0	15	0	59	448.4	0	6	3	7
SR024	76.8	11	>90		13.7	Y	N	24	1	N	N	N	Y		6	N	N	59	140	50	0	80	0	229	0	72	0	17	0	N	N	0	10	2	2
SR025	82	6.2	N		15.4	N	N	80	0.8	N	N	N	N	N	N	N	72	172	66	0	101.3	0	207	0	42	0	10	0	N	N	0	9	3	3
SR026	100	14.2	>90		12.4	N	N	25	0.7	N	N	N	Y		6	N	N	66	160	90	0	113.33	0	N	0	N	0	N	0	N	N	0	15	0	0
SR027	83	10.2	N		11.2	Y	N	21	5.2	N	N	N	N	N	N	N	88	142	70	0	94	0	222	0	53	0	6	0	N	N	4	5	4	8
SR028	89	9.1	>90		11.4	N	N	60	1.4	N	N	N	N	N	Y	N	65	117	63	0	80.33	0	208	0	43	0	14	0	N	N	0	13	1	1
SR029	103.3	9.7	>90		12.2	N	N	21	1.1	N	N	N	Y		6	Y	N	72	112	84	0	93.33	0	142	1	50	0	6	0	N	N	0	15	0	1
SR030	81	12.4	N		12.4	Y	N	30	1.3	240	N	Y	Y		1	N	N	93	148	50	0	82.67	0	282	0	58	0	13	0	35.1	266.76	2	3	4	6
SR031	108	9	N		12.8	N	N	21	0.7	N	N	N	N	N	N	N	61	150	80	0	103.33	0	N	0	N	0	N	0	N	N	0	14	1	1
SR032	116	8.2	>90	N	N	N	N	21	N	N	N	N	Y		6	N	N	82	126	73	0.5	90.67	4	211	0	55	0	8	0	N	N	0	15	0	4
SR033	92	8.1	>90		11.6	N	N	25	N	12	N	N	Y		1	N	N	88	142	69	0	93.33	0	261	0	74	0	13	0	N	N	0	15	0	0
SR034	76	14.5	48	10.8	Y	N	25	1.1	N	Y	Y	Y	Y		6	N	N	75	140	70	0	93.33	0	151	0	205	2	35	2	N	N	4	10	2	10
SR035	116	6.9	>90	N	N	N	N	21	N	N	N	N	Y		6	N	N	74	112	62	0	78.67	0	N	0	N	0	N	0	N	N	0	15	0	0
SR036	121	15.1	N		13	Y	N	21	1.4	N	N	Y	Y		6	N	N	62	162	54	5.5	90	4	175	0	43	0	7	0	N	N	4	3	4	12
SR037	103	8.5	>90		12.6	Y	N	35	1.9	N	N	Y	Y		1	N	N	82	138	62	1	86	4	200	0	61	0	6	0	N	N	4	3	4	12
SR038																																	
SR039	95	7	>90	N	N	N	N	21	N	174	N	N	Y		1	N	N	64	118	72	0	87.33	0	175	0	40	0	33	2	N	N	0	15	0	2
SR040																																	
SR041	90.2	22.5	34	11.4	Y	N		25	1.9	N	N	Y	Y		6	N	N	68	193	66	12	108.33	4	397	0	60	0	8	0	344	261.44	0	11	2	6
SR042																																	
SR043																																	
SR044	72	8	88	10.7	N	N		21	1.3	N	N	N	Y		6	Y	N	97	172	56	0	94.67	0	173	0	44	0	19	0	N	N	0	15	0	0
SR045	N	N	N	N	N	N	N	40	N	N	N	N	Y		1	N	N	78	120	76	0	91	0	490	0	67	0	12	0	N	N	0	15	0	0
SR046	N	N	N	N	N	N	21%	N	ND	N	N	N	N	N	N	N	103	135	82	N	100	4	N	0	N	0	N	0	N	N	0	15	0	4
SR047	104	8.5	56	12.5	Y	N	80%	1.4	ND	N	Y	Y			1	N	N	81	110	51	0.05	70	3	298	0	74	0	11	0	N	N	4	3	4	11
SR048																																	
SR049																																	
SR050																																	
SR051	104	9.9	ND		12.4	N	N	21	ND	ND	N	N	N	N	N	N	68	122	78	ND	92.67	4	ND	0	ND	4	ND	4	ND	N	0	15	0	12
SR052	101	16.2	>90		11.5	N	N	21	N	ND	N	N	Y		6	Y	N	75	120	78	N	92	4	268	0	50	0	11	0	ND	N	0	15	0	4
SR053	89	6.7	>90		14.3	N	N	35	1.3	ND	N	N	Y		6	Y	N	81	122	76	N	91.33	4	274	0	64	0	12	0	ND	N	0	15	0	4
SR054	86	11.8	ND		12.5	Y	N	21	1.1	ND	N	Y	N	N	N	N	74	124	44	0.06	74	3	232	0	30	0	7	0	0.58	4.408	4	3	4	11
SR055	110	11.7	ND		16.9	N	N	25	1	ND	N	N	N	N	N	N	85	111	54	ND	73	4	176	0	140	1	8	0	ND	N	0	14	1	6
SR056	ND	ND	ND	ND	N	N	N	21	N	ND	N	N	N	N	N	N	98	120	70	ND	86.67	4	225	0	119	1	9	0	ND	N	0	15	0	5
SR057	ND	ND	ND	ND	N	N	N	21	N	ND	N	N	N	N	Y	N	84	132	82	N	50	4	ND	0	ND	4	ND	4	ND	N	0	15	0	12
SR058	95	6.9	ND		11.6	N	N	25	0.89	ND	N	N	N	N	Y	N	100	146	84	N	104.67	4	316	0	48	0	ND	4	ND	N	0	15	0	8
SR059																																	
SR060	10.6	8.4	>90		11.2	N	N	21	1.1	ND	N	N	N	N	N	N	67	104	58	N	73.33	4	ND	0	85	0	4	0	ND	N	0	15	0	4
SR061	83	13.9	ND		13.3	Y	N	25	1.44	ND	N	Y	N	N	N	N	82	132	45	0.07	80	3	237	0	44	0	9	0	0.17	1.292	4	3	4	11
SR062	ND	ND	ND	ND	N	N	N	21	N	N	N	N	Y		6	N	N	70	120	70	ND	86.67	4	385	0	41	0	10	0	ND	N	0	14	1	5
SR063	83.1	8.9	ND		12.2	N	N	21	0.8	N	N	N	N	N	N	N						1		4		0		0				4		13
SR064																						1		4		0		0				4		13
SR066	87	12.7	ND		12.9	Y	N	30	1.1	ND	N	Y	Y		6	N	N	136	138	70	ND	94	4	247	0	39	0	12	0	0.65		4	3	4	12
SR067	99	8.3	ND		11	N	Y	40	0.8	ND	N	N	Y		6	N	N	88	171	69	ND	108	4	188	0	51	0	15	0	ND		4	15	0	8
SR069	130	9.1	>90		14	N	N	21	1	ND	N	N	N	N	N	N	62	122	70	ND	87	4	ND	0	ND	4	ND	4	ND		4	15	0	16
SR070	ND	ND	ND	ND	N	N	N	21	N	ND	N	N	N	N	N	N	52	120	50	ND	73.33	4	ND	0	ND	4	ND	4	ND		4	14	1	17
SR071	109	19.5	>90		14.5	N	N	30	0.9	ND	N	N	Y		6	N	N	75	136	64	ND	88	4	ND	0	49	0	16	0	ND		4	15	0	8
SR072																																	
SR073	83	7.5	>90		12	N	N	60	1.2	ND	N	N	N	N	Y	N	76	113	46	ND	54	4	235	0	53	0	15	0	ND		4	14	1	9
SR074	ND	ND	ND	ND	N	N	N	21	ND	ND	N	N	Y		6	N	N	95	130	90	ND	103	4	ND	0	ND	4	ND	4	ND		4	14	1	17
SR075																																	
SR076	116	7.7	>90		14.3	Y	Y	21	1.09	ND	N	Y	N	N	N	N	68	132	80	ND	98	4	ND	0	ND	4	ND	4	ND		4	14	1	17
SR077	121	7.8	>90		11.3	N	N	21	ND	ND	N	N	N	N	N	N	DISCHARGED					1		4		0		0				4		13
SR078	95	7.7	82		12	N	N	50	1	ND	N	N	N	N	N	N	120	115	64	0.233	81	4	311	0	58	0	6	0	0.3514		4	3	4	12

	HR	Sys BP	Dia BP	Norad	MAP	MAP SOFA	PLT	PLT SOFA	Creat	Creat SOFA	Bili	Bili SOFA	P/F	P/F	RESP SOFA	GCS	GCS SOFA	SOFA DAY 5
SR079	102	131	43	0	72.33333	0	320	0	45	0	15	0	N	#VALUE!	#VALUE!	15	0	#VALUE!
SR080	85	149	70	0	96.33333	0	344	0	58	0	20	1	N	#VALUE!	#VALUE!	14	1	#VALUE!
SR081	98	185	70	0.368	108.3333	4	200	0	60	0	10	0	0.34	2.584	4	3	4	12
BISR082	95	145	75	0	98.33333	0	547	0	46	0	25	1	0.42	3.192	4	10	2	7
SR083	95	121	60	0.021	80.33333	3	247	0	45	0	12	0	0.44	3.344	4	3	4	11
SR084	75	135	55	0	81.66667	0	263	0	53	0	16	0	N	#VALUE!	#VALUE!	15	0	#VALUE!
SR085	98	130	62	0	84.66667	0	305	0	93	0	15	0	N	#VALUE!	#VALUE!	15	0	#VALUE!
SR086	N	N	N	0	#VALUE!	0	N	0	N	0	N	0	N	N	N	N	N	#VALUE!
SR087	112	122	57	0	78.66667	0	302	0	45	0	16	0	0.34	2.584	4	3	4	8
SR088	100	136	63	0.14	87.33333	4	346	0	52	0	8	0	0.6	4.56	4	5	4	12
SR089	81	155	55	0	88.33333	0	194	0	52	0	56	2	0.353	2.6828	4	15	0	6
SR092	67	124	68	0	86.66667	0	359	0	82	0	11	0	N	#VALUE!	#VALUE!	15	0	#VALUE!
SR093	78	126	68	0	87.33333	0	N	0	N	0	N	0	N	#VALUE!	#VALUE!	15	0	#VALUE!
SR094	108	125	68	0.48	87	4	170	0	50	0	8	0	0.503	3.8228	4	3	4	12
SR096	90	124	60	0	81.33333	0	231	0	46	0	N	0	N	#VALUE!	#VALUE!	15	0	#VALUE!
SR097	86	120	70	0	86.66667	0	351	0	68	0	7	0	N	#VALUE!	#VALUE!	15	0	#VALUE!
SR098	92	132	70	0	90.66667	0	253	0	85	0	N	0	N	#VALUE!	#VALUE!	15	0	#VALUE!
SR099	77	138	80	0	99.33333	0	N	0	N	0	N	0	N	#VALUE!	#VALUE!	14	1	#VALUE!
SR101	91	132	80	0	97.33333	0	351	0	61	0	15	0	N	#VALUE!	#VALUE!	15	0	#VALUE!
SR102	75	140	68	0	92	0	325	0	48	0	6	0	0.54	4.104	4	4	4	8
SR103	77	180	100	0	126.6667	0	263	0	59	0	7	0	N	#VALUE!	#VALUE!	11	2	#VALUE!

Appendix 6 - Interleukin-8 concentration and SOFA scores

Patient BIT	IL-8 con pg/ml DAY 1	IL-8 con pg/ml DAY 5	SOFA 1	SOFA 5
SR044	27.78988201	19.56707781	10	0
SR028	12.69596745	13.45629523	3	1
SR039	37.77270423	18.70818901	2	2
SR 062	22.9744727	12.6818873	12	5
SR089	94.30448312	23.39687702	12	6
SR055	35.98452592	28.97261412	12	6
SR023	24.59368928	8.697206499	12	7
SR082	37.22357861	18.79266988	11	7
SR 067	10.62618625	16.3145645	9	8
SR 058	15.49791614	15.01919124	5	8
SR071	12.63964687	7.880558137	8	8
SR087	35.73108333	18.14498324	12	8
SR102	18.46882656	16.17376306	12	8
SR 061	20.28516516	23.5799189	12	11
SR 054	16.20192335	13.96318042	9	11
SR083	11.69627721	6.092379826	11	11
SR047	22.69286981	21.67909943	8	11
SR051	17.08897243	14.25886345	7	12
SR 057	11.96379995	14.89246994	8	12
SR078	9.485694574	13.69565768	4	12
SR088	29.57806032	9.415293853	12	12
SR094	6.54294444	10.54170539	12	12
SR066	21.08773338	10.55578553	13	12
SR036	20.86245107	22.66470953	12	12
SR 063	16.07520205	6.909028188	11	13
SR064	6.303581989	5.993818817	7	13
SR069	48.57217482	6.261341556	12	16
SR070	5.613654924	7.373672946	5	17

SR074	5.205330743	5.275731463	1	17
MRI010	34.02738588	18.32802512	4	0
MRI011	21.98886261	12.02012053	1	0
MRI014	20.65124891	20.28516516	3	0
MRI021	19.73603954	7.641195686	2	0
MRI084	9.401213708	12.24540283	4	0
MRI099	9.527935006	9.175931402	4	6
MRI009	12.62556673	7.852397849	1	1
MRI017	17.45505618	8.373363183	0	2
MRI 125	21.53829799	11.31611332	3	3
MRI 120	17.44097604	18.93347132	0	4
MRI016	8.331122751	36.06900679	5	4
MRI078	8.42968376	5.867097519	10	4
MRI 109	36.84341472	40.13816845	11	6
MRI104	10.69658697	17.01857171	5	4
MRI098	26.39594773	25.31177663	4	8
MRI102	16.70880854	22.67878967	7	8
MRI100	17.5254569	35.09747684	9	9
MRI088	41.03929768	25.15689505	11	9
MRI092	23.63623948	12.23132269	9	9
MRI091	17.62401791	32.36592887	10	10
MRI093	20.65124891	27.04363437	10	10
MRI107	18.68002872	5.810776942	13	14
MRI 064	20.93285179	10.5698658	0	13
MRI 127	42.68667455	6.134620259	0	0

Appendix 7 – TGF-β concentration and SOFA scores

Patient BIT	TGF- b con pg/ml DAY 1	TGF-beta ng/ml DAY 1	TGF-b con pg/ml DAY 5	TGF- b conc ng/ml	SOFA 1	SOFA 5
SR044	4730.803	4.730803	3745.619	3.745619	10	0
SR028	11366.31	11.36631	3325.467	3.325467	3	1
SR039	6635.975	6.635975	6201.335	6.201335	2	2
SR 062	3774.595	3.774595	3042.951	3.042951	12	5
SR089	5208.907	5.208907	2840.119	2.840119	12	6
SR041	2593.823	2.593823	2709.727	2.709727	5	6
SR055	4230.967	4.230967	4173.015	4.173015	12	6
SR023	6049.211	6.049211	3904.987	3.904987	12	7
SR 067	4332.383	4.332383	2869.095	2.869095	9	8
SR 058	3260.271	3.260271	2122.963	2.122963	5	8
SR071	3361.687	3.361687	3057.439	3.057439	8	8
SR087	7896.431	7.896431	3376.175	3.376175	12	8
SR102	10294.2	10.2942	3912.231	3.912231	12	8
SR 061	4267.187	4.267187	3021.219	3.021219	12	11
SR 054	7903.675	7.903675	5744.963	5.744963	9	11
SR083	7374.863	7.374863	3723.887	3.723887	11	11
SR047	5940.551	5.940551	4028.135	4.028135	8	11
SR051	3818.059	3.818059	3274.759	3.274759	7	12
SR 057	11091.04	11.09104	5976.771	5.976771	8	12
SR078	4564.191	4.564191	3195.075	3.195075	4	12
SR088	4955.367	4.955367	2956.023	2.956023	12	12
SR094	4593.167	4.593167	2861.851	2.861851	12	12
SR066	5143.711	5.143711	4868.439	4.868439	13	12
SR036	8222.411	8.222411	2984.999	2.984999	12	12
SR 063	4933.635	4.933635	3347.199	3.347199	11	13
SR069	7903.675	7.903675	2912.559	2.912559	12	16

SR070	5187.175	5.187175	5636.303	5.636303	5	17
SRH045	3593.495	3.593495	4462.775	4.462775	13	0
SRH046	10511.52	10.51152	9381.451	9.381451	4	4
SRH052	10612.93	10.61293	8758.467	8.758467	12	4
SRH024	6143.388	6.143388	3687.667	3.687667	8	2
SRH030	4890.171	4.890171	3368.931	3.368931	13	6
MRI 125	9480.491	9.480491	7092.347	7.092347	3	3
MRI 120	10563.73	10.56373	8316.583	8.316583	0	4
MRI 128	10573.76	10.57376	9707.431	9.707431	2	10
MRI 127	12845.56	12.84556	7592.183	7.592183	0	0
MRI 123	4527.971	4.527971	3912.231	3.912231	7	0
MRI079	5245.127	5.245127	4013.647	4.013647	1	4

Appendix 8 – Lactate con centration in m M/L in Day 1 and Day 5

BIT	Lactate conc D1	Lactate conc D5
SR044	3	1.2
SR028	1.2	0.9
SR089	5.7	1.36
SR023	1.5	1.1
SR082	4.1	1.1
SR087	6	1.9
SR102	3.9	1.1
SR 061	5	1.2
SR 054	4.6	2
SR083	2.8	0.9
SR047	1.6	1.4
SR078	0.8	1.5
SR088	5.6	0.4
SR094	1.8	0.8
SR066	3.8	1.4
SR036	2.6	0.8
MRI 125	2.4	1.7
MRI016	3.5	1.4
MRI 109	2	1.5
MRI102	0.8	0.8
MRI088	2.8	0.8
MRI107	1.2	1
MRI128	3.3	1