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MAJOR TRAUMATIC INJURY: SERUM CYTOKINE BIOMARKERS AS PREDICTORS OF POOR CLINICAL OUTCOME

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Dedication

This thesis is dedicated to the NHS, researchers and frontline health care workers who fought for us against COVID-19 pandemic.

Acknowledgements

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I undertook this project in the full knowledge that God's touching grace would see me through challenge and hurdles. Not only was I able to overcome the many challenges that came in the way, but I was also able to thoroughly enjoy the process and the opportunity afforded to me.

Abbreviations

CPR	Cardiopulmonary resuscitation
WHO	World health organisation
HIV/AIDS	Human immuno deficiency/ acquired immuno deficiency syndrome
RIDDOR	Reports from the Injuries, Diseases and Dangerous Occurrences Regulations
TARN	Trauma audit research network
SIRS	Systemic inflammation response syndrome
MODS	Multiple organ dysfunction syndrome
MOF	Multiple organ failure
PRRs	Pattern recognition receptors
PAMPs	Pathogen-associated molecular patterns
DAMPs	Damage associated molecular patterns
TLRs	Toll like receptors
CLRs	C-type lectin receptors
NLRs	NOD-like receptors
RLRs	RIG-I-like receptors
MyD88	Myeloid differentiation primary-response gene 88
TRIF	TIR-domain-containing adaptor protein inducing ifnβ
ER	Endoplasmic reticulum
HMGB1	High mobility group box 1
HSPs	Heat-shock proteins
P	Passive
A	Active
S	Surface release
RAGE	Receptor for advanced glycation end products
MSU	Uric monosodium
MAC	Multi molecular complex,
CAMs	Cell adhesion molecules
vWF	Von Willebrand factor

TxA2	Thromboxane A2
ATP	Adenosine triphosphate
ECM	Extracellular matrix
DIC	Disseminated intravascular coagulation
PCD	Programmed cell death
GPIb	Glycoprotein ib
GPVI	Glycoprotein VI
SE	Subendothelial
EC	Endothelial cells
TF:FVIIa	Tissue factor: factor VIIa
APC	Activated protein C
AT	Antithrombin
TFPI	Tissue factor pathway inhibitor
CARS	Compensatory anti-inflammatory response syndrome
ACCP	American College of Chest Physicians
SCCM	Society for Critical Care Medicine
IL	Interleukin
MDSCs	Myeloid derived suppressor cells
MARS	Mixed antagonist response syndrome
NOD	Nucleotide-binding oligomerization domain
RIG-1	Retinoic acid inducible gene 1
ARDS	Acute respiratory distress syndrome
ICU	Intensive care unit
ALI	Acute lung injury
AECC	American-European consensus conference
CPAP	Continuous positive airway pressure
Fio2	Fraction of inspired oxygen
Pao2	Partial pressure of arterial oxygen
PEEP	Positive end-expiratory pressure
MMDS	Microcirculatory and mitochondrial distress syndrome

PICS	Protein catabolism syndrome
ISS	Injury severity score
AIS	Abbreviated injury scale
APACHE II	Acute physiology and chronic health evaluation II
ICNARC	Intensive care national audit and research centre score
GCS	Glasgow coma scale
SOFA	Sequential organ failure assessment
NISS	New injury severity score
HTR	Helsinki trauma registry
HUH	Helsinki university hospital trauma unit
SCS	Simplified consciousness score
qSOFA	Quick Sequential Organ Failure Assessment
ED	Emergency department
SBP	Systolic blood pressure
CRP	C-reactive protein
TNF-α	Tumour necrosis factor
TGF-	Transforming growth factor-β
IFN-γ	Interferon-γ
ROS	Reactive oxygen species
GM-CSF	Granulocyte-macrophage colony-stimulating factor
CD	Cluster of differentiation
ILCs	Innate lymphoid Cells
JAK	Janus family kinases
STAT6	Subsequent phosphorylation of signal transducer and activator transcription 6
TYK	Tyrosine kinase

Abstract

Trauma is a leading cause of mortality and morbidity worldwide, accounting for about 5.8 million deaths annually. England alone sees approximately 25,000 cases of major trauma each year and over a fourth of these result in loss of life.

Death due to trauma is time factorial and bimodally distributed. The first phase appears within an hour of injury, presenting immediate traumatic effects due to fatal injuries and haemorrhagic shocks. The second phase is marked by onset of complications after a week, characterized by further complications such as sepsis, multiple organ dysfunction and multiple organ failure. Major trauma injuries trigger the release of cytokines, which orchestrates a disparity between pro-inflammatory and anti-inflammatory responses.

Cytokines are known, to be predominantly secreted by helper T cells and activated monocytes and macrophages and numerous distinct cell types synchronize their role as part of the immune system. Each of these cell types has a distinct role in the immune system and communicates with other immune cells using secreted cytokines. Interleukins are a large class of cytokines, which are involved in systemic inflammation and immune system modulation by stimulating humoral or cell-mediated immune responses. They play an important role in fighting infection and diseases. Interleukins are also predictive biomarkers whose concentrations are measured through multiplex bioassays.

The research study aims at investigating a panel of serum cytokines, cellular and metabolomic markers as a potential predictor of poor clinical outcome in major trauma. The study is a continuation of a research project with a larger cohort of 200 patients selectively recruited from Central Manchester Foundation Trust (CMFT) and Salford Royal Foundation Trust (SRFT).

The aim of the study was to investigate whether the selected panel of cytokines could predict trauma patients' clinical outcomes. This was achieved by measuring the concentrations of interleukin-13 and interleukin-17 through cytometric bead array methods on which statistical analysis was performed. The study analysis of the sub-cohort of 30 patients, investigated the role of IL-13 and IL-17 incepting single organ failure or multiple organ failure and evaluating them as candidate biomarkers for clinical prediction of good and poor outcomes.

In this study, IL-13 concentration increased between day 1 and day 5, post trauma ($p=0.02$). Based on the Sequential organ failure assessment scores (SOFA), day 1 concentrations were

compared to day 5 and day 8 at 2 different cut-offs, namely SOFA score <3 and ≥ 3 and SOFA score <6 and ≥6. This stratification showed no statistically significant differences meaning SOFA cut offs did not account for a clinical outcome prediction. Later Delta SOFA (day 5 SOFA – day 1 SOFA) was employed to assess whether the patients improved or got worse at the ICU. Though the comparison between IL-13 concentration on day 1 and delta SOFA did not show statistical significance, it revealed a weak negative relationship between them (r=-0.318, p=0.087), indicating that the patients got better at the ICU. Although IL-13 data did not show statistical significance based on SOFA, the data revealed a good clinical outcome when it was clustered tightly based on the movement of SOFA across concentrations.

A percentage concentration analysis was conducted between day 1 and day 5 concentrations to show how IL-13 levels changed at different SOFA scores, and whether they could predict clinical outcome. The increase in average IL-13 concentration on day 5 by 10% (3 pg/ml), tallied with decreased SOFA score, thereby indicating good clinical outcome. The analysis of IL-17 concentration showed a marginal decrease between day I and day 5 without a significant statistical difference (p=0.994). The concentration of IL-17 in day 1 was compared with SOFA score calculated for day 5 and day 8 at two different cut-offs, namely SOFA score <3 and ≥ 3 and SOFA score <6 and ≥6.

At a threshold value of 3, day 1 IL - 17 concentration, showed statistical significance in day 5 at p=0.048. The IL-17 concentration against SOFA score in day 8 returned results at the cut off 3 with p value 0.042 with a statistical significance. This shows that IL-17 levels on the day of admission could possibly predict the onset of single organ failure on case day 5. Repeating the clustering for day 1, IL-17 concentration with day 8 SOFA score at threshold 6 showed statistical significance of p=0.044, indicating the onset of multiple organ failure (MOF).

The correlational analysis between IL-13 day 1 and IL-17 day 1 concentrations and CRP day 5 showed no association. In this study, the cytokine levels were defined using CRP day 5 levels instead of SOFA scores, the data was not statistically significant. This could be because of the small sample size (n =16) made available. The CRP levels ranged between 1.1 mg/L to 197 mg/L for these 16 patients. Similarly, the average lactate concentration varied from

3.863 mM/L on day 1 to 1.010 mM/L on day 5 for only 8 data points. This extreme variation could have skewed the sample and resulted in not obtaining statistical significance.

A cross sectional comparison was conducted amongst the multiplex panel of cytokines involving on a common patient cohort (N=30) derived from the pilot project cohort (N=200). IL-13 and IL-17 on day 1 and day 5 were correlated with IL-4, IL-8, and IL-12.

The cluster plots between IL-13 and IL-17 on day 1 and day 5 showed a strong positive linear co-relationship (r = 0.575 & 0.450) respectively, expressing strong positive feedback loop between IL-13 and IL-17. The correlation analysis between IL-13 and IL-4 levels on day 1 revealed a strong positive linear association between them at r=0.537. The correlational analysis between IL-13 and IL-8 revealed negative correlations on day 1 and day 5 (r=--0.030 and r=-0.353) respectively. IL-13 and IL-12 showed a weak positive correlations on day 1 and day 5 at r=0.376 and 0.321, respectively.

The concentrations of IL-17 and IL-4 and day 5 showed a weak positive linear relationship at r=0.356 on day 1 and a strong positive correlation at r=0.518 on day 5. Correlation between IL-17 and IL-8, revealed a weak positive relationship (r=0.032) and on day 5 steeped to -0.226, indicating negative linear relationship. IL-17 and IL-12 concentrations on day 1, strongly correlated at r value 0.552. The analysis of expression levels of this spectrum of 5 cytokines indicates their synergistic relationships, interwoven in positive or negative loop of feedback mechanisms.

The results of this study show that interleukin concentrations could provide an early prediction of complications and could offer a promising direction for effective therapeutic breakthroughs.

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1 Introduction

Major trauma is defined as an injury or combination of injuries leading to debilitating consequences of prolonged disability (Glen, et al., 2016). Major traumatic injury has the potential to result in an early and untimely loss of life. Trauma can onset with severe physical injuries such as hypovolemic shock and physiological instabilities (Roden-Foreman et al., 2019), necessitating immediate restorative and medical interventions. In addition to medical first aid, cardiopulmonary resuscitation (CPR) on the site of incidence, surgical procedures and/or other emergency options to restore normal body functions to homeostasis may be required. Major trauma can cause injuries affecting either a single organ or polytrauma, in which multiple body systems are affected (McCullough et al., 2014).

1.1 Trauma: Incidence and epidemiology

Major trauma is a leading cause of mortality: estimates shows that it may be causing up to 5.8 million deaths per year across the globe (Ghaffarpasand, et al., 2020). According to the World Health Organisation (WHO), trauma accounts for 10% of deaths and 16% of disabilities worldwide - considerably more than malaria, tuberculosis and HIV/AIDS combined (Lendrum & Lockey, 2013).

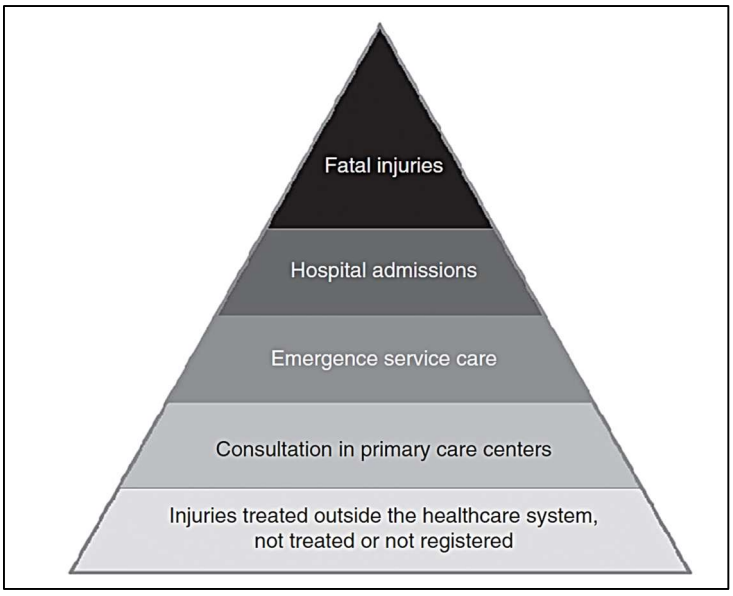


Figure 1: The injury pyramid showing the relative number of fatal and nonfatal injuries and their trail within the health-care system representing wider etiologic ranges (Alberdy et al., 2014).

The proportion of mortalities caused by traumatic injuries is rising worldwide, so much so that road traffic accidents alone are projected to be the fifth largest cause of death and disability by 2030. The peak age group of patients with traumatic injuries is in the second decade of life.

1.1.1 Types of traumatic injuries

Types of trauma are categorised according to the age, gender, and occupation of the trauma patient. The physiological reaction and psychological status of the patients after trauma differ significantly in various groups, particularly in paediatric, geriatric, and pregnant patients.

The physical characteristics of the direct object that cause the trauma consists of main mechanisms of trauma, indicating blunt, penetrating, or explosive. Other classification schemes for the mechanisms of trauma are based on the type of immediate events causing the damage in trauma. These events are classified according to the incidents such as motor vehicle traffic accidents, fall, struck by, against, other transport, firearm, stab/cut/pierce, fire or burns and machinery (Honarpisheh, 2012).

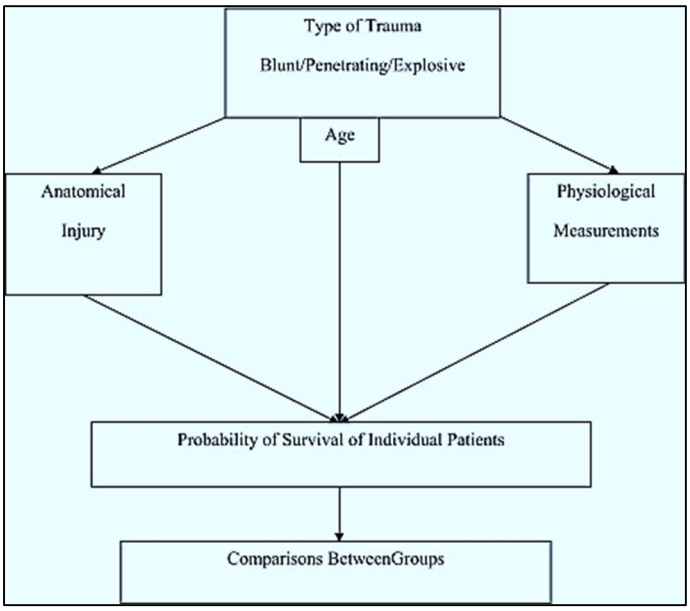


Figure 2: Outline of the main mechanisms of trauma (Honarpisheh, 2012).

Trauma is not only the leading cause of deaths during the first three decades of one's life, but it also has an enormous socioeconomic impact. According to some estimates, trauma-related deaths and injuries contribute more to costs and loss of work time than cardiovascular diseases or malignancies (Choudhry et al., 2007).

1.1.2 Traumatic incidences at a global view

Causes and consequences of injury and trauma vary from country to country. According to World Health Organization (WHO) estimates, almost 90% of deaths due to injuries occur in low- and middle-income countries making it a central global health problem in the upcoming years. Countries in North America, Western Europe, Australia, and New Zealand display the lowest rates (Penden et. al., WHO report 2002) whereas some other European countries have very high rates. Differences do exist between developed and developing countries on the injury profile in road traffic incidents, but it is a major cause of fatal injuries worldwide.

Amongst both male and female populations in the low- and middle-income countries in the Americas, interpersonal violence is the leading cause of death and disability for people in the 15 to 44 years age group. Low- and middle-income countries also suffer a high number of vehicular accidents with child or elderly pedestrians, cyclists and two-wheeler vehicle riders most prone to vehicle-related injuries. With up to a million people dying annually and about ten million seriously injured in road accidents globally, the economic and familial impacts of these accidents are serious. Murray & Lopez (1996) estimate that the financial cost associated with traumatic injuries from vehicle accidents is $500 billion.

	Low/Middle-Income Countries			High-Income Countries		
	Male	Female	Both	Male	Female	Both
Mortality						
Total deaths from all injuries (1000s)	3170	1547	4717	314	157	471
Unintentional injuries (1000s)	2096	1120	3216	202	119	321
% of total injuries	44.44	23.74	68.18	42.89	25.27	68.15
Intentional injuries (1000s)	1075	427	1501	112	38	151
% of total Injuries	22.79	9.05	31.82	23.78	8.07	32.06
DALYs (from death and disability)						
Total DALYs from all injuries (1000s)	101779	54166	155945	7692	3552	11244
% of total DALYs	7.34	3.90	11.24	5.16	2.38	7.53
Unintentional injuries (1000s)	70805	42501	113306	5189	2687	7876
% of total injuries	45.40	27.25	72.66	46.15	23.90	70.05
Intentional injuries (1000s)	30975	11665	42640	2503	865	3368
% of total injuries	19.86	7.48	27.34	22.26	7.60	29.95

Note. DALY = disability-adjusted life-years. Data were derived from the National Institutes of Health (http://www.fic.nih.gov/dcpp/gbd.html).

Figure 3: Global incidence of injuries in the low, middle, and high economy countries in the year 2001. Data derived from National Institutes of Health (Hofman et al., 2005).

Jacob et al (2002), in their Transport Research Laboratory report, summarise two major studies conducted by WHO, the World Bank and Harvard University. These reports, titled 'Global Burden of Disease' (1996), and 'World Health Report – Making a Difference' (WHO, 1999), concluded that the death or disability due to road crashes stood ninth place out of a total of over 100 separately identified causes but were projected to become the sixth most prevalent reason for deaths by 2020. Statistics published by the UK's Department for Transport has reported 1,472 road deaths, a decrease of 16% from 2019 (DfT, 2021). This is likely to be a one-off decrease due to the COVID-19 global pandemic lockdown situation.

Approximately 3-9 million injuries recorded annually even in developed countries. Over the past decade, traumatic injuries have been a major cause of death – the data indicates that around 2000 people sustain injuries and 148 deaths occur every hour due to road traffic accidents (Binkowska et al., 2015). When it comes to the nature of injuries causing fatalities, the most common cause of death are injuries to the head, chest, and abdomen because such injuries cause associated haemorrhages. Since individuals in the 20–60-year age group are the most common group of vehicle owners, this age group is worst affected by road traffic and vehicle accidents, and men are more likely to be victims than women (Binkowska et al., 2015).

WHO (2010) says that the economic cost of road traffic crashes globally is around US $518 billion and typically lead to a loss of between 1–2% of gross national product in some countries but this can increase to up to 5%. It is clear that the benefits of injury and violence prevention measures provide significant value for money, making such initiatives to have great societal benefit. There is sound evidence of the benefits of such measures in high-income countries and this makes is logical for low- and middle-income countries to adapt and implement similar schemes. Wide implementation could lower the current, unacceptably high burden of injury (WHO, 2010).

The sitution in the UK is similar. A Public Health England report (2017) predicts that road traffic crashes will become the fifth leading cause of death by 2030.

In England, the number of major trauma recorded annually go up to 20,000. Over a quarter of these result in deaths (Glen et al., 2016).

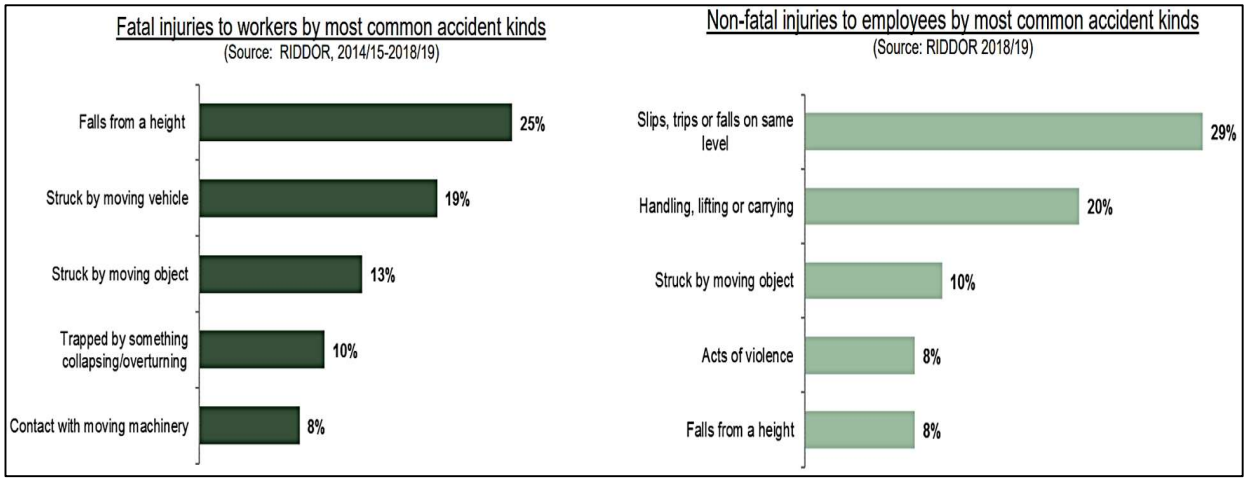


Figure 4: The charts above show the different accident types of injuries to the workers contributing to 5% or more of the total common accidents in Great Britain (Statistics, 2020).

Reports from the Injuries, Diseases and Dangerous Occurrences Regulations 2013 (RIDDOR), show that half of the fatal injuries to workers between 2014 to 2015 were accounted for two different accident types i.e., falls from a height and being struck by a moving vehicle. Falls from a height accounted for 25% of all fatal injuries (an average of 36 fatal injuries per year). Half of fall from height deaths were in the construction sector between 2014 to 2015 (annual average 18 per year). Struck by a moving vehicle accounted for 19% of all fatal injuries (an average of 27 fatal injuries per year) (Kinds of Accident statistics in Great Britain report, 2020). Almost 15,000 people die in accidents of different kinds every year in the UK. Death from road traffic accidents is a considerable proportion of overall accident-related fatalities. (NHS Commissioning Board, 2013).

In England and Wales, Trauma Audit Research Network (TARN) collects data on trauma affected requiring hospital stay for ≥72 h and those needing critical care resources or die from their injuries. The demographics, nature of trauma, medical interventions and observations about the patients are collated in TARN data and used to produce reports of the epidemiology and trauma care levels within hospitals on a monthly and quarterly basis (The Royal College of Surgeons of England, 2009). These reports indicate that ageing population and the prevalence co-morbidities amongst elderly patients makes them prone to poorer clinical outcomes and force them into hospital admissions, which have with serious consequences for the cost of healthcare.

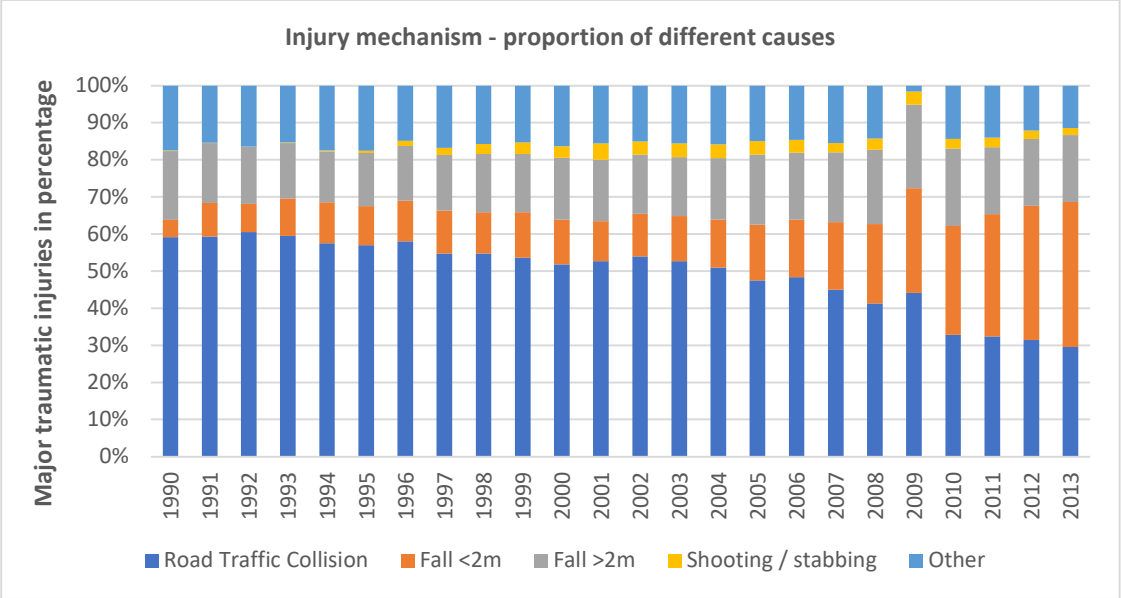


Figure 5: The major traumatic injuries vs injury mechanism (Adapted from Kehoe et al., 2015).

Data gathered by Kehoe et al. (2015) shows that the causes of trauma have changed. Road traffic collisions caused 60% of trauma incidents in 1990. But this had gone down to around 30% by 2013. Falls of less than two metres saw an increase from 4.7% to 39.1% almost a 10-fold increase. Although the different causes and the number of incidents attributable to those causes is interesting, the important finding in Kehoe et, al is that the number of trauma cases in the UK has increased exponentially since 1990. It is likely that the advancement in the medical field technology, improvement in the trauma care, identification, and early management of patients with vulnerability for complications might have improved the quality of diagnosis and treatment, but it has not been revealed in the finding, whether this is because more elderly patients are suffering injury or because the detection and reporting of injury and data recording in these groups has improved. But it does illustrate the need for sufficient research into both the onset and progression of trauma and procedures for treatment.

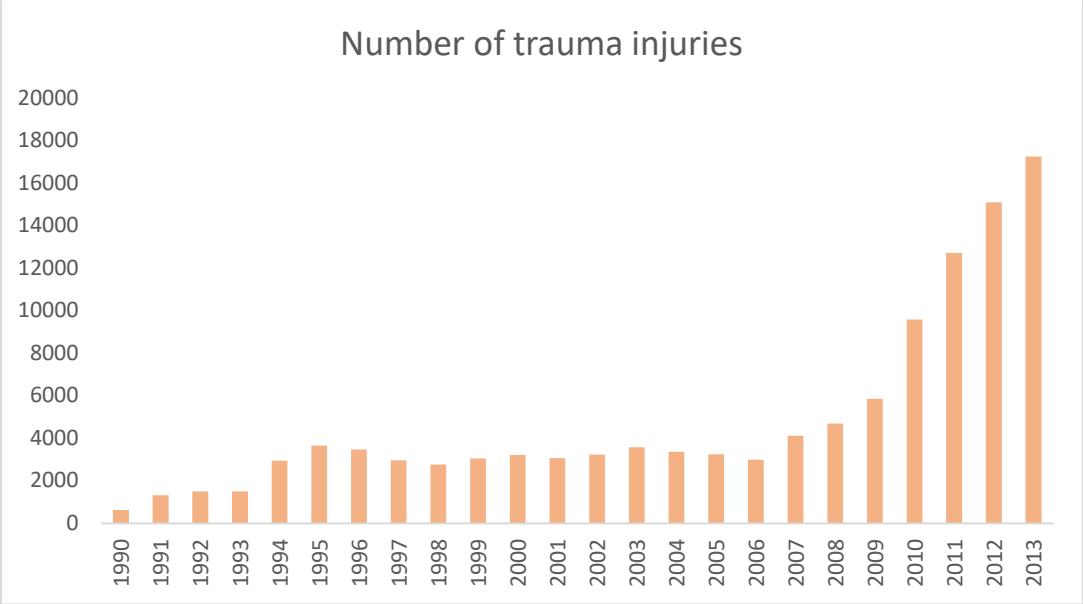


Figure 6: UK traumatic incidents between 1990 to 2006 and a drastic rise from 2007 to 2013 (Adapted from Kehoe et, al., 2015).

The global epidemiological burden of sepsis is, however, difficult to establish. It is estimated that more than 30 million people are affected by sepsis every year worldwide, resulting in potentially 6 million deaths annually. Mortality rates from sepsis, as per the data from the Surviving Sepsis Campaign 2012, were approximately 41% in Europe versus approximately 28.3% in the United States. This difference however disappeared when adjusted for disease severity. This implies that the mortality in sepsis varies according to patient characteristics as well. A multi-centre study in Australia and New Zealand that included 101,064 critical patients showed that the mortality rate in sepsis has decreased over the years from around 35% in 2000 to about 20% in 2012 (Kaukonen et al., 2014, Gyawali et al., 2019).

1.2 Pathophysiology of trauma

When someone undergoes a traumatic injury, the body initiates its natural immune response at the very moment of injury and later invokes adaptive immune response. The human immune system comprises an intricate set of innate and adaptive elements “equipped to adapt and respond to a diverse range of challenges” (Belkaid & Hand, 2014). When the initial traumatic insult crosses the threshold of immunogenic tolerance, the humoral and cellular components get activated (Huber-Lang et al., 2018). The immune system acts as a strong facilitator of homeostasis and tries to restore the normal function of the tissue (Belkaid & Hand, 2014). This objective is achieved by activating the innate immune response which in

turn triggers adaptive immune response and antigen presentation by recruiting B and T lymphocytes to the injury site. An important function of antigen presentation is to recognise the pathogen and provide the appropriate response to the stimuli. B and T cells perform two complementary functions; B cells secrete antibodies that can bind to and tag an antigen whereas T cells attack target cells (Han et al., 2015).

An initial traumatic insult disrupts macro barriers such as the skin, as well as micro barriers such as cell membranes, which causes the release of multiple danger molecules. This disruption is followed by a swift innate immune response intended to end the dangerous situation for the trauma recipient of the traumatic insult. Without this innate immune response, the trauma sufferer could face severe complications and death (Huber-Lang et al., 2018).

When there is trauma insult, the body undergoes various immunological, endocrine, metabolic changes. Traumatic injury is associated with altered host defence and hyperinflammation, an early over-activation of immune responses. This phase is followed by immunosuppression and weakened T-cell function, which causes reduction of adaptive immunity and increased vulnerability to infection, sepsis, and even organ failure (Stahel et al., 2007). The immune response is characterised by local, systemic production and release of multiple mediators such as cytokines, chemokines, coagulants and complement activation factors (Keel & Trentz, 2004).

1.2.1 Immuno- recognition and recruitment

As part of the immune response, immunocompetent cells are initiated to counter the damage at the site of tissue injury. In addition, inflammation which is a series of responses of vascularized tissues of the body to the injury, sets in at the injury site when cells related to innate and adaptive immunity are recruited and activated to get rid of infectious agents and dead tissue. (Baue et al., 1998). The clinical signs of this phenomenon can be noticed by a few different factors: increased blood flow in local blood vessels (calor and rubor), more vascular permeability, cellular infiltration (tumour) and the release of a variety of pain inducing materials at the injury (dolor) (Larsen & Henson,1983). During this inflammatory reaction, tissue damage could result from various causes; amongst them are the release of enzymes from the granules and lysosomes of infiltrating cells and the production of oxygen radicals by

these cells. The central feature of inflammatory processes is the infiltration of cells into the injured tissues.

1.2.2 Immuno activation

Two models have been proposed for the exaggerated immune-inflammatory response: 'one hit' model and ‘two hit’ model. According to the 'one hit' model, the initial tissue injury and associated shock trigger an intense Systemic Inflammation Response Syndrome (SIRS) with rapid organ injury. In the 'two hit' model, the initial SIRS is less intense and could itself usually resolve but the patient is vulnerable to a secondary inflammatory attack and reactivation of the SIRS leading to late multiple organ dysfunction syndrome (MODS), eventually causing multiple organ failure (MOF) (Smith & Giannoudis, 1997 and Namas et al., 2009).

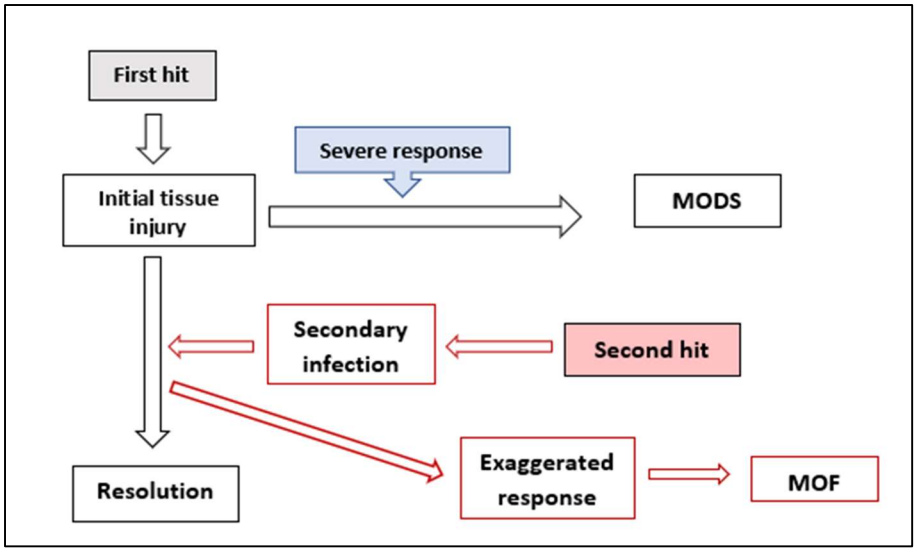


Figure 7: The ‘one-hit’ and ‘Two-hit’ paradigm of traumatic injury (Neher et al., 2011).

Inflammatory ‘one hit’ and ‘two hit’ models explain the incidents that trigger multiple organ failure (MOF) in critically ill post-surgery surgical patients even when no source of infection is detected. In the ‘one hit’ model, the initial insult is so massive that a systemic inflammatory response syndrome is triggered and leads to a very rapid MOF whereas in the ‘two hit’ model, less severely injured patients could also end with MOF as a result of a reactivation of SIRS. This could be caused by an adverse, even minor inflammatory reactions (Saadia & Schein, 1999). In other words, the pathophysiological sequelae of major injuries are characterized a cascade of immunological reactions after the initial traumatic impact or ‘first hit’. The

immunological reaction renders the patient susceptible to an adverse 'second hit' insult. The activation of innate immune response mechanisms can take place within hours or take days after the trauma and is a crucial event that marks the early phase of hyperinflammation (Neher et al., 2011).

1.3 Immuno regulatory molecules

This section provides a summary of a few well characterised immune regulatory molecules that are pivotal in the regulation of inflammation and immune responses.

1.3.1 Toll Like Receptors

Toll Like Receptors are type I transmembrane glycoproteins located at the cell surface (TLR-1, 2, 4, 5, 6, and 10) or in intracellular membranes (TLR-3, 7, 8, and 9). TLRs can recognize numerous types of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and have two key signalling adaptor proteins - the Myeloid differentiation primary-response gene 88 (MyD88) and TIR-domain-containing adaptor protein inducing IFNβ (TRIF) (Wiersinga et al., 2008).

When PAMP is recognised, pattern recognition receptors (PRRs) at the cell surface send a signal to the host about the presence of infection, which triggers pro-inflammatory and antimicrobial responses. The response is initiated by activating intracellular signalling pathways such as adaptor molecules, kinases, and transcription factors. This is followed by the synthesis of a broad range of molecules, including cytokines, chemokines, cell adhesion molecules, and immunoreceptors, which together form the early host response to infection (Mogensen, 2009).

TLRs induce the production of pro-inflammatory cytokines and type I interferons (IFNs) through MyD88 or TRIF signalling pathways (Roh & Sohn, 2018). The MyD88-dependent pathway leads to the activation of the nuclear factor kappa light chain enhancer of activated B-cells (NF-κB) and IFN regulatory factor pathways of inflammation (Frasca & Lande, 2020). In this way, PRR-induced signal transduction pathways facilitate the activation of gene expression and molecular synthesis (Krysko et al., 2011).

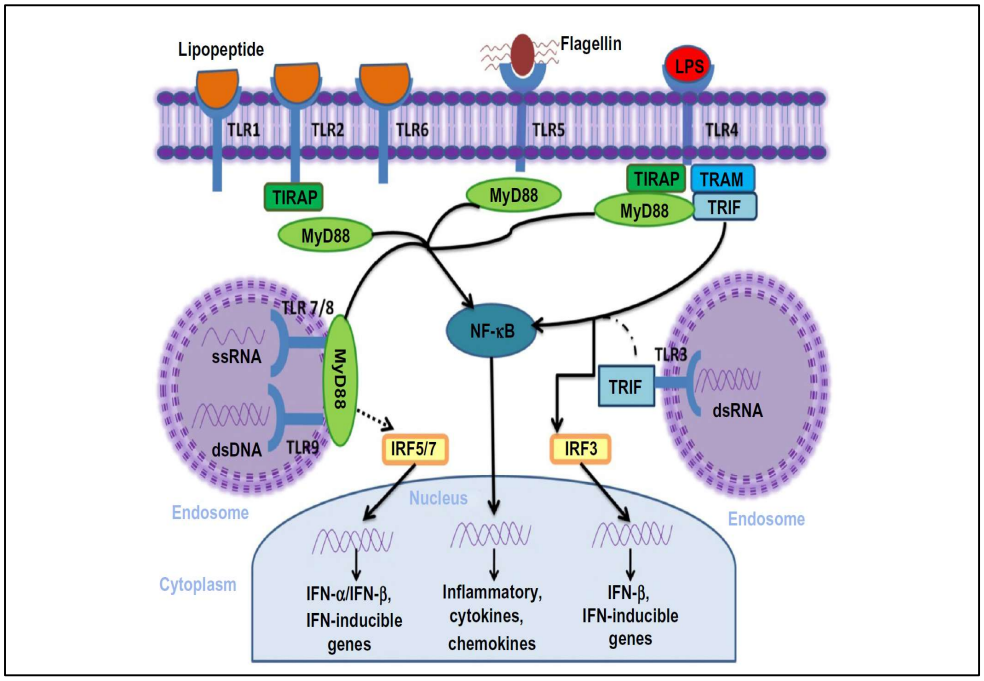


Figure 8: Pathways of Tol Like Receptors and their major signal adaptors. TRAM- TRIF related adaptor molecule, TIRAP- Toll/interleukin-1- receptor (TIR)-domain-containing adaptor protein (Bianchi, 2007).

1.3.2 Pattern Recognition Receptors (PRRs)

Pattern Recognition Receptors (PRRs) are proteins that can recognise molecules frequently associated with pathogens. PRRs identify microbes or tissue damage through specific molecular structures called pathogen-associated molecular patterns (PAMPs) or Damage Associated Molecular Patterns (DAMPs). PRRs are found in cellular membranes and endosomal membranes and are noticed as extracellular secretions in the blood stream and interstitial fluids. PRRs are classified based on their localisation, evolutionary relationships, ligand specificity and individual function. By localisation, PRRs may be divided into membrane-bound PRRs including toll like receptors (TLRs) and C-type lectin receptors (CLRs), and cytoplasmic PRRs including NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs) (Amarante-Mendes et al, 2018).

Figure 9 below shows the engagement of PRRs in response to PAMPs, wherein cell death mechanisms may be activated to promote tissue homeostasis and host-defence against pathogens. And the DAMPs form a feedback loop stimulating PRRs to engage in phagocytosis and mediate inflammatory/immune responses (Amarante-Mendes et al, 2018).

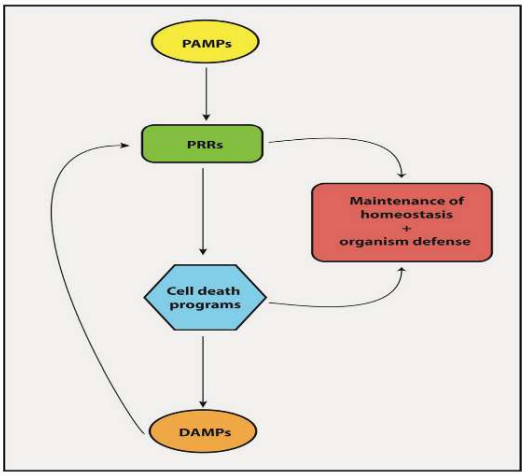


Figure 9: An Interplay between Pattern Recognition Receptors and cell death mechanisms (Amarante-Mendes et al, 2018).

1.3.3 Pathogen-associated molecular patterns (PAMPs)

Pathogen-associated molecular patterns (PAMPs) are exogenous molecules, which are identified as foreign bodies by the immune system and are evolutionarily conserved structures on the pathogens. Both innate and acquired immunity systems recognise PAMPs through PRRs such as TLRs and CLRs (Janeway, 1989 & Akira, 2006). TLRs activate several signalling pathways and appropriate cells to destroy the pathogen or pathogen-infected cells. The immunological response that is triggered produce specific T cell receptors and antibodies that are best able to recognize the pathogen on a future occasion (Bianchi, 2014).

1.3.4 Alarmins and DAMPs

Alarmins are endogenous equivalents of PAMPs and represent the collection of PAMPs for all non-pathogen danger signals arising from tissue injury. By employing using specialized secretion systems and endoplasmic reticulum (ER)-Golgi secretion pathway, the immune

system can produce and release alarmins without dying. They recruit and activate receptor-expressing cells of the innate immune system and promote the response of adaptive immunity. Alarmins can promote tissue repair and reinstate homeostasis. Together, alarmins and PAMPs represent a family of damage-associated molecular patterns (DAMPs) (Bianchi, 2007). In addition to the classical markers of tissue injury such as the S100 protein and the high mobility group box 1 (HMGB1) nuclear protein, alarmins include heat-shock proteins (HSPs), annexins and defensins. DAMPs are a newly recognised family of danger signals that can induce innate immune responses after trauma whether the trauma is caused by injury alone or leading to further infectious complications such as sepsis. The immune system uses TLRs to identify PAMPs and DAMPs. (Stahel et al., 2007).

When released from the trauma affected damaged or diseased cell, DAMPs stimulate a sterile immune or inflammatory response. DAMPs based immune responses are seen as defence strategies aimed at maintaining and restoring homeostasis. Although DAMPs have immunological benefits, they can cause harm when dysregulated or amplified. In this case, the inflammatory and tissue repairing processes could become pathogenic and can lead to serious pathologies such as sepsis, cardiovascular and neurodegenerative diseases (Relja & Land, 2019). DAMPs share some features with PAMPs and are both recognized by pattern-recognition receptors such as certain TLRs, NLRs (such as NLRP inflammasomes) and RLRs (such as RIG-I-like receptors). These receptors quickly appear at the site of infection (Tsan and Gao, 2004 and Bierhaus et al., 2005).

	DAMPs	Receptors	Release	Role in inflammation/immunity	Role in tissue repair
Nucleus	Histones	TLR2, TLR4 and TLR9	P, S and A	TLR- and inflammasome-dependent inflammatory response	N.D.
	Genomic DNA	TLR9	P	TLR9- and NALP3-mediated innate immune response, DC maturation	N.D.
	HMGB1	TLR2, TLR4, RAGE and TIM3	P and A	Recruitment/activation of immune cells	Migration/proliferation of stem cells, pro-angiogenic mediator.
	IL1a	IL-1R	P	Strong pro-inflammatory activity	Protective during early phase of inflammation
	IL33	ST2	P	Secretion of pro-inflammatory and Th2 cytokines	Epithelial cells proliferation and mucus production in the gut
Cytosol	ATP	P2Y2 and P2X7	P and A	Macrophages recruitment, IL-1β production by DC, antitumor immunity	Migration/proliferation of epithelial and endothelial cells, pro-angiogenic role
	F-actin	DNGR1	P	Contribution in recognition of necrotic cells by DC	N.D.
	Cyclophilin A	CD147	A	Inflammatory cells recruitment, inflammatory mediators release	N.D.
	HSPs	CD91, TLR2, TLR4, SREC1 and FEEL1	P, S and A	Recruitment of immune cells DC maturation, T cell-based antitumor immunity	Wound debris clearance, cell migration/proliferation and collagen synthesis in skin
	Uric acid crystals	NLRP3	P	DC maturation and neutrophil recruitment	N.D.
	S100s	TLR2, TLR4, RAGE	P	Potent immunostimulatory activity, monocytes and neutrophils recruitment	Myoblast proliferation/differentiation
Mitochondria	Mitochondrial DNA	TLR9	P	Macrophages and neutrophils activation	N.D.
	Mitochondrial trascription factor A	RAGE and TLR9	P	DC activation, type I interferon release	N.D.
ER	Calreticulin	CD91	P and S	Potent "eat me" signal, mediator of tumor immunogenicity	Cell migration/proliferation, extracellular matrix production

Figure 10: Enlisting DAMPs, and their receptors and their mode of release. P- passive release, A-active release & S-surface release, N.D- not defined (Venereau et al., 2015).

1.4 Markers of tissue injury

1.4.1 High mobility group box 1 (HMGB1)

High mobility group box 1 (HMGB1) or amphoterin is a protein that is released from myeloid cells as an immune response when the body faces infection or sepsis. Abundantly found within the cell, it was the first identified DAMP molecule (Parker et al., 2015). HMGB1 is transiently associated with nucleosomes that contain tightly bound chromatin and DNA. It binds to nuclear DNA and normally participates in transcription and DNA repair amongst other processes. When there is trauma, HMGB1 takes on the role of a strong pro-inflammatory cytokine. This different character takes place when HMGB1 is released from necrotic or activated cells. It then works through multiple cell-surface receptors including the receptor for advanced glycation end products (RAGE) and TLRs (Tang et al., 2010 and Chen et al., 2016).

HMGB1 is a nuclear protein which interacts with nucleosomes and histones. It acts an inflammation mediator and also supports gene transcription functions by interacting with transcription factors (Sharma & Naidu, 2016). It is secreted by macrophages and dendritic

cells and from the damaged tissues, it diffuses passively out of the nucleus (Klune, 2008). In a study involving 168 patients suffering severe trauma, Cohen et al. (2009), showed that plasma levels of HMGB1 increased within 30 min of severe trauma. They noticed that the levels correlated with severity of trauma, tissue hypoperfusion and onset of coagulation abnormalities alongside systemic inflammatory response and hyperfibrinolysis. Plasma levels were also found to be much higher in non survivors and those who suffered acute lung or kidney injury (Cohen et al., 2009).

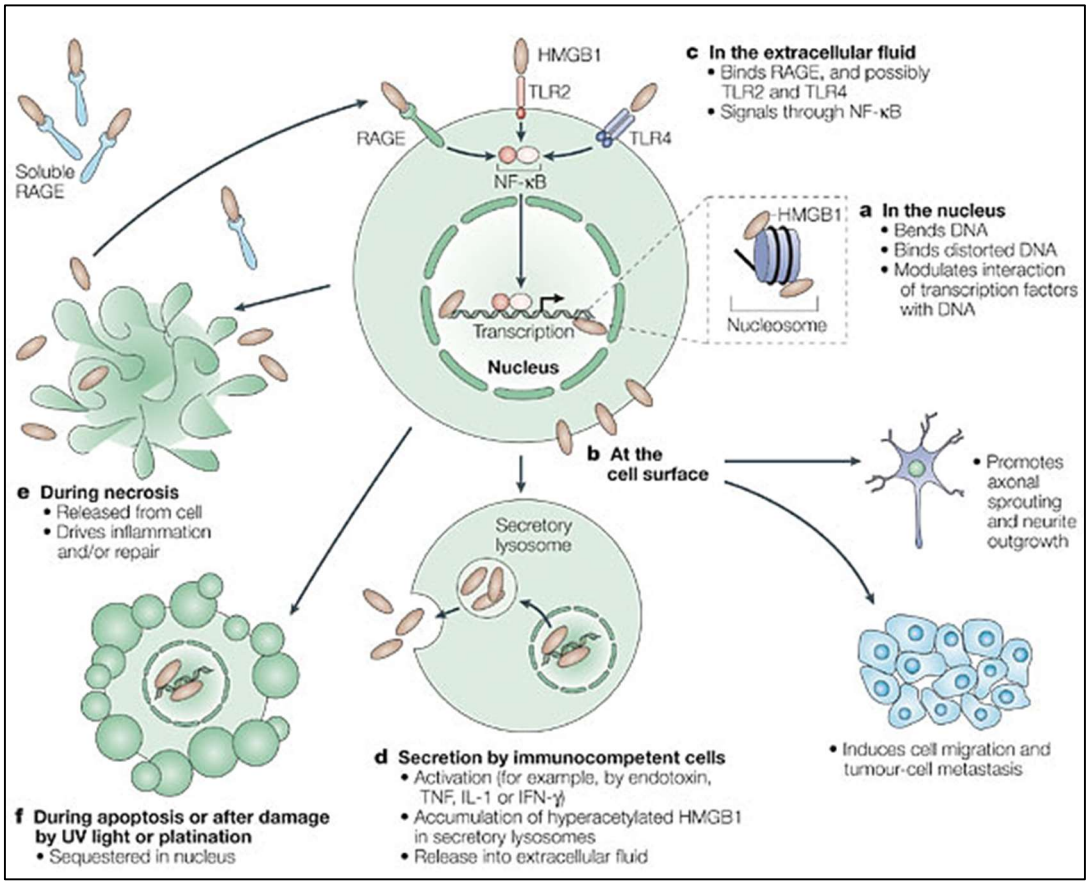


Figure 11: The Intranuclear and extranuclear roles of HMGB1 (Lotze & Tracey, 2005).

1.4.2 S100

S100 proteins or calgranulins are calcium-binding homodimeric proteins with low molecular weight. The S100 family consists of at least 25 distinct members. They are also known to be either passively released from damaged cells or actively secreted from activated cells, and they have been detected in various body fluids, such as serum, urine, sputum, cerebrospinal fluid, and faeces of patients with cancer, inflammatory and autoimmune diseases (Relja &

Land, 2019). S100 protein sub-groups present intracellular and extracellular regulatory effects and are primarily seen in cells of myeloid origin, predominantly neutrophils. S100 proteins also mediate inflammatory responses and aid in recruiting inflammatory cells to sites of tissue damage (Xia et al., 2018).

1.4.3 Uric acid

Uric acid is another DAMPs molecule produced as an end product in purine metabolism. Each person's uric acid level varies depending on age, gender, diet, physical characteristics, and renal function. Uric acid is also derived from dead cells. In their research, Yuliana et al. (2019) suggested that cells experiencing apoptosis or necrosis could secrete DAMPS molecules including HMBG1, HSP, S100, uric acid, beta-defensin and fibronectin. When there is acute kidney injury causing decreased renal function, it causes hyperuricemia and accumulation of uric acid characterised by increase in uric monosodium (MSU) crystal build up. MSU is a DAMPs molecule which could trigger an immune response. MSU is identified by the immune system on myeloid cells like macrophage or dendrite and stimulates the secretion of IL-1β cytokine and other pro-inflammatory cytokines (Yuliana et al., 2019).

1.4.4 Heat Shock Proteins

Heat shock proteins (HSPs) are proteins that are normally released from dying cells following apoptosis, necrosis, and under cellular stress (Clayton et al, 2005 & Clayton, et al, 2008). HSPs play the role of chaperones by stabilizing newly synthesized proteins and ensuring their correct folding. They also help to refold proteins that were damaged due to cellular stress (Bianchi, 2007). HSPs can act as DAMPs by interacting with TLRs following their release from the intracellular space (Vabulas, 2002). Thus, both intracellular and released products can act as DAMPs under the appropriate pro-inflammatory stimuli. In a study of 67 patients with severe trauma, Pittet et al., (2002) found that HSP72 was detected in serum within 30 minutes of injury. It was found that the levels of HSP72 did not correlate with the severity of post injury inflammatory response or organ dysfunction but patients with serum HSP72 >15 ng/mL survived whereas patients with low levels HSP72 levels died from their traumatic injuries (Sharma & Naidu, 2016).

1.4.5 Histones

Histones are a type of alkaline nuclear proteins that from spools around which DNA can wind to form structural units called nucleosomes. Extracellular histones are DAMPs involved in the pathogenesis of various trauma injury induced diseases. When there is tissue damage, it triggers histones to extracellular compartments and promotes inflammatory response. There have been many studies of mechanisms of histone-mediated injury in certain organs have been extensively studied. In 132 critically injured patients, Kutcher and team found a correlation between histone levels and injury severity score. Patients with elevated histone levels at admission had higher propensity for multiorgan failure, acute lung injury and higher mortality. Increasing histone levels from admission to 6 hours after admission also predicted the mortality (hazards ratio =1.005) (Kutcher, 2012). On the other hand, patients who showed that the histone levels were elevated on admission but started to decline after 6 hours had better outcomes (Takeuchi and Akira, 2010).

1.5 The complement system in Trauma

The complement system has a major role when there is trauma related tissue damage. It is a phylogenetic human immunity cascade system that consists of over 50 proteins that circulate as macromolecules in the blood stream. Complement system proteins are expressed on both cell surfaces and at intracellular level. Cellular and molecular effectors of the innate immune system are activated in the early phase of tissue trauma itself. This triggers complement activation, and the recruitment and activation of neutrophils (Keel and Trentz, 2005, Stahel et al., 2007).

Severe traumatic injuries could lead to high activation of the coagulation cascade, which can cause the formation of microthrombi and severe bleeding because the coagulation factors that can reduce bleeding are ingested. This scenario contributes to a downward spiral of shock and negative outcomes (Brohi et al., 2003 and Dobson et al., 2015).

The complement system plays various critical roles: it has a part in the recognition and elimination of invading pathogens. It aids in the removal of self-derived harmful cells such as apoptotic cells, supporting innate immune responses and finally by initiating general inflammatory reactions. Following traumatic tissue injury, the initiation of post-traumatic

danger response is believed to be triggered by the exposure of innate immunity to damaged cells and accumulation of molecular debris at the injury site (Satyam et al., 2019).

1.5.1 An overview of Complement activation pathways and biological effect mediated by complement products.

The activation of complement pathways takes place through three main activation cascades of the system all of which converge on multiprotein complex C3 convertase production. Figure 12 below depicts the so far known complement activation pathways and associated biological functions. In the figure, the classical pathway is depicted on the left-hand side, the lectin pathway in the middle and the alternative pathway is presented on the right-hand side. Both classical and lectin pathways share the C3 convertase, C4b2a, but this differs for the alternative pathway where it is C3bBb (Monie, 2017a).

All three activation pathways have the commonality of the formation of C3 and C5 convertases enzymatic complexes. Subsequently, two proteolytic fragments are generated by the convertases, namely the C3a and C5a anaphylatoxins (Neher et al., 2011). Both C3a and C5a respectively bind to their corresponding receptors, the C3a receptor (C3aR) for C3a convertase and two C5a receptors (C5aR and C5aR2) to initiate pro-inflammatory signalling by binding to both myeloid and non-myeloid cells. C5a attracts neutrophils and recruits immune cells to the site of injury. It then activates cellular attack processes like oxidative burst and lysosomal enzyme release (Haas and van Strijp, 2007, Ward, 2004).
C3a and C5a anaphylatoxins aid the degranulation of mast cells and start the upregulation of adhesion molecules on endothelial cells thereby enabling smooth muscle contraction and improving the liver's acute phase response. Another anaphylatoxin, C3b is generated at a later stage. C3b is an opsonising component that helps phagocytosis through removal of bacteria and cell debris (Bordron et al., 2019). A similar cleavage of C5 leads to the formation of C5b, which initiates the multimolecular complex, the MAC (C5b-9) that can perforate bacteria membrane and nucleated cells thereby causing rapid cell lysis and death (Mollnes and Fosse, 1994, Morgan, 1999).

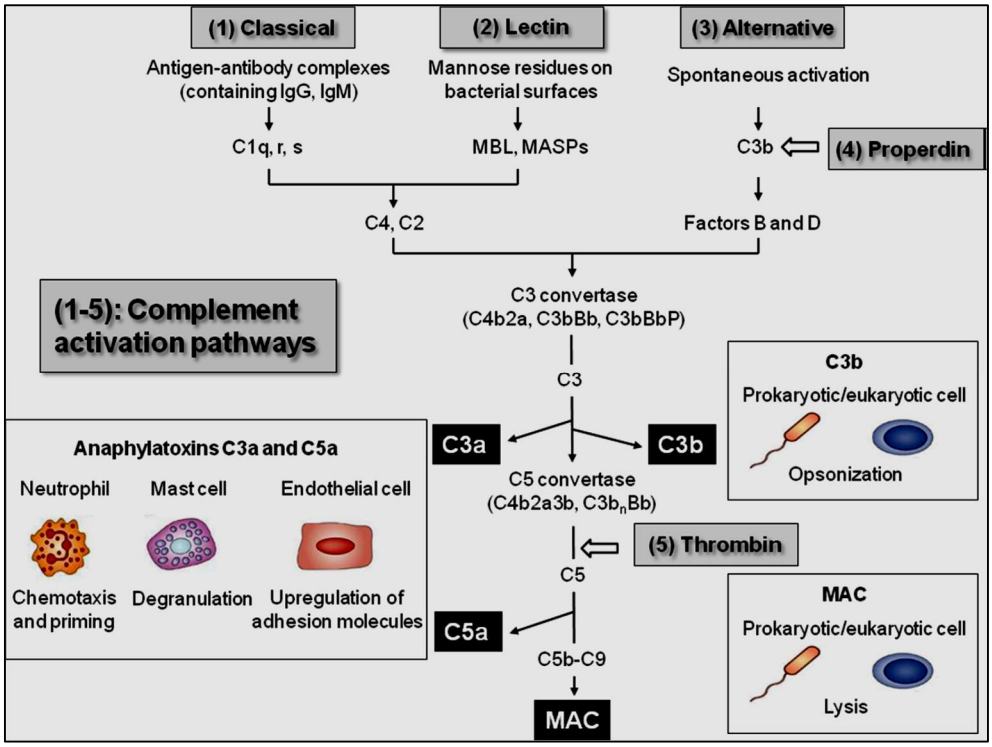


Figure 12: Representation of the three main activation cascades of the complement system (Monie, 2017b).

The alternative activation pathway has a second initiation mechanism called properdin pathway. This new pathways is able to recognise several DAMPs and PAMPs on foreign and apoptotic cells, which allows C3 convertase assembly on the target (Spitzer et al., 2007). Properdin pathway also functions as a stabilizer for C3 convertase complexes of the alternative pathway. Yet another complement activation pathway has been recently described by Gros et al., (2019) who identified clotting factor thrombin as a C5 convertase. Thrombin is capable of generating C5a in the absence of C3, thus providing a direct link between the complement and coagulation system (Gros et al., 2019).

Coming to post-trauma complement activation, there is a bridging of the varying cellular responses of the innate and the adaptive immune system. (Chakraborty et al., 2018). Figure 13 below shows the alteration to the cellular responses of neutrophils, monocytes, and B-cells. This takes place by modulating complement regulatory proteins on T-cells and by robust activation of complement factors.

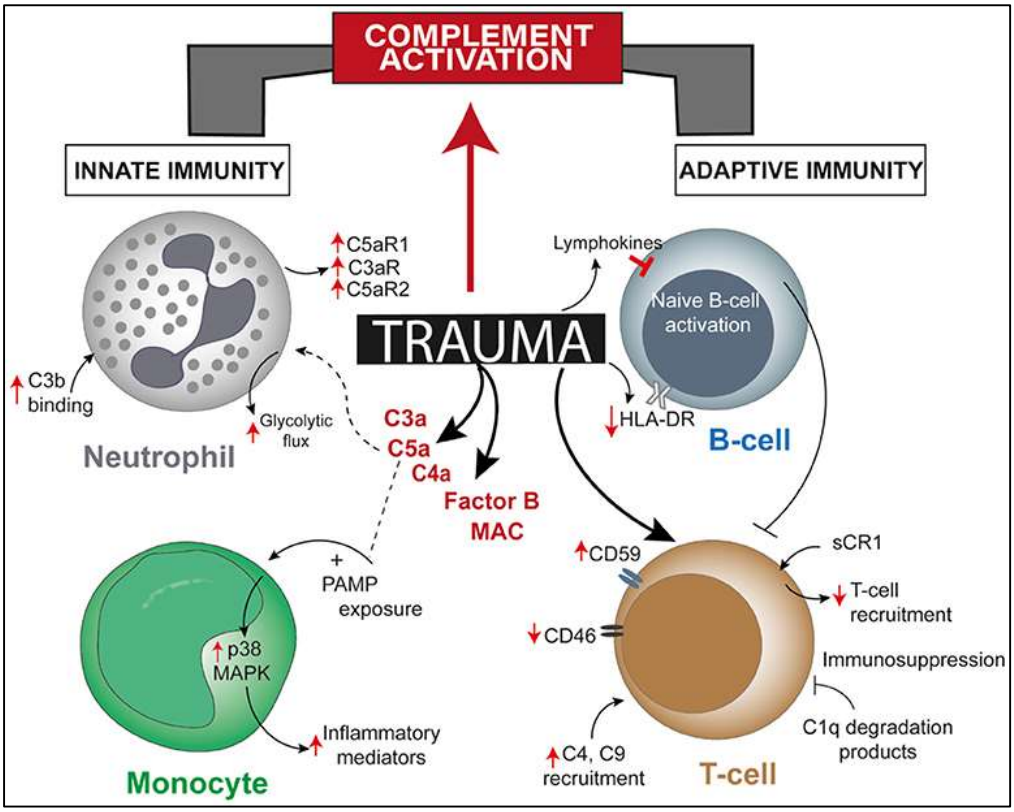


Figure 13: Activation of post-traumatic complement system connecting modulated (Chakraborty et al., 2018).

1.6 Mechanism of haemostasis

Haemostasis is a mechanism to respond to haemorrhage by preventing excessive bleeding and retaining the blood within vessel walls even if they are damaged. It is a sophisticated process that involves various elements such as platelets, plasma coagulation cascades, fibrinolytic proteins, and cytokines (Fogelson and Neeves, 2015). When a vascular injury occurs, the blood flow pressure can widen the injury and cause severe bleeding. As a response, vasoconstriction sets in to reduce the blood flow (Periayah et al., 2017). The aim of homeostasis is, therefore, to seal the vascular injury and curtail blood loss. It is thus a critical function and governs the body's ability to quickly initiate robust injury response. Obtaining arterial and venous thrombosis can prevent fatal injury and morbidity.

To attain haemostasis, immune system employs vascular and extravascular receptors to seal off the impairments. Triplett (2000) describes the primary haemostasis process that results from the formation of an initial 'platelet plug' as a consequence of interactions between thrombocytes, vessel wall and cell adhesion molecules (CAMs) like laminin, thrombospondin

and vitronectin. The endothelial and sub-endothelial linings of the vessel wall display different characteristics. The former could show antithrombotic properties because of the presence of negatively charged heparin-like glycosaminoglycans, neutral phospholipids, synthesis and secretion of platelet inhibitors, coagulation inhibitors and fibrinolysis activators. The sub-endothelial layer is highly thrombogenic as it contains collagen, Von Willebrand factor (vWF) and CAMs.

When a vascular insult occurs, reflex neurogenic mechanisms set in. Local mediators like endothelin and platelet-derived thromboxane A2 (TxA2) are released leading to persistent contraction of the blood vessels i.e., arteriolar vasospasm (Triplett, 2000). At the same time, the injury to the blood vessel triggers vascular spasm to constrict the blood vessels, which could eventually stop the blood flow. Such a response is limited to local area and is usually attained within 30 minutes of the injury. Alongside vasoconstriction, the exposed collagen fibres release adenosine triphosphate (ATP) and other inflammatory mediators so as to recruit macrophages. In addition, the extracellular matrix (ECM) becomes thrombogenic and encourages platelet adhesion and aggregation (Periayah et al., 2017). The complement cascade is also rapidly activated, which allows to target and clear both damaged tissue and pathogens. However, where there is either excessive or insufficient activation of the complement, it results in dysfunctional immune response. The complement activation also induces and modulates various remote organ effects (Huber-Lang et al., 2020).

1.7 Dysregulation of homeostasis

Homeostasis overlaps with inflammatory pathways when the patient suffers from severe trauma. At this stage, microbial molecules and endogenous danger signal molecules can regulate homeostasis. The adverse impact of simultaneous activation of the inflammatory pathway and coagulation cascades can vary from mild thrombocytopenia to fulminant disseminated intravascular coagulation (DIC). There are many causes for the dysregulation of coagulation in sepsis. One is hypercoagulability, which is thought to be caused by endothelial cell release of tissue factors (Remick, 2007). There have been in instances where in vitro experimental models of endotoxemia and bacteraemia have totally inhibited inflammation-induced thrombin production (King et al., 2014).

The onset of inflammation is followed by immunosuppression as an adaptive response to the stimuli from inflammation factors. Stearns-Kurosawa et al. (2011) suggest that process is

mediated by PD-1 (programmed cell death-1) expressed on both T cells and B cells. The exhaustion of T cells invariably leads to immunosuppression (Swieringa et al., 2018).

The anti-inflammatory immune response can alter the equilibrium between procoagulant and anticoagulant status of the host immune status (Cavaillon and Giamarellos-Bourboulis, 2019). Coagulation is triggered by endotoxemia or bacteraemia and by circulating pro-inflammatory cytokines. A procoagulant state could then develop in the vascular system depending on the tissue factor. Sepsis patients often present disseminated intravascular coagulation (DIC). Alongside, the fibrinolytic system is reduced, and suppression of activated fibrinolysis is often a predictor of microbial infection, septic shock, and mortality. The consequent inflammatory reactions may be beneficial but are often more harmful (Annane et al., 2005). If the infectious agent overwhelms the immune response, it leads to organ dysfunction. While the possible benefits of coagulation inhibitors have been shown by assays performed in animal models, there is no conclusive evidence that their benefits for patient survival (Adib-Conquy and Cavaillon, 2009). However, treatment with activated Protein C has been shown to improve survival in patients presenting septic shock (Adib-Conquy and Cavaillon, 2009, Iba and Levy, 2020).

1.8 Coagulation cascade following trauma.

Inflammation and coagulation are tightly linked defence mechanisms following injury and auto-strengthen by co-stimulus (Hotchkiss et al., 2016). The activation of the coagulation cascade is one of the earliest events initiated after tissue injury. Thus, the primary function of the coagulation cascade is to promote haemostasis and limit blood loss in response to tissue injury. This is achieved by plugging damaged blood vessels and to prevent blood loss by generating short-term clots. These clots consist of cross-linked fibrin strands that bind and stabilize weak platelet haemostatic plugs. Their formation is critically dependent on the action of thrombin, and is generated after the stepwise activation of coagulation zymogens (Chambers and Scotton, 2012).

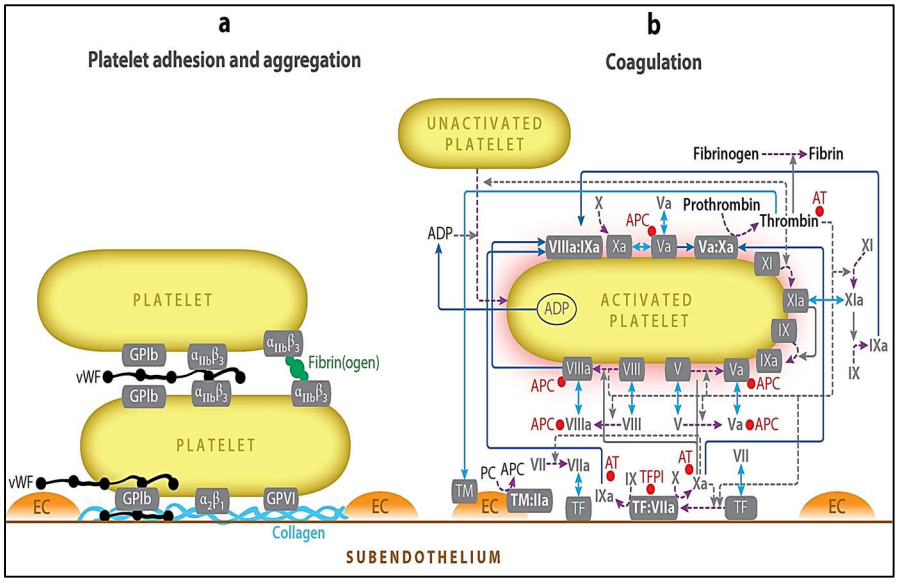


Figure 14: Platelet aggregation and coagulation (Fogelson and Neeves, 2015).

The Figure 14 shows the cellular or chemical activation (purple lines), movement in fluid or along a surface (dark blue lines), enzyme action in a forward direction (solid grey lines), the feedback action of enzymes (dashed grey lines), binding to or unbinding from surface (light blue double-headed arrows), and chemical inhibitors (red circles) (Fogelson and Neeves, 2015).

The coagulation proteins are the core components of the coagulation system that lead to a complex interplay of reactions resulting in the conversion of soluble fibrinogen to insoluble fibrin strands (Palta et al., 2014). Figure A shows platelet adhesion receptors and their ligands. Each platelet's surface bears approximately 25,000 platelet surface protein called Glycoprotein Ib (GPIb) receptors that bind to surface-bound von Willebrand factor (vWF).

Also, about 50,000 integrin αIIbβ3 receptors bind to fibrinogen and vWF. Approximately 4,000 glycoprotein VI (GPVI) receptors and 1,000–4,000 integrin α2β1 receptors bind to several types of collagen. The integrins will be activated to form strong and stable bonds. Collagen is a major constituent of the subendothelial (SE) matrix. Fibrinogen is an abundant plasma protein and vWF is adsorbed to subendothelial collagen that circulates in plasma which is secreted by endothelial cells (ECs).

Figure B is showing coagulation reactions following a major trauma. Majority of clotting factors are the precursors of proteolytic enzymes known as zymogens that circulate in an

inactive form. The activation of each zymogen is termed by suffix letter “a” to the Roman numeral identifying that specific zymogen (Palta et al., 2014) & (Satyam et al., 2019).

The rapid amplification of thrombin as an essential step in the development of a stable clot, and the interdependence of coagulation factors and cellular elements. It builds on the classical cascade in several ways. One such way is activation of both factor X and factor IX by the tissue factor : factor VIIa (TF:FVIIa) complex (Marlar et al., 1982).

Thrombomodulin (TM) on ECs is a cofactor for thrombin in producing the inhibitor activated protein C (APC). Other major inhibitors are antithrombin (AT) and tissue factor pathway inhibitor (TFPI). Surface-bound enzyme complexes TF:VIIa, VIIIa:IXa, Va:Xa, and TM:IIa and other surface-bound species are shown in boxes. The cellular or chemical activation (purple lines), movement in fluid or along a surface (dark blue lines), enzyme action in a forward direction (solid grey lines), the feedback action of enzymes (dashed grey lines), binding to or unbinding from surface (light blue double-headed arrows), and chemical inhibitors (red circles) (Fogelson and Neeves, 2015).

The coagulation enzyme-cofactor complexes form on SE and platelet surfaces possess enzymatic efficiencies 10^5– 10^6 fold to those of the enzymes alone. The activation of a coagulation protein takes place by proteolysis of the precursor by another enzyme (Swieringa et al., 2018).

Involvement of cellular elements, namely, activated platelets, in the final two phases acts by, both providing a negatively-charged phospholipid surface on which reactions can occur, and by providing a localizing surface in direct proximity to the area of damage upon which most of the necessary elements for successful coagulation could be formed (Adams and Bird, 2009).

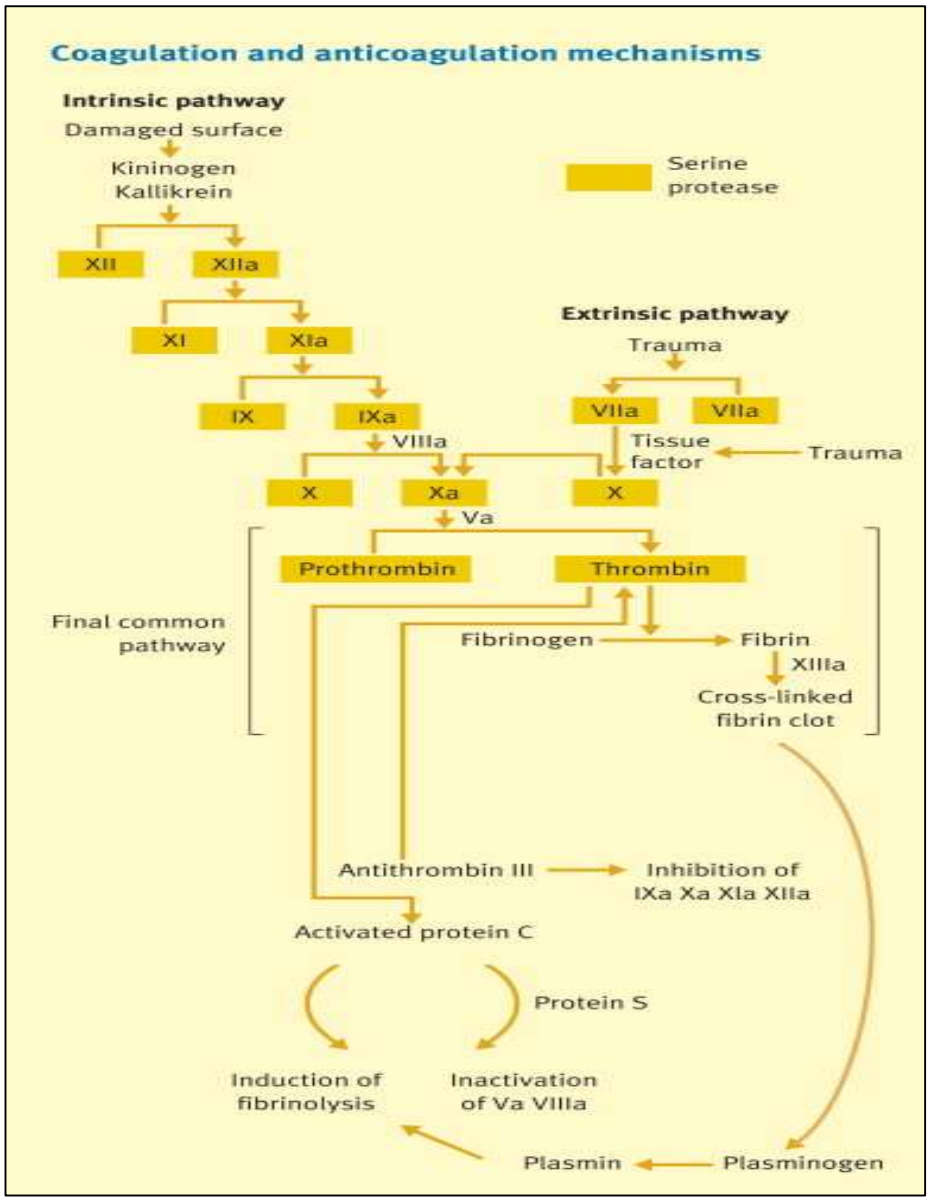


Figure 15: The coagulation cascade model showing maintenance of blood fluidity through balanced activity of pro- and anticoagulation enzymes (Saville and Brown, 2007).

The coagulation system consists of three subsystems. A procoagulant subsystem provides the rapid, localized response to the injury and because of the enmeshing of the platelets by fibrin, a haemostatic plug that is spatially constrained and mechanically stable. An anticoagulant subsystem modulates two of the key reactions of the procoagulant system, prothrombin, and factor X activation, by inactivating cofactor proteins that are critical components in making these reactions rapid and local to the injury site. The anticoagulant subsystem, through inhibitors of the clotting proteases acts to shut down the process. The fibrinolytic subsystem, by proteolytic digestion of the fibrin at reinforces the haemostatic plug, is responsible for the

temporary nature of the haemostatic plug. Digestion of the fibrin occurs after tissue repair has commenced and haemorrhage is no longer a threat (Bhagavan and Chung-Eun, 2002). Sustained coagulation is achieved when thrombin synthesized through the initial TF–FVIIa–FXa complex catalyzes the activation of FXI, FIX, FVIII, and FX (Teller and White, 2011).

1.9 Immune response following a major traumatic injury

As explained in the above sections, traumatic injuries induce an acute immunological dysfunction. This process is characterised first by over-activation of innate immune responses or hyperinflammation, followed by mitigation of adaptive immunity with decreased T-cell function (immunosuppression) causing increased susceptibility to infection and multiple organ failure (Stahel et al., 2007).

When faced with traumatic injury, two phases of immune response are triggered: the hyper-inflammatory systemic inflammatory response syndrome (SIRS) and the hypo-inflammatory compensatory anti-inflammatory response syndrome (CARS). During both phases, the injured patients are highly prone to “second hits” that could aggravate the pathophysiological cascade and cause sepsis, MOF and morbidity (Baue, 2006). When there is imbalance between these two phases of immune responses, with predominant release of any one type of mediator the possibility of sepsis, immunosuppression and MODS is much higher (Bone, 1996, Toliver-Kinsky et al., 2018). In the established SIRS-CARS model, post-traumatic sepsis and possible death is the result of a dysregulation of early innate immune response. In this model, overproduction of pro-inflammatory mediators and cytokines weakens endothelial integrity leading to inadequate perfusion and MOF. If patients survive the early SIRS event, a CARS response results. If there are additional insults such as nosocomial infection, there could be a late ‘second-hit’ and recurrent SIRS.

Other studies (Singer et al., 2016) that have considered SIRS and CARS along with genetic studies have expressed the view that a SIRS “second hit” is unlikely. (Xiao et al., 2011) confirm this view saying simultaneous occurrence of SIRS and CARS is based on gene expression as mediators of the immune response. Based on study of leukocyte genomic expression patterns Xiao et al., (2012) showed that there is a simultaneous induction both pro-inflammatory and anti-inflammatory genes and suppression of adaptive immunity genes.

Therefore, there is minimal genomic or clinical evidence for SIRS 'second-hit' (Gentile et al., 2012, Xiao et al., 2011).

1.9.1 Systemic inflammatory response syndrome (SIRS)

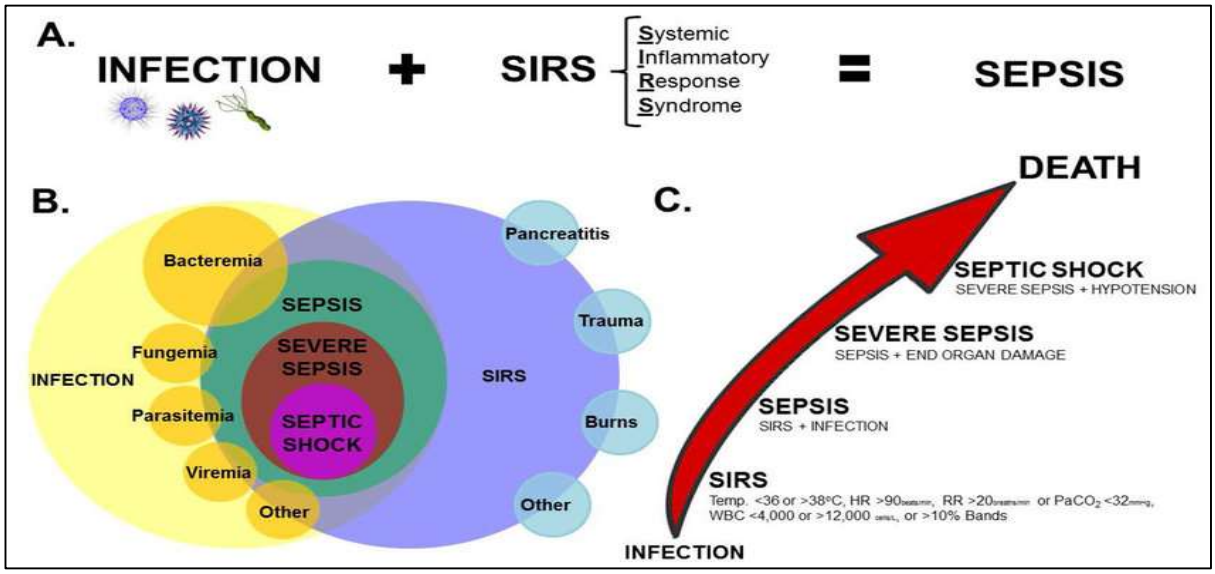


Figure 16: Representation of an earlier conceptual view and definition of systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic shock (Delano and Ward, 2016).

The term systemic inflammatory response syndrome (SIRS) was decided at the 1992 conference of the American College of Chest Physicians/Society for Critical Care Medicine (ACCP/SCCM). SIRS describes the systemic inflammatory process irrespective of the cause for the response. The introduction of SIRS was intended to define a clinical response to a non-specific insult, either infectious or non-infectious in origin. When SIRS has a suspected infection source, it is termed sepsis. It is not mandatory to confirm infection with positive cultures in the early stages. Severe sepsis is when there is failure of one or more organs. When severe sepsis is accompanied by hemodynamic instability despite replenishing intravascular fluid volume, it is called septic shock. These conditions, depicted in figure 16, together represent a physiological chain of events with progressive deterioration of the balance between the body's pro- and anti-inflammatory responses (Chakraborty and Burns, 2019).

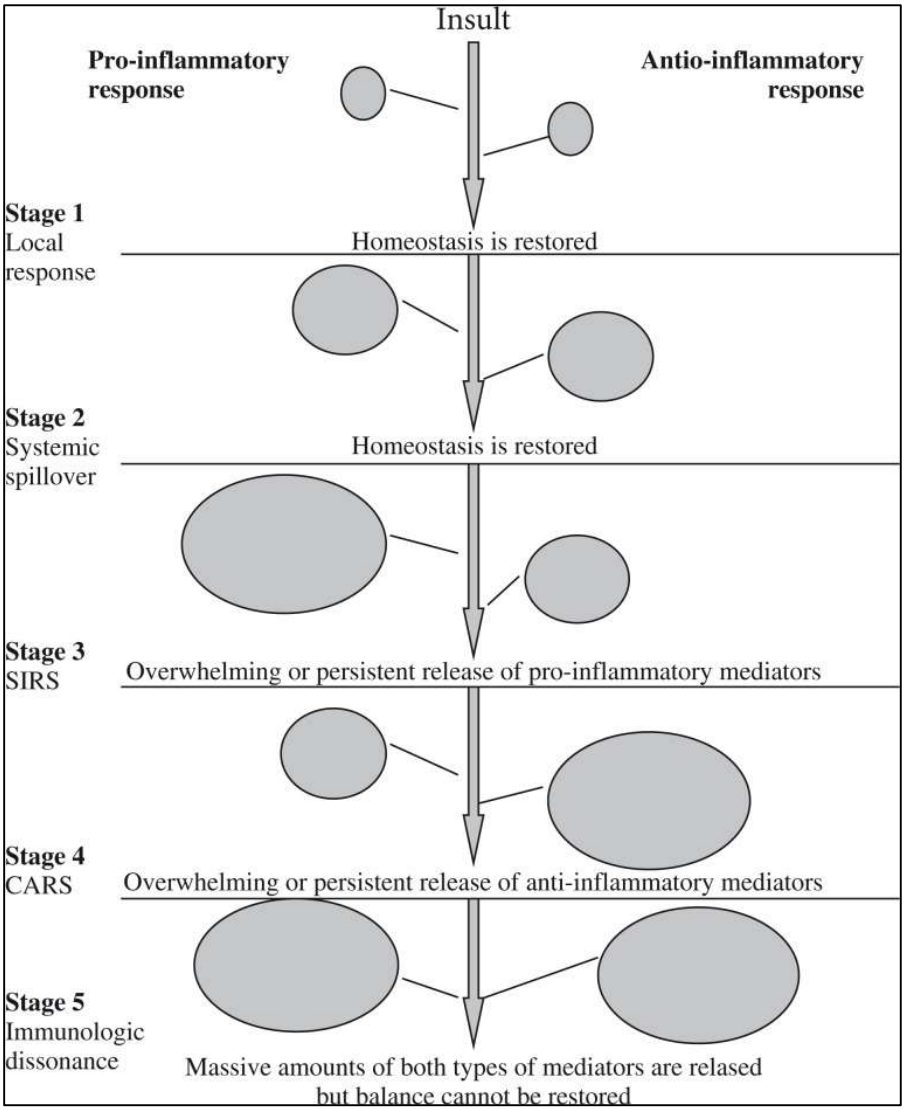


Figure 17: A hypothetical model of pro-inflammatory (SIRS) and anti-inflammatory response (CARS) to trauma and infection, which can lead to multiple organ failure (Binkowska et al., 2015).

SIRS	SEPSIS	SEVERE SEPSIS	SEPTIC SHOCK	MODS
Two or more of the following criteria: ➢ Temperature >38° or <35° ➢ Heart rate >90 per min ➢ Respiratory rate >20 per min or AP CO2 <32 mm Hg ➢ White Blood Count >12,000; <4,000 mm 3 or more than 10% of immature forms	With documented infection	Sepsis associated with organic dysfunction, hypoperfusion or hypotension	Sepsis with refractory hypotension to appropriate reanimation and hypoperfusion manifestations	Failure of two or more organs so homeostasis cannot be sustained without support

Figure 18: Clinical parameters for SIRS (Miller et al., 2008).

SIRS is confirmed on the basis of the presence of at least two out of four clinical criteria: fever (> 38.0 °C) or hypothermia (< 36.0 °C), tachycardia (> 90 beats/min), tachypnea (> 20 breaths/min), leukocytosis (> 12 × 109/L), or leukopenia (< 4 × 109/L) (Varela et al., 2018).

1.9.2 The cellular mechanisms involved in SIRS

SIRS involves interactions among haemostatic, inflammatory, endocrine, and neurological systems. It worsens the initial damage caused by hypoperfusion and reperfusion. When SIRS sets in, endothelium exposed to inflammatory cytokines loses integrity from hypoperfusion and becomes more porous, which permits mediators of tissue damage to enter the intercellular space. This could lower the ability to fight infection, leading to sepsis and further activation of the destructive inflammatory response. The activation of coagulation and neuroendocrine pathways enables humoral and cellular factors to cause damage to tissue even far from the injury site, which leads to the extracellular release of DAMPs. Thus, an inflammatory response is triggered even when there is no infection. If SIRS is persistent, it can cause MOF. Organ damage and sepsis in turn produce further exposure to PAMPs and DAMPs, thereby setting off a vicious cycle of continued inflammation and immune activation (Lord et al., 2014).

1.9.3 Compensatory anti-inflammatory response syndrome (CARS)

In a seminal study, Prof Bone (Bone,1996) described the onset of post inflammatory immunosuppression and labelled this process as compensatory anti-inflammatory response syndrome or CARS. Being an anti-inflammatory response, CARS is opposite to SIRS and is characterised by increased appearance of anti-inflammatory cytokines such IL-10 and IL-6 amongst others and also cytokine antagonists (Gentile et al., 2012).

While CARS do oppose SIRS, it does pose the risk of septic complications. Left unresolved, SIRS and CARS combine in a catabolic syndrome that leads to clinical complications, MODS and fatality. (Lord et al., 2014, Arlati, 2019) present an updated understanding CARS in which is the magnitude of cytokine release depends on the patient's own premorbid immune-inflammatory status.

1.9.4 Modified SIRS-CARS model

The modified SIRS-CARS model is derived from a wide range of genetic studies. TLRs with the exception of TLR3 and TLR7, haptoglobins, collagenases and cytokines (IL-1Ra, IL-4, IL-6, IL-8, IL-10) were assessed in T and B cells, leukocytes, neutrophils, and genes encoding proteins responsible for apoptosis. In the genetic study Xiao et al., (2011), it was found that the activation or suppression varied in conjunction with injury severity and MOF. It was also found that traumatic injury can induce the production of inflammatory mediators and activate receptor protein genes involved in the recognition of PRR. This then suppresses those receptor genes that are responsible for antigen presentation, proliferation of T cells and apoptosis. This mechanism, named "gene storm" is a synchronised response and shows that the immune system undergoes rapid adaptive changes in response to trauma. Xiao et al., (2011) concluded that changes in gene expression cause post-traumatic complications. Gene storm lasts a long period – over 28 days - in patients with complications. In contrast, where patients are without complications, gene transcription is silenced within 7-14 days (Tompkins, 2015).

The outcome of these gene studies was that SIRS was shown not to be short-term and transient because, in severely ill patients, the expression of genes involved in the immune response could be much prolonged after the injury. This can also result in the production of immature bone marrow cells called myeloid derived suppressor cells or MDSCs, which carry a

strong immunosuppressive action (Cuenca et al., 2011). This modified model is depicted in figure 19 below.

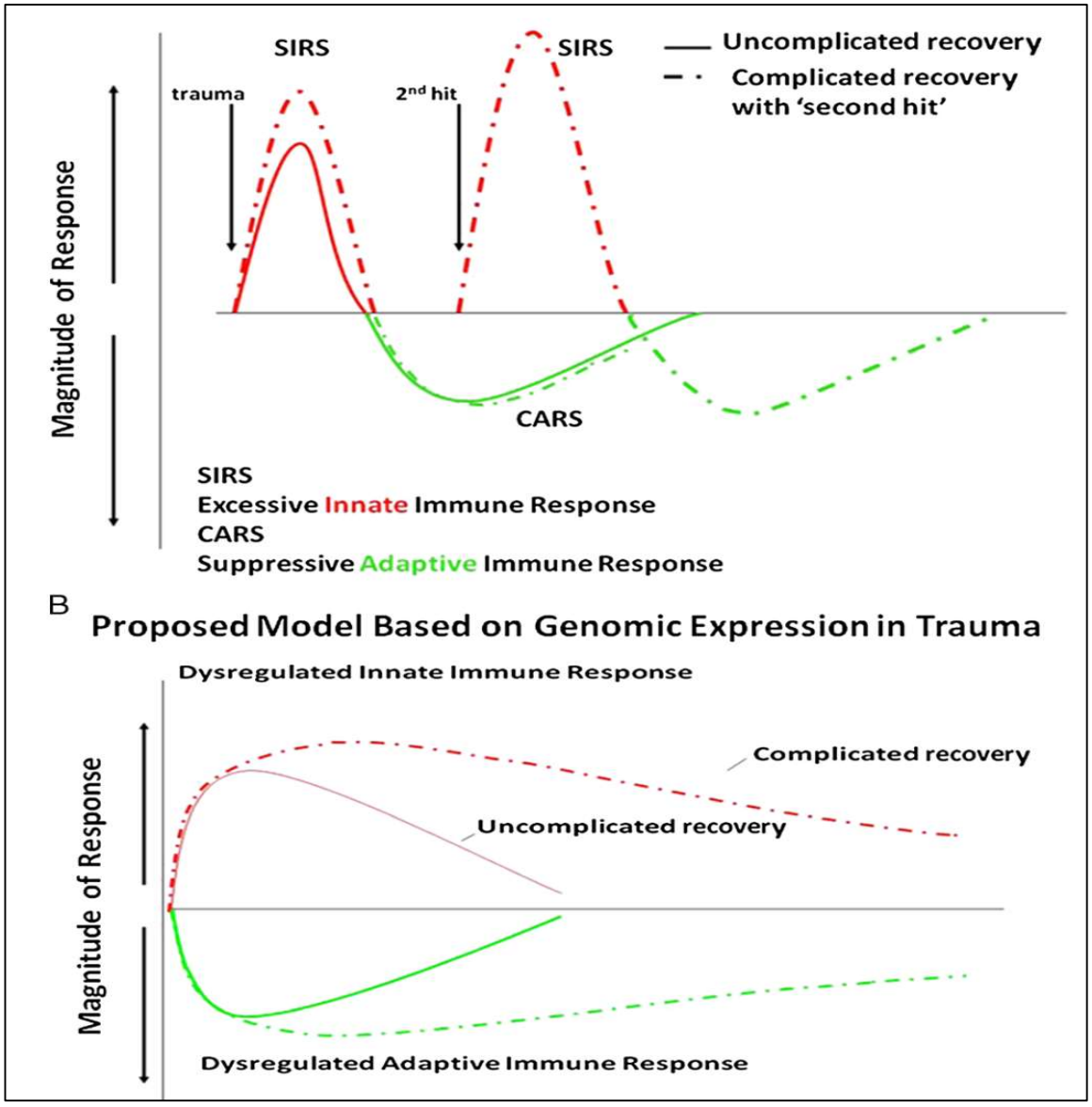


Figure 19: A modified SIRS-CARS model to adapt multiple hits
(Binkowska et al., 2015, Gentile et al., 2012).

1.9.5 Mixed antagonist response syndrome (MARS)

From the gene studies referred to in section 1.8.4, there is evidence to suggest that SIRS and CARS develop simultaneously rather than in sequence as previously believed. The mixed antagonist response syndrome (MARS) was developed to reflect the balance between SIRS

and CARS (Osuchowski et al., 2012, Leong and Yi, 2019). Figure 20, taken from Arlati (2019) shows the chronological profile of pro-inflammatory and anti-inflammatory cytokines in MARS. Here SIRS and CARS are ongoing occurrences of antagonist response syndrome.

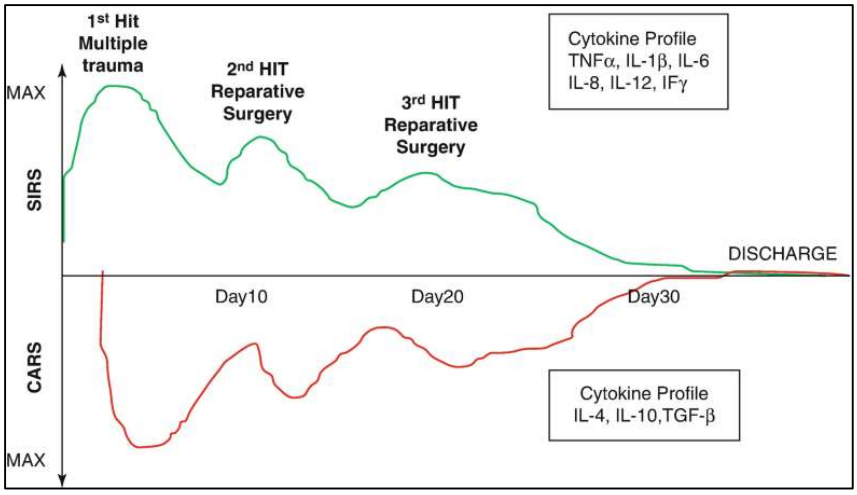


Figure 20: Chronological profile of MARS (Arlati, 2019).

1.10 Pathophysiology of sepsis

Sepsis is an inflammatory disease brought about by the activation of the innate immune system which leads to an excessive and irregular host response to an existing infection. The annual number of cases of sepsis across the globe has been estimated to be over 30 million. Of this, over 6 million patients die. Sepsis is the leading cause of mortality in hospital intensive care units. Worse, sepsis leaves long-term consequences in survivors including serious disability and additional infections which may require rehospitalization and/or long-term medical care. Thus, sepsis places a heavy burden on the healthcare systems (Monneret et al., 2019).

With SIRS at the beginning, an excessive pro-inflammatory condition occurs and is followed by the excessively anti-inflammatory CARS. SIRS can result in MODS and fatality, while advanced CARS is characterised by immunosuppression. Sepsis can lead to mortality due to secondary lethal infections. It was believed that pathogen invasion is responsible for sepsis damage but later research has shown that substantial damage is caused by excessive uncontrolled host response (Schouten et al., 2008).

Two key findings characterize the innate immune response in sepsis: initiation by infection related microbials and subsequent complement related endogenous danger signals and

specific cell-surface receptors (Takeuchi and Akira, 2010). These cells include immune, epithelial and endothelial that constantly act upon their local environment. The binding of PAMPs and DAMPs complement with other receptors to induce a complex intracellular signalling system with complementary activities, which collectively cause sepsis damage (Jaén et al., 2020).

Sepsis patients could have deranged coagulation and this is an important predictor of clinical outcome (Saracco et al., 2011). When there is dysregulation of homeostasis combined with sepsis, the related coagulopathy could vary from mild alterations to severe disseminated intravascular coagulation (DIC). Acute coagulopathy could be evident early in victims of major trauma (Brohi et al., 2007). Septic patients with DIC can often exhibit thromboembolic disease as purpura fulminans or the less apparent microvascular fibrin deposition. These characterise multiple organ dysfunction and the patient could worsen to organ failure. Therefore, patients presenting coagulopathy have higher chances for mortality than those who have similar injury patterns but do not present coagulopathy (Spahn et al., 2019).

1.10.1 Sepsis formation

Sepsis formation originates due to inadequacy of non-adaptive host factors. The deterioration in host defence mechanisms such as anatomic barriers, cellular immunity and humoral defences reduce the ability to protect against infections and paves the way for further local or systemic infections. Polat et al., (2017) showed that the host is not passive in sepsis. There is a vicious cycle because complement system induces the indigenous inflammatory mediators in organ damage and non-infectious triggers, which then leads to the same inflammatory response. The clinical response can be maintained even though the infection can be eradicated (Polat et al., 2017).

Sepsis characterises the trauma recipient's response to a severe infection. It is a very serious clinical condition that has a very high mortality rate (Gyawali et al., 2019). Sepsis arises because of inappropriate regulation of the normal physiologic responses that eradicate pathogens causing a medical emergency with potential for end-stage organ dysfunction and death. Despite significant advancements in both the understanding of sepsis pathophysiology and clinical interventions and hemodynamic monitoring tools sepsis is still a major cause of morbidity and mortality in critically ill patients. (Gyawali et al., 2019).

1.11 Innate immunity and inflammatory mediators

Immuno-response to pathogens begins with activating the innate immune cells including macrophages, monocytes and neutrophils (Vincent et al., 1996). This occurs via PAMPs binding in these cells in response to bacterial toxins or β-glucans from fungal sources. Immuno-response can also involve DAMPs binding to specific receptors on monocytes and macrophages such as TLRs, CLRs, nucleotide-binding oligomerization domain type receptors (NOD) and retinoic acid inducible gene 1 (RIG-1) like receptors. These interactions result in the activation of intracellular signal transduction pathways and release pro-inflammatory cytokines like TNFα, IL-1 and IL-6. Pattern recognition receptors, such as the NOD-like receptor group, aggregate into larger protein complexes called inflammasomes that are involved in producing cytokines such as IL-1β and IL-18. Pro-inflammatory cytokines cause build-up of leukocytes, activation of the complement system and upregulation of endothelial adhesion molecules. When sepsis occurs, the above immune response is magnified leading to the death of host cells and tissues (Gyawali et al., 2019).

1.12 Trauma induced complications

1.12.1 Acute respiratory distress syndrome

Acute respiratory distress syndrome (ARDS) is a respiratory condition that is characterized by hypoxemia and stiff lungs. ARDS is life-threatening because the patient would be dependent on mechanical ventilation, without which they die. ARDS evolves in different phases as a response to different traumatic insults (Fanelli et al., 2013). While trauma injury is not the only cause of ARDS – it is also caused by pneumonia amongst other conditions – traumatic injury and resultant sepsis are major causes of ARDS. In such cases, ARDS is often seen in conjunction with sepsis and MOF. The physiological characteristics of ARDS include pulmonary oedema, severe arterial hypoxemia and impaired CO_2 excretion (Matthay et al., 2012).

In a review of ARDS cases, (Siegel, 2016) estimated that up to 15% of patients admitted to intensive care units have ARDS. Incidence of ARDS also varies with older patients more prone to it: only 16 out of 100,000 persons in the 15 to 19 age group develop ARDS but this increases to 306 per 100,000 persons in the 75 to 84 years age group. Worldwide, nearly 3 million people per annum develop ARDS and it accounts for 23% of ICU patients who require mechanical ventilation (Kaku et al., 2019, Bellani et al., 2016). The LUNG-SAFE study looked

into that mortality associated with ARDS: 34.9% for patients with mild ARDS died while it was 40% for those suffering moderate ARDS. But this increased to 46.1% for those with severe ARDS (Matthay et al., 2012). Understandably, many different interventions have been attempted to treat ARDS but few have shown effectiveness. (Pham and Rubenfeld, 2017) take the view that means to reduce ventilator dependency are more effective than pharmacological interventions especially because mechanical ventilation can itself aggravate lung damage although it may be necessary to provide ventilation to save the patient's life (Fan et al., 2013).

An important function of the lung is carbon dioxide excretion and oxygen transfer across the distal alveolar–capillary unit. The uninjured lung has a lining of endothelial cells linked by plasma membrane structures which serves as a selective barrier to fluid and solutes (Bhattacharya and Matthay, 2013). When the patient suffers ARDS, it results in clinical and physiological abnormalities because the permeability of pulmonary tissue increases (Sharma, 2010). ARDS pathophysiology develops through acute, proliferative stage and fibrotic stages (Mackay & Al-Haddad, 2009).

A similar but less severe form of acute respiratory failure is Acute Lung Injury (ALI). The key difference between ARDS and ALI is in the degree of hypoxemia (Rezoagli et al., 2017). Both ARDS and ALI have assessed based on scoring systems. In proposing a new "expanded definition" for ARDS, Murray et al., (1988), created the Murray Lung Injury Score. The score is based on four different variables each of which is assigned a score of between 0-4 and a final score is arrived by adding the scores for the four different variables. (Rezoagli et al., 2017, Murray et al., 1988, Fanelli et al., 2013). The American-European Consensus Conference (AECC) defined ARDS as the acute onset of respiratory failure and presented criteria for assessing ALI and ARDS. Raghavendran and Napolitano (2012) provide an overview of the different scoring systems, who pose interesting question about which factors to use and weigh in determing ALI and ARDS. The AECC definition has been questioned over the years as new findings about the disease were made. Subsequently, the diagnostic criteria for ARDS was updated via the 2012 Berlin definition (Fan et al., 2018).

	Limitations of the AECC definition	Proposals of the Berlin definition
Time	The time of the disease was not defined	The time corresponding to ''acute'' was specified
Acute lung injury category	Erroneously interpreted when the PaO_2/FiO_2 ratio is between 201 and 300 mmHg, resulting in misperception	• Three ARDS subgroups were included, according to the severity, which are mutually exclusive • The term ''acute lung injury'' was removed
Oxygenation	Inconsistency in the PaO_2/FiO_2 ratio due to the effects of PEEP and/or FiO_2	• Minimum level of PEEP was added to each subgroup • FiO_2 effects are less important in the most severe subgroup
Chest X-ray	Poor reliability of chest X-ray interpretation	• The radiographic criterion was clarified • Examples of X-rays were created[a]
PCP	• High PCP and ARDS can coexist • Poor interobserver reliability in PCP measurement and the clinical assessment of left atrial hypertension	• Need to measure PCP was removed • Hydrostatic edema is not the primary cause of respiratory failure • Clinical elements[a] were created to help rule out hydrostatic edema
Risk factors	Were not formally included	• Included • When there are no risk factors, it is necessary to objectively rule out hydrostatic edema

Figure 21: AECC definition and criteria (Fioretto and Carvalho, 2013).

	Acute Respiratory Distress Syndrome
Timing	Within 1 week of a known clinical insult or new or worsening respiratory symptoms
Chest imaging[a]	Bilateral opacities—not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (eg, echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation[b]	
Mild	200 mm Hg $<$ PaO_2/FIO_2 $\leq$ 300 mm Hg with PEEP or CPAP $\geq$5 cm H_2O[c]
Moderate	100 mm Hg $<$ PaO_2/FIO_2 $\leq$ 200 mm Hg with PEEP $\geq$5 cm H_2O
Severe	PaO_2/FIO_2 $\leq$ 100 mm Hg with PEEP $\geq$5 cm H_2O

Abbreviations: CPAP, continuous positive airway pressure; FIO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure.
[a] Chest radiograph or computed tomography scan.
[b] If altitude is higher than 1000 m, the correction factor should be calculated as follows: [$PaO_2/FIO_2 \times$ (barometric pressure/760)].
[c] This may be delivered noninvasively in the mild acute respiratory distress syndrome group.

Figure 22: The Berlin definition (Thompson et al., 2017).

The Berlin definition classifies ARDS as mild, moderate and severe according to the value of PaO_2/FiO_2 ratio considered with a CPAP or PEEP value of at least 5 cmH_2O (Force et al., 2012).

1.12.2 Multiple Organ Dysfunction Syndrome

Multiple Organ Dysfunction Syndrome or MODS is a clinical condition marked by the development of sepsis and/or acute insult onset progressive physiological dysfunction in two or more organs or organ systems. Some critically injured patients survive the initial insult but could develop MODS. MODS is associated with poor clinical outcomes and its persistence after the initial inflammatory surge is a key determining factor in host survival (Fröhlich et al., 2014). Many trauma patients with MODS could further regress into multiple organ failure (MOF). MODS is, therefore, a contributor to patient mortality and its treatment accounts for a large share of the cost of acute trauma care.

MOD could arise as a systemic inflammatory response to both infectious and non-infectious insults (Rosenthal and Moore, 2016) because of cellular damage induced by sepsis. Blood coagulation and fibrinolysis systems, which normally aid the preservation of systemic and organ circulation against injuries, tend to dysfunction during sepsis and can cause DIC. DIC induces organ dysfunction and is closely associated with higher mortality (Fujishima, 2016).

The mechanism of sepsis can damage mitochondria. A damaged mitochondrion shows increased membrane permeability prompting autophagic removal and leading to mitochondrial dysfunction. This is the origin of sepsis induced cell damage (Rosenthal and Moore, 2016). Severe loss of mitochondria results in low cellular energy stores, necrotic cell death and increased inflammation because of the release of HMGB1 (Crouser et al., 2008). In severe sepsis and septic shock, microcirculatory dysfunction and mitochondrial depression cause regional tissue distress. The tissues are then unable to metabolise oxygen, a condition called microcirculatory and mitochondrial distress syndrome or MMDS (Jang et al., 2019).

The primary form of MODS refers to patients who require ICU support at the beginning but are then able to recover with relatively low overall resource requirement. There is a secondary form of MODS which refers to patients whose fail to recover from MODS. These patients require prolonged ICU stay, often suffer high rates of infection and immunosuppression, and carry higher risk of mortality (Gentile et al., 2012). Some researchers have named the latter protein catabolism syndrome or PICS (Gentile et al., 2012) and this is complicated form of MODS (Vanzant et al., 2014, Xiao et al., 2011). Better prediction of MOF, and enhanced individual monitoring and therapy could contribute to

clinically better outcomes in severely injured patients (Fröhlich et al., 2014). (Fröhlich et al., 2014) also cite other studies and data, which show that overall incidence of MODS is 32.7% in multiple trauma patients. The mortality rate is also high, approaching close to 50% (Miao et al., 2020).

Multi-trauma is defined as injury to at least two body regions with total injury severity score (ISS) < 16 usually in conjunction with SIRS on at least one day during the first 72 hours whereas MODS-MOF show both sepsis and ISS of ≥25. Previous studies found several correlations between onset of MODS after trauma with factors such as older age, high trauma scoring, the presence of shock, base deficit <8 mEq/L, hyperlactatemia>2.5 mmol/L in the first 24 h after trauma, requirement of blood transfusion of over 6 bags of packed red blood cells, elevated IL-6 level amongst clinical conditions or the need for resuscitation (Rendy et al., 2017).

Organ	**Clinical measurement**	**Upper reference limit (URL)**	**Lower reference limit (LRL)**
Heart	Troponin I	> 0.056 ng/mL	
Kidney	Estimated glomerular filtration rate (EGFR)	NA	60 mL/min/1.73 m^2
Liver	aspartate transaminase (AST) *or*	> 3 times of > 114 IU/L	NA
Liver	alanine transaminase (ALT)	> 3 times of > 105 IU/L	NA
Kupffer cells in the liver, spleen, and bone marrow.	Bilirubin	> 1.3 mg/mL	NA
Liver	Albumin	NA	< 3.5 mg/dL

Table 1: The end- stage organ dysfunction was defined with the following criteria. Adapted from (Zymliński et al., 2019).

After trauma, a pro-inflammatory systemic reaction such as SIRS is stimulated by pro-inflammatory cytokines. To restore the equilibrium, the body triggers an anti-inflammatory response. If there is good outcome, the homeostasis can be achieved. However, a dominance of anti-inflammatory response can lead to CARS or MARS.

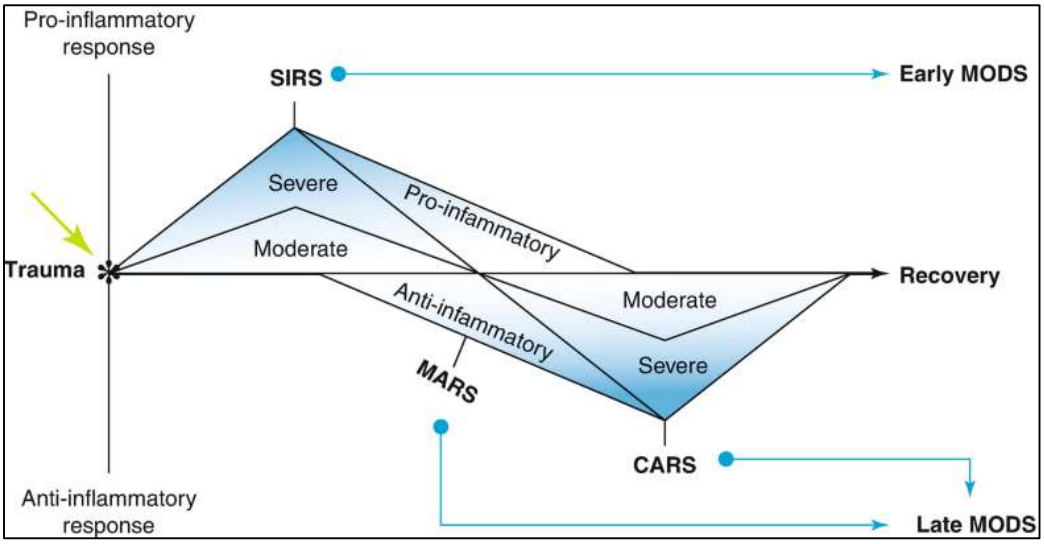


Figure 23: The inflammatory response after trauma (Hietbrink et al., 2008).

The bold blue arrow in figure 23 above shows progressive occurrence of immunosenescence according to age. The dashed line represents the level required for effective immune surveillance. This line intersects with the immunosenescence line at around age 65 i.e., onset of sepsis at age 65 or more is more difficult for the older patient to respond to. Younger patients have rapid recovery, so the length of immunosuppression and risk of infection remain less (Reinhart et al., 2017). In older patients, lengthy spell of immunosuppression may and associated increased risk of infection may mean that a full recovery might be virtually impossible. Therefore, it could be said that sepsis amplifies immunosenescence, which in the above figure would cause the blue line to slope down further and more rapidly (Monneret et al., 2019).

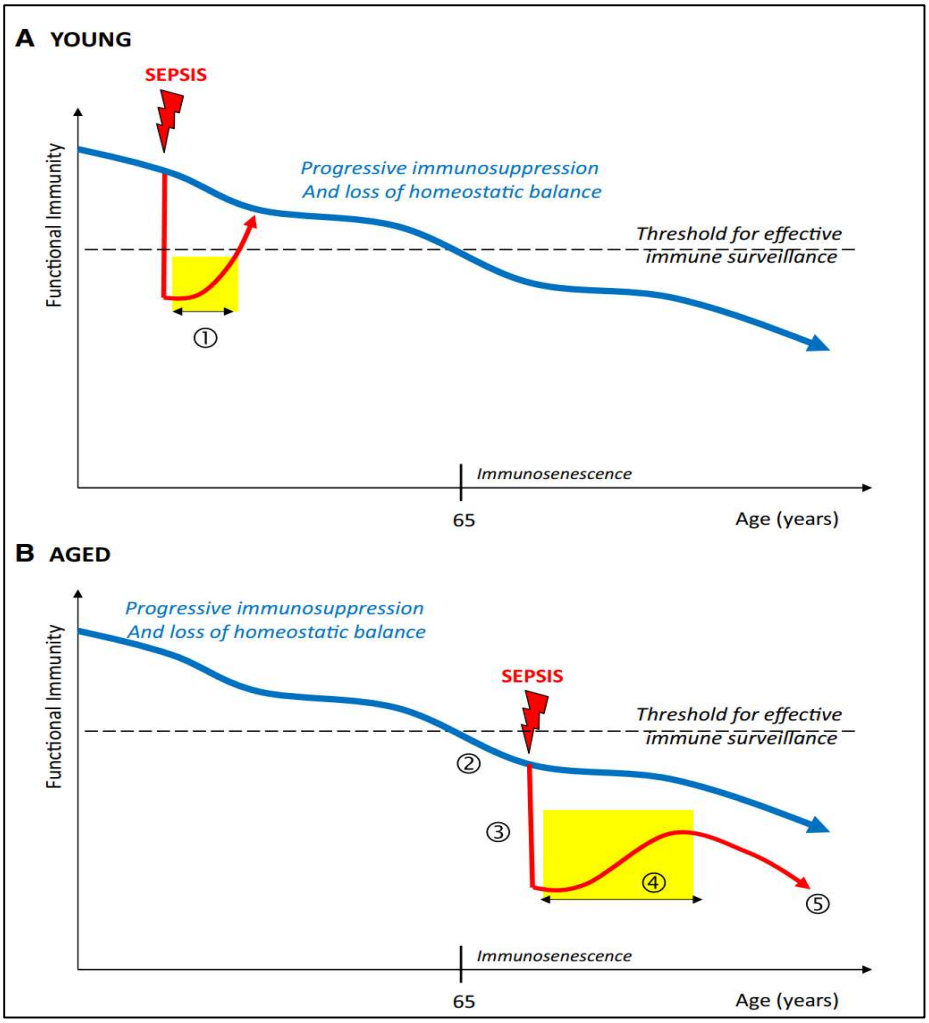


Figure 24: Immune trajectories after sepsis in young and elderly subject (Monneret et al., 2019).

1.13 Evaluating the severity of traumatic injury

The previous sections discussed the pathophysiology of severe traumatic injury. Considering how quickly patients may descend to MODS and possible negative outcomes including mortality, identifying serious injuries early and intervening appropriately could prevent further complications and save the patient's life (McLymont and Glover, 2016). Trauma scores are a useful tool to evaluate whether the patient's injuries are life threatening (Bilgin et al., 2005). Alongside inflammatory parameters, trauma scoring systems can be used prognostically to calculate the risk to the patient from clinical signs and symptoms. The rationale behind using scoring systems is that crossing the scoring threshold can determine the initiation of interventions even before clinical symptoms manifest. Earlier interventions

have been shown to improve patient outcome and reduce the possibility of mortality and morbidity (Mariani et al., 2019).

Different clinical scoring systems, based on clinical aspects and patient characteristics including psychological factors are used because scoring helps in predicting various outcomes ((Gortzis et al., 2008). Scoring is almost always used in intensive care settings but could also be used by emergency response teams to measure the severity of illness of patients. Clinically, they could also be used to understand actual patient outcome against the predictions based on the scoring. From a research perspective, the usage of severity scores enable comparison of outcomes between patients in different cohorts of patients (Bouch and Thompson, 2008). There are different scoring systems, each with relative advantages and disadvantages (Pohlman, 2020). Some scoring systems are based on anatomical description of the injuries, some give weighting to physiological parameters but the most widely used systems predicting patient outcome following traumatic injuries combine anatomical and physiological parameters relating to the injury (Chawda et al., 2004, Fuchs et al., 2019). There is a lot of ongoing research into this area and it has been claimed that future scoring systems could include a more granular measurement of molecular level data and predicting long-term outcomes (Wunsch and Kramer, 2016).

Some commonly used scoring systems are Abbreviated Injury Scale (AIS), Injury Severity Score (ISS), APACHE II, Intensive Care National Audit and Research Centre Score (ICNARC), Glasgow Coma Scale (GCS) and Sequential Organ Failure Assessment (SOFA). The following section provides descriptions of common scoring systems, in this research, the primary scoring system used was SOFA. ISS was also calculated for the cohort of patients at the time of admission.

1.13.1 Abbreviated Injury Scale and Injury Severity Score

The AIS was originally developed for use in vehicle crash investigators. Although it was later extended to be more relevant for medical audit and research, it does not by itself provide outcome prediction (Bågenholm, 2020, Palmer et al., 2016). The AIS has had several updates and now has an extended and sophisticated set of injury descriptions along with injury coding (Petrucelli et al., 1981). The injury score ranges from 1 for minor injuries to 6 for fatal injuries (Ruge et al., 2020). AIS provided the foundation for ISS and many other injury scoring systems.

Injury severity score is used as a standard injury measurement for outcome prediction in trauma. It is internationally recognized anatomical scoring system to assess trauma severity that provides an overall score for patients with multiple injuries (Loftis et al., 2018).

ISS scores range from 1 to 75. A patient will an AIS score of 6 is automatically assigned an ISS score of 75. The key threshold is 15; any patient with ISS of greater than 15 is considered as having major trauma (Brown et al., 2017, Andelic et al., 2010). Some researchers believe that there are some deficiencies in ISS (Stevenson et al., 2001) because it limits the total number of contributing injuries to a total of three, one each from the three most injured regions, which has some disadvantages. One, it may be unable for account for multiple injuries in one body region and two, the trauma injury suffered by the patient could be underscored if there are serious injuries in more than three regions and third, there is the possibility that the anatomical severity of the patient's injury severity could be underestimated, particularly if the injury is penetrating trauma (Chawda et al., 2004).

AIS Severity	Ordinal Description
1	Minor injury
2	Moderate injury
3	Serious injury
4	Severe injury
5	Critical injury
6	Virtually unsurvivable injury

AIS = Abbreviated Injury Score.

Figure 25: Abbreviated Injury Score components (Becher et al., 2013).

(Osler et al., 1997) proposed the New Injury Severity Score (NISS) with some improvements and some researchers (Chawda et al., 2004, Balogh et al., 2000) believe that NISS is better in predicting MOF after trauma.

1.13.2 Acute Physiology and Chronic Health Evaluation score

The Acute Physiology and Chronic Health Evaluation or APACHE score is extensively used for risk prediction (Wong and Knaus, 1991). It is a sophisticated scoring system that considers 34 different variables over three patient factors that influence acute illness outcome - pre-

existing disease, patient reserve, and severity of acute illness. These are combined with a chronic health evaluation , and the two combined to produce the severity score (Bouch and Thompson, 2008). The data required for APACHE II score are collected within 30 minutes of admission specialist nurses and a Microsoft Excel tool is used to convert the parameters into the APACHE II score (Akavipat et al., 2019). Thus, APACHE II is thus a mechanism for clinicians to use almost real-time patient data to obtain a dynamic assessment of the patient's condition.

	Total Acute Physiology Score								
	4	***3***	***2***	***1***	***0***	***1***	***2***	***3***	***4***
Physiological variable									
Temperature – rectal (°C)	⩾41	39–40.9		38.5–38.9	36–38.4	34–35.9	32–33.9	30–31.9	⩽29.9
Mean arterial pressure (mmHg)	⩾160	130–159	110–129		70–109		50–69		⩽49
Heart rate	⩾180	140–179	110–139		70–109		55–69	40–54	⩽39
Respiratory rate	⩾50	35–49		25–34	12–24	10–11	6–9		⩽5
Oxygenation (aDO_2 or PaO_2)	⩾500	350–499	200–349		⩽200				
					$pO_2 > 70$	pO_2 61–70	$pO_2 < 55–60$		$pO_2 < 55$
Arterial pH	⩾7.7	7.6–7.69		7.5–7.59	7.33–7.49		7.25–7.32	7.15–7.24	<7.15
Serum sodium (mEq/l)	⩾180	160–179	155–159	150–154	130–149		120–129	111–119	⩽110
Serum potassium (mEq/l)	⩾7	6–6.9		5.5–5.9	3.5–5.4	3-3.4	2.5–2.9		<2.5
Serum creatinine (mg/dl) [double score for acute renal failure]	⩾3.5	2–3.4	1.5-1.9		0.6-1.4		<0.6		
Haemocrit (%)	⩾60		50–59.9	46–49.9	30–45.9		20–29.9		<20
White blood count (total/mm^3)	⩾40		20–39.9	15–19.9	3–14.9		1–2.9		< 1
Glasgow Coma Scale Score = 15 minus actual GCS									

Age points	***0***	***1***	***2***	***3***	***4***	***5***	***6***
Age (years)	⩽44		45–54	55–64		65–74	⩾75

Chronic health score. The following points are assigned for severe organ system insufficiency or immunocompromise (10):

Points	***Patients***
2	Elective
5	Emergency

Figure 26: Acute Physiology and Chronic Health Evaluation II (APACHE II) score (KNAUS et al., 1985).

The above figure 26 shows the various parameters and criteria used in APACHE II. Once calculated, the score can be used to predicted mortality rate. A score of 0–4 shows 4% death rate, but this increases to 75% when the score is 30–34 and to 85% when APACHE II exceeds 34. In other words, higher score values are associated with increasing risk of hospital death (Sam et al., 2009, Chand et al., 2007a).

APACHE II can be combined with accurate disease descriptions to prognostically stratify acutely ill patients to help investigators compare not only the relative success of different therapies but also the effectiveness of ICU treatment in different medical centres (KNAUS et al., 1985). Despite its many advantages, Chand et al. (2007) and Ho (2007) point out to a

potential limitation in that APACHE II is focused on the first 24 hours the patient spends in ICU but does not take into account either the subsequent course of the patient's illness or the medical therapies delivered to the patient (Ho, 2007, Chand et al., 2007b).

1.13.3 Glasgow coma scale

Glasgow Coma Scale or GCS is used as a means to arrive at an objective description of the extent of impaired consciousness in acute medical and trauma patients. Used in multiple settings such as ICUs and emergency response by paramedics, the scale assesses patients under three areas of responsiveness: eye-opening, motor, and verbal responses (Kurniawan et al., 2020). These are each separately reported to obtain a clear, communicable picture of the patient's state. Impairment of consciousness is a dangerous condition as it could be an indicator of decline in patient's condition and increased risk of mortality (Jain et al., 2019).

Criteria		GCS score	SCS score
Eye opening	Spontaneous	4	-
	To speech	3	
	To pain	2	
	None	1	
Verbal response	Oriented	5	2
	Confused conversation	4	
	Inappropriate word	3	
	Incomprehensible sounds	2	1
	None	1	
Motor response	Obeys commands	6	2
	Localizes pain	5	
	Normal flexion (withdrawal)	4	1
	Abnormal flexion (decorticate)	3	
	Extension (decerebrate)	2	
	None	1	
Total score		3–15	2–4

Figure 27: Glasgow coma scale (GCS) and simplified consciousness score (SCS) (Kim et al., 2018).

The total score is the sum of the scores in the above three categories. GCS scores range from 3–15. Scores of 3–8 are usually associated with patients in coma.

There are some limitations in the contexts to which GCS score can be applied. For example it is not a scale that can accurately reflect extracranial injuries (Offner et al., 1992). GCS also has limitations in its ability to accurately score those patients who are intubated and mechanically ventilated or those who may be under the influence of alcohol or drugs.

1.13.4 Sequential organ failure assessment (SOFA) score

Sequential Organ Failure Assessment or SOFA is a sophisticated scoring system for assessing the risk of organ dysfunction and organ failure following traumatic injuries (Vincent et al., 1996). Developed in 1994, SOFA is based on six separate scores for the respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems. For each scoring area, the values can range from 0 to 4, with higher SOFA scores indicating worsening organ dysfunction. (Marshall et al., 1995). SOFA is extremely useful in creating a score-based assessment of the degree of organ dysfunction or failure over time for both individual patients and, for groups of patients.

Table 2. Sequential (sepsis-related) organ failure assessment (SOFA) score.[8,9]

System	Score				
	0	1	2	3	4
Respiration					
PaO_2/FIO_2, mmHg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory support
Coagulation					
Platelets, $\times 10^3$ μL^{-1}	≥150	<150	<100	<50	<20
Liver					
Bilirubin, mg dL^{-1} ($\mu mol\ L^{-1}$)	<1.2 (20)	1.2–1.9 (20–32)	2.0–5.9 (33–101)	6.0–11.9 (102–204)	>12.0 (204)
Cardiovascular	MAP ≥ 70 mmHg	MAP < 70 mmHg	Dopamine < 5 or dobutamine (any dose)[a]	Dopamine 5.1–15 or epinephrine ≤ 0.1 or norepinephrine ≤ 0.1[a]	Dopamine > 15 or epinephrine > 0.1 or norepinephrine > 0.1[a]
Central Nervous System (CNS)					
Glasgow Coma Scale score[b]	15	13–14	10–12	6–9	<6
Renal					
Creatinine, mg dL^{-1} ($\mu mol\ L^{-1}$)	<1.2 (110)	1.2–1.9 (110–170)	2.0–3.4 (171–299)	3.5–4.9 (300–440)	>5.0 (440)
Urine output, mL per day				<500	<200

FIO_2: fraction of inspired oxygen; MAP: mean arterial pressure; PaO_2: partial pressure of oxygen.
[a]Catecholamine doses are given as $\mu g\,kg^{-1}\,min^{-1}$ for at least 1 h.
[b]Glasgow Coma Scale scores range from 3 to 15; higher score indicates better neurological function.

Figure 28: Sequential (sepsis-related) organ failure assessment (SOFA) score (Singer et al., 2016, Gyawali et al., 2019).

SOFA score is usually calculated on admission to ICU and could be repeated at every following 24- hour period. If the physiological parameters do not match any row, zero points are given. In cases where the physiological parameters match more than one row, the row representing the highest score is selected (Lambden et al., 2019). Calculating SOFA does require sufficient data and there may be practical challenges in accurately assessing SOFA score due to its complexity (Seymour et al., 2016). Alternatives methods such as Quick Sequential Organ

Failure Assessment (qSOFA) score was recently introduced as a novel method to make a quick estimation of mortality risk of patient admitted in hospital with suspected infection but are treated outside of ICU (Jawa et al., 2017). The qSOFA score has also been useful in predicting mortality in assessments conducted in emergency departments. Therefore, qSOFA may help the medical team make decisions about the need for ICU care and aid better resource allocation in hospitals (Jawa et al., 2017, Singer et al., 2017). Research into qSOFA has shown that it can help identify patients potentially at risk of dying from sepsis (Marik and Taeb, 2017). In their research, Tian et al., (2019) showed that the qSOFA score ≥2 might identify higher risk of mortality, regardless of whether the patient is septic or not.

Quick Sequential Organ Failure Assessment (SOFA) score

qSOFA (Quick SOFA) Criteria	Points
Respiratory rate ≥22/min	1
Change in mental status	1
Systolic blood pressure ≤100 mmHg	1

Figure 29: Criteria for quick SOFA (Tian et al., 2019).

In a study of 64 multiple trauma patients, Tranca et al., (2016) showed that all severity score systems aid in predicting not only the mortality rate but also the risks of occurrence of sepsis in multiple trauma patients. They offer significant advantages over using sole markers such as CRP, albumin or lactate performance (Basile-Filho et al., 2019). Not only do they allow physicians to direct efforts towards patients who would benefit most following initial triage, but they can also help organizational and management choices by evaluating the level of care given in individual departments, centres etc for comparison.

1.14 Cytokines

Cytokines are small glycoproteins that synchronize the development of the body's immune and inflammation responses. They are secreted mainly by lymphocytes, monocytes, and macrophages. Cytokines affect the interactions between cells and when released, function as signals to trigger immune system response. Released in several paracrine, autocrine and

endocrine pathways, cytokines are potent mediators of inflammation and pathogen elimination by displaying both pro-inflammatory and anti-inflammatory mechanisms (Monastero and Pentyala, 2017) and display their pleiotropic nature in different contexts (Kassab et al., 2019).

At less than 40kDa, cytokines are small in size and are categorised as interleukins, chemokines, interferons, and tumour necrosis factors depending on the structural homologies of their receptors (Dinarello, 2007). They are non-structural and extracellular glycoproteins that participate in the host defence mechanism by acting on the cells which express complementary receptors (Oppenheim, 2001, Rodney et al., 2018a). Generally, cytokines act locally at the area of the injured target organ or organ system where they are generated but the production of substantial amounts of cytokines may cause them to stream into the circulation and make them detectable in serum samples. Therefore, measurements of serum cytokine levels can serve as biomarkers of disease severity and provide an indication of the inflammation mediated traumatic injury (Mack, 2007).

Broadly, cytokines express robust modulation during progressive stages of inflammation, immune response, and repair mechanisms and thus cytokines are widely preferred means of biomarker discovery (Tarrant, 2010).

1.14.1 The role of Cytokines in inflammation

Cytokines play an important role in mediating the inflammatory response to tissue injury. Cytokines initiate some of the systemic changes which occur (Desborough, 2000). After major traumatic injuries, the concentration of pro- and anti-inflammatory cytokines is altered. A lot of researchers have attempted to study how immuno-modulators can predict clinical evolution and outcome in patents who have suffered major trauma. Cytokines such as TNF-α, IL-6, IL-10 and IL-8 have been studied in some detail before (Valparaiso et al., 2014).

Since cytokines are effective mediators of inflammation and their measurement can help understand disease severity and progress. Cytokines are usually generated and act in the local area or organ that has suffered injury but when they are produced in large amounts, they stream into the circulatory system and are detectable within the serum (Mack, 2007).

Although the immunological response to severe trauma largely depends on the expression patterns of secreted cytokines there could be wide changes in cytokine expression at various

time points following trauma (Jastrow III et al., 2009). However, a better understanding of cytokines and the factors that determine their production is required to make early predictions of clinical outcomes of critically ill patients, and to also design cytokine based therapeutics that could be used to modify the host immune response in severe trauma (Oberholzer et al., 2000).

The initial cytokine mediated response of the innate immune system is pro-inflammatory. At this stage, cytokines such as IL-1, IL-6, IL-8, IL-17 and interferon-γ are released causing the illness to worsen. Subsequently, there is an anti-inflammatory comeback with the release of IL-4, IL-10, IL-13 and transforming growth factor-β to reduce the inflammation and promote healing. Pro-inflammatory cytokines are produced by activated macrophages and engage in up-regulating inflammatory reactions. Some pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are involved in the process of pathological pain (Zhang and An, 2007).

Cytokine production stimulates its target cell to secrete additional cytokines leading to a cascade which determines the inflammatory response to both injury and subsequent healing (Rodney et al., 2018a). Pro-inflammatory cytokines trigger a cytokine storm, which activates endothelial cell dysregulation and increases its permeability causing extravasation of large molecules and fluid into the interstitium. The endothelial cells then acquire a prothrombotic tendency and form microthrombi. Vasodilation sets in when the endothelium releases nitric oxide and further perpetuates hypotension, hypoperfusion and reduced oxygen delivery (Frankenstein et al., 2006). Consequent cellular hypoxia and mitochondrial dysfunction could culminate in multiorgan dysfunction syndrome (MODS) and even death. A pro-inflammatory cytokine storm is thus associated with the development of MODS and higher risk of mortality.

Cytokine-mediated immunity response is critical to the pathophysiology of sepsis. (Brown et al., 2006). Like many hormones, cytokines act by binding to a receptor on the target cell and alters the function of the target cell (Corwin, 2000). A patient in this condition suffers weakened immunity and faces the risk of secondary infections and mortality. (Delano and Ward, 2016, Galley and Webster, 1996). The subsequent anti-inflammatory response tries to mitigate this by reducing the number of circulatory lymphocytes and monocytes, as well as their functional ability.

The consequences of inflammatory response are determined by the balance between pro- and anti-inflammatory mediators. In many infectious diseases and autoimmune and allergic conditions, there is imbalance between pro-inflammatory and anti-inflammatory cytokines. Cytokines exhibit a lot of variability and have short half-lives and it could be difficult to precisely identify whether pro- or anti- inflammatory mediators of inflammatory response are predominant. If the patient exhibits MODS or extreme immunosuppression, consideration of other clinical parameters may be needed to understand which mediators are predominant. Elenkov and Chrousos., (2002) express the view that further research into the regulation of pro- and anti-inflammatory cytokine balance is needed to obtain a better understanding of many diseases.

The panel of cytokines chosen for the analysis in the pilot study are IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17 and TGF- β. The following sections discuss IL-13 and IL-17. One of the objectives of this research is to make comparative analyses of IL-13 and IL-17 with three other cytokines: IL-4, IL-8 and Il-12. More information about the latter three cytokines have been included in the appendices 7.10.

1.14.2 Interleukin-13 (IL-13)

IL-13, an anti-inflammatory cytokine has a vital role in monocyte maturation and proliferation and the production of inflammatory mediators such as cytokines and chemokines. In humans, the IL-13 gene is located on chromosome 5q31-33 in a cluster of genes encoding a few different interleukins and the granulocyte-macrophage colony-stimulating factor (GM-CSF). IL-13 was originally cloned from activated human T-lymphocytes (Marone et al., 2019) and produced by stimulated Th2 cells, B lymphocytes, CD8+ cells , type 2 Innate lymphoid Cells (ILCs), alveolar macrophages, human mast cells and basophils (Ochensberger et al., 1996, Redrup et al., 1998, Borriello et al., 2015, Jia et al., 2016). IL-13 promotes B-cell proliferation and IgE synthesis and induces the deposition of extracellular matrix proteins in fibroblasts.

The gene encoding IL-13 is an upstream of the IL-4 gene. These two cytokines have 25% homology commonality and share some functional properties. IL-4 and IL-13 both act on hematopoietic immune cells and non-hematopoietic immune cells. Together, these actions are critical in the phenotypes of allergic diseases such as AD, asthma, and Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) (Matsunaga et al., 2020).

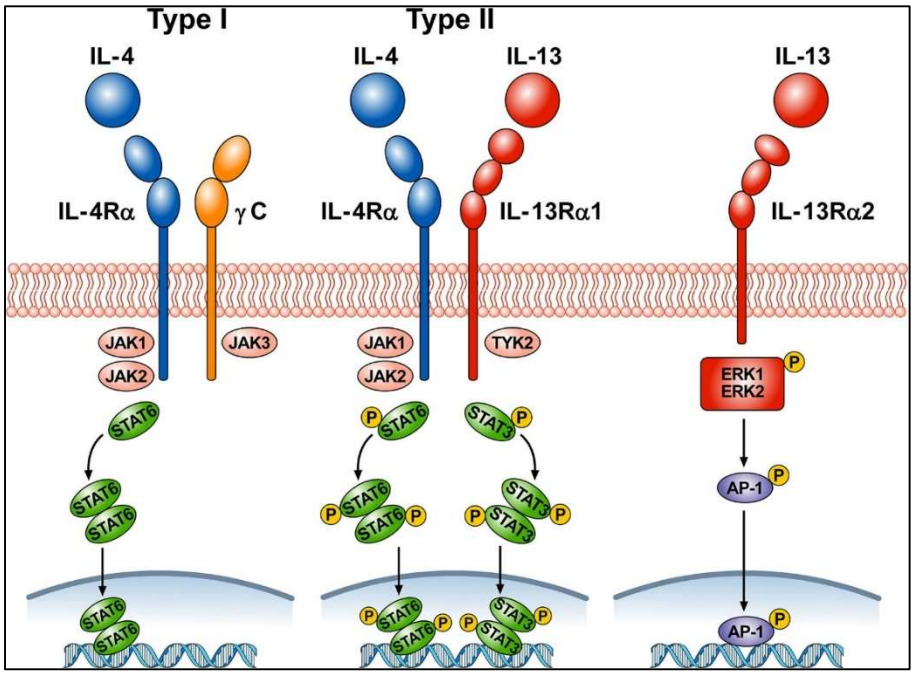


Figure 30: Schematic representation of IL-4 and IL-13 signalling pathways and the three receptors that bind IL-4, IL-13, or both (Marone et al., 2019).

IL-13α1 receptor binds to the type 2 IL-4R receptor (Matsunaga et al., 2020) and the receptor complex leads to activation of JAK1 and JAK2 Janus family kinases, and tyrosine kinase 2 (TYK2). Kinase activation triggers STATs recruitment, phosphorylation, and dimerization. The STAT dimers bind specific DNA elements and activate downstream genes (Marone et al., 2019). IL-13 uses only its type II receptor for signalling (Seyfizadeh et al., 2015). IL-13 also binds to a third IL-13Rα2 and this IL-13 using IL-13Rα2 causes phosphorylation of ERK1/2 in a STAT6-independent manner. This form of the dimeric transcription factor AP-1, which upon phosphorylation translocate to the nucleus and bind to specific DNA elements (Marone et al., 2019).

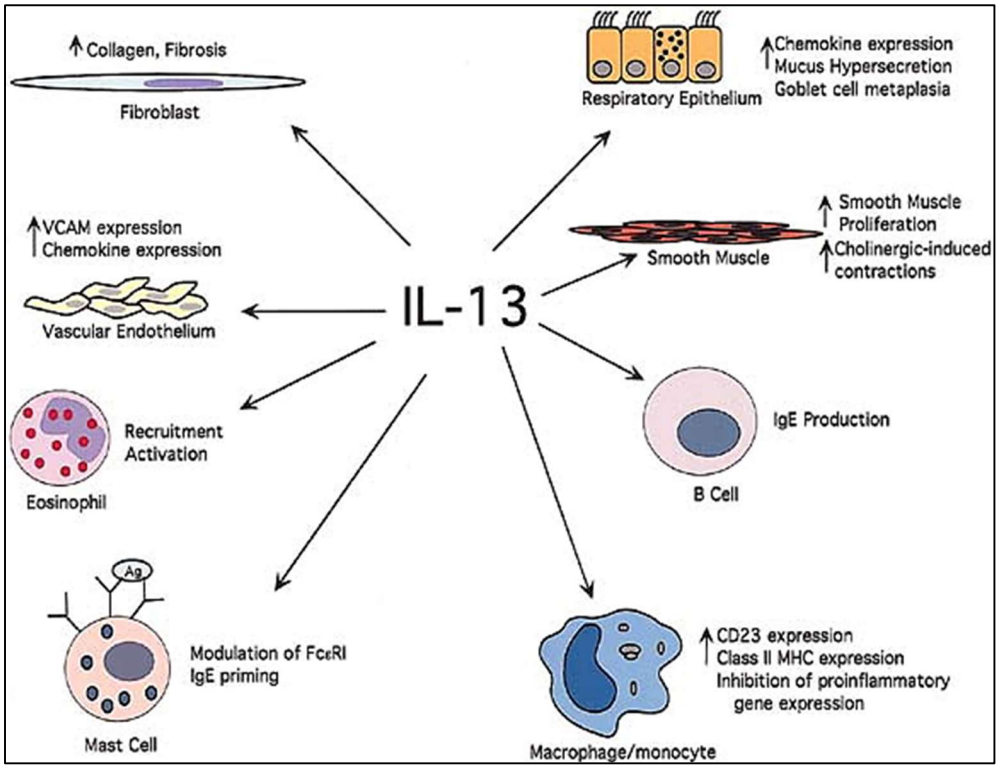


Figure 31: An overview of varied actions of IL-13 on hematopoietic and nonhematopoietic cells (Hershey, 2003).

IL-13 has diverse functions relevant to the pathogenesis of allergic disorders (Hershey, 2003). It impacts a wide variety of cell types demonstrating the potent immunoregulatory role of IL-13. In human B cells, IL-13 shows similar effects as IL-4. IL-13 promotes B-cell proliferation and induces class switching to IgG4 and IgE along with CD40/CD40 ligand co-stimulation. It also induces the expression of surface antigens, including the low-affinity IgE receptor CD23 (Akdis et al., 2016). In monocytes and macrophages, IL-13 up-regulates the expression of many integrins such as CD11b, CD11c, CD18, and CD29. IL-13 inhibits prostaglandins, reactive oxygen and nitrogen intermediates, which are all pro-inflammatory mediators produced by monocytes and macrophages (Doherty et al., 1993). IL-13 also has important functions on nonhematopoietic cells, including endothelial cells, smooth muscle cells, fibroblasts, and epithelial cells. When faced with inflammation, blood monocytes leave the blood stream and develop into inflammatory macrophages. The inflammatory macrophages are recruited to identify pathogens and clear cellular debris within the tissues (Jakubzick et al., 2017, Chousterman et al., 2017).

In endothelial cells IL-13 is a potent inducer of vascular cell adhesion molecule, which is important in the recruitment of eosinophils. IL-13 enhances proliferation and cholinergic-induced contractions of smooth muscle cells in vitro and induces type I collagen synthesis in human dermal fibroblasts (Wills-Karp, 2001). Stimuli such as allergens, pollutants and viral and bacterial infections activate epithelial cells which release cytokines. Cytokine mediators activate a variety of immune cells including type 2 innate lymphoid cells, Th2 cells, mast cells, macrophages, basophils, eosinophils, B cells, all of which produce several cytokines including IL-13. In epithelial cells, IL-13 is a potent inducer of chemokine expression (Marone et al., 2019) by inducing various changes that are mediated by the engagement of type 2 IL-13 receptor. These changes include goblet cell hyperplasia, mucus production, smooth muscle cell hyperplasia in airways, triggering fibroblasts, activating B cell and producing IgE. IL-13 and IL-4 can both initiate sensory neurons by engaging type 2 receptor (Marone et al., 2019, Giuffrida et al., 2019).

Various animal studies conducted by (Zurawski and de Vries, 1994, Zhu et al., 1999, Zheng et al., 2000, Hershey, 2003) and Han et al. (2015) have shown the role IL-13 plays in allergy induced inflammation and also that IL-13 is an effector module in asthma and other obstructive pulmonary disorders.

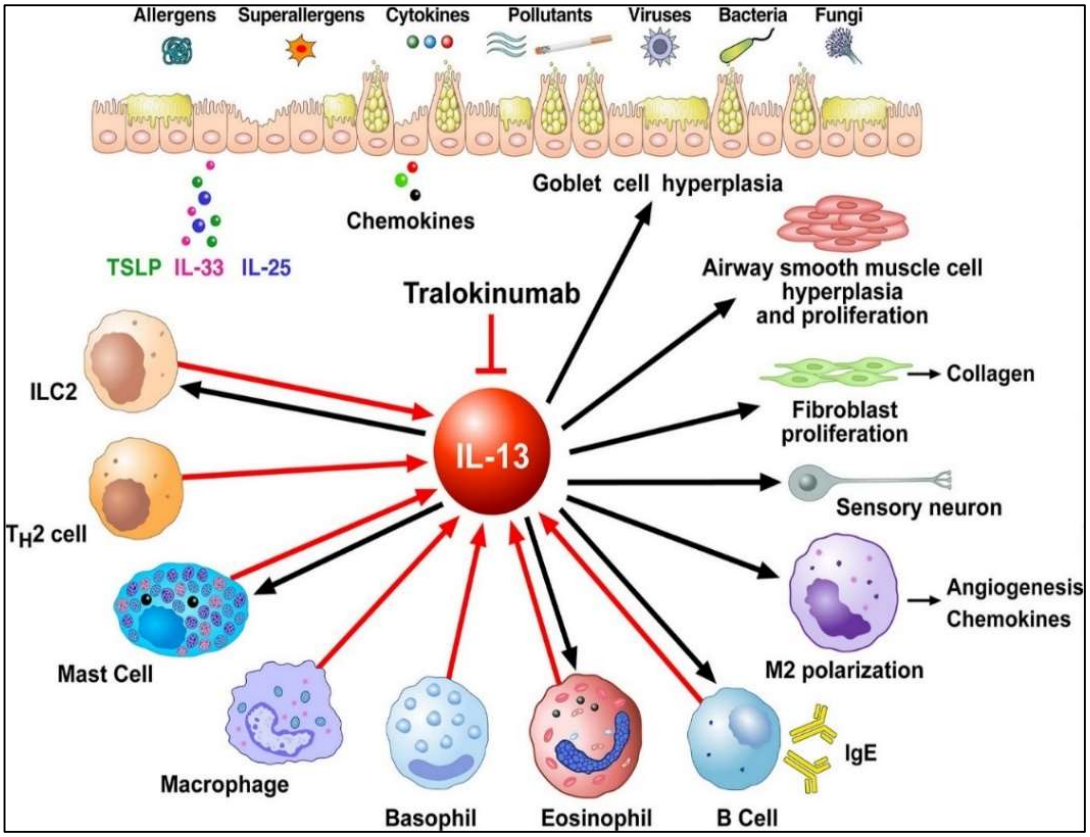


Figure 32: Schematic representation of the cellular sources of IL-13 (red arrows) and its effects of immune and structural cells in asthma (black arrows) (Marone et al., 2019).

1.14.3 Interleukin-17 (IL-17)

IL-17 is a pleiotropic pro-inflammatory cytokine that is mainly produced by Th17 cells, CD8+ cells, innate immune cells such as lymphocytes ($\gamma\delta$ T cells). IL-17 is also produced by natural killer T (NKT) cells, T cell receptor TCR β+ cells and natural Th17 cells (Amatya et al., 2017). IL-17 is a topic of active research because of the pathogenic role it plays in inflammatory conditions such as ischemia, reperfusion injuries and chronic inflammatory diseases such as asthma, inflammatory bowel disease, arthritis and autoimmune disorders (Dai et al., 2015, Ge et al., 2020). As a pro-inflammatory cytokine, IL-17 links T cell activation to the mobilization and activation of neutrophils. IL-17 cytokine is also called IL-17A to denote the fact that it is the primary member of the IL-17 family that has six structure-related cytokines IL-17 A to F (Weaver et al., 2007).

The IL-17 cytokine family has a great capacity for synergism and their potency could be amplified by the cytokine milieu found at the inflamed site. From a clinical and therapeutic

perspective, the behavioural properties of IL-17 are an area of research because a comprehensive understanding of the mechanisms of tissue-specific functions of the IL-17 family is required. Therapeutic considerations include blocking more than one cytokine, but Brembilla et al. (2018) posit that rational drug design needs a better understanding of the relationship between different members of the IL-17 family.

Although its specific behaviour is dependent on the environment in which it is produced, IL-17, like some other cytokines, has functions related to T cells, epithelial cells, endothelial cells and fibroblastic cells (Martin et al., 2014, Abdel Galil et al., 2015). All IL-17 cytokines produce similar effects in target cells but could elicit very different and even opposite functions in some tissue-specific contexts. It can trigger multiple pro-inflammatory mediators including IL-1, IL-6, TNF-α and chemokines (Kolls and Lindén, 2004). IL-17 has been associated with the pathogenesis of infectious diseases, cancer and inflammatory diseases (Zenobia and Hajishengallis, 2015). It also contributes to the pathogenesis of autoimmune diseases including psoriasis, rheumatoid arthritis, inflammatory bowel disease and systemic sclerosis. Ge et al.'s (2020) recent research shows that IL-17 is both a biomarker and a therapeutic target in sepsis. IL-17 recruits and activate neutrophils and mediates protective innate immunity against pathogens (Ebrahim et al., 2019).

IL-17 signals through the IL-17R receptor family which exhibits a broad expression pattern with IL-17RA being ubiquitous (Gaffen, 2011). IL-17R subunits are single transmembrane chains and the extracellular region of IL-17R subunits contains two fibronectin III-like (FN) domains to mediate the protein to protein interactions and ligand binding (Brembilla et al., 2018). All cytokines in the IL-17 family signal using five different heterodimeric receptors, which share the common cytoplasmic signal domain called SEFIR, which stands for similar expression of fibroblast growth factor (SEF/IL17R). IL-17 binds to IL-17RA and IL-17RC receptors to mediate signalling (Amatya et al., 2017). The two receptors create the conserved SEFIR signal domain, which initiates the Act1 adaptor (the ubiquitin ligase enzyme). Act1 recruits TRAF6 and activates the nuclear factor-κB (NF-κB) pathway. Act1 is an up-stream of the CCAAT/Enhancer Binding Protein (C/EBP)-β and C/EBP-δ, and mitogen-activated protein kinase (MAPK) pathways, which act in tandem to control target gene expression. Most IL-17 downstream genes have NF-κB and C/EBP binding sites, and in many cases both are necessary for IL-17-mediated promoter activity (Onishi and Gaffen, 2010).

Cytokine	Other names	Cellular source	Receptor	Major functions
IL-17	IL-17A, CTLA-8	T cells (memory)	IL-17R (also known as, IL-17AR)	Inflammation, neutrophil recruitment, cytokine secretion, bone metabolism
IL-17B		Multiple organs	IL-17BR (also known as, IL-17Rh1/Evi27)	Cytokine secretion, inflammation
IL-17C		Unknown	Unknown	Regulation of Th1 cytokines
IL-17D	IL-27	Multiple organs	Unknown	Cytokine secretion
IL-17E	IL-25	Th2	IL-17BR (also known as, IL-17Rh1/Evi27)	Regulation of Th2 cytokines
IL-17F	ML-1	$CD4^+$ T cells and monocytes	IL-17R?	Angiogenesis, cytokine secretion, regulation of Th1 cytokines
HVS13	vIL-17	Herpesvirus saimiri infected cells	IL-17R?	Unknown (not required for cellular transformation)

Figure 33: The family of IL-17 group of cytokines with their cellular source, receptors and characteristic functions (Brembilla et al., 2018).

IL-17 cytokines can function as homodimers and also form heterodimers. They use the IL-17 A–E receptor subunits. IL-17A, A/F and F dimers bind with IL-17 receptors A–C to promote tissue repair and provide immunity against bacteria and fungi. The interaction of IL-17E with IL-17 receptors A–B complex induces humoral immune responses, which are required for protection from parasites. Pappu et al. (2011) express the view that some aspects of IL-17 receptor subunits are still unknown. For example, it is not fully understood whether IL-17B behaves like the other family members and uses IL-17 receptor A. The IL-17 receptor D is an orphan receptor and while it is understood that there is interaction between IL-17B and IL-17RB, the biological function of this interaction is not yet understood. (Pappu et al., 2011). It has, however, been found that IL-17C interacts with IL-17RA and IL-17RE to trigger host defence. Likewise, IL-17E binds to IL-17RA and IL-17RB complex to mediate Th2 response. It has also been identified receptor binding molecule for IL-17A, IL-17F, IL-17C and IL-17E mediated signalling is Act1 (Song and Qian, 2013).

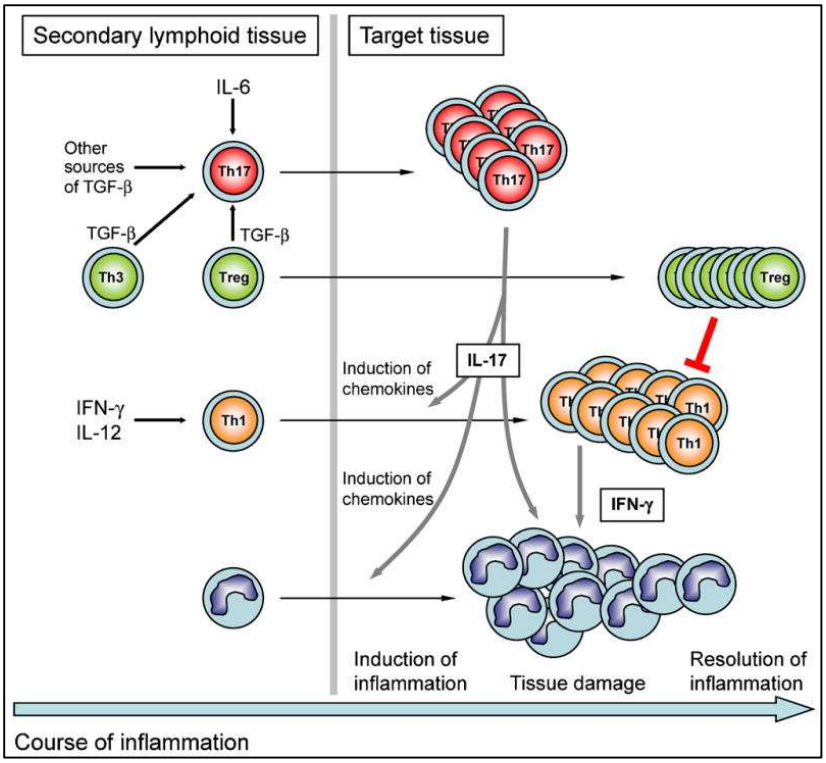


Figure 34: Development of tissue inflammation driven by Th17 cells (Korn et al., 2007).

Th17 cells, which are amongst the main sources of IL-17, are strong inflammatory cells that cause inflammation of tissues and induce the infiltration of other inflammatory cells into the target organ. As part of IL-17 mediated immunity response, antigen specific T cells primed in secondary lymphoid tissue are sent to the Th17 developmental pathway alongside IL-6 and TGF-β. The activated Th17 cells infiltrate the target organ and by inducing chemokines trigger a secondary wave of inflammatory response involving recruiting mononuclear cells and Th1 cells to the inflamed tissue. IL-17 produced by Th17 and IFN-γ produced by Th1 both act on myeloid cells to induce their effector functions that eventually leads to tissue damage. Whereas Th17 cells are prone to apoptosis, Th1 cells might finally be suppressed by T-reg cells that accumulate in the target organ and thus induce the resolution of inflammation.

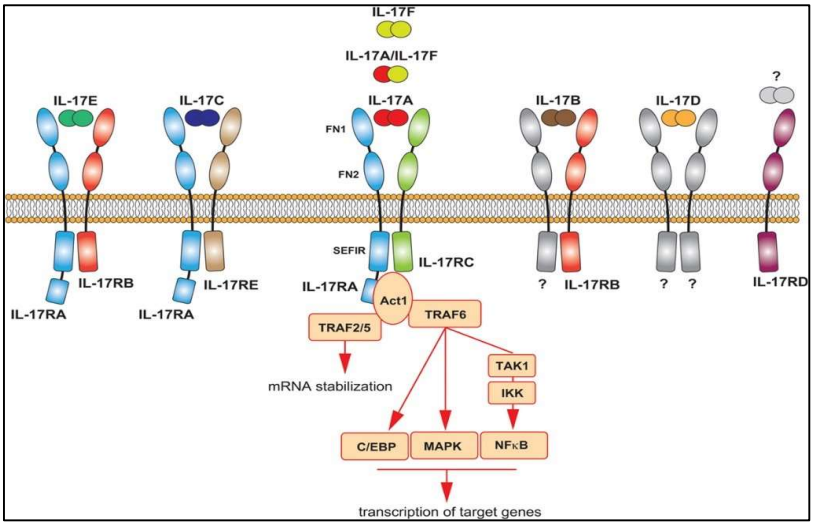


Figure 35: IL-17 family of cytokines with their receptors (Brembilla et al., 2018)

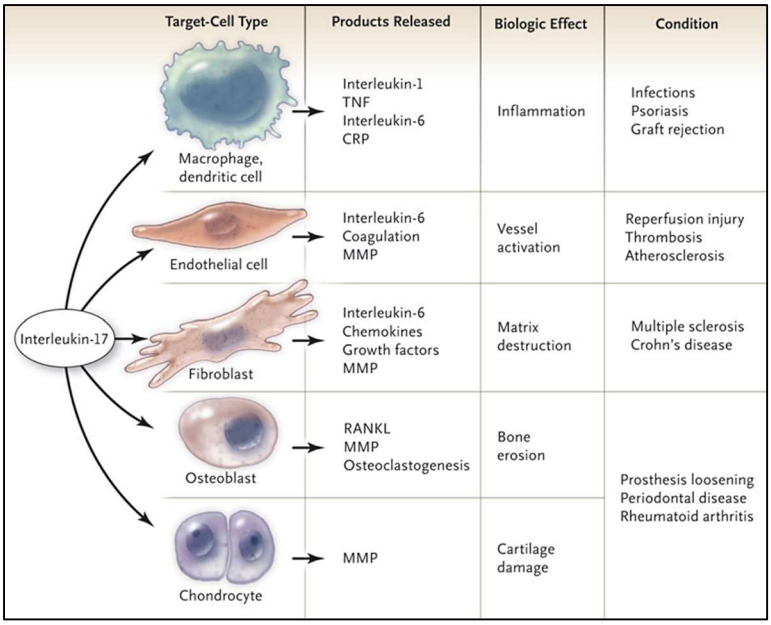


Figure 36: Effects of IL-17 on cell functions and Its role in the pathophysiology of diseases (Miossec et al., 2009).

For each key effect of interleukin-17, the target-cell type involved, and the products released in response to interleukin-17 are shown. Each biologic effect is linked to examples of conditions in which an association with the presence of interleukin-17 has been observed. CRP denotes C-reactive protein, MMP matrix metalloproteinase, RANKL receptor activator of nuclear factor-κB ligand, and TNF tumor necrosis factor (Miossec et al., 2009).

Cytokines are crucial in maintaining the innate or cell-mediated immune response. Dysregulated production of cytokines could suggest a disease development and diagnosis. It is important to quantify the level of cytokines in a rapid, accurate and sensitive manner. There have been numerous methods employed to measure the proteins and other biomolecules pivotal to a disease and diagnosis. Assays which enable “rapid, simple, sensitive, selective, and cost-effective detection of the proteins discovered are of significant importance for the understanding, diagnosis, treatment, and prevention of disease” (Yang et al., 2005).

1.15 Methods involved in cytokine detection

In assaying cytokines, there are some essential requirements. The assay method must be simple and sensitive. Since cytokines give an indication of the immunological risk factors of the patient, and thereby, critical for diagnosis, treatment, disease prevention (Yang et al., 2005), the measurement of cytokine concentration is done upon admission and repeated at periodic intervals. The assay method must, therefore, allow rapid and cost-effective detection of the proteins.

The cellular actions of cytokine molecules potent and the readings from bioassays could be highly sensitive. Yet, obtaining adequate sensitivity to measure normal concentrations in biological fluids by immunoassay can be challenging owing to both external factors, such as equipment calibration or sample preparation, and physiological factors such as the patient’s pre-existing medical condition. The ‘normal range’ for cytokines vary a lot and it is uncertain whether they can even be defined accurately (Heney and Whicher, 1995).

Current assay techniques “based on RT-PCR, immunoassays, or bioassays, are limited in their use in the clinic, in particular because of the long time (1–3 hours) required to carry out the assays” (Boyle et al., 2006). The most widely employed methods of bioassays include blotting techniques, Enzyme-linked immunosorbent assays (ELISA) and the bead-based and electrochemiluminescence-based multiplex assays (Leng et al., 2008).

1.15.1 Enzyme-linked immunosorbent assay

Enzyme-Linked Immuno Sorbent Assay (ELISA) was designed as an alternative search to replace radio immunoassays which used radioactive isotopes. which were used in (RIA). There

are four different types of ELISA assays. In direct ELISA, the plate surface directly coated with the antibody or antigen. Then a washed and appropriate substrate is added to the medium. This produces a signal through colouration and enables the measurement of the amount of the antigen or antibody (Engvall, 2010). Indirect ELISA uses a two-step process for detection in which an antigen specific primary antibody specific binds to the target. In the second step, a labelled secondary antibody binds to the primary antibody for detection. A third method is the sandwich ELISA, which requires two antigen specific antibodies called matched antibody pairs are used. One of these is coated on the plate to prevent mobilization of the antigen. The other antibody in the pair helps detect the antigen. In competitive ELISA, the sample antigen and reference antigen compete to bind with the labelled antibody. The reference antigen is precoated on a multi-well plate and the sample preincubated with labelled antibody. The sample to be measured is placed into the well along with enzyme-tagged antigen or antibody. Once the wells are washed and enzyme substrate added, the resulting coloration enables quantifying the concentration (Aydin, 2015). Figure 37 below has a pictorial depiction of the four types of ELISA.

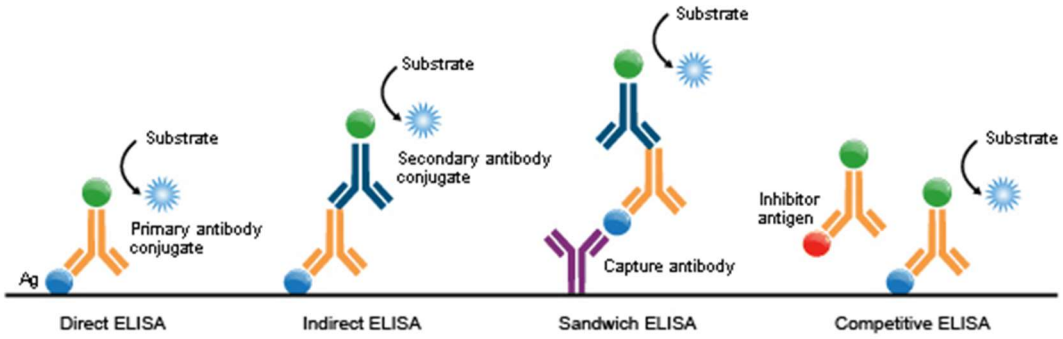


Figure 37: Types of ELISA studies
Adapted from - http://www.abnova.com/support/resources.asp?switchfunctionid={70196CA1-59B1-40D0-8394-19F533EB108F})

1.15.2 Flow Cytometric Methods

Flow cytometric multiplex arrays are currently the most popular assay method. This method, also known as cytometric bead array (CBA) is a multiplex assay that allows simultaneous measurement of multiple proteins (McKinnon, 2019), which is a major advantage over ELISA, which allows only one cytokine to be measured at a time (Morgan et al., 2004, Leng et al.,

2008). CBA methods decrease the time needed to obtain the results. CBA methods can be used to measure different types of analytes (Medeiros and Gomes, 2019b).

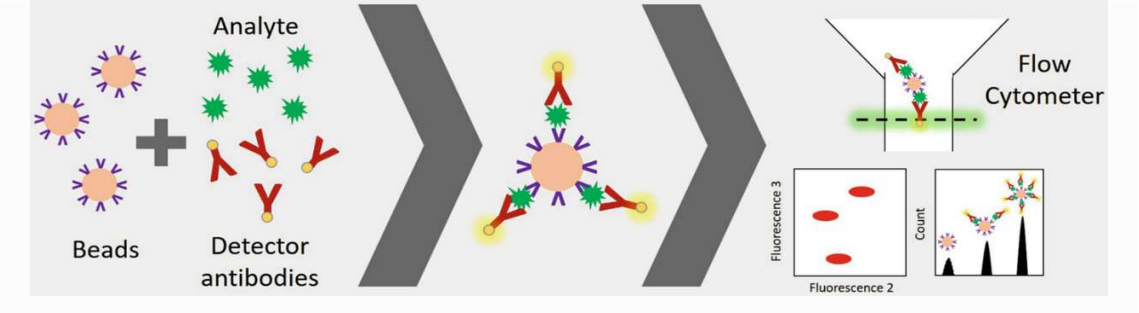


Figure 38: An overview of Cytometric Bead Array (Medeiros and Gomes, 2019a).

In CBA, the sample is incubated with capture beads and detector antibodies are incubated in a process that leads to the creation of sandwich complexes. If multiple proteins have to analysed, the sample is incubated with a variety of beads. The sandwich complexes are analysed with flow cytometry to detect fluorescent particles (Medeiros and Gomes, 2019b). Each type of bead has a specific protein-capturing antibody, and each antibody has its own specific fluorescent intensity. Based on the fluorescence intensity of the beads, they capture particular proteins (Elshal and McCoy, 2006). The fluorescence intensity is usually analysed using specialised software (Depince-Berger et al., 2016a).

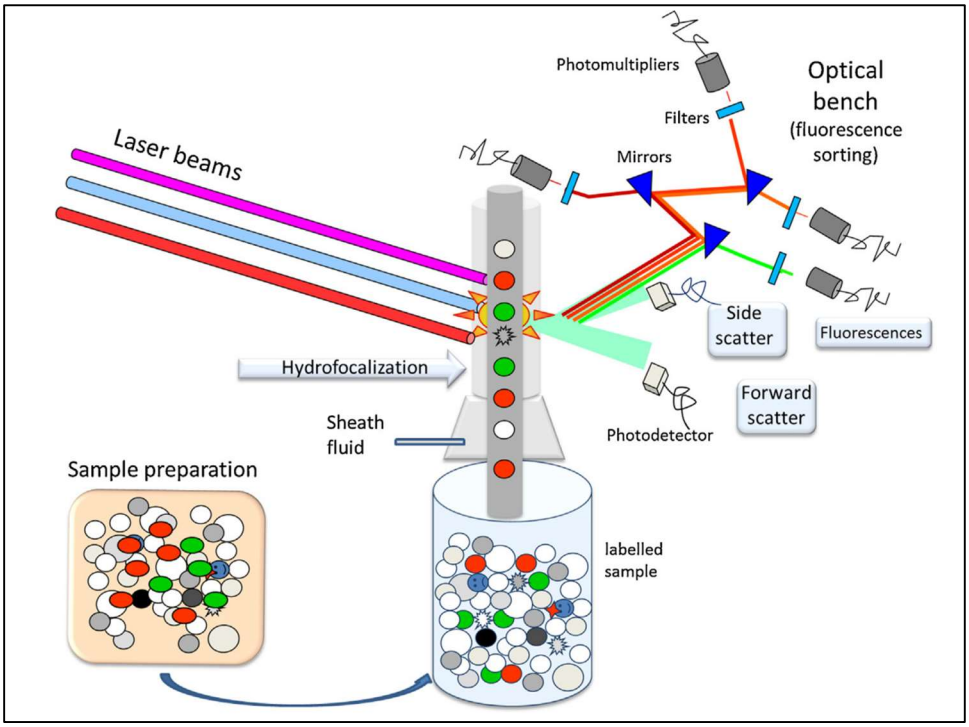


Figure 39: Flow cytometry showing cells of interest labelled with fluorochromes
Adapted from- https://tw.sinobiological.com/category/fcm-facs-what-is-fcm.

The cell suspension and sheath fluid are run through the cytometer. The sample is injected through a channel enclosed by an outer sheath containing fluid that flows faster. The sheath fluid moves and alters the velocity of the central fluid by creating a drag effect. The flow front creates a parabolic effect and causes hydrodynamic focusing. In which a single line of particles is focused at the nozzle. In optimal conditions, the central fluid and sheath fluid do not mix. When the cells scatter light, they are detected both in front and side respectively by forward scatter and side scatter detectors. Positively stained cells emit fluorescence, which is then measured by fluorescence detectors (Cossarizza et al., 2019).

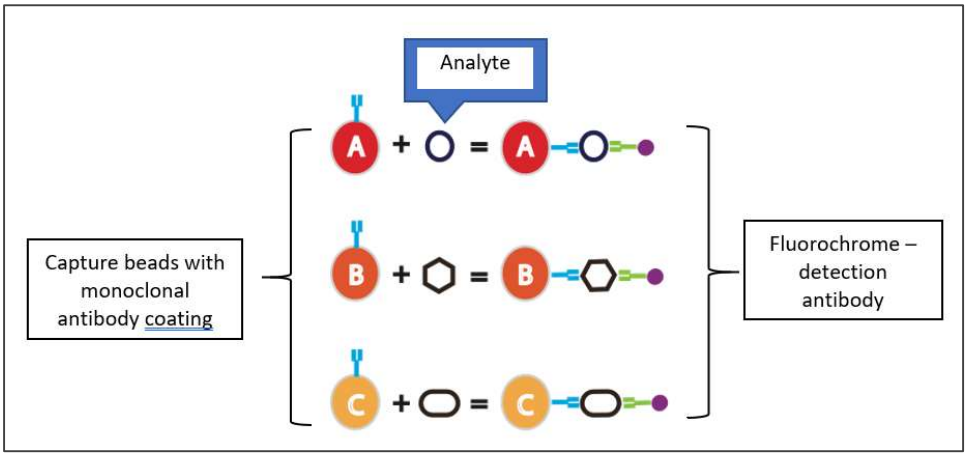


Figure 40: Schematics for a CBA assay

The analysis of flow cytometry assays requires specialist software for accurate readings of cell parameters such as size, volume, refractive index and fluorescence caused by the interactions between fluorescent probes and cytoplasmic molecules or antigens (Aebisher et al., 2017). Research into the use of flow cytometry in medicine is an area of interest because CBA methods deliver a higher level of accuracy in measurement and detection, thus allowing broader applications especially in various disciplines such as hemopathies, immunology and even drug discovery (Depince-Berger et al., 2016b). Moreover, multiplex assays like CBA are more reliable than a uniplex assay assuming there are no errors is sample preparation and handling and measures have been taken to minimise non-reactivity to other antibodies and minimize such cross-reactions. Occasionally, some problems do arise when a varying range of proteins are assayed together (Leng et al., 2008).

Intrinsic properties	Extrinsic properties
Size and granularity	surface antigen content, total protein content, lectin binding, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) content, intracellular pH, nuclear and intracellular antigen content

Table 2: Cellular properties measured through flow cytometry.

1.16 Aims of the study

As a continuation of a larger part of the study, to look for just not cytokines, but also cellular, immune and metabolomic markers as potential predictors of poor clinical outcome in major trauma. This thesis focuses on defining the relationship between the cytokines and cytokine profiles defined for the trauma patients in the first, third and fifth days after admission. The full study covered a cohort of 200 patients, whose clinical data was to be assessed statistically.

The principal aim of this study is to identify and investigate any associated patterns amongst a cluster of eight global cytokines: IL-4, IL-6, IL-8, IL-1, IL – 12, IL-13, IL-17 and TGF-β with a stance as biomarkers that have potential to predict good and poor clinical outcome in patients with major traumatic injuries. The patients recruited were those admitted to Central Manchester Foundation Trust (CMFT) and Salford Royal Foundation Trust (SRFT) between 2017 and February 2019. The aim of this study is intended to be achieved through the following objectives:

1. Collating and managing the patients' meta data on four different time points after admission - days 1, 3, 5 and 8. The metadata consists of clinical and physiological parameters and were provided to be utilised in calculating injury severity and SOFA scores.
2. Recruitment and sampling of a smaller patient cohort (N=30) from the larger cohort of 200 trauma patients admitted to CMFT and SRFT.
3. The cytokine profile for IL-13 and IL-17 will be compared and measured through cytometric bead array methods, measured from the source data of the same patient cohort derived by unpublished data from Matthew Alan Jones (Jones, 2017), Renata Georgeta Apreutesei (Apreutesei, 2019) and Luhaib Abbood.
4. To measure the concentrations of interleukin-13 and interleukin-17 through cytometric bead array methods and to assess whether these concentrations vary in patient cohort, indicating an early prediction (day 1) of a poorer outcome and a later prediction (day 5).
5. To conduct a cross-sectional comparative analysis of data using clinical meta data on days 1, 5 and 8 to assess the trend in cytokine concentration.

6. Comparing cytokine concentration with clinical metadata to evaluate clinical outcome using ISS and SOFA scores in addition to the levels of CRP and lactate.

2 Literature survey

2.1 Summarised literature survey of cytokine research in trauma and related fields

The introduction chapter covered various topics such as the pathophysiology of trauma and sepsis, immuno-regulatory mechanisms, and the trauma induced complications such as ARDS, MODS and MOF. It also exemplified that different scoring systems such as APACHE II, ISS and SOFA to evaluate the severity of traumatic injury. There is extant literature on the topic of cytokines as biomarkers and various researchers have studied interleukins in contexts of acute inflammatory responses, chronic allergic responses, traumatic injuries, complications of sepsis, septic shock, morbidity, and mortality associated. To provide a snapshot of available research, a scan of literature was conducted, and a table has been compiled showing the related studies of cytokine biomarkers for the past 18 years.

This thesis covers an analysis of IL-13 and IL-17 and their possible role as a biomarker for major trauma incidents. With reference to that there are innumerable studies quoted in the table below. Collighan et al, (2004) showed that higher levels of IL-13 on day 3 correlated with fatalities in patients with septic shock. Whereas Mimasaka et al., (2007) observed undetectable ranges of IL-13, IL-4 and IL-10 levels in the post-mortem of major trauma, Silosi et al. (2016) noted significant higher serum concentrations of IL-13, IL-17, anti-Cyclic citrullinated peptide (anti-CCP), and IgM-RF in patients with extra-articular rheumatoid arthritis (eRA), compared to controls. IL-17 increased proportional to the disease activity of eRA while IL-13 concentration was inversely proportional with CRP. Compared with those in controls, higher concentrations of IL-13 and IL-17 confirms that these markers, found with high specificity, showed their involvement in the pathogenesis of eRA (Siloşi et al., 2016). In a study by Ali et al., (2018) day 1 IL-17 levels in polytrauma patients showed an independent susceptibility for sepsis. Though there are lot more available, it is not feasible to show them all here.

The comparative data analysis presented in chapter 4 also included assessment of IL-13 and IL-17 against IL-4, IL-8, and IL-12. There is precedence for that type of comparisons. As an example, Bozza et al., (2007) observed a positive correlation between IL-13 and IL-8 on day 1 as day 1 was predictive of worsening condition of patients into septic shock and organ dysfunction to improve on day 3. (More details are found in chapter 5 – discussion).

In the last 12-13 months, we have been facing a pandemic COVID-19 and recently a few studies have been published looking into the possible link between the role of interleukins and COVID-19. This is an emerging and ongoing area of research. It is expected that there will be a lot more studies into this, but as an early set of references, the table includes five papers involving a broad spectrum of cytokines such as IL-1B, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17 for their diverse roles.

The below table contains a snapshot of 40 key papers as relevant references. In addition to IL-13 and IL-17, research studies on multiplex analysis of various inflammatory cytokines that are relevant to our pilot study is also detailed.

	Authors	Year of publication	Country conducted in	Scoring system used	Number of participants	Cytokines (Biomarkers) measured	Method used	Timepoints measured	Trauma Type	Key findings	Reference
1	Damas et al.,	1992	Belgium	APACHE II	40	IL-1β, TNFα, IL-6	Two-step immunoradiometric assay (IRMA)	Days 1-12	Sepsis	Peaked IL-6 levels found on day 1 within 4 hours and on day 7 and CRP levels raised after 20hours correlating with acute phase response of trauma.	Damas, P., Ledoux, D.I.D.I.E.R., Nys, M., Vrindts, Y.V.O.N.N.E., De Groote, D.O.N.A.T., Franchimont, P. and Lamy, M., 1992. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. Annals of surgery, 215(4), p.356.
2	Hensler et al.,	2000	Germany	Injury severity score (ISS)	SHT=32, multiple injuries with SHT = 50, multiple injuries without SHT = 39	IL-10, IL-13	ELISA	Days - 1 to 10, 14, 21, and 28. Additional samples were drawn at every 6 hours during the first three days	severe head trauma (SHT), multiple injuries,	Peaked IL-10 levels within 3 hours post trauma in all 3 injury groups. i.e., Severe head trauma (SHT) multiple injuries with SHT and multiple injuries without SHT. IL-13 levels showed no increase post trauma in none of the groups.	Hensler T, Sauerland S, Riess P, Hess S, Helling HJ, Andermahr J, Bouillon B, Neugebauer EA. The effect of additional brain injury on systemic interleukin (IL)-10 and IL-13 levels in trauma patients. Inflammation Research. 2000 Oct 1;49(10):524-8.
3	Varedi et al.,	2000	USA	not applicable	control Sprague-Dawley male rats & ratswith 50% of their body surface receiveing scald-burn	TGF-β, IL-4	ELISA	Day1 - Day 8 , at 1st hour after rats were sacrifised	thermally injured rats	Increased TGF-β levels seen after an hour, post injury in both control and burn rats. Significant increase in TGF-β level indicated immue suppression & sepsis in burn rats. No changes in IL-4 levels all through day 1 – day 8 in both groups.	Varedi M, Jeschk, MJ, Englander EW., Herndon DN and Barrow RE, 2001, Serum TGF-β in thermally injured rats, *Shock, 16 (5), pp, 380-382*
4	Kivioja et al.,	2001	Sweden	Qubec classification	27	IL-6, IL-10, TNF-α, TNF-β	Enzyme-Linked Immunospot (ELISPOT) Assays,	Day 1-3 & Day 14	Whiplash injury and whiplash-associated disorders (WAD)	Peaked IL-6, IL-10 & TNF-α levels following a whip-lash trauma (day 3). On day 14 follow up, the cytokine levels seemed normal. A similar observation was noted on control group with ankle sprain indicating the cytokine profile did not differentiate between the two group.	Teche, Stefania P, Rovaris, Diego L, Aguiar, Bianca W, Hauck, Simone, Vitola, Eduardo S, Bau, Claiton H.D, Freitas, Lucia H, and Grevet, Eugenio H. "Resilience to Traumatic Events Related to Urban Violence and Increased IL10 Serum Levels." *Psychiatry Research* 250 (2017): 136-40
	Collighan et al.,	2004	United Kingdom	APACHE II, SOFA	Sepsis patienrts=31, sex matched healthy controls=24	IL-13, IL-2, TNF-α & Monocyte Human Leukocyte Antigen – DR isotype (HLA-DR)	ELISA & flow cytometry	Days 1, 3, 5 and 7 post trauma	sepsis or septic shock	Peaked IL-13 levels in the septic shock group on day 3 and eventually lowered, levels similar to non-shocked group. Higher IL-13 levels were observed in non-survival patients. IL-13 (anti-inflammatory) correlated with TNF-α (pro-inflammatory) expression levels (p=0.017).	Collighan N, Giannoudis PV, Kourgeraki O, Perry SL, Guillou PJ, Bellamy MC. Interleukin 13 and inflammatory markers in human sepsis. Br J Surg. 2004 Jun;91(6):762-8.
6	Mimaska et al.,	2007	Japan	Abbreviated injury scale (AIS) & injury severity score (ISS)	43 autopsy subjects, traumatic death = 20 & non-traumatic death controls = 23	Post mortem serum samples of granulocyte-macrophage colony stimulating factor (GM-CSF), interferon (INF)-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, and tumour necrosis	Multiplex immunoassay	within 48 hours of death	post mortem traumatic and non traumatic	IL-4, IL-10 and IL-13 levels were undetectable. IL-6 ($p < 0.001$), and IL-8 ($p < 0.01$) showed significant statistical difference between traumatic group and non-traumatic group.	Mimasaka, S., Funayama, M., Hashiyada, M., Nata, M., & Tsunenari, S. (2007). Significance of levels of IL-6 and IL-8 after trauma: a study of 11 cytokines post-mortem using multiplex immunoassay. Injury, 38(9), 1047-1051.
7	Bozza et al.,	2007	Brazil	APACHE II, SOFA	60	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-γ, G-CSF, monocyte chemoattractant protein [MCP]-1, macrophage inflammatory protein-1 and tumour necrosis factor [TNF]-α	ELISA	Day 1 & day 3	Sepsis	IL-1β, IL-6 (p=0.007), IL-7, IL-8 (p=0.01), IL-10 (p=< 0.001), IL-13 (p=0.008), IFN-α, MCP-1 and TNF-α showed significant increase in septic shock as compared to severe sepsis. IL-1β, IL-6, IL-8, IL-10, MCP-1 and G-CSF positively correlated with organ dysfunction, as assessed by the day 1 SOFA score and on day 1 were predictive of worsening organ failure or dysfunction to improve on day 3.	Bozza, F.A., Salluh, J.I., Japiassu, A.M. *et al.* Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. *Crit Care* 11, R49 (2007).
8	Lausevic et al.,	2008	Serbia	injury severity score (ISS)	65	C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-10 (IL-10) and phospholipase A2 group II (PLA2-II)	ELISA	T1=within 24 hours after injury, T2= day 2, T3= day 3, T4= day 7, T5= day 10	multiple organ failure (MOF)	Statistically significant levels of CRP, PLA2II were observed on all days in both groups of patients – with MOF and without MOF. IL-6 and IL-10 levels stayed significantly high. In the study, 45% of patients with IL-6 > 250 pg/l and > 500 pg/l indicated bad outcome.	Lausevic, Z., Lausevic, M., Trbojevic-Stankovic, J., Krstic, S., & Stojimirovic, B. (2008). Predicting multiple organ failure in patients with severe trauma. Canadian journal of surgery. Journal canadien de chirurgie, 51(2), 97–102.

	Authors	Year of publication	Country conducted in	Scoring system used	Number of participants	Cytokines (Biomarkers) measured	Method used	Timepoints measured	Trauma Type	Key findings	Reference
9	Frangen et al.,	2008	Germany	ISS	71	IL-17, IL-6	ELISA	Within 4 days of trauma incidence, evryday morning.	polytrauma	The results was obtained in 2 stages. Amogst 71 patients, in 62 people, IL-17 was not detected (group A). The rest 9 patients (group B) showed a peak in IL-17 (above healthy threshold in plasma 45 pg/ml). Further correlations obtained from group B IL-17 was significant with IL_6 at	Frangen TM, Bogdanski D, Schinkel C, Roetman B, Kälicke T, Muhr G, Köller M. Systemic IL-17 after severe injuries. Shock. 2008 Apr;29(4):462-7
10	St Ledger K etal.,	2009	USA		healthy = 60, asymptomatic = 26, and symptomatic = 96	IL-13	Erenna immunoassay		healthy, asymptomatic, and symptomatic asthma subjects.	Very low concentrations of circulating serum IL-13 were measured as low as 0.007pg/ml, which far exceeds the sensitivity of detecting cytokines, using conventional methods. This helps in identifying the baseline IL-13 levels in healthy subjects which would otherwise result in collecting the uncountable numbers of asymptomatic patients samples.	St Ledger, K., Agee, S.J., Kasaian, M.T., Forlow, S.B., Durn, B.L., Minyard, J., Lu, Q.A., Todd, J., Vesterqvist, O. and Burczynski, M.E., 2009. Analytical validation of a highly sensitive microparticle-based immunoassay for the quantitation of IL-13 in human serum using the Erenna immunoassay system. Journal of immunological methods, 350(1-2), pp.161-170.
11	Hergenroeder et al.,	2010	USA	APACHE II, GCS & ISS	36 (22 healthy patients & 14 polytrauma parients with TBI	IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, and IL-1ra	ELISA	Days 1-5/ 10 hours or 20 hour time point	Polytrauma patients with traumatic Brain Injury (TBI)	IL-6 levels in all the TBI patients. At a cut-off of <5 pg/ml, IL-6 levels identified 100% healthy group & at a cut-off of >128 pg/ml, IL-6 levels promptly distinguished 85% of TBI patients who developed complications like intra cranial pressure & amongst orthopedic patients, Il-6 peaked between the range of healthy and TBI.	Hergenroeder, G.W., Moore, A.N., McCoy, J.P. *et al.* Serum IL-6: a candidate biomarker for intracranial pressure elevation following isolated traumatic brain injury. *J Neuroinflammation* 7, 19 (2010).
12	Sousa et al.,	2010	North Portugal	ISS	99	TNFα , IL-6, IL-10, HMGB-1, and ICAM-1	ELISA	24, 48, and 72 hours after injury	severe polytrauma	IL-6 & IL-10 levels peaked between 48-62 hours post trauma. Th1 ratio was less than Th2 between 24-72 hours post trauma, suggesting bad outcome such as MODS and death. The simultaneous occurrence of pro- and anti-inflammatory responses could help in assessing the outcome.	Sousa, António, Raposo, Frederico, Fonseca, Sara, Valente, Luís, Duarte, Filipe, Gonçalves, Moura, Tuna, Diana, and Paiva, José-Artur. "Measurement of Cytokines and Adhesion Molecules in the First 72 Hours after Severe Trauma: Association with Severity and Outcome." *Disease Markers* 2015 (2015): 747036-8. Web
13	Cohen et al.,	2011	Israel	Abbreviated Injury Scale, AIS	48	IL-4, IL-6, IL-8, IL-10, TGF-β	Bead based immunoassay	T1- Day 1 to Day 5 & T2 - after a month	Acute Stress Symptoms (ASS) & Posttraumatic Stress Symptoms (PTSS)	IL-6, IL-8 & TGF-β showed higher concentrations in the ASS and PTSS group when compared to the control. The ASS and PTSS scored positively correlated. IL-4 and IL-10 levels did not show any significance with the same.	Cohen, Miri, Meir, Tamar, Klein, Ehud, Volpin, Gershon, Assaf, Michael, and Pollack, Shimon. "Cytokine Levels as Potential Biomarkers for Predicting the Development of Posttraumatic Stress Symptoms in Casualties of Accidents." *International Journal of Psychiatry in Medicine* 42.2 (2011): 117-31.
14	Tsalik et al.,	2012	USA	Not mentioned	336	Procalcitonin (PCT), IL-6 & CRP	Il-6 & PCT: electrochemiluminescent immunoassay. CRP: chemiluminescent immunoassay.	Jul 2003 to Dec 2003 and Dec 2006 to Dec 2007	Complicated sepsis	PCT, IL-6, and CRP highly correlate with several infection parameters. However, a single measurement at the time of initial presentation does not predict LOS or need for discharge to a higher level of care.	Tsalik, Ephraim L, Jaggers, L. Brett, Glickman, Seth W, Langley, Raymond J, Van Velkinburgh, Jennifer C, Park, Lawrence P, Fowler, Vance G, Cairns, Charles B, Kingsmore, Stephen F, and Woods, Christopher W. "Discriminative Value of Inflammatory Biomarkers for Suspected Sepsis." *Journal of Emergency Medicine* 43.1 (2012): 97-106.
15	Volpin et al.,	2014	Israel	Abbreviated injury scale (AIS)	Total participants=71, healthy controls=13, moderately injured=31, severe orthopaedic trauma= 27	IL-1β, Il-2, IL-4, IL-6, IL-8, IL12, TGF-β, INFγ, TNFα	ELISA	Day 1 (with in few hours of injury)& Day 180 (after 6 months)	moderate - severe trauma	IL-6($p<0.001$), TGFβ ($p<0.01$) and IL-8 ($p < 0.05$) were detected in highest levels in the severely injured group and significantly low levels of IL-4 ($p = 0.044$) in all injured patients. High serum levels of these cytokines can be used as potential reliable biomarkers for predicting the development of (SIRS) in patients with multiple trauma	Volpin, G., Cohen, M., Assaf, M., Meir, T., Katz, R. and Pollack, S., 2014. Cytokine levels (IL-4, IL-6, IL-8 and TGFβ) as potential biomarkers of systemic inflammatory response in trauma patients. *International orthopaedics* , *38* (6), pp.1303-1309.
16	Greenberg et al.,	2015	USA	Not mentioned	106 children (1 month -18 years)	IL-6, IL-10	Immunoassay- Randox Evidence Investigator	Day 1 (preop) and Day 3 (postop)	Acute Kidney Injury	IL-6 and IL-10 levels on day 1 increased after cardiac bypass surgery in children indicating AKI. IL-10 level decreased on day 3. IL-6 seems to predict stage 2 and 3 AKI preoperatively.	Greenberg, J.H., Whitlock, R., Zhang, W.R., Thiessen-Philbrook, H.R., Zappitelli, M., Devarajan, P., Eikelboom, J., Kavsak, P.A., Devereaux, P.J., Shortt, C. and Garg, A.X., 2015. Interleukin-6 and interleukin-10 as acute kidney injury biomarkers in pediatric cardiac surgery. *Pediatric Nephrology* , *30* (9), pp.1519-1527.

	Authors	Year of publicatio	Country conducted in	Scoring system used	Number of participants	Cytokines (Biomarkers) measured	Method used	Timepoints measured	Trauma Type	Key findings	Reference
17	Dai and Zhang	2015	China	SOFA	Total participants = 56, n = 18, SAP without organ dysfunction,n = 18, SAP with organ dysfunction, controls=20	IL-17, IL-6	ELISA	0,6,12 and 24 hours	Severe acute pancreatitis (SAP)	IL-6 & IL-17 concentrations positively correlated with fatalities with high SOFA and high base line of IL-17 levels predicted longer LOS, MOF and death. continuous veno-venous hemofiltration (CVVH) employed can shrink related systemic complications by removing inflammatory cytokines from serum samples.	Dai SR, Li Z, Zhang JB. Serum Interleukin 17 as An Early Prognostic Biomarker of Severe Acute Pancreatitis Receiving Continuous Blood Purification. *The International Journal of Artificial Organs* . 2015;38(4):192-198.
18	Aisiku et al.,	2016	USA	APACHE II, GCS & ISS	200	IL-1β, IL-6, IL-8, IL-12, TNFα, IL-10	multiplex immunoassay	0,5,10,15 and 20 hours	severe traumatic head injury	Peaked IL-6 , IL-8 & IL-10 levels in ARDS compared to non-ARDS group, indicating an onset of an early phase acute TBI	Aisiku, I. P., Yamal, J. M., Doshi, P., Benoit, J. S., Gopinath, S., Goodman, J. C., & Robertson, C. S. (2016). Plasma cytokines IL-6, IL-8, and IL-10 are associated with the development of acute respiratory distress syndrome in patients with severe traumatic brain injury. Critical care, 20(1), 288.
19	Sapan et al.,	2016	Indonesia	ISS	54	IL-6 & Il-10	ELISA	48, 72, 120 hours after trauma	multiple organ dysfunction syndrome (MODS) & Polytrauma	Elevated cytokine levels of IL-6, IL-10, and IL-6/IL-10 ratio are directly proportional with MODS and mortality. IL-6 & then followed by IL-10 level peaked in fatalities than among survived group, while in fatality group IL-10 level peaked only if IL-6 level was <50 pg/ml	Sapan, H.B., Paturusi, I., Jusuf, I., Patellongi, I., Massi, M.N., Pusponegoro, A.D., Arief, S.K., Labeda, I., Islam, A.A., Rendy, L. and Hatta, M., 2016. Pattern of cytokine (IL-6 and IL-10) level as inflammation and anti-inflammation mediator of multiple organ dysfunction syndrome (MODS) in polytrauma. *International journal of burns and trauma* , *6* (2), p.37.
20	Silosi et al.,	2016	USA	NA	30 patients and from 28 controls (healthy persons)		ELISA	T1- Fasting state blood sample drwan in the morning	Rheumatoid arthritis	IL-13 and IL-17 might be of better usefulness in the prediction of eRA activity status than IgM-RF and anti-CCP. The serum concentrations of IL-13, IL-17, anti-CCP, and IgM-RF were statistically significantly higher in patients with eRA, compared to controls. IL-17, increased proportionally with the disease activity of eRA. IL-13 concentration significantly negatively correlated with CRP. The presence of higher IL-13 and IL-17 serum levels in patients, compared with those of controls, confirms that these markers, found with high specificity, might be involved in the pathogenesis of eRA.	Siloşi, I., Boldeanu, M.V., Cojocaru, M., Biciuşcă, V., Pădureanu, V., Bogdan, M., Badea, R.G., Avramescu, C., Petrescu, I.O., Petrescu, F. and Siloşi, C.A., 2016. The relationship of cytokines IL-13 and IL-17 with autoantibodies profile in early rheumatoid arthritis. *Journal of immunology research* , *2016* .
21	Ali et al.,	2018	Egypt	APACHE II	100	IL-17, IL-6, and TNF-α	ELISA	T1-3 hours within surgical intervention	major trauma admitted to the surgical ICU	Onset of sepsis amogst older patients with high APACHE II score, significamnty high IL-17 levels on day 1 in patients who developed sepsis compared to those who did not [72 (45–176) pg /mL vs. 37 [28–53] pg/ mL, P < 0.0001].	Ali, M.A., Mikhael, E.S., Abdelkader, A., Mansour, L., El Essawy, R., El Sayed, R., Eladawy, A. and Mukhtar, A., 2018. Interleukin-17 as a predictor of sepsis in polytrauma patients: a prospective cohort study. European Journal of Trauma and Emergency Surgery, 44(4), pp.621-626.
22	Shimazui *et al* .,	2017	Japan	SOFA	92 (5 Trauma patients)	IL-6	Rapid measurement system	Days 1-5	ICU admissions	IL-6 found to be at peak on D1 and decline over a 5 day period, Maximum patient IL-6 score significantly differed when patients were grouped into lowest (1-8), Intermediate (9-16) and high (17-24) highest SOFA scores	Shimazui, T., Matsumura, Y., Nakada, T. A., & Oda, S. (2017). Serum levels of interleukin-6 may predict organ dysfunction earlier than SOFA score. *Acute medicine & surgery* , *4* (3), 255–261. https://doi.org/10.1002/ams2.263
23	Sapan *et al* .,	2017	Indonesia	ISS, AIS & SOFA	54	IL-6, IL-10	ELISA, RT-PCR	Not defined	Polytrauma	- SOFA only used to ascertain if patients have organ dysfunction. All other analysis based on ISS and mortality. IL6/IL10 ratio decreased in more severe trauma, Higher IL6/IL10 ratio was found in survivors compared to non surviviors	Sapan, H. B., Paturusi, I., Islam, A. A., Yusuf, I., Patellongi, I., Massi, M. N & Hatta, M. (2017). Interleukin-6 and interleukin-10 plasma levels and mRNA expression in polytrauma patients. *Chinese Journal of Traumatology* , *20* (6), 318-322.
24	Diaz et al.,	2017	Spain	Web based research	3390 refernces	Twenty genetic markers are described: four associated with bacteremia (TLR-1, TLR-2, Protein C and Selectin-E), nine with sepsis (IL-1B, IL-1A, IL-6, TNF-α, TLR-1 , MBL-1, Hsp70, PAI-1 and MIF-1), seven with severe sepsis (IL-1RN, IL-10, TNF-α, CD14, TREM-1, Caspase 12 and DEFB-1), five with septic shock (TNF-B, TLR-4, Hsp70, MBL-1 and CD14), and three with multiorgan dysfunction (TLR-1, PAI-1 and Protein C	Summary of other researches	Records over 14 years in PubMed, NEJM & ILLIAC	diagnosis and progression of sepsis.	The panel of cytokines serve as a prognostic biomarkers of sepsis with promising results	Alfredo Prado-Díaz, Andrés Castillo, Diana Marcela Rojas, and Mónica Chávez-Vivas. "Molecular Markers in the Diagnosis and Prognosis of Sepsis, Severe Sepsis and Septic Shock." *Revista De La Facultad De Medicina, Universidad Nacional De Colombia* 65.1 (2017): 145-55.

	Authors	Year of publication	Country conducted in	Scoring system used	Number of participants	Cytokines (Biomarkers) measured	Method used	Timepoints measured	Trauma Type	Key findings	Reference
25	Hall et al.,	2017	USA	N/A	*in vitro,* murine models and healthy human donors for nasal mucosa ans nasal epithelial cells (NEC)	IL_13 and IL-17A	Cell culture and Flow cytometry	Days 0, 3 and 6	Severe asthma	IL-17A enhances IL-13-induced STAT6 activation, leading to increased IL-13-driven transcripts and lung pathology. IL-17A-mediated enhancement of IL-13 activity is observed in both mouse and human cells, suggesting that IL-17A may directly contribute to asthma severity.	Hall, S. L., Baker, T., Lajoie, S., Richgels, P. K., Yang, Y., McAlees, J. W., van Lier, A., Wills-Karp, M., Sivaprasad, U., Acciani, T. H., LeCras, T. D., Myers, J. B., Kovacic, M. B., & Lewkowich, I. P. (2017). IL-17A enhances IL-13 activity by enhancing IL-13-induced signal transducer and activator of transcription 6 activation. The Journal of allergy and clinical immunology, 139(2), 462-471
26	Ali et al.,	2018	Egypt	APACHE II	100	IL-17, IL-6 & TNF-α	RT- PCR, ELISA	within 3 h after ICU admission and always before the first surgical procedure	major trauma admitted to the surgical ICU	Serum IL-17 levels were significantly higher (P<0.0001) on day 1 in sepsis patients versus patients who did not. TNF-α & IL-6 did not vary significantly between the groups. In polytrauma, IL-17 showed independent association with susceptibility for sepsis.	Ali, M.A., Mikhael, E.S., Abdelkader, A., Mansour, L., El Essawy, R., El Sayed, R., Eladawy, A. and Mukhtar, A., 2018. Interleukin-17 as a predictor of sepsis in polytrauma patients: a prospective cohort study. *European Journal of Trauma and Emergency Surgery* , *44* (4), pp.621-626.
27	Khurana et al.,	2018	India	Not stated	Total cohort=80, Trauma patients with susupected sepsis=40, age matched healthy controls=40	m IL-6, IFN-γ, TNFα, IL-17A, IL-17F, and IL-4 & IL-13	Bead-based cytometric analysis	Day 0 & Day 4	polytrauma with sepsis	Significantly increased levels of serum IL-4, IL-6, IL-17A, IL-17F, IFN-γ and TNFα was noticed in patients with sepsis. Lowered levels of IL-13 and lowered levels of IL-4 & IL-2 significantly correlated with patient recovery post antimicrobial treatment (p <0.005).	Khurana, S., Kumari, M., Bhardwaj, N., Kumar, S., Sagar, S., Malhotra, R. and Purva Mathur, P., 2018. T-helper-17, Regulatory T-helper Cells Related Serum Markers and IL-13 in the Outcome of Polytraumatic Patients with Bacteremia. *Iranian Journal of Immunology* , *15* (4), pp.302-308.
28	Wang, et al.,	2018	China	Not stated	122 sepsis patients, 106 healthy controls with no sepsis	peptidoglycan recognition protein (PRP), cluster of differentiation (CD)64, procalcitonin (PCT), NF-κB-p65, inhibitor of NF-κB (IκBα), IL-1, IL-17, TNF-α and IL-6	ELISA, Flow cytometry	Blood drawn at the ICU	Sepsis	Significantly elevated IL-1, IL-17, TNF-α and IL-6 levels (p=0.001) amogst sepsis patients compared with those in healthy individuals. The upregulation of pro-inflammatory cytokines in the serum of patients with sepsis was demonstrated.	Wang, L., Zhao, H. and Wang, D., 2018. Inflammatory cytokine expression in patients with sepsis at an intensive care unit. Experimental and therapeutic medicine, 16(3), pp.2126-2131.
29	Potjo et al.,	2019	South Africa	SOFA	68	circulating IL-1R, IL-10, C-reactive protein (CRP), procalcitonin (PCT	Laser immunonephelometry, Immunoluminescence and Bio-Plex suspension bead array system	Not stated	sepsis or systemic inflammatory response syndrome (SIRS)	Significant increase in Il-1Ra & IL-10 levels (P ≤ 0.05) in septic patients versus patients with SIRS.	Potjo, M., Theron, A. J., Cockeran, R., Sipholi, N. N., Steel, H. C., Bale, T. V., ... & Tintinger, G. R. (2019). Interleukin-10 and interleukin-1 receptor antagonist distinguish between patients with sepsis and the systemic inflammatory response syndrome (SIRS). Cytokine, 120, 227-233.
30	Robak et al.,	2019	Poland		45	IL-17A, IL-17B, IL-17E and IL-17F	Luminex bead-based immunoassays		systemic sclerosis	No difference was found between patients with SSc and the control group as regards the serum concentration of IL-17A. However, IL-17B and IL-17E levels in patients with SSc, and its types diffuse and limited were higher ($p < 0.001$) compared to the control. The serum level of IL-17F was higher in SSc ($p < 0.005$) and lSSc ($p < 0.05$) compared to the control. Serum concentration of IL-17B was elevated in SSc patients with renal abnormalities ($p < 0.05$) compared to those without. Serum levels of IL-17B correlated with the levels of IL-17E in patients with SSc ($r = 0.54$, $p < 0.05$).	Robak, E., Gerlicz-Kowalczuk, Z., Dziankowska-Bartkowiak, B., Wozniacka, A. and Bogaczewicz, J., 2019. Serum concentrations of IL-17A, IL-17B, IL-17E and IL-17F in patients with systemic sclerosis. Archives of medical science: AMS, 15(3), p.706.
31	Patel et al.,	2019	USA	The International Society of Thrombosis and Hemostasis (ISTH) scoring algorithm	Healthy control = 50 & patients with sepsis and suspected DIC = 137	IL-6, IL-8, IL-10, and TNFα, Procalcitonin (PCT)	ELISA	Upon admission to ICU & prior to receiving any treatment.	Disseminated intravascular coagulation (DIC) sepsis	IL-6 & PCT showed strongest correlation of the measured markers with DIC score. PCT may actually be more useful than some of the other less specific inflammatory markers such as IL-8, IL-10, and TNFα.	Patel, P., Walborn, A., Rondina, M., Fareed, J. and Hoppensteadt, D., 2019. Markers of inflammation and infection in sepsis and disseminated intravascular coagulation. Clinical and Applied Thrombosis/Hemostasis, 25, p.1076029619843338.
32	Mors et al.,	2019	Germany	ISS & AIS	204	Systemic fbrinogen, IL-6, and IL-10	Il-6 & IL-10-ELISA & Fibrinogen- Clauss method	D1 (with in few hours of admission)	geriatric trauma	Systemic fbrinogen levels were signifcantly increased in geriatric trauma patients, while IL-6 showed a clear trend to enhanced levels in this group as well. Fibrinogen levels correlated positively with age. Systemic IL-10 levels were signifcantly lower in the geriatric group, showing a negative correlation with age.	Mörs, K., Wagner, N., Sturm, R., Störmann, P., Vollrath, J.T., Marzi, I. and Relja, B., 2019. Enhanced pro-inflammatory response and higher mortality rates in geriatric trauma patients. *European Journal of Trauma and Emergency Surgery* , pp.1-8.

	Authors	Year of publicatio	Country conducted in	Scoring system used	Number of participants	Cytokines (Biomarkers) measured	Method used	Timepoints measured	Trauma Type	Key findings	Reference
33	Crawford et al.,	2019	USA	motor Glasgow Coma Score (mGCS) < 6, Abbreviated Injury Scale	57	IL-4, IL-5, IL-8, and IL-10, IFN-γ, IL-7 and IL-17	Multiplex beads assay	For every 6 hours, for 72 hours (12 time points)	Traumatic Brain Injury with thoracic trauma	Cytokine levels in the patients with traumatic brain injury who had received chest trauma showed higher concentrations of IL-4, IL-5, IL-8, and IL-10 and those without chest injury showed lower concentrations of IFN-γ and IL-7.	Crawford, Angela M. MD; Yang, Shiming PhD; Hu, Peter PhD; Li, Yao PhD; Lozanova, Petya MD; Scalea, Thomas M. MD; Stein, Deborah M. MD, MPH Concomitant chest trauma and traumatic brain injury, biomarkers correlate with worse outcomes, Journal of Trauma and Acute Care Surgery: July 2019 - Volume 87 - Issue 1S - p S146-S151
34	Liu et al.,	2019	China	Murray Score	12 patients with 2019-nCoV infections, 8 patients pneumonia, 8 patients with H7N9 influenza and 8 healthy controls	M-CSF, IL-10, IFN-α2, IL-17, IL-4, IP-10, IL-7, IL-1ra, G-CSF, IL-12, IFN-γ, IL-1α, IL-2, HGF, and PDGF-BB,	qRT-PCR	24 hours of blood sample collection from laboratory-confirmed 2019-nCoV cases upon admission	ARDS and 2019-nCoV	2019-nCoV viral load was highly positively associated with the plasma levels of 16 cytokines (M-CSF, IL-10, IFN-2, IL-13, IL17, IL-4, IP-10, IL-1β, IL-7, IL-1ra, G-CSF, IL-12, IFN-γ, IL-1α, IL-2, and HGF), and negatively associated with PDGF-B. The findings suggest that 2019-nCoV infection was associated with an elevated production of a wide array of cytokines/chemokines in the plasma of 2019-nCoV infected patients.	Liu, Y., Zhang, C., Huang, F., Yang, Y., Wang, F., Yuan, J., Zhang, Z., Qin, Y., Li, X., Zhao, D. and Li, S., 2019. novel coronavirus (2019-nCoV) infections trigger an exaggerated cytokine response aggravating lung injury. *ChinaXiv (2020)*
35	Bagaria et al.,	2020	India	ISS, Thoracic trauma severity score (TTSS)	865	IL-1β, IL-6, IL-8, IL-10, and TNF-α	ELISA	Not mentioned	Blunt chest trauma	Peaked levels of IL-1β and IL-10 were seen in the serum and broncho alveolar lavage (BAL) fluid of patients. The baseline and peaked levels of IL-6, IL8, and TNF-α indicated mortality.	Bagaria, V., Mathur, P., Madan, K., Kumari, M., Sagar, S., Gupta, A., Soni, K.D., Bhattacharjee, H. and Kumar, S., 2020. Predicting Outcomes After Blunt Chest Trauma—Utility of Thoracic Trauma Severity Score, Cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α), and Biomarkers (vWF and CC-16). Indian Journal of Surgery, pp.1-7.
36	Huang, et al.,	2020	China (Wuhan)	Not stated	41 2019-nCoV patients & 4 healthy controls	IL1B, IL1RA, IL2, IL4, IL5, IL6, IL7, IL8 (also known as CXCL8), IL9, IL10, IL12p70, IL13, IL15, IL17A, Eotaxin (also known as CCL11), basic FGF2, GCSF (CSF3), GMCSF (CSF2), IFNγ, IP10 (CXCL10), MCP1 (CCL2), MIP1A (CCL3), MIP1B (CCL4), PDGFB, RANTES (CCL5), TNFα, and VEGFA	RT-PCR	Not stated	2019-nCoV	Early studies have shown that increased amounts of proinflammatory cytokines in serum (eg, IL1B, IL6, IL12, IFNγ, IP10, and MCP1) were associated with pulmonary inflammation and extensive lung damage in SARS patients.22 MERS-CoV infection was also reported to induce increased concentrations of proinflammatory cytokines (IFNγ, TNFα, IL15, and IL17).23	Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X. and Cheng, Z., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet* , *395* (10223), pp.497-506.
37	Wan et al.,	2020	China	Not stated	123	CD4 + T, CD8 + T, IL-6 and IL-10	flow cytometry, multiple microsphere flow immunofluorescence	Not stated	novel coronavirus pneumonia (NCP)	There was no significant linear correlation between the lymphocyte subsets and cytokines, while significant differences were noticed between the two groups in CD4 + T, CD8 + T, IL-6 and IL-10.	Wan, S., Yi, Q., Fan, S., Lv, J., Zhang, X., Guo, L., Lang, C., Xiao, Q., Xiao, K., Yi, Z. and Qiang, M., 2020. Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). MedRxiv.
38	Leija-Martínez	2020	Mexico	multiple reseach studies	4,103	TNF-α, IL-6 and IL-17			obese patients with COVID-19	TNF-α and IL-17A are more elevated in patients with obesity and COVID-19, and consequently, they have a greater probability of developing ARDS and death. IL-17A stimulates M1 macrophages, which respond with the increased synthesis of IL-1β, IL-6, IL-15 and TNF-α, which subsequently activate more Th17 lymphocytes. Therefore, there is a positive immunological feedback loop between M1 macrophages and Th17 lymphocytes; for this reason, a Th1-Th17 immune profile predominates in obesity with an increase in serum concentrations of IL-17A and TNF-α. Elevated level of IL-6.	Leija-Martínez, J.J., Huang, F., Del-Río-Navarro, B.E., Sanchéz-Muñoz, F., Muñoz-Hernández, O., Giacoman-Martínez, A., Hall-Mondragon, M.S. and Espinosa-Velazquez, D., 2020. IL-17A and TNF-α as potential biomarkers for acute respiratory distress syndrome and mortality in patients with obesity and COVID-19. *Medical hypotheses* , *144* , p.109935.
39	Lucas et al.,	2020	USA	Clinical Severity Score	135	IL-1α, IL-1β, IL-5, IL-4, IL-6, IL-12, IL-13, IL-16, IL-17A, IL-21, IL-23, IL-33, IFNλ, eotaxin, IFNα, thrombopoietin (TPO)	ELISA, Flow cytometry	Measurements are divided into three time-periods: 0–11 days after symptom onset, 12–19 days after symptom onset, and ≥20 days after symptom onset. If an individual had more than one measurement of a biomarker during any particular time period	COVID-19 of varied severity	Although COVID-19 patients with severe illness in the first few days from symptoms onset exhibited augment of IL-6 and IL-10, at later days from symptoms onset, they showed increased levels of IFN-α and IL-13, IL-17, IL-22, eotaxin, IFN- λ and a reduction of IL-6. Severe patients displayed an increase of monocytes with down-regulation of HLA-DR.	Lucas, C., Wong, P., Klein, J., Castro, T.B., Silva, J., Sundaram, M., Ellingson, M.K., Mao, T., Oh, J.E., Israelow, B. and Takahashi, T., 2020. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature, 584(7821), pp.463-469.
40	Pham et al.,	2021	Denmark	fracture classification of injury according to Arbeitsgemeinschaft für Osteosynthesefragen (AO) standards	47	IL-1β, IL-2, IL-6, IL-8, IL-12p70, TNF-α, IFN-γ, MMP-1, MMP-3, and MMP-9, IL-1RA, IL-4, IL-10, and IL-13, TGF-β1 and TGF-β2,	ELISA		Intra-articular fractures and posttraumatic osteoarthritis (PTOA) at the ankle.	Simultaneous elevations of both, pro-inflammatory cytokines IL-1β, IL-2, IL-6, IL-8, IL-12p70, TNF-α, IFN-γ, MMP-1, MMP-3, and MMP-9 and anti-inflammatory cytokines IL-1RA, IL-4, IL-10, and IL-13 in intra-articular ankle fractures versus healthy contralateral joints. White blood cell analysis of Synovial Fluid in acute ankle fracture joints showed an initial upregulation of neutrophils after injury. The neutrophil level then decreased in the following days. In contrast, the monocyte level was initially low and increased over the following days. The ratio of pro- and anti-inflammatory cytokines levels in the joint at a certain time point after injury may play an important role in the imbalance of metabolism leading to PTOA development.	Pham, T.M., Frich, L.H., Lambertsen, K.L., Overgaard, S. and Schmal, H., 2021. Elevation of Inflammatory Cytokines and Proteins after Intra-Articular Ankle Fracture: A Cross-Sectional Study of 47 Ankle Fracture Patients. Mediators of Inflammation, 2021.

3 Methodology

3.1 Experimental design, materials, and methods

The research study was designed to identify and analyse the relationship and interaction between a panel of immune biomarkers that have a significant impact on clinical outcome in a cohort of patients suffering major trauma. The study was approved UKCRN-NIHR Portfolio status (BIT 19377), which supported research nurse funding for clinical activities in Central Manchester Foundation Trust (CMFT) and Salford Royal Foundation Trust (SRFT). It was determined that 200 patients had to be recruited from both hospitals to meet the objectives of the wider study.

This Masters by Research study employed clinical data of patients with traumatic injuries from both hospitals to analyse the concentration of cytokines IL-13 (N=30) and IL-17 (N=30) from a wider panel of cytokines studied in the project. The panel included IL-4 (N=80), IL-6 (N=80), IL-8 (N=57), IL-10 (N=80), IL- 12 (N=80) and TGF-β (N=39). These cytokines were analysed by research colleagues at Prof. Nirmalan's laboratory and shared, along with clinical data obtained from the hospitals, amongst all of Prof Nirmalan's research students. In addition to the analysis of IL-13 and IL-17, this study covers a comparative analysis with IL-4, IL-8 and IL-12 for common patients i.e., patients from the cohort for whom concentration analysis was done for all of the above five cytokines. The comparative analysis was made using statistical data analysis methods to determine the correlation amongst the biomarkers in predicting patient outcome.

3.2 Ethical aspects

The research study received the ethical approval of Local Ethics Research Committee Manchester, NHS/HSC research and development offices- IRAD ID 172620- and the Ethical Committee, University of Salford, under ethics code ST1617-17.

3.3 Recruitment strategy

Both female and male ICU patients between the age group 16-90 from MRI and SRFT who underwent, or with immediate requirement for, surgical treatment were considered suitable to participate in the study so long as they had ISS above 15.

For the study, a team of research nurses from Central Manchester Foundation Trust (CMFT) and Salford Royal Foundation Trust (SRFT) recruited patients meeting the criteria. The patients themselves, or their next of kin, were required to fill in an informed consent form. They were given up to 48 hours to read it. Patients had the freedom to withdraw from the study at any time. The blood samples drawn from patients who withdrew were swiftly destroyed from the biobanks. To record patient consent, a consent form was put in place prior to drawing and storing blood samples (Appendix 1).

According to the protocol set for this study, blood samples were drawn at three points from recruited patients starting with a 20 ml of venous blood collected within 24 hours of the injury and then repeated on the third and fifth days after traumatic injury. A set of standard operating procedures were implemented, and training was also provided to all researchers to ensure consistency and reliability in the process.

Table 12) The clinical data collection sheet used for each of the four clinical data collection points.

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	____/N/A		
Treated with antibiotics	Y / N	Source of sepsis		_____/empirical/unknown	

Abbreviations: HR, Heart rate; Hb, Heamoglobin; eGFR, Estimated glomerular filtration rate; FiO^2, Fraction of Inspired Oxygen; BP, Blood pressure; WCC, White cell count; CRP, C Reactive Protein; GCS, Glasgow Coma Score; PLT, Platelet count; CVVH, Continuous Veno-Venous Heamofiltration.

Figure 41: The information sheet containing the clinical details, at each time point of blood sample collection.

Beside blood sampling, clinical details mentioned in figure 41 were also collected. This information sheet was filled in, at each time point in the study: days 1, 3, 5 and an additional sample in day 8. Based on the clinical data, ISS, SOFA, Δ-SOFA scores were calculated for each patient. A team of trained research nurses followed a unified standard protocol between MRI and SRFT and were responsible for drawing the bloods and entering the clinical parameters.

SOFA and ISS scores were then used in clinical and immunological evaluation studies by comparing the concentrations of the cytokines in the panel used in the study for predicting the patient's clinical outcome, which could be good, bad, or fatal.

3.4 Sample collection and transportation

At each time point, 20ml of venous blood was collected and stored on ice bath. On collection, the research nurse team from the hospitals informed our research team at the University of Salford. A courier was arranged to pick up the samples from the hospitals to bring to the university lab within 3-6 hours for sample preparation.

3.4.1 Separation of Serum

Of the 20ml blood sample, 10ml was used for serum preparation by transferring it into two 15ml polypropylene centrifuge tubes and centrifuged for five minutes at 2000rpm (644xg). The serum was obtained at the top layer and was transferred as aliquots of 300µl into 8 labelled cryovials. These were then stored in the blood components bank at -80°C. Cytokine measurement and quantification were done using the separated serum.

3.4.2 Separation of PBMCs

Peripheral blood mononuclear cells (PBMCs) play an important part in human immunity by giving selective responses to the immune system. A PBMC is any blood cell that consists of a round nucleus; examples include lymphocytes, monocytes, or macrophages. These blood cells are an essential element in the immune system fighting infection and responding to intruders. Separation of PBMCs from whole blood is done using Lymphoprep, a hydrophilic polysaccharide that separates layers of blood based on cellular density differences (Boujtita, 2008). The gradient centrifugation separates the blood layers with plasma on the top layer, a lower layer of PBMCs below followed by a fraction of polymorphonuclear cells (such as neutrophils and eosinophils) and finally erythrocytes at the bottom layer (Pourahmad and Salimi, 2015).

A 5 ml of blood sample was carefully layered with 5 ml of Lymphoprep (1:1 ratio) and centrifuged for 20 minutes at 1800 rpm (522xg) at 20°C. PBMCs form at the interface between serum and Lymphoprep after centrifugation. The PBMCs layer was harvested and transferred into a 15ml centrifuge tube. The PBMCs were given a phosphate-buffered saline (PBS) wash by centrifuging at 1200 rpm for 10 minutes. The PBMCs pellet formed at the

bottom of the tube was re-suspended in cryopreservation media. An ml of cryopreservation media was prepared from combing 900µl foetal bovine serum (FBS) and 100µl of dimethyl-sulfoxide (DMSO). The re-suspended pellet is transferred into cryovials placed in the container “Mr. Frosty” containing isopropyl alcohol. This was then cryopreserved at -80°C into the deep freezer and were utilised for cellular studies by all research students in the team.

3.5 Cytometric bead array – preparation of standards from known concentrations

The Cytometric Bead Array kits used in this study were purchased from Becton Dickinson (BD) Biosciences company. Each kit contains the BD CBA Human Soluble Protein Flex Set and Human Soluble Protein Master Buffer Kit. BD Fluorescence-Activated Single Cell Sorting (FACS) Verse flow cytometer with both 488nm and 640nm lasers was used for detecting cytokines. The CBA Human Soluble Protein Flex Set can generate the standard curve separately for each cytokine. The CBA Human Soluble Protein Flex Set also contains a vial of capture beads and PE detection reagent of 50X concentration, which needs dilution prior to use.

Becton Dickinson (BD) Cytometric Bead Array (CBA) standards are provided in lyophilized form and standards must be reconstituted and serially diluted for usage. The manufacturer’s instructional manual was followed for kit protocol. For cytometric analysis, the cryovials containing patient serum were first set to thaw at room temperature after being retrieved from the deep freezer. For the analysis, 50µl of patient serum was added to each tube followed by 50µl of mixed capture beads and mixed gently. The tubes were placed at room temperature for an hour for incubation. All serum samples were analysed in triplicate.

After the serum sample tubes had incubated for one hour, 50µl of PE detection reagent was added to each tube. The tubes were left to incubate for two hours at room temperature but also protected from direct light. After the two-hour incubation, 1ml of wash buffer was added to each tube and centrifuged at 200xg for 5 minutes. After the supernatant was aspirated, the pellet was resuspended in 300µl of wash buffer for flow cytometric analysis.

Three more reagents – one each for measuring standard cytokine concentration, mixed capture beads, and PE detection were required prior to the cytometric bead array assay. A standard curve was prepared using known standard concentrations and used to determine

the concentration of IL-13 and IL-17 in patients' samples. For preparing the standards, vials of lyophilised standard spheres from each CBA flex set were pooled into one tube. Following this, standard spheres were reconstituted in 4mls of assay diluent and left to equilibrate at room temperature for 15 minutes. Meanwhile, nine additional tubes were lined with 500µl of assay diluent and labelled with the standard concentrations, shown in Table 3. A negative control filled with just assay diluent was prepared independently from the serial dilution.

Tube label	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 3: Standard concentrations of Interleukin-13 and Interleukin-17A through serial dilution.
(Created following a serial dilution of standard spheres from the BD Bioscience flex sets).

3.6 Detection and analysis of IL-13 and IL-17 through flow cytometry

Serum samples from the recruited patients were analysed to detect the concentration of IL-13 and IL-17 using FACS Verse flow cytometer from BD Bioscience. The accompanying FAC Suite application was used to operate the analyser and evaluate the data.

In the FACS Verse flow cytometer, the sample is hit with a laser to identify fluorescent antibodies. The captured fluorescence was expressed as median PE fluorescence intensity (MFI) and used for analysis. MFI was then converted to the concentration of IL-13 and IL-17 using the standard curves generated during optimisation of the cytometric bead array method. The concentrations were then evaluated against patients' clinical data to determine if they have potential to predict patient outcome and the development of

complications. The criteria laid out in the following table used to categorise good and poor outcomes in patients.

Good consequences
• A SOFA score of <3, 5 days after traumatic injury.
Poor consequences
• A SOFA score of ≥ 3, 5 days after traumatic injury. • The patient remaining hospitalised 8 days after injury.
Multiple organ failure
A SOFA score of ≥6, indicating the onset of sepsis and increased in-hospital mortality rate.
Abbreviations: SOFA, Sequential organ failure assessment.

Table 4: The definitions of good and poor consequences for trauma patients used in this study.

3.7 Flow chart summarising the study design

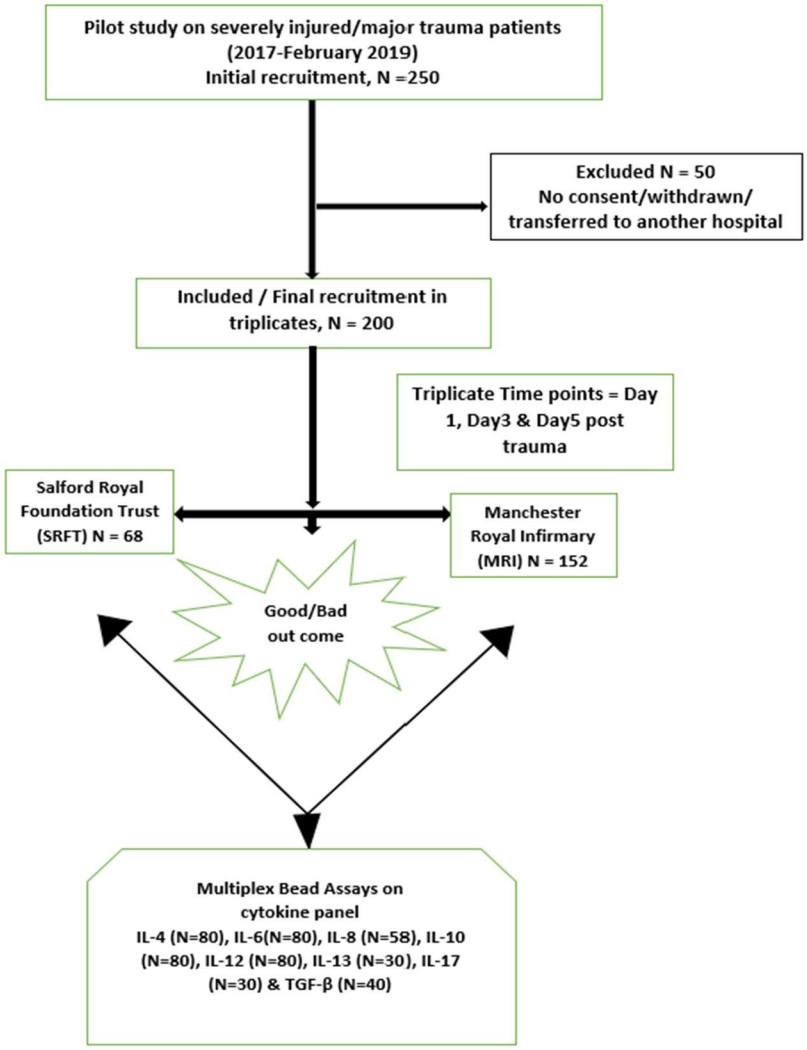


Figure 42: Flow chart of study design

Setting up cytometric bead array- fluorescence recompense

This study utilised cytokine assays on IL-13 and IL-17 with duplex samples for days 1 and 5 from recruited patients. For these two specific interleukins, a cohort of 30 patients with major traumatic injury were selected from the overall 200 patients recruited from Central Manchester Foundation Trust and Salford Royal Foundation Trust. The sample size n=30 was selected based on the latest patients recruitment and to match the ongoing analysis of IL-4, IL-8 and IL-12, to be utilized later for comparative analysis as the last stage of project since n=30 is an adequate sample number, it was also useful to draw statistical conclusions of trends.

Patients' clinical data was gathered for days 1, 3, 5 and 8, which allowed comparison of interleukin concentrations with a range of clinical and biochemical parameters. This report

focuses on interleukin concentrations on day 1 and day 5. The raw data from the study can be found in chapter 7- Appendices.

3.7.1 Optimisation of the cytometric bead array

Several experiments designed up to optimise the cytometric bead array for IL-13 and IL-17. These required setting up the correct gating for the capture beads to distinguish every population of capture bead to build the standard curves used for of IL-13 and IL-17 concentration. This process was undertaken for all from 30 patients' serum samples.

3.7.2 Optimizing IL-13 cytometric bead array

IL-13 capture beads were independently analysed to optimise the gating for this population. Gates were added around the general bead population before identifying the IL-13 capture beads (Figure 43).

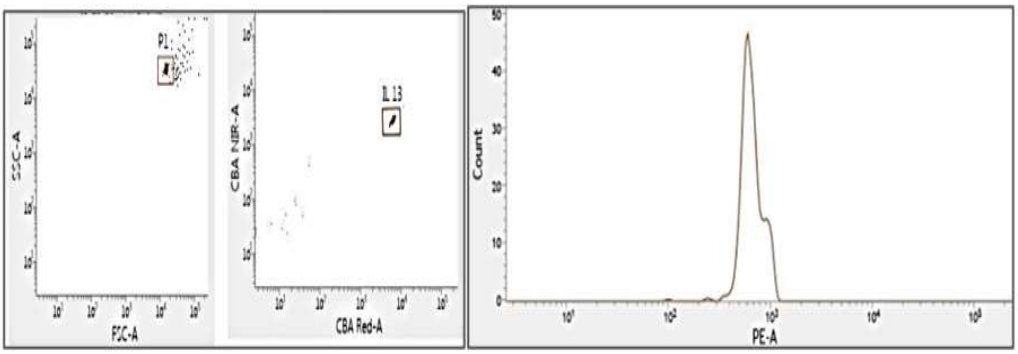


Figure 43: The flow cytometry gating (P1) to identify the interleukin-13 capture bead population by measuring the median fluorescence intensity

Once the gating was optimised for IL-13, a standard curve was generated using the standard concentration.

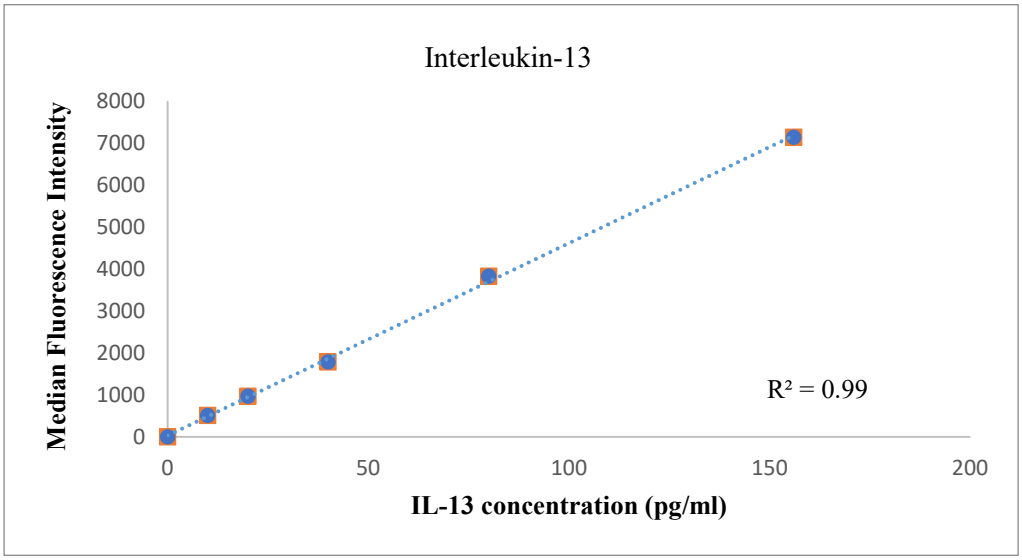


Figure 44: The standard curve generated for IL-13 from a single capture bead population

3.7.3 Optimising interleukin-17 cytometric bead array

Subsequently IL-17 capture beads were independently analysed, to optimise the gating for IL-17 population of capture bead. Gates were added around the general bead population before identifying the IL-17 capture beads arrays (Figure 45).

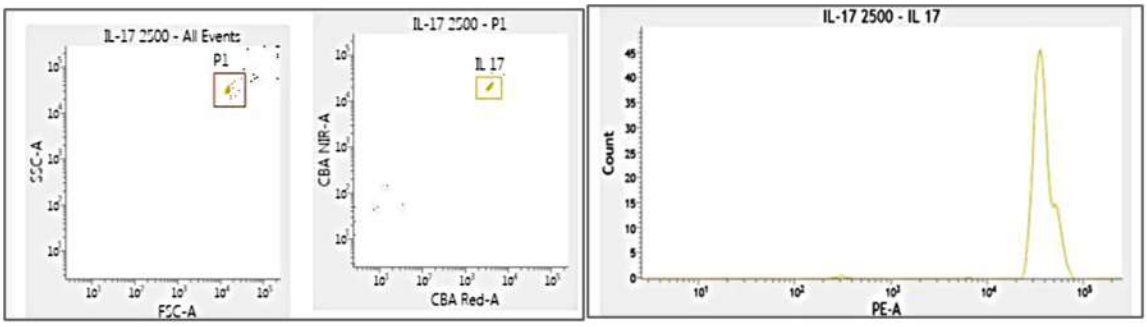


Figure 45: showing the flow cytometry gating (P1) in identifying the interleukin-17 capture bead population to measure the median fluorescence intensity.

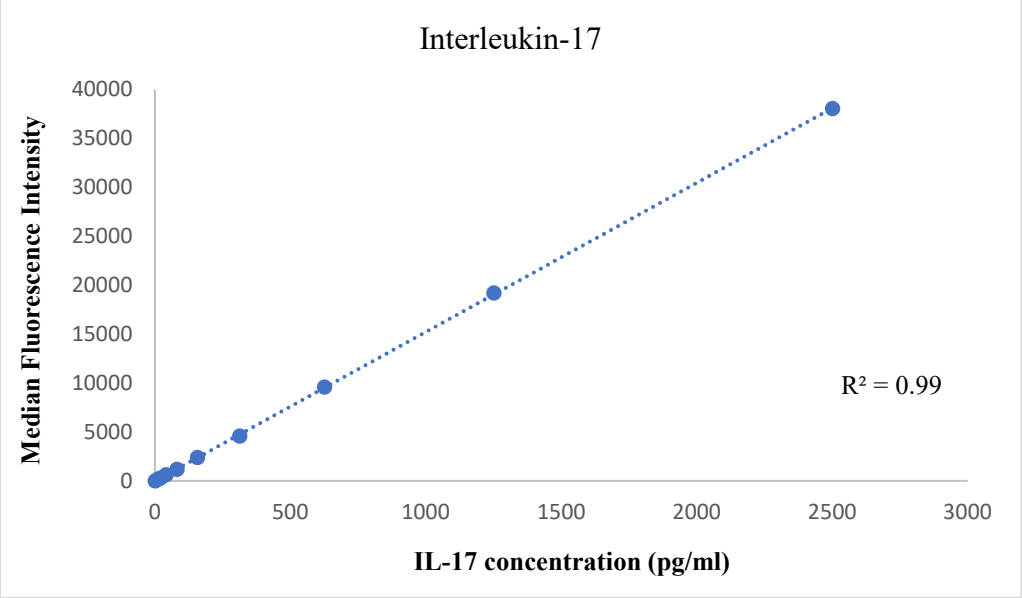


Figure 46:The standard curve generated for IL-17 from a single capture bead population

The standard curves were derived through a range of pg/ml concentrations of the standards on the x axis/and plotted PE-median fluorescence intensity on y-axis to deduce cytometric measurements. Once the gating was optimised for IL-17, a standard curve was generated using standard concentrations.

3.8 Assessment by Statistical analysis

For meeting the objectives of this research study, data was analysed in two ways. First, assessment was carried out to understand the pattern variations between serum cytokines levels in samples drawn on days 1 and 5. Second, the concentrations of cytokines were compared with both clinical parameters and scores such as ISS and SOFA. For this purpose, statistical analysis was performed using Microsoft Excel 2019 and SPSS version 20. The normality was tested using two sample T-test to determine the parametric or non-parametric nature of the data (Wang and Lee, 2020). For the data set, a pairwise comparison was carried out by using Mann-Whitney U-test and Spearman Rho correlation coefficient test. Statistical significance was defined as $p<0.05$. The data was summarised using mean, standard deviation (SD), standard error (SEM) (Ennos, 2007). The cytokine concentration used for analysis were the average of the results measured on days 1 and 5. Every sample was analysed in triplicate and the average was used as the final reading for cytokine concentration in

pg/ml. Since mean was used to obtain cytokine concentration, further analysis was also done using mean and SEM.

3.8.1 Analysis of total patient cohort

As on the 4th of February 2019, the study had received a total of 250 patient samples collectively from Manchester Royal Infirmary and Salford Royal Hospital. Those patients who could not provide samples at the desired time points, withdrew consent, or were transferred to a different hospital were removed from the study (Figure 47).

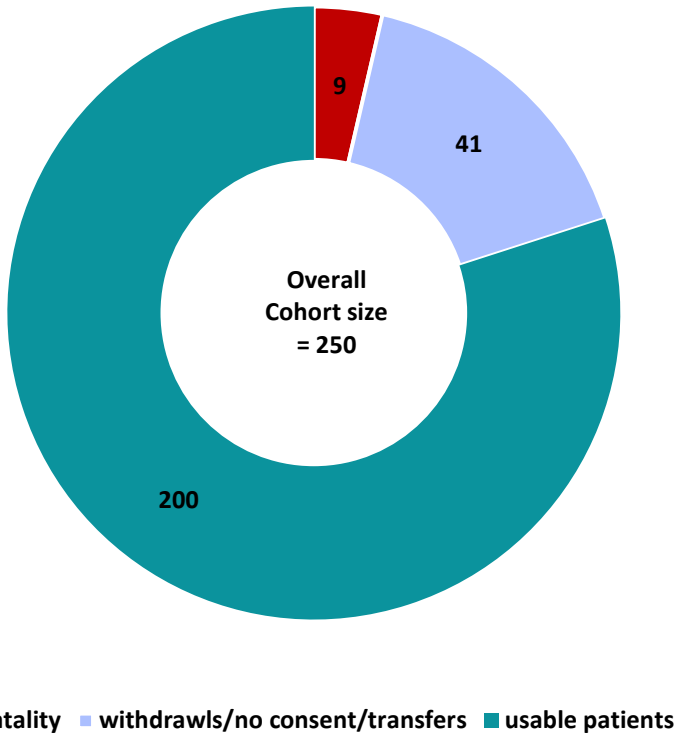


Figure 47: Patient cohort - Analysis of patient cohort

From samples received from the two hospitals, Manchester Royal Infirmary and Salford Royal, 200 samples were found to be usable for study. The chart in figure 47 shows the count of patients by category.

Following receipt of day 8 clinical data, the patient was put into good or poor outcome categories. The categorisation was based on the patient's day 5 SOFA score and whether the patient remained in hospital past day 8 following traumatic injury – the standard criteria in differentiating good and poor outcome patients shown in table 4 in Chapter 3.

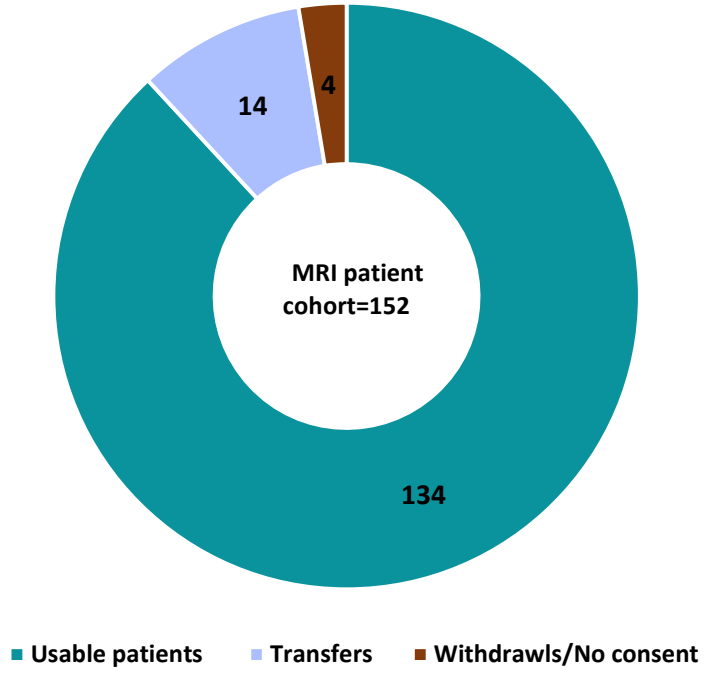


Figure 48: Patient cohort from Manchester Royal Infirmary.

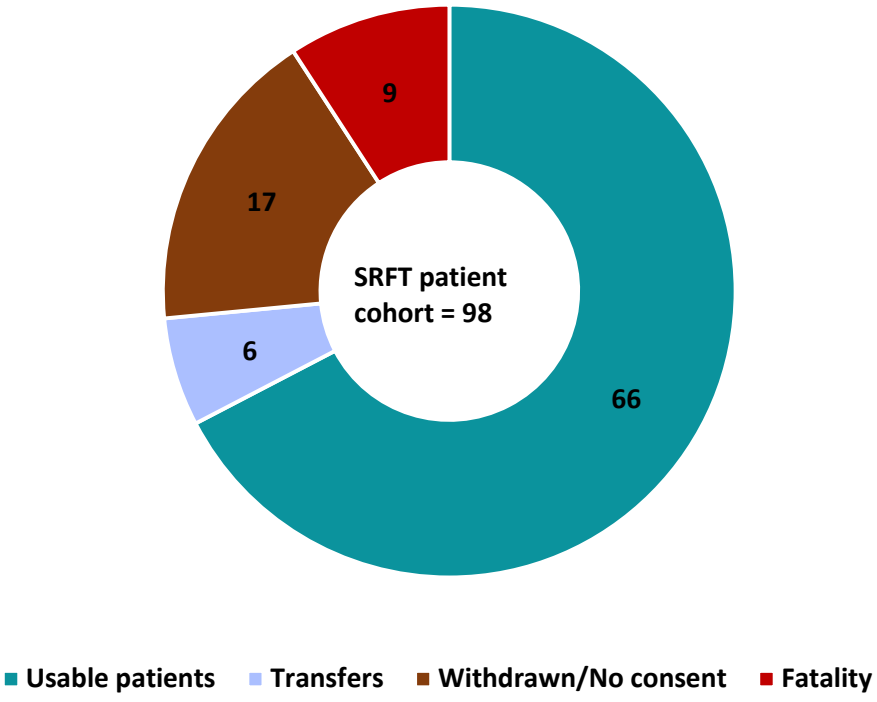


Figure 49: Patient cohort from Salford Royal Hospital

Of the 250 patients, 152 were recruited from MRI and 98 from SRFT. The above two charts (figures 48 and 49) show the count of patients whose samples were used in the study and those who were withdrawn, transferred out or withdrew consent. That left 134 patients from MRI and 66 patients from SRFT.

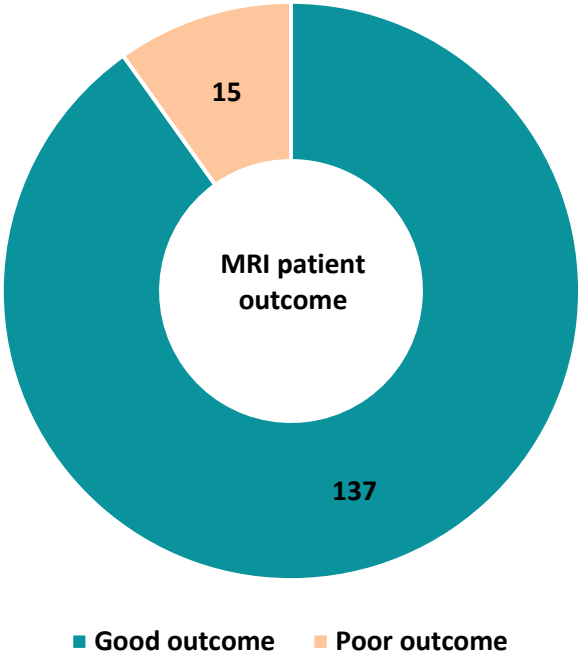


Figure 50: A comparison of good and poor outcome patients from Manchester Royal Infirmary

Figure 50 and 51 shows the results obtained from analysing the patient samples received from Manchester Royal Infirmary and Salford Royal Hospital, respectively. A comparison was made as good and poor outcome for entire cohort, based on the conditions mentioned in table 3.

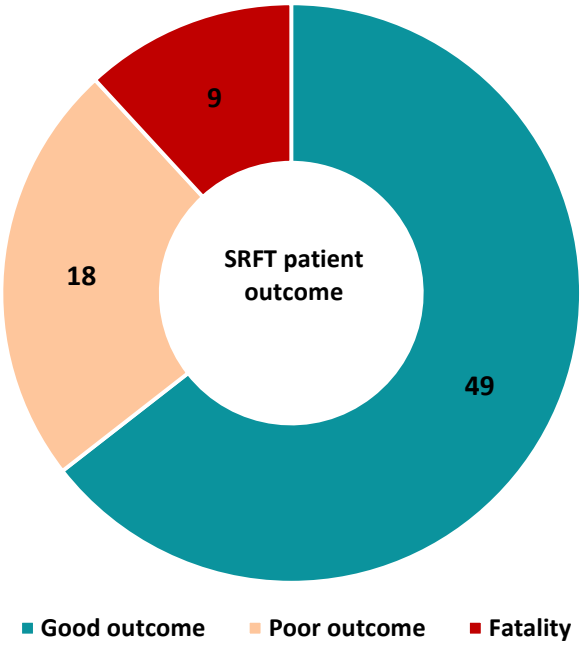


Figure 51: A comparison of good and poor outcome patients from Salford Royal Hospital.

Out of the samples of 200 patients, 30 duplicate sets (days 1 and 5) were used for the analysis of IL-13 and IL-17. Of these, 17 were from Manchester Royal infirmary and the rest from Salford Royal. Although day 3 samples were used in the larger study by other research colleagues looking into IL-6 and IL-10, it was subsequently omitted because of funding limitations. The duplicate sets were also used for analysing IL-4, IL-8, IL-12 and TGF-β. Subsequently, statistical analysis for the wider cohort of patients was conducted by other researchers (Jones, 2017, Apreutesei, 2019) to evaluate whether the panel of cytokines could be used as biomarkers for predicting patient outcome and possibility of the patients' developing further complications such as MODS or MOF.

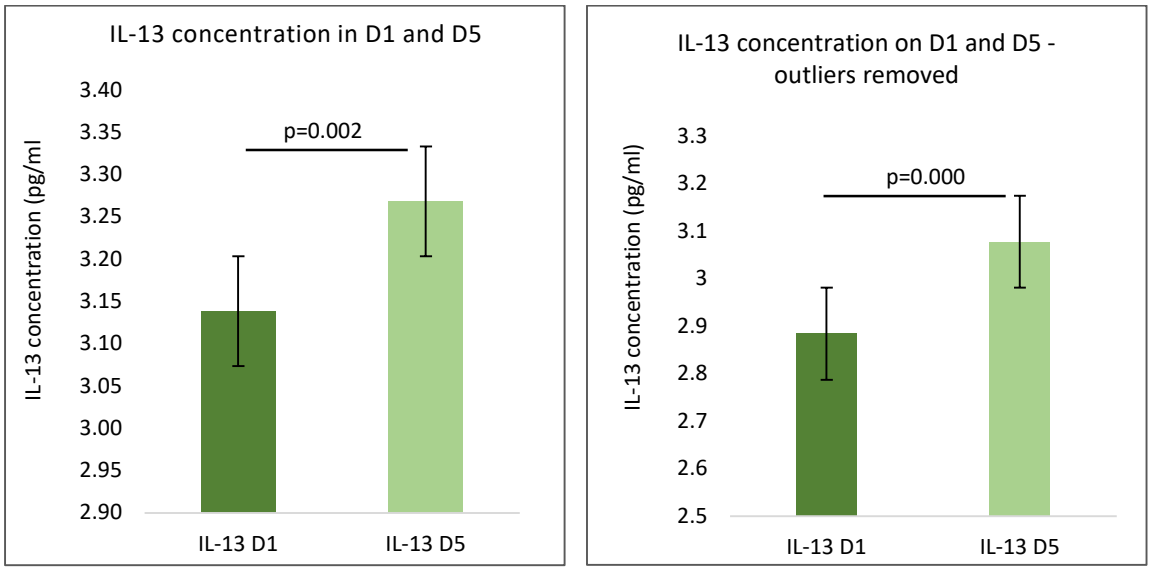
4 Results

4.1 Interleukin-13 as a predictor of patient outcome

Interleukin-13 was measured in patients' day 1 and day 5 serum samples using CBA (Figure 43). Measuring IL-13 levels in the serum samples of patients with trauma could predict the clinical outcome of the patient over the days of hospital stay indicating deteriorated heath or recovery.

4.1.1 Interleukin-13 concentrations in trauma patient serum samples

A) B)



Figures 52A & B: Mean IL-13 concentrations (D1/D5 comparison) with standard error bars.

IL-13 levels on day 1 ranged between a minimum of 2.459 pg/ml to a maximum of 5.568 pg/ml. For this range, the standard deviation was 0.7095 and standard error 0.1295. On day 5, the minimum IL-13 was noted as 2.680 pg/ml and a maximum value of 5.844 pg/ml. The standard deviation for this range was 0.6844 with a standard error of 0.1250.

The average IL-13 concentration, measured as both mean and median, in days 1 and 5 showed a minor variation. Median concentrations were 3.081 pg/ml and 2.942 pg/ml. Figure 52A shows the average (mean) concentration on day 1 and day 5 are shown, which were 3.138 pg/ml and 3.268 pg/ml, respectively. The significance or 'p' value calculated

using Mann-Whitney U-test, shows a statistically significance at 0.002. Figure 52B shows average concentrations for days 1 and 5 but with outlier data were removed to avoid skews. With outliers removed, the average (mean) concentration on day 1 was 2.884 pg/ml and increased to 3.077 pg/ml in day 5. Of the 30 patients, for IL-13 day1, there were three outlying values with an average concentration of more than 50% of the mean for the cohort. On the same basis, there were two outlying value for IL-13 day 5, which were removed.

IL-13 concentration for the cohort after removing outliers is shown in figure 52B. When the outliers were removed, the p value improved to 0.000 showing a statistical significance.

4.2 Comparison of IL-13 concentration in D1 with D5 SOFA score (threshold of 3)

This section shows the comparison of IL-13 concentration in day 1 with day 5 SOFA score. The comparison is made with day 5 SOFA score because it is a better indicator of the patient's medical outcome. The threshold for SOFA score to determine good (<3) and poor outcomes (≥ 3) is as laid out in table 4.

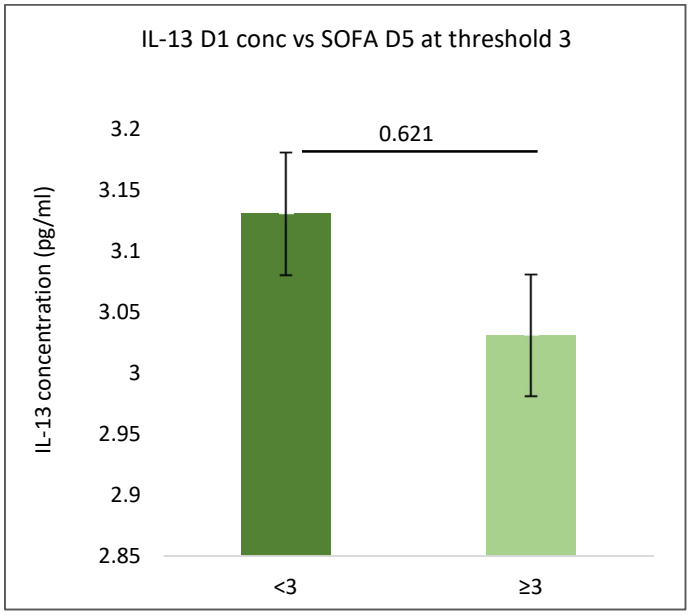


Figure 53: Concentration of day 1 IL-13 compared to day 5 SOFA score at threshold 3. Mann-Whitney U-test was used to compare the data clustered in the groups of SOFA <3 and SOFA ≥ 3). The data is presented as mean values with standard error bars.

4.3 Comparison of IL-13 concentration in D1 with D5 SOFA score (threshold of 6)

This section shows the comparison of IL-13 concentration in day 1 with day 5 SOFA score but uses a different threshold cut-off of <6 and ≥6. The threshold of 6 was chosen as it indicates that there is more than one organ starting to fail and increases the risk to develop

sepsis with subsequent increased risk of death. This has been done because SOFA score 6 indicates MOF. In this way we can compare cytokine concentration at different SOFA thresholds.

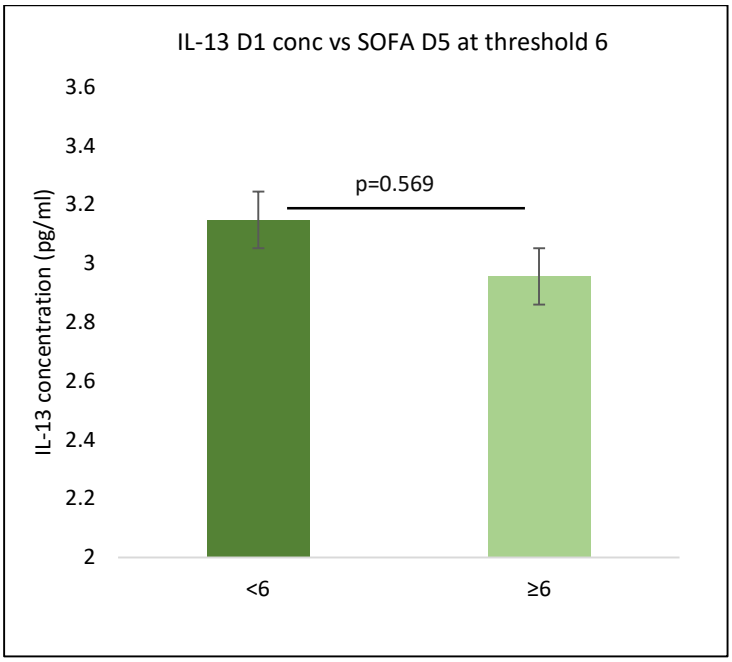


Figure 54: Concentration of day 1 IL-13 compared to day 5 SOFA score at threshold 6. Mann-Whitney U-test was used to compare the data clustered in the groups of SOFA <6 and SOFA ≥ 6). The data are presented as mean values with standard error bars.

The same set of data was verified under two separate thresholds to assess whether the pattern changes. When the threshold was changed from <3 and ≥3 to <6 and ≥6, the p value changed slightly but did not show statistical significance in either scenario. For the former threshold, the p value was 0.621 but this decreased to 0.569 for the latter. Neither of these p values show significance between the two ranges despite the downward movement when the threshold was changed.

4.4 Comparison of 1L-13 concentration in D1 with D8 SOFA score

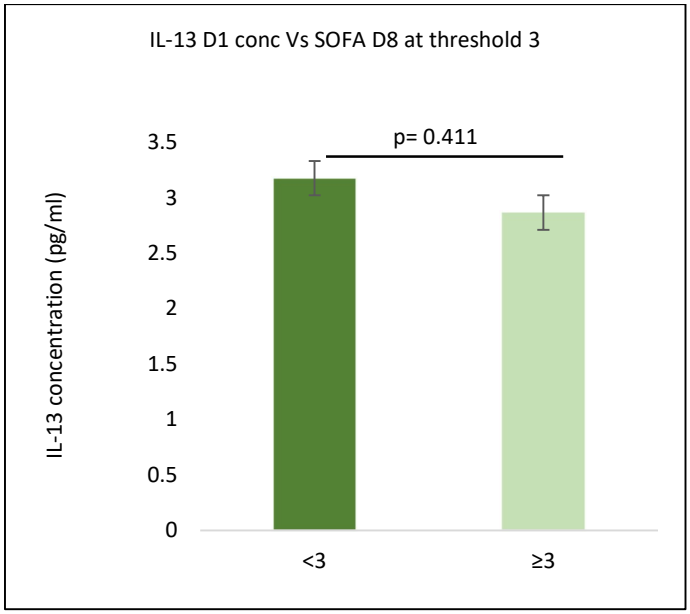


Figure 55: Concentration of day 1 IL-13 compared to day 8 SOFA score at threshold 3. Mann-Whitney U-test was used to compare the data clustered in the groups of SOFA <3 and SOFA ≥ 3) The data are presented as mean values with standard error bars.

Figures 55, 53 show the average concentration of day 1 IL-13 that were assessed against day 8 SOFA score. This was also put into the two different thresholds, <3 and ≥3 and <6 and ≥6 to understand whether the pattern changes. When the threshold was changed, the p value increased but neither thresholds showed significance. For the <3 and ≥3 threshold, the p value was 0.411 but increased to 0.517 for the <6 and ≥6 group. Neither of these p values show significance between the two ranges.

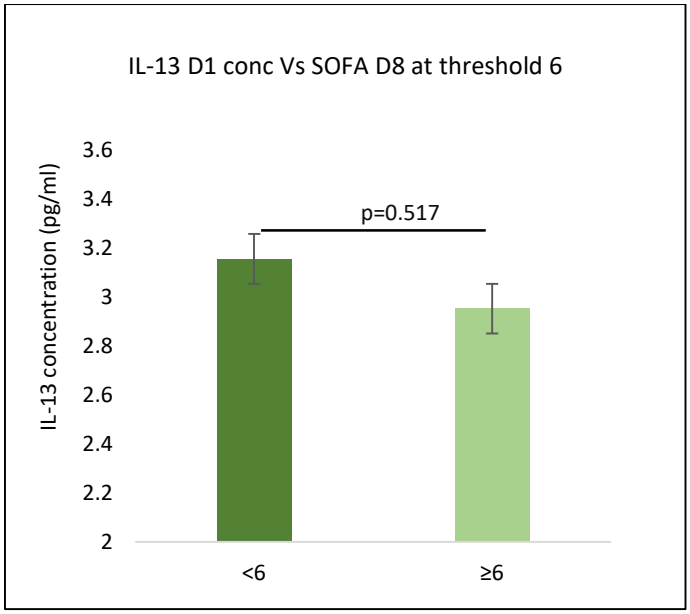


Figure 56: Concentration of day 1 IL-13 compared to day 8 SOFA score at threshold 6.

Mann-Whitney U-test was used to compare the data clustered in the groups of SOFA <6 and SOFA ≥ 6). The data is presented as mean values with standard error bars.

4.5 D1/D5 ratio for IL-13 concentration and SOFA score in D5, as predictor of clinical outcome

A) B)

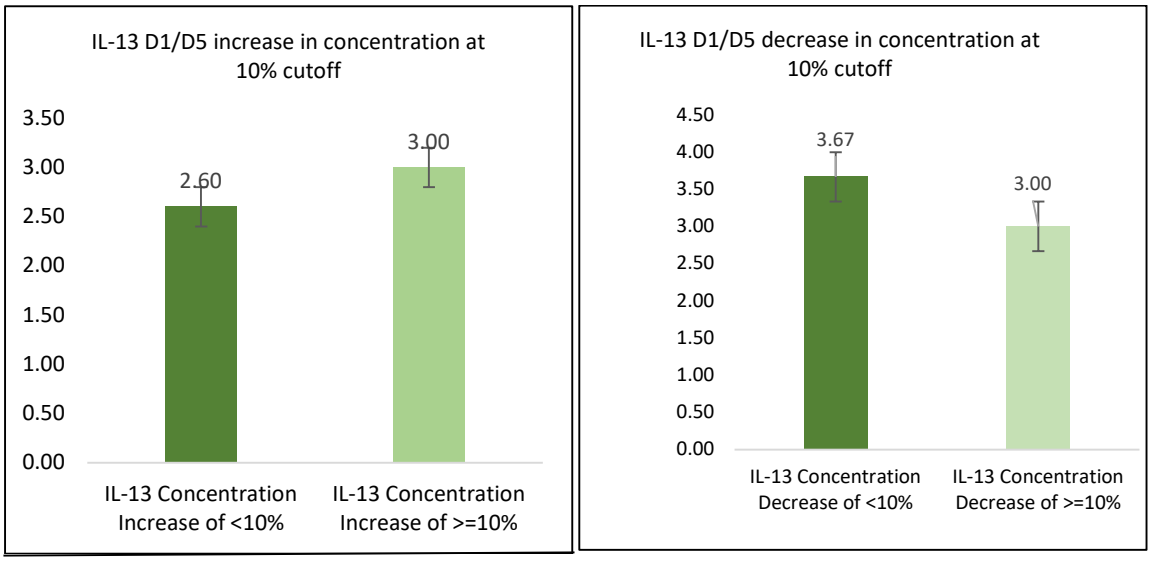


Figure 57A & B: IL-13 D1/D5 concentration ratio displayed in day 5 SOFA score

This test is performed mainly to see by what percentage the concentration between the day 1 and day 5 changes and how they show changes at different SOFA scores and whether this could indicate patient outcome on day 5 post trauma. As a first step, the change of concentration for all the 30 patients was calculated by noting the difference in day 5 concentration minus day 1 concentration of IL-13. This change was noted in percentages.

To arrive at the appropriate percentage ratio, the data has been segregated into four categories: category 1 where the concentration has increased from day 1 to day 5 by more than 10%, category 2- where the concentration of IL-13 from day 1 to day 5 has increased by up to 10%, category 3- shows the concentration of IL-13 has decreased by less than 10% and category 4 showing a decrease by more than 10%. The maximum increase is 26.54% and the minimum increase is 1.36 and the average of this change in percentage for IL-13 concentration is 9.32%. Therefore, a 10% cut-off was chosen as it is closest to the mean value and which is not significantly different from the average, in order to avoid the skewness in the data and the median increase is 7.24%.

As a second step, delta SOFA was calculated (day 5 SOFA – day 1 SOFA). The following different observations were noted pertaining delta SOFA scores within the sub-cohort of 30 - an increase in SOFA score between day 1 and day 5 (4 patients), decrease in SOFA score from day 1 to day 5 (21 patients) and no difference in the SOFA score at all (5 patients). Percentage of delta SOFA was calculated. The following observations were noted for percentage of change in concentration and corresponding change in percentage delta SOFA at 10% cut-off value – a change in the concentration for decrease in SOFA, change in the concentration for increase in SOFA, change in the concentration with no change in SOFA at all.

The averages were calculated for each category and represented in the figures 57A and 57B. These graphs represent, amongst the patients with increased IL-13 concentration between Day 1 and Day 5, the SOFA score on Day 5 has also slightly increased to 2.6. IL-13 concentration with an increase ≥ 10% is 3.0. As shown in figure 57A, the increase in the IL-13 concentration on D5 by 10%, tallies with decreased SOFA score. Since lower SOFA scores are

associated with better clinical outcomes, the increase in IL-13 concentration could be an indication of good clinical outcome. Correlation between day 1 IL-13 and Delta SOFA

The below graph shows the relationship between Δ SOFA and IL-13 concentration in pg/ml on DAY 1. A Spearman correlation test showed that there is a negative relationship between them (r=-0.318, p=0.087). This implies that as IL-13 concentration increases delta SOFA reduces, which indicates better clinical outcomes with higher IL-13 concentration. This reinforces the findings depicted in figures 57A and 57B which show that higher levels IL-13 concentration is associated with decrease in SOFA score, and thereby good clinical outcomes.

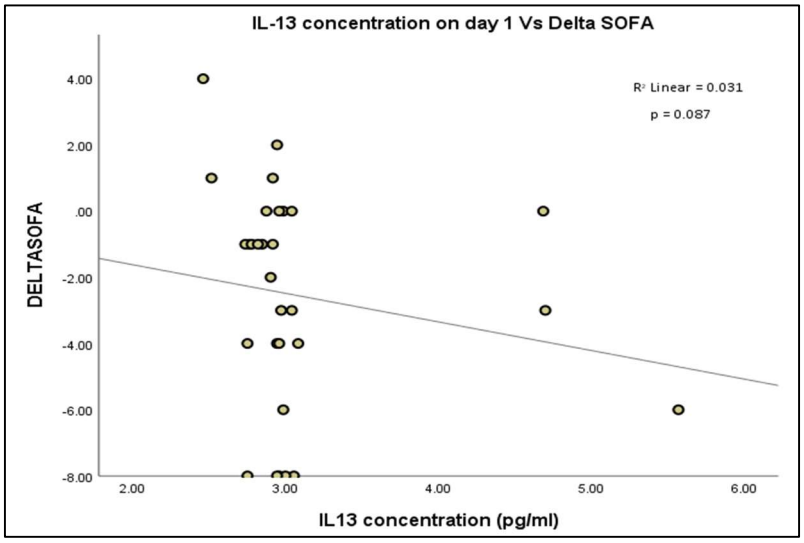


Figure 58: concentration of day 1 IL-13 compared with delta SOFA.

4.6 Interleukin-17 as a predictor of patient outcome

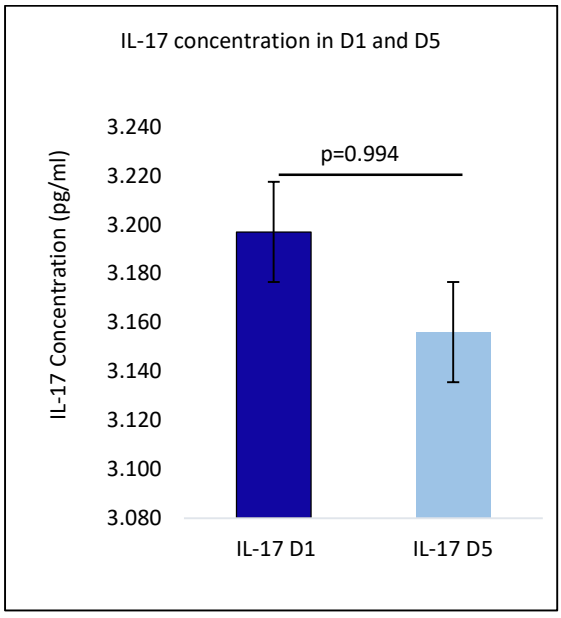
Measuring IL-17 levels in the serum samples of patients with trauma could predict the clinical outcome of the patients over the days of hospital stay indicating deteriorated heath or recovery. Using CBA, IL-17 was measured in duplex on patient serum samples from days 1 and 5.

4.6.1 Interleukin-17 concentrations in trauma patient serum sample

IL-17 concentration on day 1 ranged from a minimum of 2.55 pg/ml to a maximum of 4.71 pg/ml. For this range, the standard deviation was 0.46 and standard error was 0.08. For day 5, the minimum IL-17 concentration was 2.83 pg/ml and the maximum 4.63 pg/ml. The standard deviation was 0.33 and the standard error was 0.06. Mean IL-17 concentration for

day 1 was 3.19 pg/ml, whereas in day 5 it was 3.15 pg/ml. This is shown in figure 59A. The median concentration for day 1 was 3.15 pg/ml, whereas for day 5 it was 3.07 pg/ml. Statistical analysis showed no significant difference between days 1 and 5. The p value obtained Mann-Whitney U-Test was 0.99 (Figure 59A). Figure 59B was generated after removing the outliers. Without the outliers, the minimum value on day 1 was 2.555 pg/ml and maximum concentration was 3.772 pg/ml with an average concentration of 3.099 pg/ml. On day 5 the minimum was 2.832 pg/ml, and the maximum was 3.468 pg/ml with a mean of 3.105 pg/ml. The p value for these ranges was 0.817. This shows a slight change but there is no statistical significance between the average concentrations of IL-17 in day 1 and day 5 with or without outliers but indicating a movement in the concentrations. Patients with an average concentration of more than 50% of the mean for the cohort were treated as outliers and removed. Of the 30 patients, there were two outlying values for day 1 and one outlying value for day 5. IL-17 concentration for the cohort after removing outliers is shown in figure 59B.

A)



B)

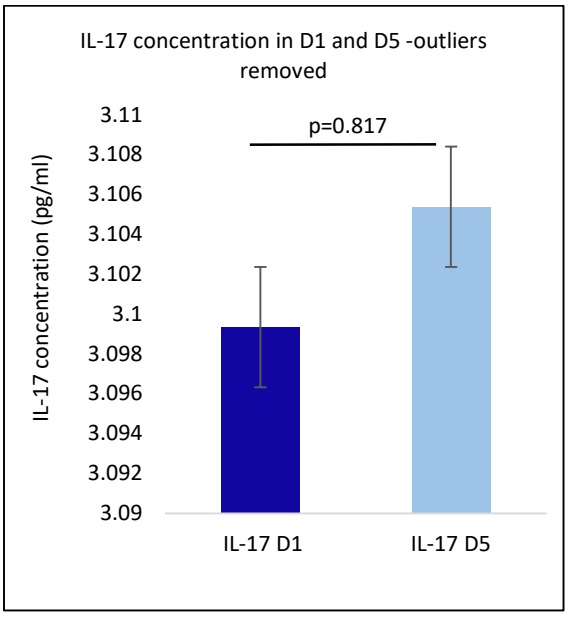


Figure 59A & B: Mean IL-17 Concentrations (D1/D5 comparison).

4.7 Comparison of IL-17 concentration in day 1 with day 5 SOFA score at threshold 3

A) B)

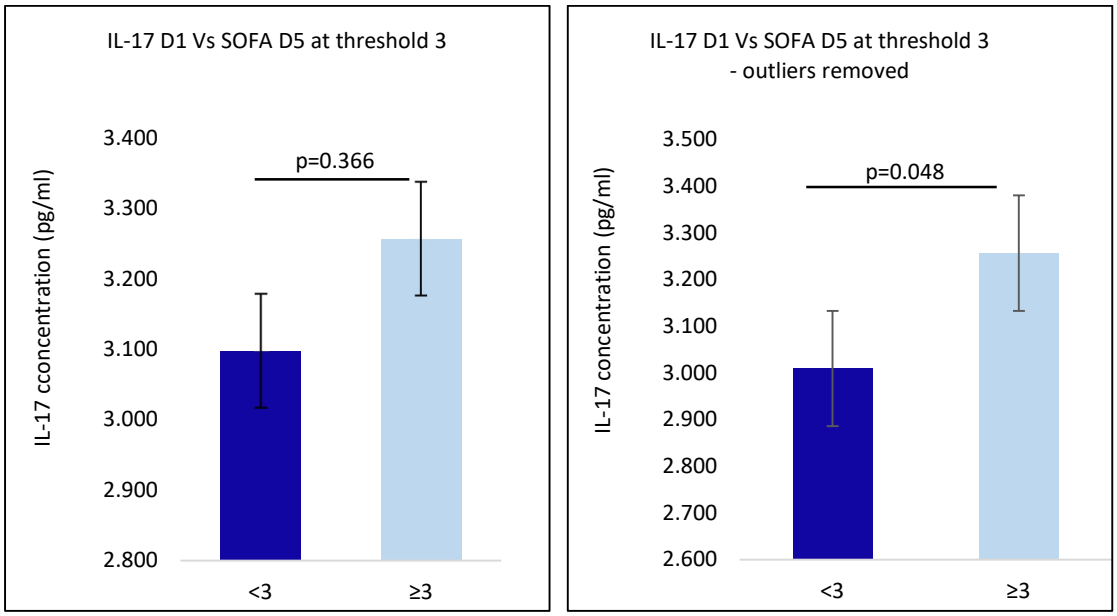


Figure 60A & B: Comparison of day 1 IL-17 concentration with day 5 SOFA score (threshold of 3).

4.8 Comparison of IL-17 concentration in day 1 with day 5 SOFA score at threshold 6

A) B)

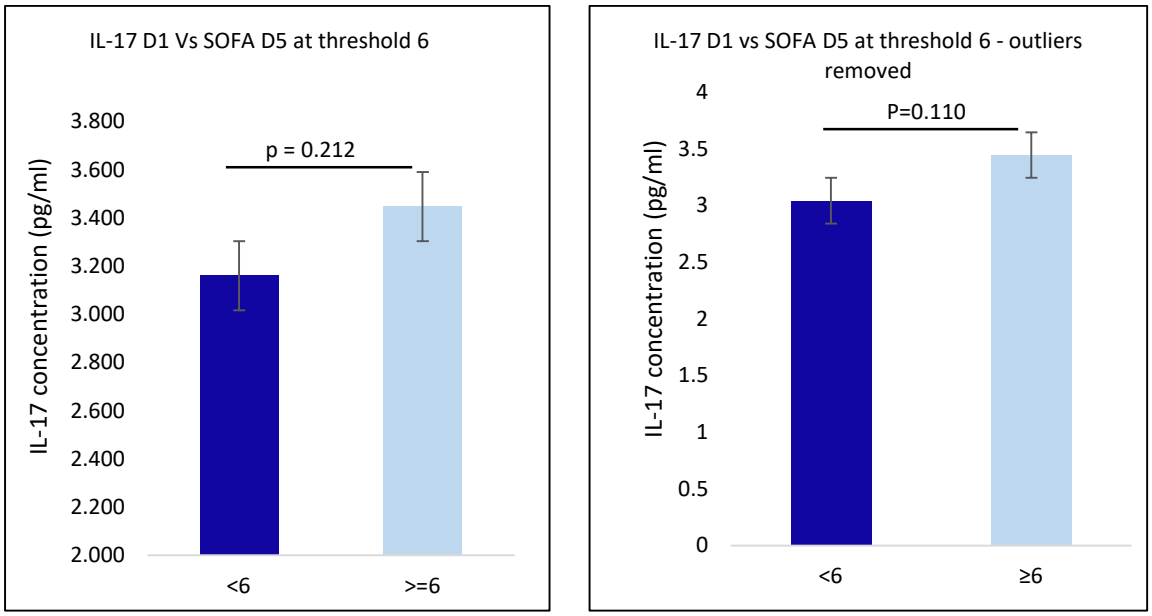


Figure 61A& B: Comparison of day 1 IL-17 concentration with day 5 SOFA score (threshold of 6).

(Mann-Whitney U-test was applied to assess the data clustered in two groups for SOFA <6 and ≥6).

The same set of data was verified under different thresholds, to see whether the pattern changes. When the threshold was changed from <3 and ≥3 (p=0.366) and p changed to 0.048 when outliers were removed. With cut-off at <6 and ≥6 (p=0.212) and the p value decreased to 0.110 when the outliers were removed. No statistical significance was observed in either of ranges.

4.9 Comparison of IL-17 concentration in D1 with D8 SOFA score at threshold 3 and 6

A) B)

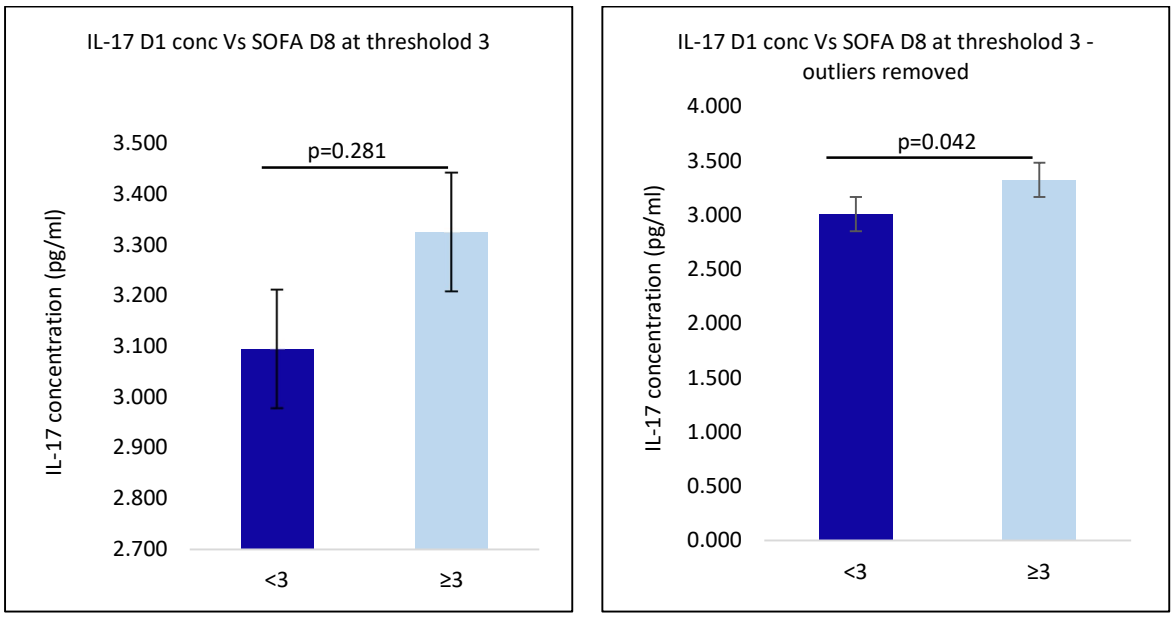


Figure 62A& B: Comparison of day 1 IL-17 concentration with D8 SOFA score (threshold of 3). (Mann-Whitney U-test was applied to assess the data clustered in two groups for SOFA <3 and ≥3).

A) B)

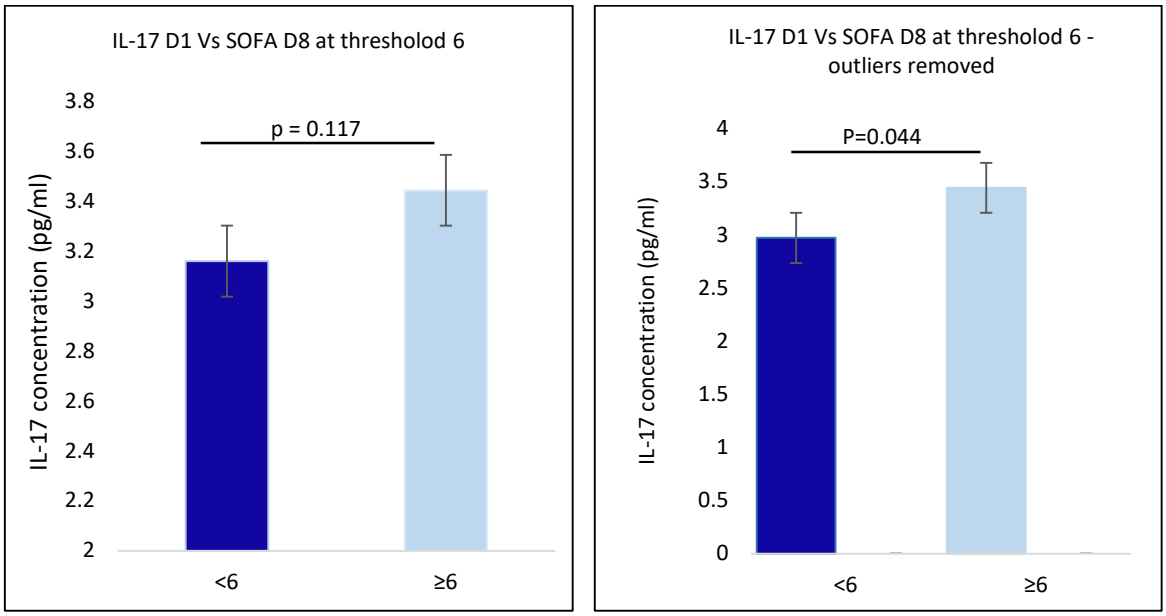


Figure 63A & B: comparison of day 1 IL-17 concentration with D8 SOFA score (threshold of 6). (Mann-Whitney U-test was applied to assess the data clustered in two groups for SOFA <6 and ≥6).

Repeating the SOFA score threshold-based grouping for IL-17 concentration by day 8 SOFA score showed the following results - The p value for <6 and ≥6 threshold was 0.117 for the full range. On removing the outliers, IL-17day 1 concentration showed a statistical significance with SOFA scores on day 8 at p=0.044.

4.10 D1/D5 ratio for IL-17 concentration and SOFA score in D5, as predictor of clinical outcome

On examining the data presented in the figures 64A& B, showed that the maximum increase of IL-17 between D1 and D5 within cohort of 30 patients is 26.54%. The minimum increase for those patients with IL-17 concentration between D1 and D5 is 1.3593%. The average increase is based on mean which is 9.32%. The average is based on median which is 7.24%. The cut off 10% was ideal because it is closest to the mean increase and slightly above the median increase, and this avoids any skewness in the data.

A) B)

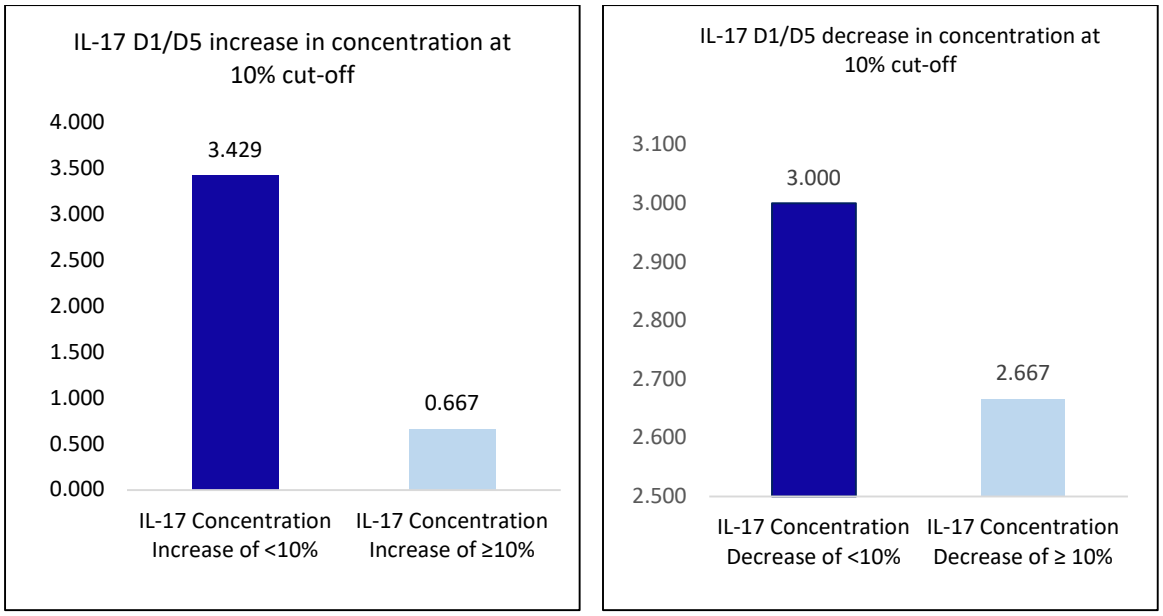


Figure 64A & B: IL-17 D1/D5 concentration ratio displayed in D5 SOFA score

This test is performed mainly to see by what percentage the concentration between the day 1 and day 5 concentration changes and how they show change at different SOFA score and if this could indicate patient outcome on day 5 post trauma. As a first step, the change of concentration for all the 30 patients was calculated by noting the difference in day 5 concentration minus day 1 concentration of IL-17. This change was noted in percentages.

To arrive at the appropriate percentage ration, this has been segregated into four categories: category 1 where the concentration has increased from day 1 to day 5 by more than 10%, category 2- where the concentration of IL-17 from day 1 to day 5 has increased by up to 10%, category 3- shows the concentration of IL-17 has decreased by less than 10% and category 4 showing a decrease by more than 10%. The maximum increase is 19.46% and the minimum increase is 0.46% and the average of this change in percentage for IL-17 concentration is 6.41%. Therefore, a 10% cut-off was chosen as it is closest to the mean value and which is not significantly different from the average, in order to avoid the skewness in the data.

As a second step was based on delta SOFA (day 5 SOFA – day 1 SOFA). The following different observations were noted pertaining delta SOFA scores within the sub-cohort of 30 - an increase in SOFA score between day 1 and day 5 (17 patients), decrease in SOFA score

from day 1 to day 5 (13 patients) and no difference in the SOFA score at all (zero patients). Percentage of delta SOFA was calculated. The following observations were noted for percentage of change in concentration and corresponding change in percentage delta SOFA at 10% cut-off value – a change in the concentration for decrease in SOFA, change in the concentration for increase in SOFA, change in the concentration with no change in SOFA at all.

The averages were calculated for each category and represented in the charts. Figure 64A shows that amongst the patients with increased IL-17 concentration between day 1 and day 5, with an increase ≥ 10% is 3.0. Figure 64B shows that SOFA score on day 5 has also slightly decreased to 2.7. The decrease in IL-17 concentration on day 5 by more than 10%, tallies with increased day 5 SOFA, which could indicate poor clinical outcome.

4.11 Logarithmic chart for the panel of cytokines

	IL-4	IL-6	IL-8	IL-10	IL-12	IL-13	IL-17	TGF-β
D1 Cytokine Vs D5 SOFA (<3)	3.52	73.13	39.02	7.91	6.73	3.13	3.10	6878.18
D5 Cytokine Vs D5 SOFA (<3)	3.63	14.69	17.36	20.28	7.04	3.32	3.12	4623.19

Table 5: Day 1 and day 5 concentrations of cytokines with day 5 SOFA at threshold 3

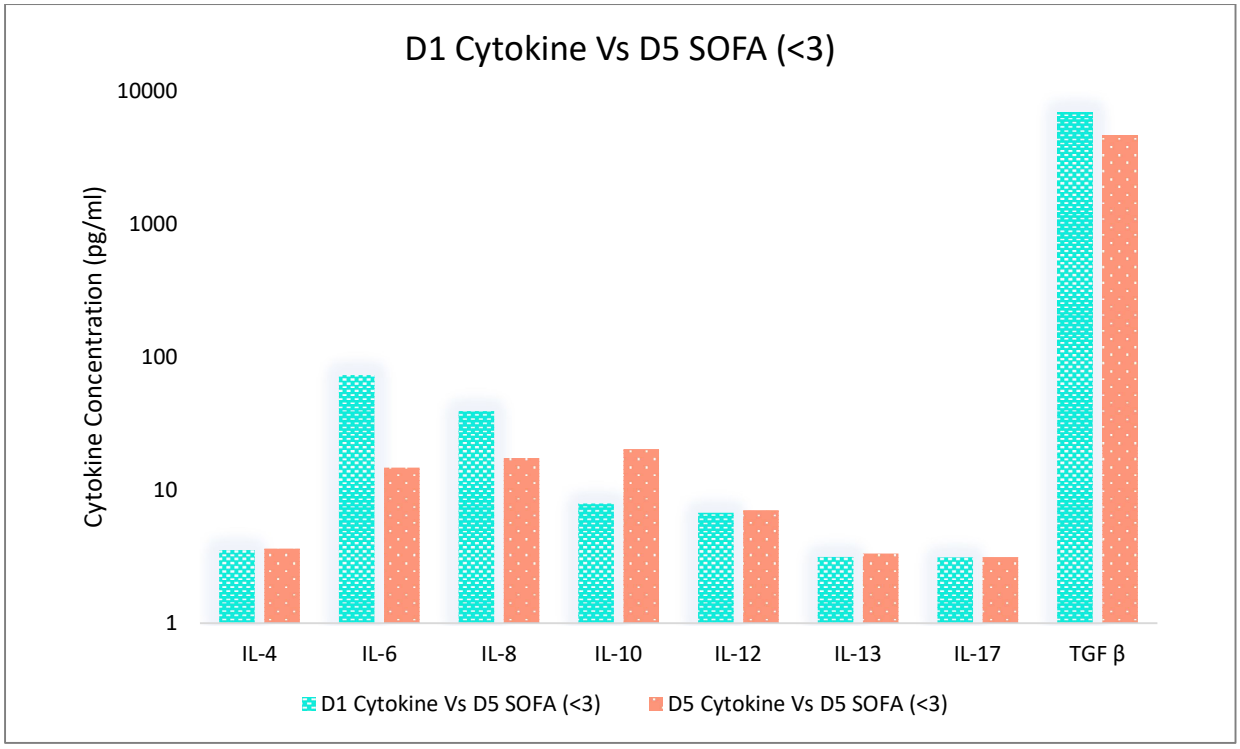


Figure 65: An outline of day 1 and day 5 concentrations of IL - 4, IL - 6, IL-8, IL-10, IL-12, IL-13, IL-17 and TGF-β associated with D5 SOFA score <3

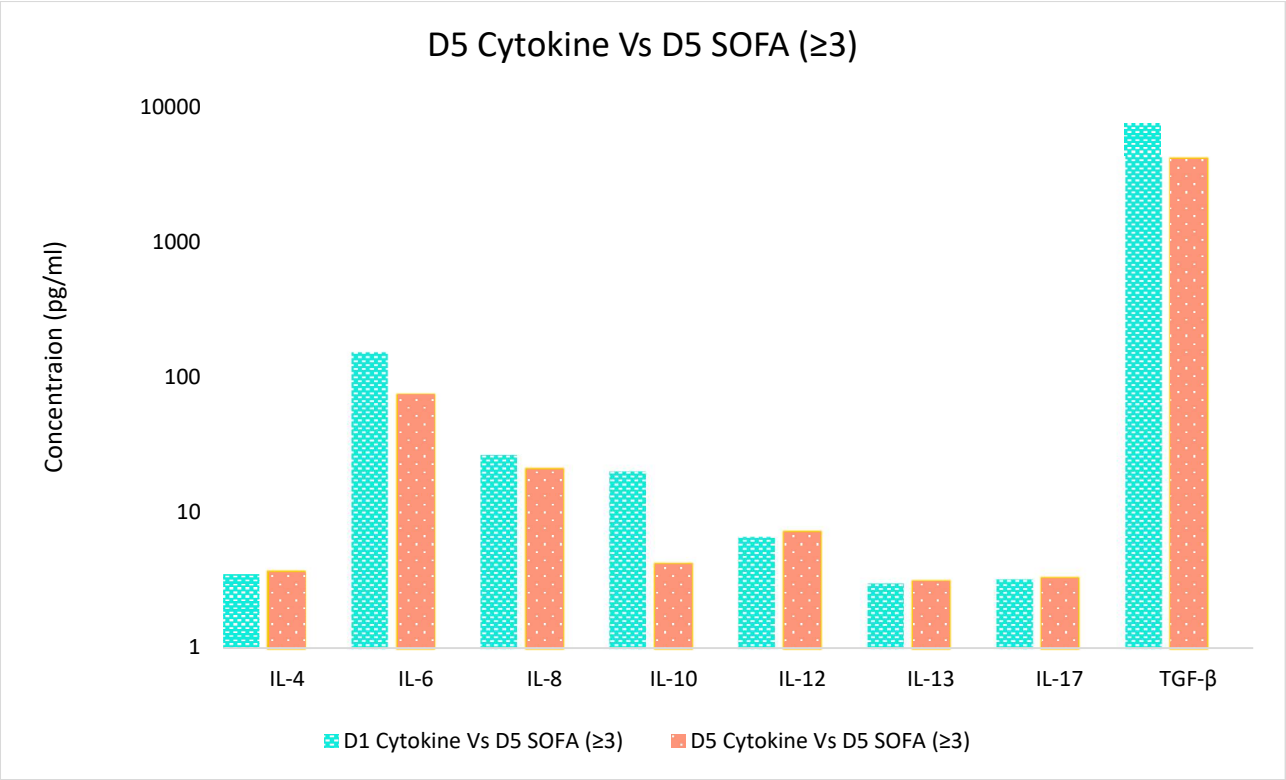


Figure 66: An outline of day 1 concentrations of IL - 4, IL - 6, IL-8, IL-10, IL-12, IL-13, IL-17 AND TGF-B associated with day 5 SOFA score ≥3.

	IL-4	IL-6	IL-8	IL-10	IL-12	IL-13	IL-17	TGF-β
D1 Cytokine Vs D5 SOFA (≥3)	3.51	156.74	26.91	20.28	6.69	3.03	3.26	7633.42
D5 Cytokine Vs D5 SOFA (≥3)	3.63	74.78	20.93	4.15	7.12	3.10	3.28	4120.64

Table 6: Day 1 and day 5 concentrations of cytokines with day 5 SOFA score at thrshold 6

The bigger pilot study for the full cohort of 200 patients covered eight cytokines: IL-4, IL-6, Il-8, IL-10, IL-12, IL-13, IL-17 and TGF-β although not all cytokines were measured for all 200 patients. The above graphs show the change in average concentrations for all the cytokines for those patients for whom the relevant cytokines were measured. The above graphs show the variations in the average concentrations between day 1 and day 5 post trauma, based on the SOFA threshold of <3 and ≥3.

This thesis focuses on a smaller cohort of approximately 30 patients for whom IL-13 and IL-17 were measures. For this cohort, concentrations of cytokines IL-4, IL-8 and IL-12 were also measured. Therefore, the cross-sectional comparative analysis in section 4.15 covers correlation between IL-13 and IL-17 with IL-4, IL-8, IL-12 for these common patients.

4.12 Analysis of clinical data for IL-13 and IL-17 cohort

4.12.1 Lactate concentration for all samples analysed in IL-13 and IL-17 cohort

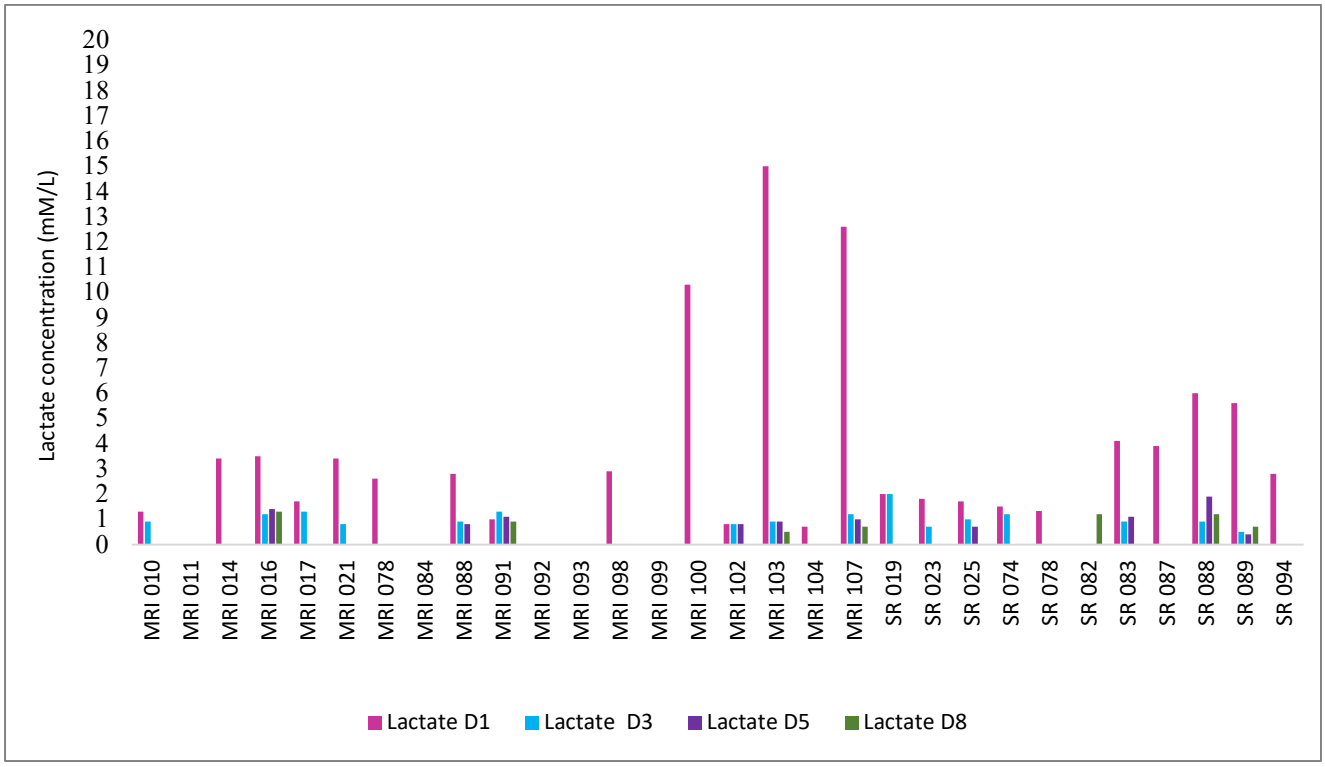


Figure 67: The concentration of lactate for all samples (N=30) analysed for IL-13 and IL-17 levels.

Day 1 lactate concentration was found to have an average of 3.86333 mM/L ± 0.74311 mM/L, with the concentrations ranging between 0.7 mM/L and 15.0 mM/L. From this peak in day 1, the average concentration went down in day 3 to 1.03125 mM/L. In day 5, lactate concentration went down further to 1.0100 mM/L and then to its lowest level of 0.92857 mM/L in day 8.

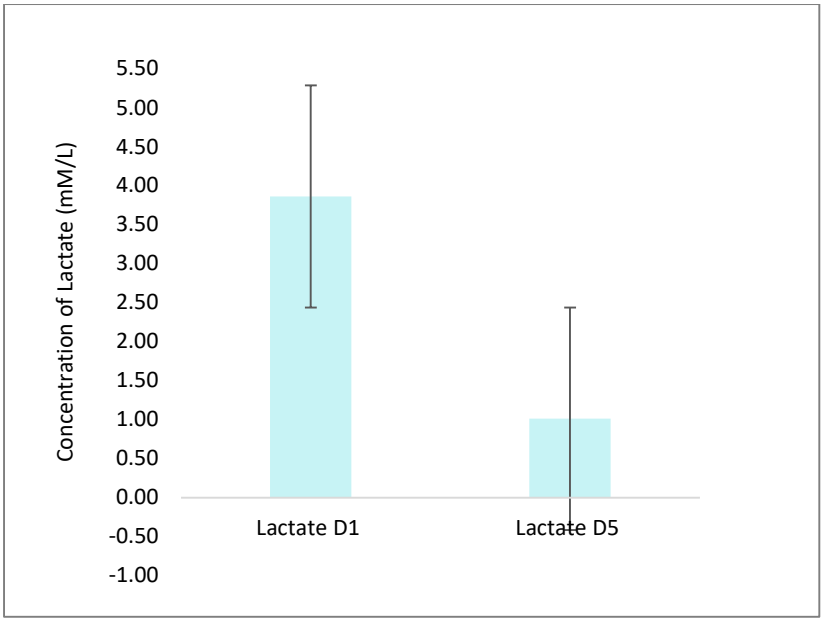


Figure 68: The average lactate concentration for all samples (N=30), at each time point following traumatic injury.

4.12.2 The C Reactive Protein concentration for all samples analysed in IL-13 and IL-17 cohort (N=30)

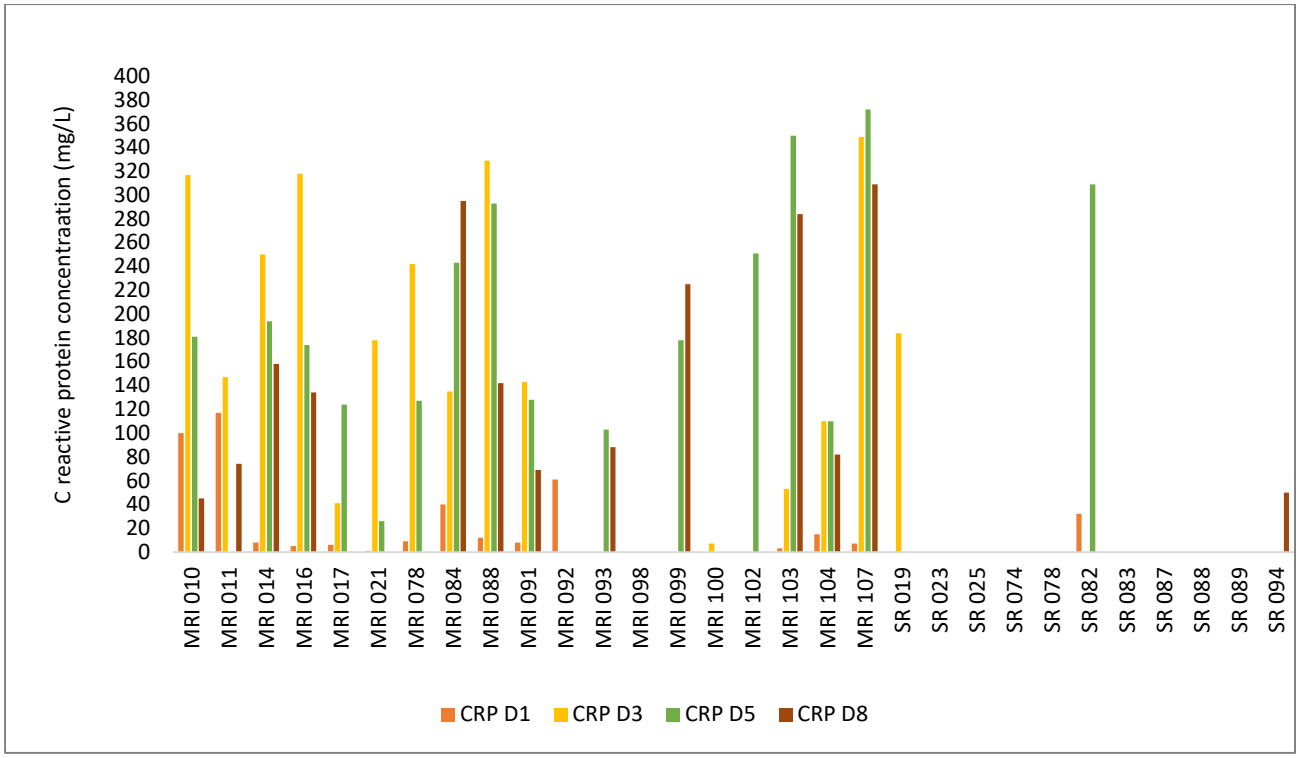


Figure 69: The C Reactive Protein concentration for all samples (N=30) analysed for IL-13 and IL-17 cohort.

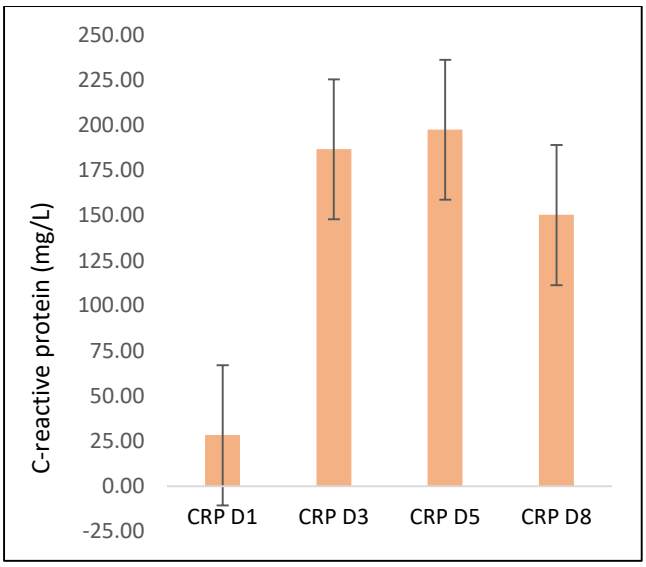


Figure 70: The average C Reactive Protein concentration for all samples (N=16), at each time point following traumatic injury.

Day 1 C Reactive Protein was found to have an average concentration of 28.27 ± 9.13 mg/L, with the values ranging from 1mg/L to 117mg/L. The average concentration went up in day 3 to 186.87 ± 27.60 mg/L, and again increased to a peak of 197.68 ± 23.60 mg/L in day 5. Subsequently, it went down to 150.39 ± 25.75 mg/L in day 8.

4.12.3 Comparative analysis of IL-13 day 1 with C-reactive protein day 5

C-reactive protein (CRP) is an acute-phase protein that is used as infection marker. Neumaier et al., (2006) showed that CRP is linked with the onset of SIRS. Immediately after traumatic injury, CRP levels increase very rapidly and indicate inflammation and infection. Once the condition is resolved, CRP levels show a similar rapid decline too meaning CRP should ideally be measured before the injury is resolved (Lee et al., 2005). Based on this factor, CRP was measured on day 5 but the hospitals made data available only for 16 out of the 30 patients in the cohort.

When cytokine levels were defined using Day 5 CRP levels instead of SOFA scores, the data was not statistically significant. This could be because of the small sample size (n =16), where CRP data was sparsely available.

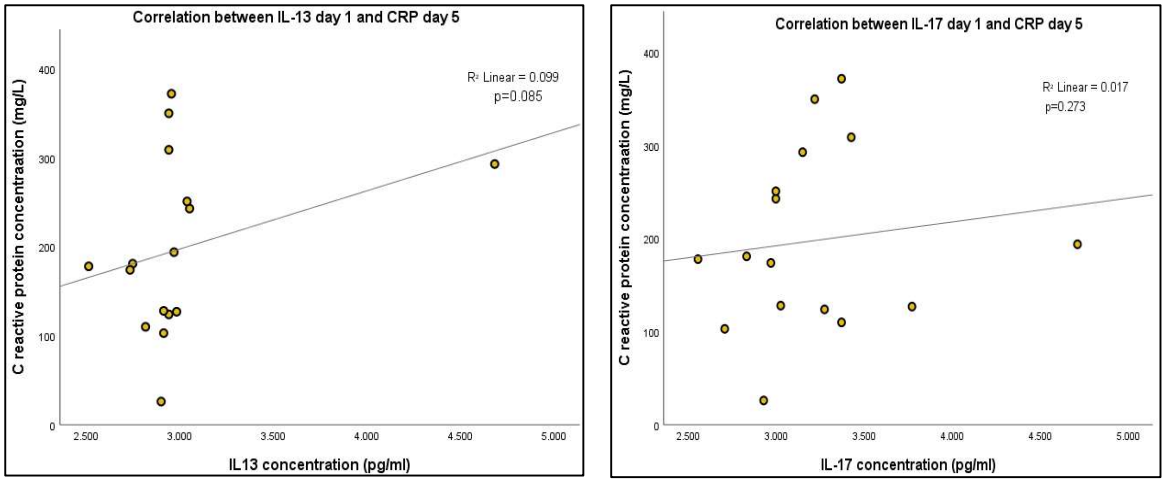


Figure 71A & B: Correlation between day 1 IL-13 concentration and day 5 CRP and day 1 IL-17 concentration and day 5 CRP.

Figure 71A above plots day 1 concentration of IL-13 and day 5 CRP. The chart shows that there is a positive correlation between IL-13 concentration and CRP. When the concentration for IL-13 is higher, CRP is also high. This indicates positive correlation with r= 0.443. However, p=0.085 meaning there is no statistical significance. Figure 71B shows the comparison between IL-17 concentration in day 1 and day 5 CRP. The chart shows r = 0.292 (positive linear relationship) and p=0.273. This indicates no statistical significance.

4.13 Comparative analysis of cytokines – IL-13, IL-17 with IL-4, IL-8, and IL-12

A multiplex panel of cytokines could indicate synergistic relationships amongst the cytokines and intertwined in positive or negative loop of feedback mechanisms. These might govern the good predictive value for the early stratification of major trauma patients for focussed clinical intervention and improvement of clinical outcome. For all the tests below a Spearman's rank-order correlation test was applied on both groups – with or without outliers, to obtain the correlation co-efficient at statistical significance p=0.05. The subsequent sections show the comparison between IL-13 and IL-17, and individual comparisons with the other cytokines in the chosen panel. In sections 4.16.1 to 4.16.8, references to outliers indicates outlying values identified in section 4.3.1. Likewise, references to outliers in sections 4.16.1, 4.16.2 and 4.16.9 to 4.16.14 are the outlying values identified in section 4.9.1.

4.13.1 Correlation between IL-13 and IL-17 on day 1 and day 5

A) B)

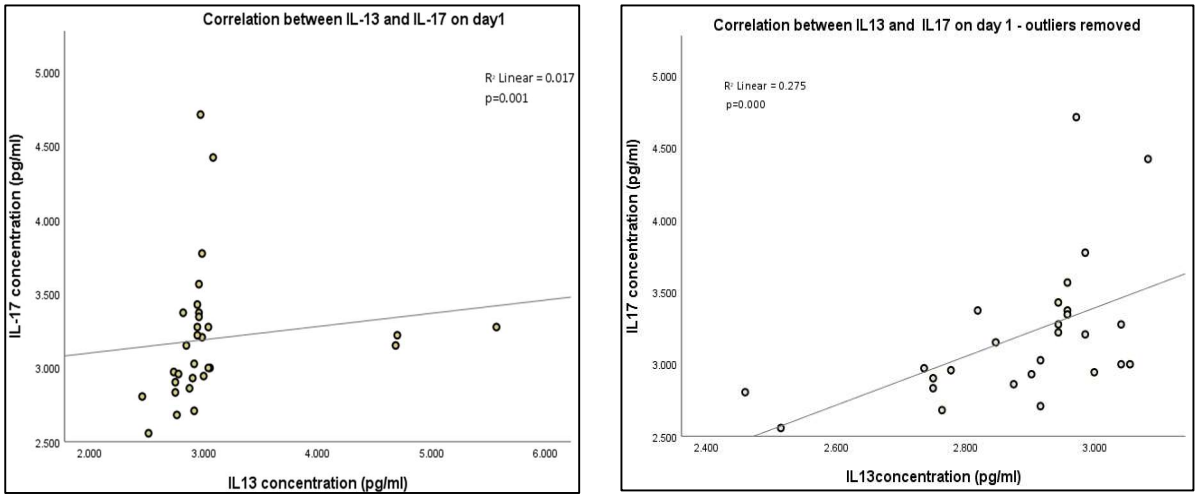


Figure 72A & B: Cluster plot showing the correlation between IL-13 and IL-17 concentrations on day 1.

The above chart plots day 1 concentration of IL-13 and IL-17. The chart shows that there is a strong positive correlation between the values for IL-13 and IL-17. When the concentration for IL-13 is higher, the concentration of IL-17 is also high but there are indeed some outliers. The average day 1 concentration for IL-13 is 3.138 pg/ml and the average day 1 concentration for IL-17 is 3.1972 pg/ml. The charts show that the values are clustered around the average concentration values and show good statistical significance with p value of 0.001 (figure 72A). In this chart, correlation coefficient 0.575 indicates a strong positive linear relationship. When this process is repeated after excluding outliers (Figure 72B), the data again shows strong significance with p=0.000 and positive linear relationship with a correlation coefficient of 0.625. The scales for the X and Y axes are used as default generated by the SPSS graph generator.

4.13.2 Correlation between IL-13 and IL-17 on day 5

A) B)

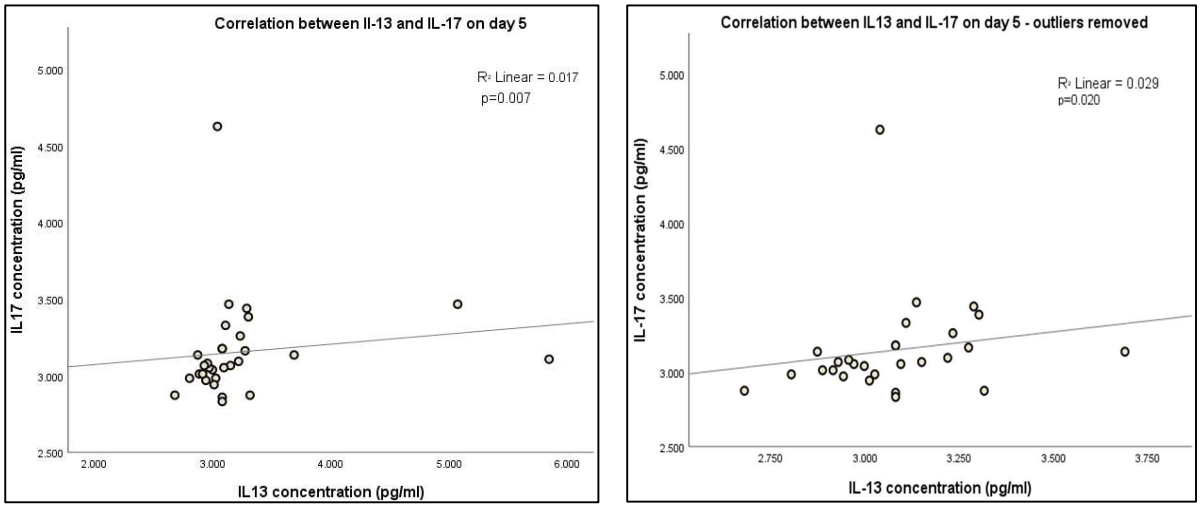


Figure 73A & B: Correlation between IL-13 and IL-17 concentrations on day 5.

The above chart plots day 5 concentration of IL-13 and IL-17. The chart shows that there is a positive correlation between the day 5 concentrations for IL-13 and IL-17. When the concentration for IL-13 is higher, the concentration of IL-17 is also high. The average day 5 concentration for IL-13 is 3.268 pg/ml and the average day 5 concentration for IL-17 is 3.156 pg/ml. The charts show clustering around the average concentrations but there are some outliers. Despite the outliers, there is statistical significance with a p value of 0.007 (figure 73A). The correlation coefficient of 0.480 indicates a positive linear relationship. Figure 73B shows the data with outliers excluded depicts a very interesting picture. Firstly, the p value of 0.020 shows statistical significance and correlation coefficient of 0.436 shows positive linear relationship. Normally, the p value would be expected to decrease (showing increase in significance) when outliers are removed. In this instance, the opposite has happened. This could be because outliers in the IL-13 range plotted in the X axis were removed but there is a lone outlier in the IL-17 range plotted in the Y axis. Nonetheless, there is good statistical significance both with outliers and also when outliers are excluded.

4.13.3 Correlation between IL-13 and IL-4 on day 1

A) B)

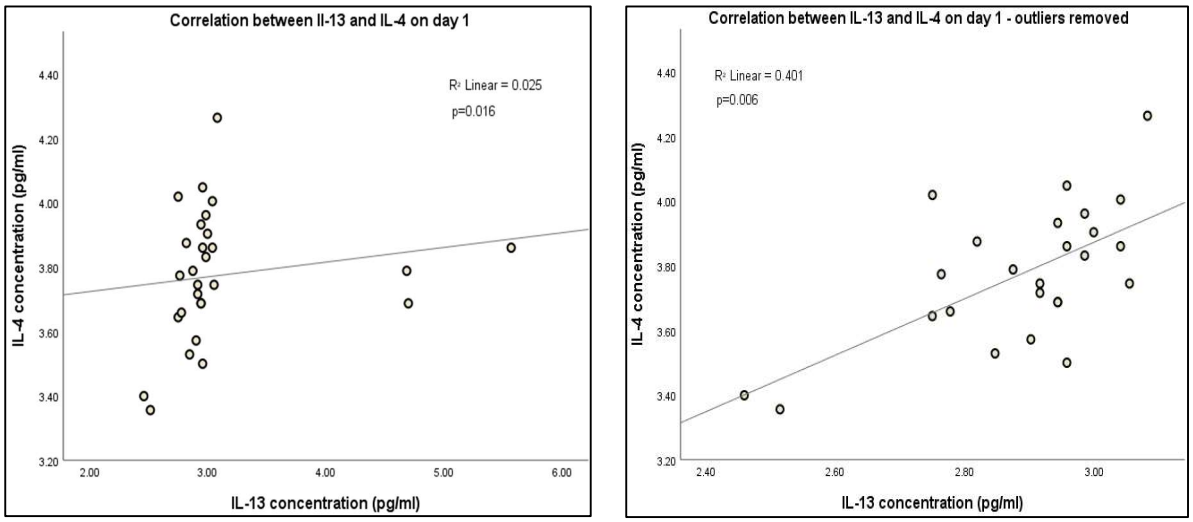


Figure 74A & B: Correlation between IL-13 and IL-4 concentrations on day 1.

The above chart plots day 1 concentration of IL-13 and IL-4. The chart shows that there is a positive correlation between the values for IL-13 and IL-4. The average day 1 concentration for IL-13 is 3.138 pg/ml and the average day 1 concentration for IL-4 is 3.5083 pg/ml. Figure 74A shows the correlation graph with the data showing statistical significance at p=0.016 and correlation coefficient 0.450. Figure 74B shows the same two ranges but with outliers excluded. The p value decreased to 0.006 showing a further increase in significance. The correlation coefficient 0.537 indicates a positive linear relationship.

4.13.4 Correlation between IL-13 and IL-4 on day 5

The below charts plot day 5 concentration of IL-13 and IL-4. The chart shows that there is a positive correlation for the IL-13 and IL-4 ranges. The average day 5 concentration for IL-13 is 3.268 pg/ml and 3.6233 pg/ml for IL-4. The scatter plots shows that the values are clustered around the respective average. The charts shows that the values are clustered around the average values with correlation.

A) B)

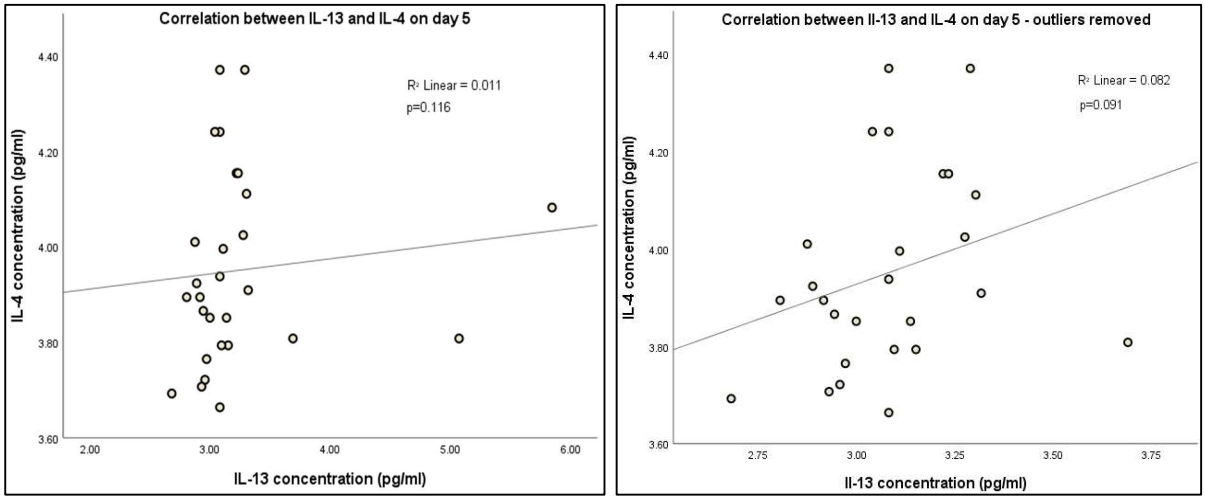


Figure 75A & B: Correlation between IL-13 and IL-4 concentrations on day 5

However, the day 5 concentrations do now show statistical significance. Figure 75A shows the full range having p=0.116, which means there is no statistical significance. When outliers were removed (figure 75B), significance slightly moved up to 0.091 meaning there is no statistical significance even with outliers taken out. The correlation coefficients of 0.304 and 0.338 for the two charts indicate positive linear relationship.

4.13.5 Correlation between IL-13 and IL-8 on day 1

A) B)

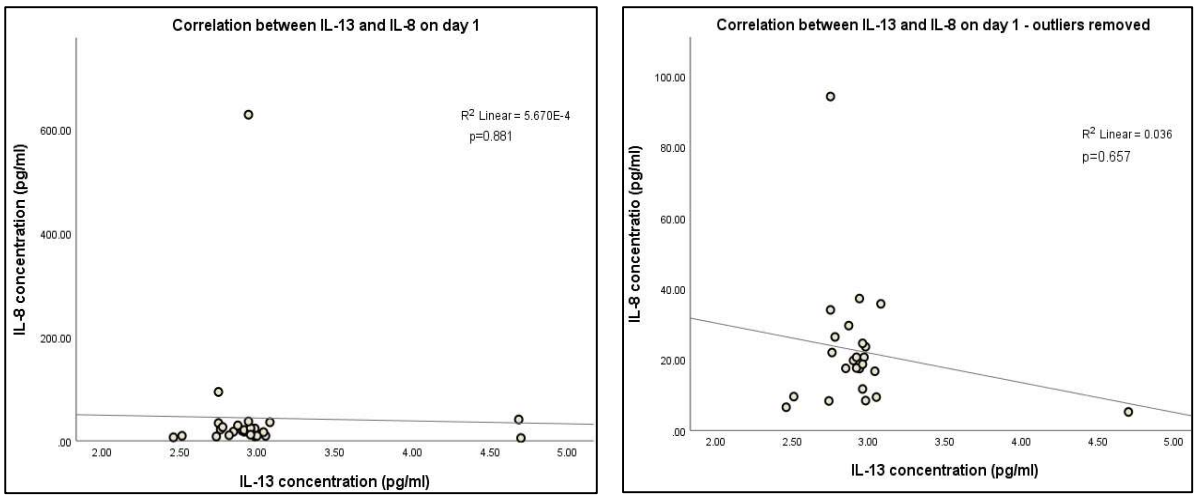


Figure 76A & B: Correlation between IL-13 and IL-8 concentrations on day 1.

The above two charts show the concentrations of IL-13 and IL-8 on day 1. Figure 76A shows that there is a weak negative correlation between IL-13 and IL-8 ranges. The correlation coefficient is -0.030 for the first chart and -0.096 for the second chart (76B) showing the range after excluding outlying values. The average day 1 concentration for IL-13 is 3.138 pg/ml and 34.102pg/ml for IL-8. The day 1 concentrations do now show statistical significance for the full range or after excluding outliers. Figure 76A shows the full range having p=0.881, which means there is no statistical significance. When outliers were removed (figure 76B), p value decreased slightly to 0.657, again showing no statistical significance.

4.13.6 Correlation between IL-13 and IL-8 on day 5

A) B)

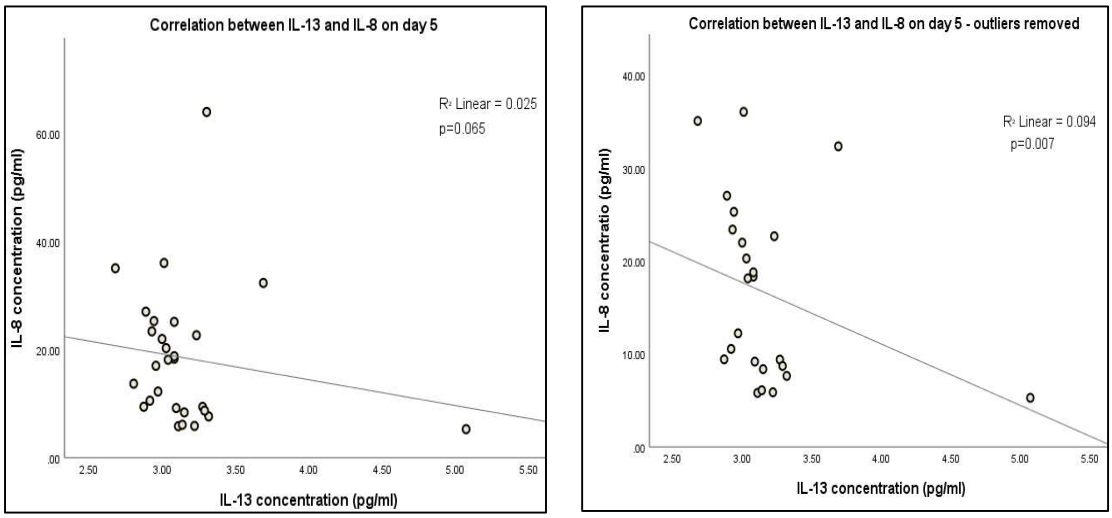


Figure 77A & B: Correlation between IL-13 and IL-8 concentrations on day 5.

The above two charts show the concentrations of IL-13 and IL-8 on day 5. Both charts (figure 77A and 77B) show that there is strong negative correlation between IL-13 and IL-8 day 5 concentrations. The correlation coefficient is -0.353 for the full range and -0.533 after excluding outlying values. The average day 5 concentration for IL-13 is 3.268 pg/ml and 18.191pg/ml for IL-8. The day 5 concentrations do now show statistical significance with a p value of 0.065. However, when outliers were removed the p value decreased significantly to 0.007 showing strong linear statistical significance.

4.13.7 Correlation between IL-13 and IL-12 on day 1

A) B)

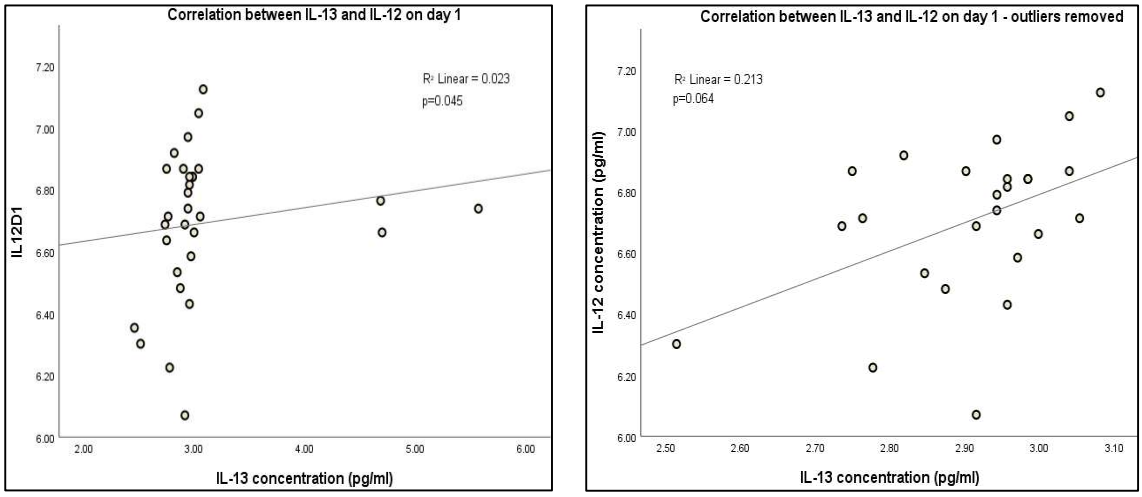


Figure 78A & B: Correlation between IL-13 and IL-12 concentrations on day 1.

The chart plots day 1 concentrations of IL-13 and IL-12. Figure 78A shows that there is both positive correlation and statistical significance for day 1 IL-13 and IL-12 concentrations. When the concentration for IL-13 is high, the concentration of IL-12 is also high. The average day 1 concentration for IL-13 is 3.138 pg/ml and the average day 1 concentration for IL-12 is 6.704 pg/ml. For the full range, the p value is 0.045 and correlation coefficient 0.368. Figure 78B, without outliers shows day 1 IL-13 and IL-12 levels. The data is not significant (p=0.064) although the correlation coefficient of 0.376 indicates a moderate positive linear relationship.

4.13.8 Correlation between IL-13 and IL-12 on day 5

A) B)

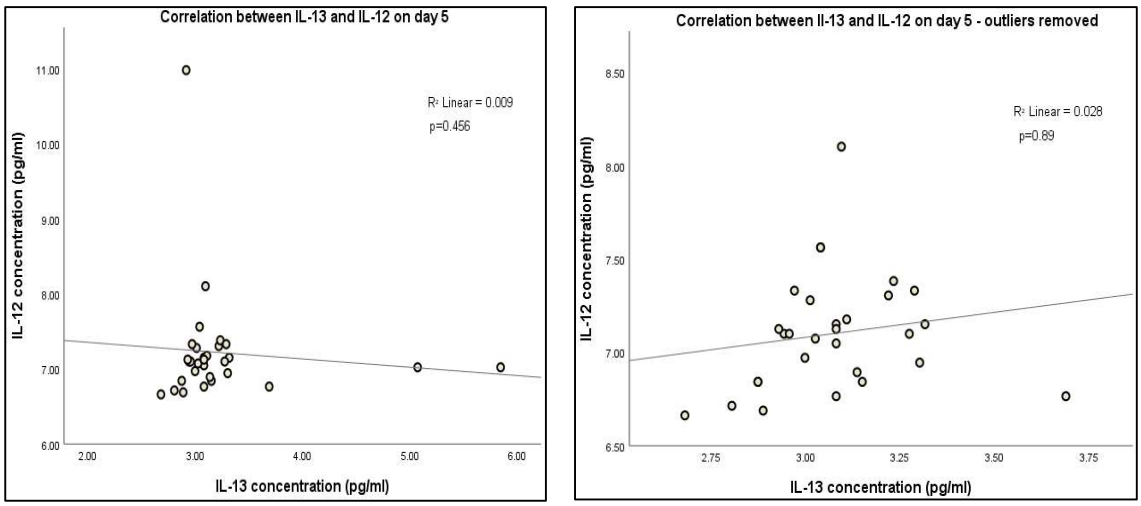


Figure 79A & B: Correlation between IL-13 and IL-12 concentrations on day 5.

The above charts show day 5 concentration of IL-13 and IL-12 and present an interesting contrast. The average day 5 concentration for IL-13 is 3.268 pg/ml and the average day 5 concentration for IL-12 is 7.041 pg/ml. Figure 79A, showing the full range does not have statistical significance (p=0.456) and correlation coefficient 0.141 indicates poor linear relationship. Figure 79B plotting the data without outliers also shows no significance (p=0.089), and the correlation coefficient of 0.321 indicates a weak positive linear relationship.

4.13.9 Correlation between IL-17 and IL-4 on day 1

The below charts show day 1 concentrations of IL-17 and IL-4. Both charts show positive correlation but no statistical significance between the concentration ranges for IL-17 and IL-4. The average day 1 concentration for IL-17 is 3.197 pg/ml and the average day 1 concentration for IL-4 is 3.508 pg/ml. The p value for the full range is 0.063 showing no significance (figure 80A). When outliers are taken out (figure 80B), the p value was 0.155. The correlation coefficients are 0.356 and 0.281 respectively for figure 80A and 80B.

A) B)

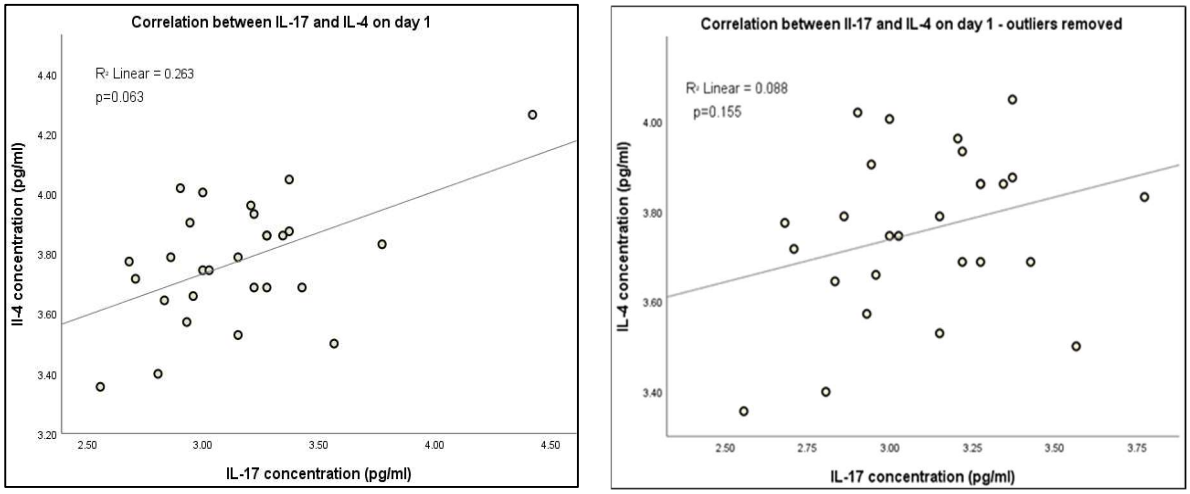


Figure 80A & B: Correlation between IL-17 and IL-4 concentrations on day 1.

4.13.10 Correlation between IL-17 and IL-4 on day 5

A) B)

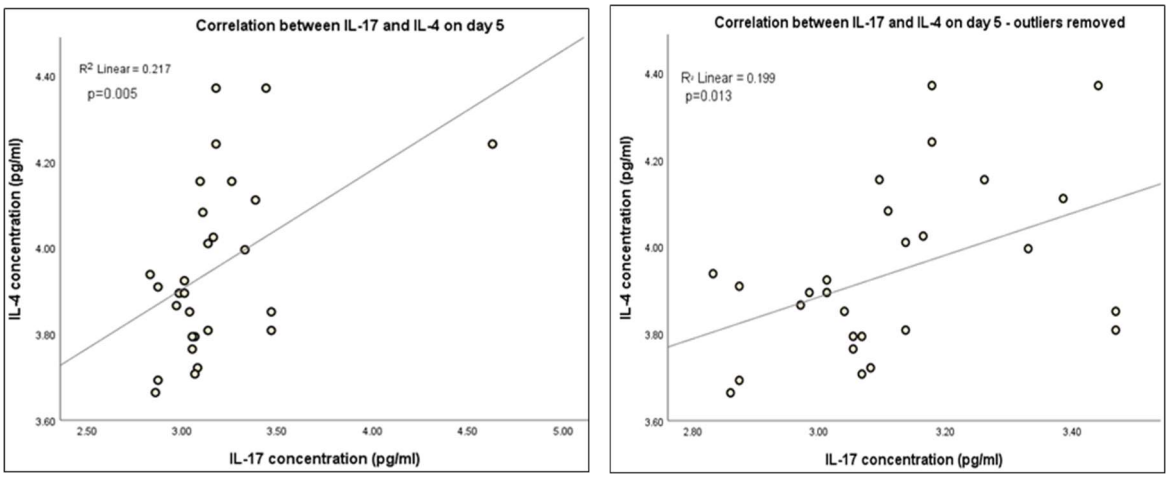


Figure 81A & B: Correlation between IL-17 and IL-4 concentrations on day 5.

The above two charts show day 5 concentrations of IL-17 and IL-4. The average day 5 concentration for IL-17 is 3.156 pg/ml and the average day 5 concentration for IL-4 is 3.623 pg/ml. For the full data set, p value is 0.005 showing statistical significance. The correlation coefficient is 0.518 showing strong positive correlation. The data without outliers shows

significance with p value of 0.013 and correlation coefficient is 0.471, showing positive linear relationship, as shown in the figure 81B.

4.13.11 Correlation between IL-17 and IL-8 on day 1

A) B)

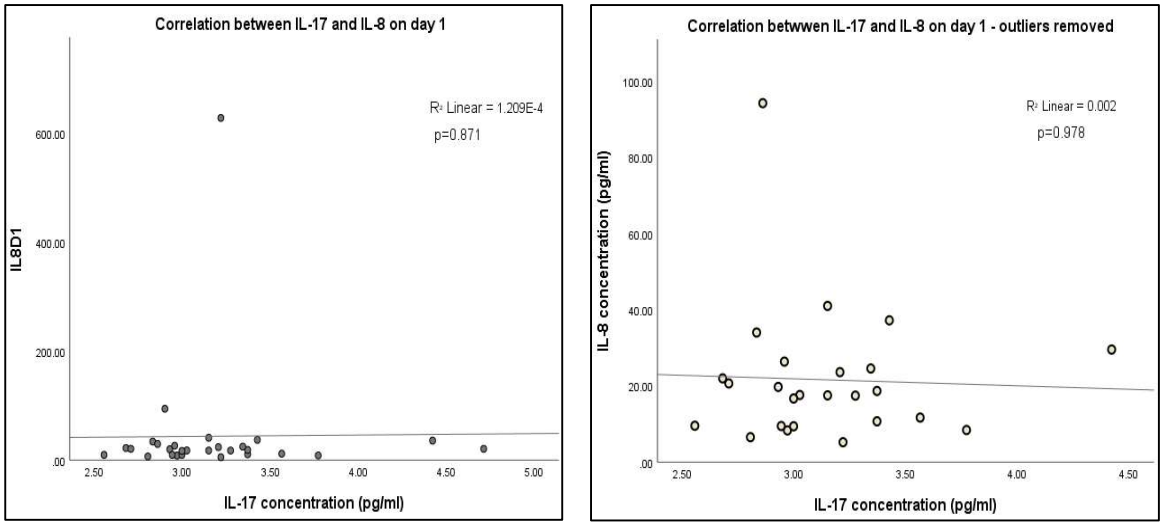


Figure 82A & B: Correlation between IL-17 and IL-8 concentrations on day 1.

The above charts show day 1 concentrations of IL-17 and IL-8. The charts show that there is weak correlation and no statistical significance between the concentration ranges for IL-17 and IL-8. The average day 1 concentration for IL-17 is 3.197 pg/ml and the average day 1 concentration for IL-8 is 34.102 pg/ml. Figure 82A on the left shows the full range whereas the data without outliers is shown in the chart on the right in figure 82B. The two charts show p=0.871 and 0.978 respectively meaning there is no statistical significance. As stated, there is weak correlation with coefficients of 0.032 and 0.006.

4.13.12 Correlation between IL-17 and IL-8 on day 5

A) B)

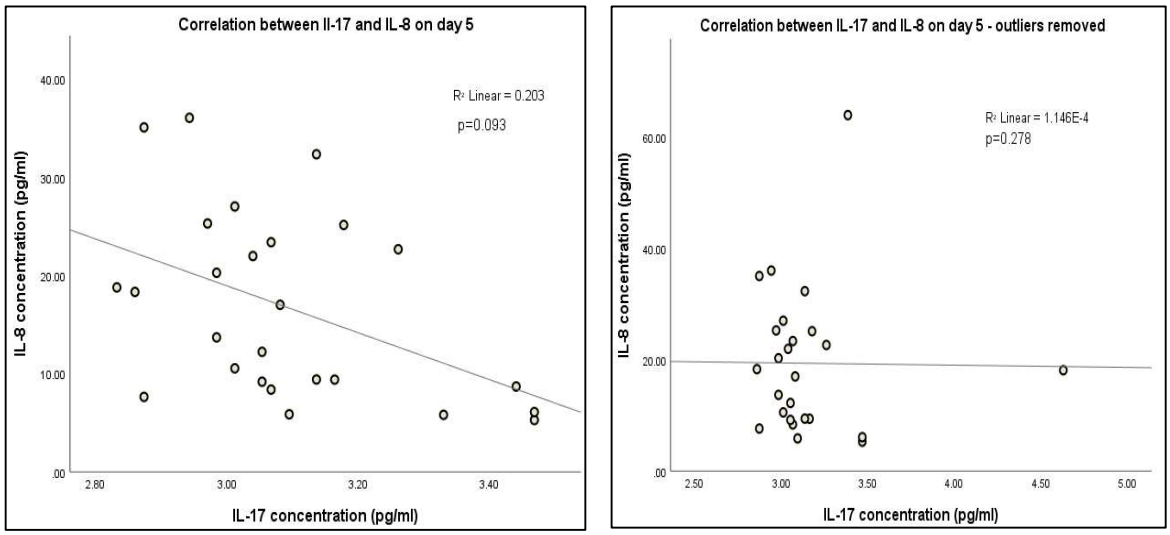


Figure 83A & B: Correlation between IL-17 and IL-8 concentrations on day 5

The above charts show day 5 concentrations of IL-17 and IL-8. The charts show that there is no statistical significance between IL-17 and IL-8 day 5 concentrations but presents a contrasting picture for correlation. The average day 5 concentration for IL-17 is 3.156 pg/ml and the average day 5 concentration for IL-8 is 18.191 pg/ml. Figure 83A on the left shows the full range, which has a p value of 0.093. For these two ranges, the correlation coefficient is -0.324, which explains why the line is sloping down as IL-17 concentration increases. The data without outliers has p value of 0.278 and is shown in the chart on the right in figure 83B. The correlation coefficient is -0.226, showing negative linear relationship.

4.13.13 Correlation between IL-17 and IL-12 on day 1

A) B)

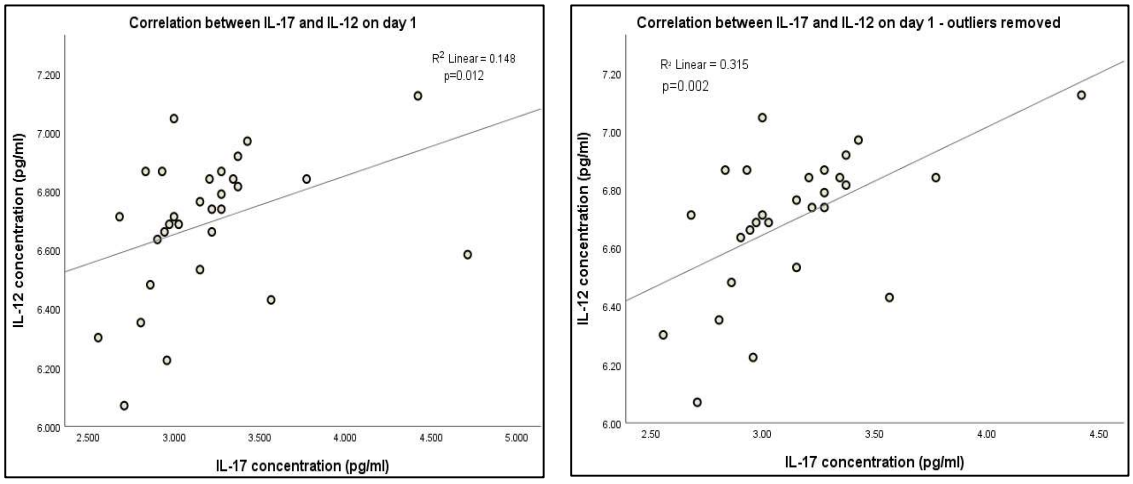


Figure 84A & B: Correlation between IL-17 and IL-12 concentrations on day 1

The above charts show day 1 concentrations of IL-17 and IL-12. Both charts show positive correlation and statistical significance between the concentration ranges for IL-17 and IL-12. The average day 1 concentration for IL-17 is 3.197 pg/ml and the average day 1 concentration for IL-12 is 6.704 pg/ml. The p value for the full range is 0.012 showing significance (figure 84A). When outliers are taken out (figure 84B), the p value decreased to 0.002 showing further increase in significance. The correlation coefficients are 0.453 and 0.552 respectively for figures 84A and 84B.

Correlation between IL-17 and IL-12 on day 5

A) B)

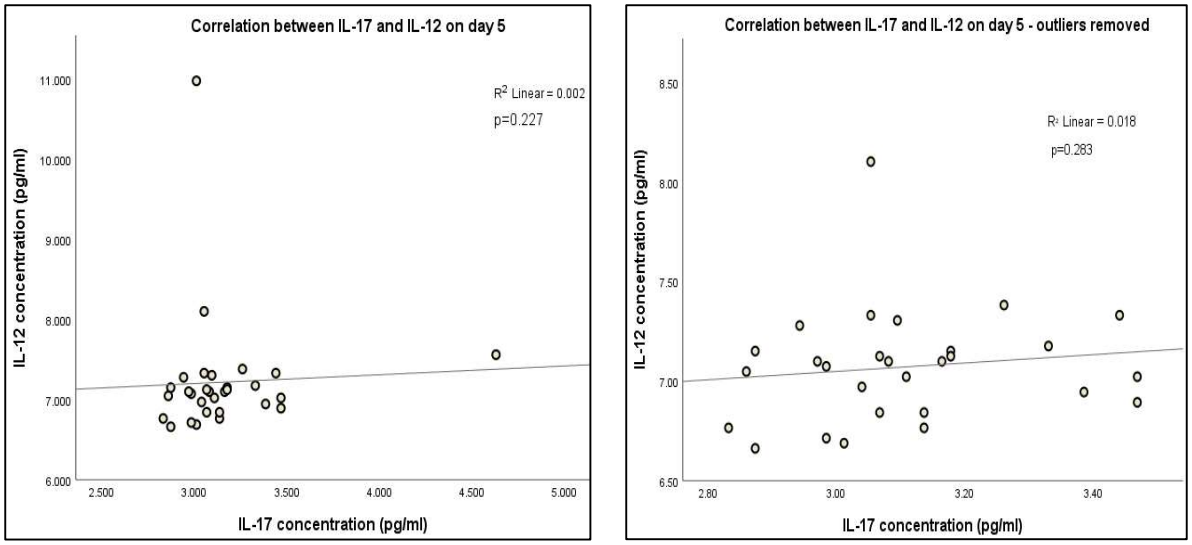


Figure 85A & B: Correlation between IL-17 and IL-12 concentrations on day 5

The above two charts show day 5 concentrations of IL-17 and IL-12. The average day 5 concentration for IL-17 is 3.156 pg/ml and the average day 5 concentration for IL-12 is 7.042 pg/ml. For the full data set, p value is 0.227 which means there is no statistical significance. The correlation coefficient is also 0.227 showing weak positive correlation, as shown in figure 85A. The data without outliers shows even less significance with p value of 0.283 and is shown in the chart on the right in figure 85B. The correlation coefficient is 0.210, showing weak linear relationship.

4.14 Verification of patient outcome based on SOFA score stratification.

4.14.1 Analysis of IL-13 concentration for patient outcome

A) B)

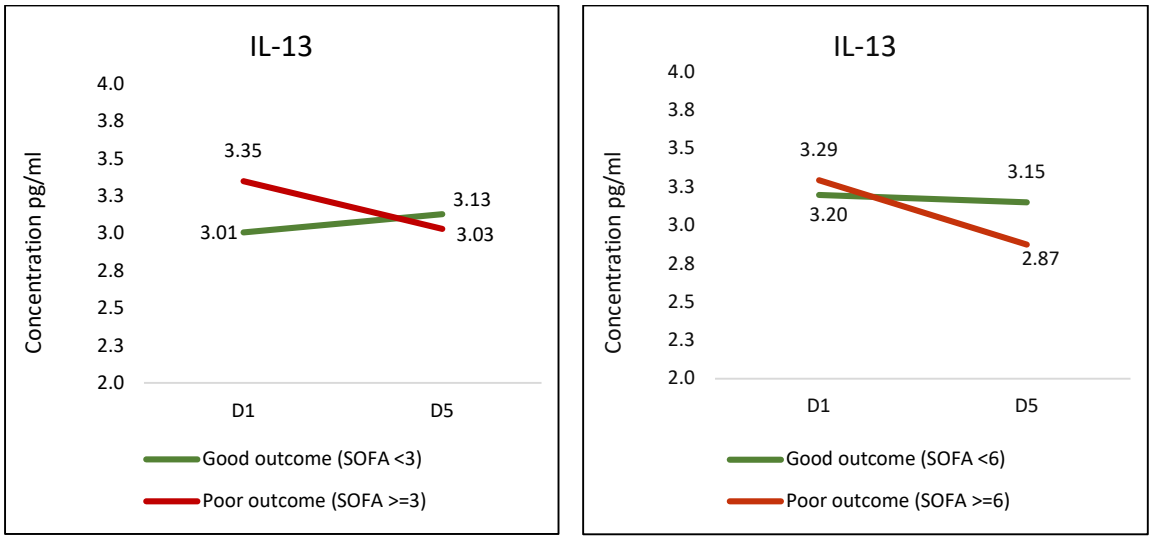


Figure 86A & B: IL-13 for both good outcome and poor outcome patients between day 1 and day 5 at a threshold of 3 and 6

The above charts show the change in IL-13 concentrations for the two cut-off thresholds of 3 and 6. In figure 86A, which shows cut-off threshold 3, it can be seen that the concentration of IL-13 has increased between days 1 and 5 for those patients whose SOFA value is less than 3. This shows the concentration of IL-13 has increased for those patients who had good clinical outcome. On the contrary, the concentration of IL-13 decreased between days 1 and 5 for those patients who had poor clinical outcome represented by SOFA scores 3 or above. Figure 86B shows IL-13 concentrations at cut-off threshold 6. At this threshold, the IL-13 concentrations for both patient groups show a decline between days 1 and 5. Patients with poor outcome show a considerable drop between days 1 and 5. When patients were clustered around a higher SOFA, anti-inflammatory characteristic of IL-13 seems subdued.

4.14.2 Analysis of IL-17 for patient outcome concentration

A) B)

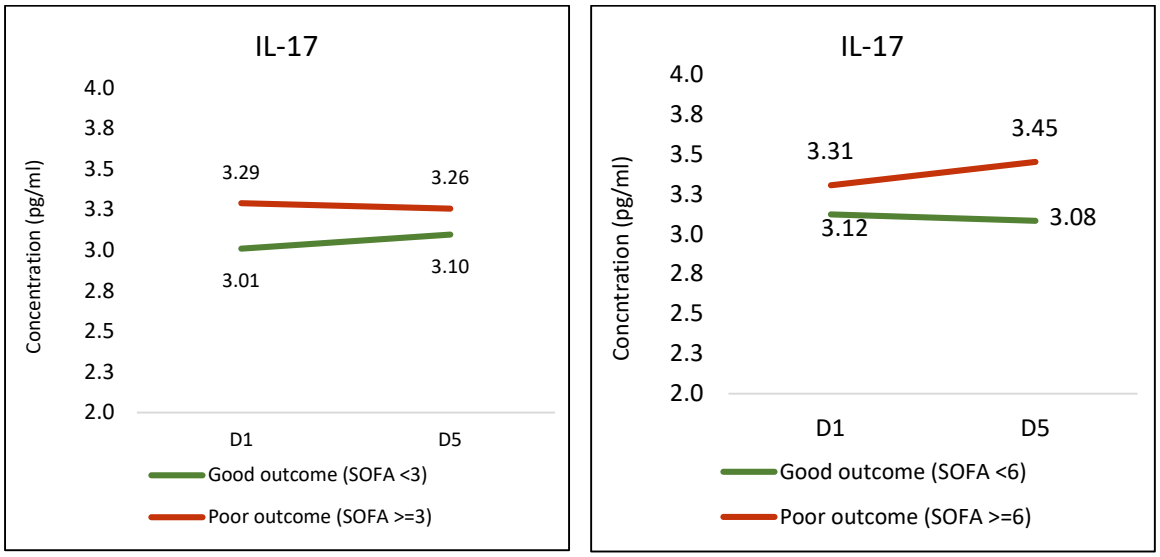


Figure 87A & B: IL-17 for both good outcome and poor outcome patients between day 1 and day 5 at a threshold of 3 and 6

The above charts show the change in IL-17 concentrations for the two cut-off thresholds of 3 and 6. In figure 87A, which shows cut-off threshold 3, it can be seen that the concentration of IL-17 has increased for those patients who had better clinical outcome. The concentration of IL-17 has decreased between days 1 and 5 for those patients whose SOFA value is more than 3 – the poor outcome patients. Figure 87B shows IL-17 concentrations at cut-off threshold 6. At this threshold, the IL-17 concentrations for patient group with SOFA less than 6 show a slight decrease between days 1 and 5. Patients with poor outcome (SOFA ≥ 6) show a small increase between days 1 and 5. The pro-inflammatory nature of IL-17 seems to play the role when patients were clustered around a higher SOFA.

5 Discussion

Traumatic injuries are a public health concern because they are a leading cause of mortality and morbidity causing nearly about 5.8 million deaths annually (Lockey, 2018). As a cause of mortality, trauma is one of the most prevalent non-contagious illnesses and injuries (Vos et al., 2020). In England, a quarter of patients with major trauma lose their lives (Glen et al., 2016). The NHS Commissioning Board (2013) states that major trauma is the leading cause disability in those who are below 45 years of age. Some patients become chronically ill with poor wound healing and recurrent infections - a syndrome called 'persistent inflammation, immunosuppression and catabolism syndrome' (PICS) sets in (Mira et al., 2017). Patients who develop PICS, tend to require prolonged and frequent rehospitalisation, putting more burden on healthcare resources despite, its low incidence (Hesselink et al., 2020).

Diagnosis of trauma requires detailed investigation including imaging. Accidents, including falls and road traffic collisions, are increasing in number thereby giving rise to more major trauma sufferers (Binkowska et al., 2015, Kehoe et al., 2015, Trauma audit & research network, 2017, Moran et al., 2018). Given the scale of the problem, it is unsurprising that the National Audit Office (NICE report, 2017), estimated that major trauma care costs the UK up to £3.7 billion or 7% of NHS national budget (paper, 2017). The emphasis is, therefore, on early detection of life-threatening situations and timely, efficient medical interventions that can contribute to speedy recovery and prevent further deterioration or complications. NICE (2014) has rightly identified that late or inadequate investigation and/or poor treatment increases the risk of mortality and morbidity.

A traumatic injury invokes the innate immune response from the very moment of injury. This is followed by the adaptive immune response. Once the initial traumatic insult has crossed the verge of immunogenic tolerance, the humoral and cellular components get activated (Huber-Lang et al., 2018). This network collectively regulates homeostasis as an attempt to restore normal functioning of the tissue (Belkaid & Hand, 2014).

Two phases of immune response are triggered after traumatic injury: a pro-inflammatory systemic reaction such as SIRS (section 1.9.1) and an anti-inflammatory CARS (section 1.9.3). During both phases, injured patients are highly prone to "second hits" that could aggravate the pathophysiological cascade and cause sepsis, MOF, and morbidity (Baue, 2006).

However, a dominance of anti-inflammatory response can lead to CARS or MARS (section 1.9.4). The activation of coagulation and neuroendocrine pathways enables humoral and cellular factors to cause damage to tissue even far from the injury site, which leads to the extracellular release of DAMPs. Complement system is an important part of the 'danger response' and assists in clearing DAMPs and PAMPs. In some instances, there can be maladaptive immune response, which could cause subsequent MODS (Karasu et al., 2019). Traumatic injury is associated with altered host defence and hyperinflammation, which trigger initial activation of immune response. This phase is followed by immunosuppression and weakened T-cell function, which causes reduction of adaptive immunity and increased vulnerability to infection, sepsis, and even organ failure (Stahel et al., 2007). The immune response is characterised by local, systemic production and release of multiple mediators such as cytokines, chemokines, coagulants and complement activation factors (Keel & Trentz, 2004).

The broad objective for this study was to examine early cytokine expression in critically injured trauma patients and assess their value in predicting development into MOF. We hypothesized that early changes in cytokine production could identify patients at risk for MOF. Once the risk to the patient has been assessed, there could be early and assertive intervention with antibiotics and other drugs to prevent complications from developing. Conversely, this could mean that overuse of antibiotics could be avoided. Targeted intervention to prevent complications and reduce hospital stays could also provide cost savings to the NHS.

The study was part of the main trauma research project, which recruited a total of 200 patients. This thesis focused on a smaller sub cohort of 30 patients with primary focus on IL-13 and IL-17 cytokines. As a part of comparative data analysis, patterns of serum concentrations of IL-13 and IL-17 were analysed in relation to a broader spectrum of cytokines, namely IL-4, IL-8 and IL-12 for the sub cohort of 30 patients.

5.1 Results & Outcomes

For the study, hospitals provided clinical meta data about the patients and their clinical outcomes. Cytokine concentration was measured on day 1 and day 5 post-trauma of admissions. SOFA scores were recorded on days 1, 3, 5 and 8 to gauge injury severity and organ dysfunctions; for the comparative analysis SOFA scores in day 1 and day 5 were used. From the SOFA scores, Delta SOFA (day 5 SOFA – day 1 SOFA) was calculated. Parameters

such as C-reactive protein and lactate levels were used to assess inflammation. These data were analysed and compared with clinical metadata and used to establish the type of outcome for the patient cohort. For the analysis, patients were grouped into good outcome and poor outcome categories using the criteria specified in table 4. Cytokine concentrations were measured for their inflammatory marker characteristics and through this multi-step comparative analysis, the potential of cytokines to serve as biomarkers was assessed.

5.1.1 Interleukin-13

To understand the IL-13 expression pattern in the patients' serum after a major trauma, the day 1 concentration of IL-13 (N= 30) were compared with day 5 samples (Figure 52A). The day 1 samples showed a mean value of 3.138 pg/ml, and the day 5 samples showed a mean value of 3.268 pg/ml. The results showed an increase of cytokine levels on day 5 with statistical significance obtained at p=0.002, indicating that day 1 concentration could predict the patient outcome on day 5. A lower level of IL-13 at admission initially indicated onset SIRS condition and are linked to severe injury and pro-inflammatory cytokines increase in attempt to battle the infection. The increase in IL-13 concentration in later stages expressing the anti-inflammatory characteristics, manifests CARS and is correlated with an attempt of IL - 13 to supress pro-inflammatory mediators and restore the homeostasis, indicating a better patient outcome in day 5.

The pattern obtained on 30 patient cohort analysis is consistent with the study by Collighan et, al., (2004), showing an increase in the serum IL-13 levels between day 0 and day 1, amongst sepsis patients who survived (Collighan et al., 2004). The meta data provided by both hospitals provided patients demography (wherever available) recorded that all of the 30 patients survived eventually (see appendix), indicating upregulating expressions of anti-inflammatory biomarkers.

Minty et al. (1993) proved that IL-13 in PBMC cells in the presence of bacterial lipo polysaccharides, which is an important PAMPs, inhibited cytokines such as IL-6, IL-1beta, IL-8. They observed IL-6 mRNA accumulation was rapidly inhibited (within 4 hours) showing IL-13's direct action upon monocytes. Monocytes and macrophages secretion indicates chronic inflammation and initiate the recovery response of the body. This process is indicative of IL-13's anti-inflammatory behaviour that relates to the elevated concentrations in day 5 post

trauma. In their research on multiple sclerosis patients, a similar pattern of elevated concentration was noticed (Martins et al., 2011). The patients in Martin et al.'s study also showed good outcome meaning high levels of anti-inflammatory IL-13 is a biomarker for good patient outcome. In our cohort of patients there were no fatalities.

In another study, Khurana et al (2004) showed that serum IL-13 levels of poly trauma patients who were discharged correlated to a prognostic patient outcome (Khurana et al., 2018) showing the anti-inflammatory properties of IL-13. A similar pattern of patient outcome has been recorded for this sub-cohort of 30 trauma patients all of whom eventually survived the traumatic event irrespective of their nature of injury.

To determine the predictive characteristics of IL-13 that reflect on the clinical outcome, this study went on to explore the correlation between the IL-13 concentrations and the SOFA score. The patient cohort was split into two groups based of SOFA evaluations at two different threshold ranges: SOFA of <3 and ≥ 3, and SOFA of <6 and ≥ 6 to observe the trend pattern amongst the two groups. IL - 13 concentration in D1 showed no correlation significance with SOFA score in day 5 for these patients at both cut-offs ($p = 0.621$ and $p = 0.569$ respectively). Using the same threshold, IL-13 concentration showed no statistical significance with day 8 SOFA score with p values 0.411 and 0.517. Gaulitz et al., (2008) has shown in their study that though altered levels of IL-13 concentrations were observed in burn patients with respiratory injuries, it did not show any significance when compared to the healthy control group (Gauglitz et al., 2008). This was observed on the second time point of 5-7 days after the burn injury, which coincides with the day 5 of post trauma in this thesis.

In this study, IL-13 concentration increased between day 1 and day 5, from 3.1384 pg/ml to 3.2682 pg/ml ($p=0.02$). This shows that the anti-inflammatory response had triggered since hospital admission. In section 4.4, IL-13 concentrations at the threshold for SOFA score (<3 for good and ≥ 3 for poor outcomes) showed that IL-13 concentration was lower in patients with SOFA score of ≥3 (figure 55). As further discussed in section 4.5, those patients for whom IL-13 concentration increased also had decreased SOFA score. Since lower SOFA scores are associated with better clinical outcomes, it can be concluded that increase in IL-13 concentration could be an indication of good clinical outcome.

This was also similar to the findings of the multiplex cytokine analysis conducted by Bozza et al. (2007), which amongst other aspects showed that the levels of IL-13 concentration were elevated on day 1 but decreased in subsequent time points. These findings were associated with the progression of organ dysfunction on day 3 (Bozza et al., 2007) unlike this study in which all the patients in the cohort of 30 survived. The findings of this study are also comparable with Hensler et al., (2000) wherein IL-13 levels showed no increase post trauma in none of the groups. St Ledger et al., (2009) found that IL-13 targeted therapies are useful in treating asthma. Although the assay method and patient characteristics in their study were different, it is evident that IL-13 has a role in inflammatory regulation.

Further evaluation was made using delta SOFA, which was obtained by measuring the change in SOFA score between days 1 and 5. Delta SOFA was used along with clinical parameters to assess whether the patients improved or got worse. The difference in IL-13 concentration was compared with delta SOFA to be used as a reliable tool for risk stratification into better outcome, worse outcome, or no change in the patients' condition. In this study, IL-13 concentration levels on day 1 and day 5 were individually compared with delta SOFA. The comparison between IL-13 concentration on day 1 and delta SOFA did not show statistical significance. The r=-0.318 and p value was 0.087 and revealed a very weak negative relationship between them. As the IL-13 concentration increased, the delta SOFA decreased indicating good patient outcome.

The data was further evaluated to determine the reliability of clinical outcome obtained through delta SOFA. The percentage cut off test (please see section 4.6) was performed to see by what percentage the concentration between the day 1 and day 5 concentration had changed at different SOFA scores. The increase in the mean IL-13 concentration on D5 was 10%. This corresponded with a decrease in day 5 SOFA score (figure 57A). As the average mean IL-13 concentration at 10% cut off decreased, the day 5 SOFA also decreased (57B). Together, they indicated a better clinical outcome amongst the patients.

Most studies on the role of IL-13 in sepsis performed to date have been in mice. In Matsukawa's murine model of sepsis, neutralization of IL-13 was detrimental to survival after cecal ligation and puncture (CLP), indicating that endogenous IL-13 serves as a protective cytokine during the evolution of septic peritonitis. These studies have shown that IL-13 is important in regulating organ-specific inflammation, by controlling the production of

tissue levels of specific cytokines (Matsukawa et al., 2000). From the findings of this study on human serum samples, we can infer that higher levels of IL-13 in day 5 might be used as potential biomarker for predicting the onset of CARS in traumatic injury reflecting a better clinical outcome.

5.1.2 Interleukin-17

Current studies suggest that IL-17 is a potent pleiotropic biomarker for sepsis and trauma (Ge et al., 2020). It can activate and recruit neutrophils and play a protective role in innate immunity against pathogens by contributing to the pathogenesis of inflammatory diseases (Zenobia and Hajishengallis, 2015).

The day 1 concentration of IL-17 from (N= 30) patient serum samples were compared with day 5 samples. The mean IL - 17 value for day 1 was 3.197 pg/ml, whereas in day 5 was equal to 3.156 pg/ml, showing a negligible difference. Statistical analysis showed no significant difference between day 1 and day 5 ($p=0.994$). Even when the outlier values were removed the significance changed negligibly to 0.817 (65B), showing no statistical difference.

In adults, major trauma initiates a two-fold compromise with hyper-inflammation during the acute response to injury and subsequent immunosuppression. Post traumatic hyper inflammation is characterised by local and systemic release of pro-inflammatory cytokines, metabolites and acute phase proteins leading to SIRS (Keel and Trentz, 2005). Anti-inflammatory cytokines are released as a counter balance, whose hyper-secretion can bring in CARS, conferring susceptibility to infection and septic complications and eventually MOF in adults (Ahmed Ali et al., 2018). In their cohort of 100 polytrauma patients, Ahmed Ali et al., (2018) found that the level of IL-17 on the day of injury was elevated although measurement on subsequent days were not made.

To determine the relationship between IL-17 concentrations within the clinical parameters of the 30 patients and its prognostic abilities for a clinical outcome, the concentration of IL - 17 in day 1 was tested against SOFA score calculated for day 5 and day 8 at two different cut-offs, namely SOFA score <3 and ≥ 3 and SOFA score <6 and ≥ 6. SOFA score of <3 is indicative of single organ failure. SOFA score at threshold 6 indicate multiple organ failure with an increased chance of mortality.

At threshold 3, day 1 IL - 17 concentration, statistically correlated with SOFA score in day 5 at p=0.048 (excluding outliers). The IL-17 concentration against SOFA score in day 8 returned similar results at the cut off 3 with p value 0.042 with a statistical significance. This shows that IL-17 levels on the day of admission could predict the onset of clinical complications and poor outcome for the patients.

Repeating the grouping for day 1 IL-17 concentration with day 5 SOFA score, at threshold 6 showed no significance (p=0.123) and analysis between day 1 IL-17 and day 8 SOFA score yielded a p value at a statistically significant difference of p=0.044 (outliers removed). The significance obtained with SOFA cut off 6 on day 8, indicates an onset of multiple organ failure. This finding is consistent with the results obtained by Dai and Zhang (2015) with IL-17 levels positively correlating with fatalities of high SOFA and high base line of IL-17, predicted longer period of hospital stay, MOF and death. This slight decrease in IL-17 levels in day 1 is suggestive of a good clinical outcome. This also indicates the role of anti-inflammatory cytokines downregulating the proinflammatory cytokine IL-17.

The upregulation and downregulation of inflammatory response occurs through the interplay amongst the cytokines within the network, achieved through positive or negative feedback mechanisms. To understand, this complexity and their contribution in immuno-regulation, correlation tests were conducted. Cytokines interact closely with each other and play a crucial role in the progression of conditions such as sepsis. This study focussed on the associations of a cytokine network with prognosis and disease severities in sepsis and other poor outcomes.

5.1.3 CRP

C-reactive protein (CRP) is an acute-phase protein that is used as infection marker. Neumaier et al., (2006) showed that CRP >50mg/ml is associated with the onset of SIRS. Immediately after traumatic injury, CRP levels increase very rapidly and indicate inflammation and infection. Once the condition is resolved, CRP levels show a similar rapid decline, indicating the CRP should ideally be measured before the injury is resolved (Lee et al., 2005). Based on these factors, CRP was measured on day 5 but the hospitals made data available only for 16 out of the 30 patients in the cohort.

Day 1 CRP concentration ranged between 28.26667 ± 9.12796 mg/L and on Day 5, the concentrations ranged between 1 mg/L and 117 mg/L, it increased to a peak of 197.68750 ± 23.60229 mg/L, without appreciating a statistically significant difference. The correlational analysis between IL-13 day 1 and IL-17 day 1 concentrations and day 5 CRP showed no association. In this study, the cytokine levels were defined using Day 5 CRP levels instead of SOFA scores, the data was not statistically significant. This could be because of the small sample size (n =16). CRP data was not provided by the hospitals for all patients. The CRP levels ranged between 197 mg/L to 1.1 mg/L for these 16 patients. This extreme variation could have skewed the sample and resulted in not obtaining statistical significance.

5.1.4 Lactate

The lactate concentration on day 1 was found to have an average of 3.86333 mM/L. In day 5, lactate concentration went down to 1.0100 mM/L and then to its lowest level of 0.92857 mM/L in day 8. However, statistical significance was not seen in this analysis since only eight data points were available for the sub-cohort of 30 patients.

5.1.5 Comparative analysis

A multiplex panel of cytokines could indicate synergistic relationships amongst the cytokines and intertwined in positive or negative loop of feedback mechanisms. These might govern the good predictive value for the early stratification of major trauma patients for focussed clinical intervention and improvement of clinical outcome. Thus, a cross sectional comparison was made amongst the cytokines (IL-4, IL-8, and IL-12) with common patient cohort with IL-13 and IL-17 on day 1 and day 5 to analyse their expression levels.

The cluster plots on day 1 showed correlation coefficient, r = 0.575 (p=0.001), revealing a strong positive linear co-relationship between IL-13 and IL-17. When the concentration for IL-13 was higher, the concentration of IL-17 also peaked. Similar results were obtained on day 5 tests. IL-13 and IL-17 on day 5 showed a moderate linear significance at correlation coefficient of 0.480. Nevertheless, the average concentrations of IL-13 and IL-17 on both days showed a marginal difference**.** IL-17 downregulation could have exerted pleiotropic effects on lymphocyte promotion and tissue destruction to act as a pro-inflammatory cytokines and were at similar levels (3.1972 pg/ml) as IL-13 (3.138 pg/ml). This result was similar to the results of study by Feng et al. (2014). In their study, the concentration of pro-inflammatory cytokines IL-6 and IL-17 increased alongside the anti-inflammatory cytokines

IL-13 and TGF-β in the serum samples of patients with ulcerative colitis. In another study by Silosi et al. (2016), the presence of higher IL-13 and IL-17 serum levels in extra-articular rheumatoid arthritis patients (eRA), when compared with those of controls confirmed that these markers might be involved in the pathogenesis of eRA. (Siloşi et al., 2016).

Clausen et al. (2019) studied a panel of cytokines for neuroinflammatory responses in TBI patients. The elevated IL-13 levels were measured through circulating Th2 cells. IL-17A also showed a spike as a rapid response of Th17 production. Both IL-13 and IL-17A's levels elevated within the first 6 hours, indicating a rapid upstream signalling of the circulating immune cells (Th2 and Th17) and corresponding to positive feedback loop between IL-13 and IL-17A (Clausen et al., 2019).

The correlation analysis between IL-13 and IL-4 levels on day 1 revealed a strong positive linear association between them. This could be due to the upregulated expression of the IL-4 and IL-13. A similar pattern of IL-4 and IL-13 concentrations were found to be statistically significant with $p < .0001$ in research into atopic dermatitis patients (Neis et al., 2006). In a study by Punnonen et al., (1993), IL-13 did not have any additive or synergistic effects on IL-4-induced IgE synthesis when both cytokines were added at saturating concentrations. This could be explained by similarities in the signalling pathways of these two cytokines. IL-13 and IL-4 are the Th2 cytokines that share a functional receptor IL-4Rα and exhibit their anti-inflammatory properties through Jak/Stat (STAT6 transcription factor) signalling pathways that mediate and regulate several inflammatory genes expression especially in Th2 associated in the pathogenesis of allergic diseases such as asthma (Bhattacharya and Matthay, 2013). The gene encoding IL-13 is an upstream of the IL-4 gene. These two cytokines have 25% homology commonality and share some functional properties. IL-4 and IL-13 both act on hematopoietic immune cells and non-hematopoietic immune cells. Together, these actions are critical in the phenotypes of allergic diseases such as asthma and atopic dermatitis (Matsunaga et al., 2020).

Correlation between IL-13 and IL-8 day 1 concentrations showed a weak negative correlation. The correlation coefficient was -0.030. On day 5 correlation showed a strong linear negative correlation at coefficient of -0.533 and $p=0.007$. This behaviour is a classical indication of the pro-inflammatory effect of IL-8 against which a counter regulation of anti-

inflammatory properties of IL-13 were expressed. This is consistent with the results obtained by Rodney et al., (2018).

In this study, the correlation between IL-13 and IL-12 cytokine levels, the concentrations of both IL-13 and IL-12 seemed high. In this study, the average day 1 concentration for IL-13 was 3.138 pg/ml and the average day 1 concentration for IL-12 was 6.704 pg/ml. For the full range, the p value is 0.045 and correlation coefficient 0.368, showing a moderately stronger linear relationship. Day 5 concentrations indicated a correlation coefficient of 0.321 exhibiting a weak regressive relationship at p=0.45.

The correlation between IL-17 and IL-4 on day 5 showed a positive linear significance at correlation coefficient 0.518. This was in concordance with a study by Baumann et al., (2018) which showed an elevated levels of IL-4 and IL-17 amongst patients with allergic rhinitis at the time point of 5 hours (Baumann et al., 2013). This could be linked to the differentiation of helper T 2 type (Th-2) cells in the presence of IL-4 and IL-13 leading to IgE production and marking allergic inflammation. This in turn activated epithelial cells to undergo apoptosis mediated by Th17 (IL-17) and Th1 neutrophil recruitment. This entire chain of events describes (Akdis et al., 2016) how the cytokines considered in this thesis, interplay with each other to mediate an allergic inflammation and tissue injury.

The study further analysed IL-17 and IL-8 levels for the 30 patient sub-cohort. The correlation on day 1 at 0.032, showing a weak positive correlation. On day 5, it was -0.324 showing statistical significance at a weak negative correlation. This can be compared to a Matsumoto et al.'s (2018) hierarchical clustering and network visualisation study, who also obtained a correlation between IL-17 and IL-8. Increased cytokines were compared to those of the controls and the connections are shown to represent networks with major impact. This could be because the cytokine and plasminogen activator inhibitor 1 (PAI-1) is a marker for clot formation after an injury incidence (Matsumoto et al., 2018). The overall pattern matches the result of this study although it did not cover plasminogen activator inhibitor 1 (PAI-1).

The average day 5 concentration for IL-17 is 3.156 pg/ml and the average day 5 concentration for IL-12 is 7.042 pg/ml. For the full data set, p value was 0.227 which means there is no statistical significance. The correlation coefficient is also 0.227 showing weak

positive correlation. This study was similar to findings by Nielsen et al. (2004). They showed that the elevated expression of both IL-12 and IL-17 mRNA induced active ulcerative colitis and with Crohn disease may be involved in sustaining the intestinal inflammation in irritable bowel disease (Nielsen et al., 2003).

5.2 Conclusion

In this study, IL-13 significantly increased on day 5 whereas IL-17 showed a marginal increase. In trauma patients, if pro-inflammatory cytokines peaks on a later stage, then it indicates a potential two hit. Alternatively, it could indicate that their anti-inflammatory regulation drops but might elevate eventually. To verify this hypothesis, different SOFA cut-off criteria was considered. No major changes were noted for IL-13 concentrations. Thus IL-13 and IL-17 could be used as potential biomarkers as SOFA scores are calculated commonly, in the days preceding other days subsequent to the actual injury. There is a possibility that if the blood samples were drawn and analysed within a short duration of injury, different results could be expected.

The IL-13 concentration increased in the days after the injury. When the analysis was done using the two thresholds of <3 and ≥3, and <6 and ≥6, there was no significance between the ranges with the p values always above 0.4. Yet, the results obtained showed a clear shift between day 1 and day 5 with an increased level of IL-13 in day 5, which is likely to have contributed to the good patient outcome and provides further evidence of IL-13 being a suitable biomarker.

Those patients who did not develop sepsis had lower levels of IL-17 concentration compared to those who developed sepsis. This indicates that lower levels of IL-17 concentration contribute to better patient outcome. In our study, the decrease between day 1 and day 5 of IL-17 is important. Although the decrease was marginal, it is to be noted that all the patients in the cohort survived and there were no fatalities. In conjunction with the statistical significance noticed during the data stratification based on SOFA score, it can be concluded that IL-17 is a potential biomarker for understanding clinical outcomes in patients suffering traumatic injuries. Indeed, studies that recruited a larger number of patients did show statistical significance for IL-17. For example, Bozza et al., (2007) (N=60), St Ledger et al., (2009) (N=122) and Khurana et al., N=80 (2018).

A limitation encountered during the research was that clinical meta data such as the nature of injury and injury severity scores, length of stay and details of surgical intervention could not be retrieved from the hospitals for all patients. It was also not certain whether the patients had received blood transfusion before the bloods were drawn for analysis. If so, blood transfusion might have altered the active phase of expression and true representation of cytokine profiles. Nevertheless, analysis of IL-13 and IL-17 cytokines in a larger cohort might reflect the cytokine accuracy and increase the statistical power of the study. Employing the current assay methods, rapid turnaround test kits could be developed in future for better point of care facilities. Such test kits could allow testing and make it possible to identify, within a few minutes, patients who are likely to be severely injured and more at risk of organ failure.

This chapter has referred to numerous relevant studies although they do not all study trauma patients. That is not considered a weakness because they provide support in establishing this panel of cytokines as biomarkers of tissue injury with diagnostic abilities. They reflect the concepts of immune-regulation and the role played by the panel of cytokines, and their expression patterns relevant to this thesis. Overall, the results obtained in this study indicate the predictive abilities of IL-13 and IL-17 as inflammatory biomarkers of clinical outcomes.

5.3 Achieving Research Objectives

This study had six research objectives. The following sections summarise how those objectives were met and present the key findings from the research.

5.3.1 Collating and managing clinical data (objective 1)

The principal aim of this study was to investigate whether the chosen panel of eight cytokines could serve as biomarkers that can predict clinical outcome in patients with major traumatic injuries. The full study covered a cohort of 200 patients recruited CMFT and SRFT. Research nurses from the two hospitals recruited patients and obtained consent forms. Patients' blood samples were drawn at three points: first within 24 hours of the injury and repeated on days three and five. In addition to blood samples, clinical data was collected for days 1, 3, 5 and 8. Based on the gathered clinical data, ISS, SOFA, Δ-SOFA scores were calculated for each patient.

5.3.2 Analysis of IL-13 and IL-17 concentration (objectives 2 and 3)

From the 200 patients recruited overall a smaller cohort of 30 patients was chosen for the analysis of IL-13 and IL-17. As per the protocol for the project, blood samples and clinical data were gathered for this cohort of 30 patients. Serum samples from the recruited patients were analysed to detect the concentration of IL-13 and IL-17 using FACS Verse flow cytometer from BD Bioscience. Standard curves for IL-13 and IL-17 concentrations were built using correct gating for the capture beads of these two cytokines. IL-13 and IL-17 concentrations were measured in patients' day 1 and day 5 serum samples using CBA.

5.3.3 Evaluation of IL-13 and IL-17 as biomarkers (objective 4)

For meeting this objective, comprehensive data analysis was conducted on the gathered data. This included assessment to understand the pattern variations between serum cytokines levels in samples drawn on days 1 and 5 and subsequent comparison of cytokine concentrations with clinical parameters and SOFA score. The comparison of IL-13 concentrations in days 1 and 5 showed statistical significance but no significance was found when comparing IL-17 concentrations in days 1 and 5. IL-13 is an anti-inflammatory cytokine whereas IL-17 is pleiotropic. As such, the behaviour of IL-17 can vary in different situations. Moreover, the concentration of IL-13 was higher in those patients who also had higher concentrations of IL-17. This pattern could be explained by the pleiotropic nature of IL-17.

5.3.4 Cross-sectional comparative analysis (objective 5)

Average (mean) concentrations of IL-13 and IL-17 were compared against each other and then with mean concentrations of IL-4, IL-8, and IL-12. Comparison of IL-13 with IL-17 showed that, with the exception of some outlying values, concentration for IL-13 was high in those patients who also had higher concentration of IL-17. The comparison revealed positive linear relationship and statistical significance with p value of 0.000 in day 1 and 0.007 in day 5. Comparison of IL-13 with IL-4 showed positive linear relationship in both days 1 and 5. Whilst day 1 concentration showed statistical significance, day 5 concentrations did not show statistical significance. Comparison of IL-13 with IL-8 showed no statistical significance in either day 1 or day 5. Comparison of IL-13 with IL-12 followed varying patterns in days 1 and 5. While day 1 concentration showed statistical significance, there was no statistical significance identified in day 5. Comparison of IL-17 with IL-4 showed positive linear relationship in both days 1 and 5. Whilst day 1 concentration showed no statistical

significance, day 5 concentrations did show statistical significance. Comparison of IL-17 with IL-8 showed no statistical significance in either day 1 or day 5. Comparison of IL-17 with IL-12 followed the same varying patterns seen with IL-13 / IL-12 comparison. While day 1 concentration showed statistical significance, there was no statistical significance identified in day 5. The reasons for these different patterns have been analysed and discussed in section 5.1. The findings of the comparative analysis match the results reported by other researchers. However, there isn't extant literature on the link between cytokine concentration and clinical outcome in major trauma. Therefore, this study is an important contribution to understanding the behaviour of cytokines and their utility as biomarkers in predicting clinical outcome in patients who have suffered major traumatic injuries.

5.3.5 Comparing cytokine concentration with clinical metadata (objective 6)

The concentrations of IL-13 and IL-17 were measured against CRP and lactate. The comparison did not show statistical significance, which is believed to be caused by the small number of patients for whom lactate, and CRP measurements were available for all days.

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7 Appendices

7.1 Appendix 1 - Patient Information Sheet

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 purchase 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.

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 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 to take part 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 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, post code 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 trail and the doctors will continue to provide you with whatever medical treatment is needed.

Will this affect the way I am treated in the 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 apart?

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 ne needle, which might result in some minor discomfort during collection and possibly a small bruise.

Will the information from this study be kept confidential?

Any information, including personal information which is collected about you during the course of 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 Trail subject number, date of birth and initials, so that you cannot be recognised from it. This is the exception of information obtained from the Health and Social Care Information Centre as described earlier. Only the researchers and the 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 the Manchester Royal Infirmary and the University of Salford.

Who has reviewed the study?

All research in the NHS is looked by an independent group of people called a Research Ethics 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 for independent advice.

Further Information

Thank you for considering participating in this study. If you have any questions about this research, 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 2766777
Emergency Telephone	0161 2764712

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, (Fore name and Surname) …….. freely agree to take part in the study.
- ☐ I confirm that I 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 be 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 the information held and managed by The Health and Social Care Information Centre and the 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.

Patient: *Date:* *Signature:* *Printed Name:*	*Person responsible for collecting the informed consent:* *Date:* *Signature:* *Printed Name:*

7.2 Appendix 2: Clinical data collection sheet

Day			Date		
HR		BP	__/__	Temp	__C◦
Hb		WCC		PLT	
eGFR		Creatinine		Billirubin	
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			
Antibiotic treatment	Y/N	Source of sepsis		__empirical/unknown	

7.3 Appendix 3: Pilot study -Clinical data for 200 patients from Central Manchester Foundation Trust – Day 1

	Day 1	Sys BP	Dia BP	MAP	Temp	Hb	WCC	PLT	eGFR	Creat	Bili	PT	Intubated	NIV / CPAP	FiO2	P/F	Lactate	Norad	CRP	CVVH/HD	Sedated	GCS	Antibiotics	Septic source	MAP SOFA	PLT SOFA	CREAT SOFA	BILI SOFA	RESP SOFA	GCS SOFA	SOFA DAY 1
BIT001	115	110	60	76.6667	37.8	134	31.6	356	42	132	34	15.6	N	N	0.21	N	1.2	0	19	N	N	15	Y		0	0	1	2	0	0	3
BIT002	125	122	84	96.6667	38	122	18.6	142	50	134	28	16.6	N	N	0.28	38.2	3.9	0	63	N	N	15	Y		0	1	1	1	2	0	5
BIT003	94	70	45	53.3333	36	123	11.5	180	55	116	6	15.4	N	N	0.3	N	N	0	2	N	N	15	Y		1	0	1	0	0	0	2
BIT004	129	101	61	74.3333	36.5	112	16.2	213	76	85	7	14.4	N	N	0.21	63.1	0.9	0	102	N	N	15	N		0	0	0	0	0	0	0
BIT005	82	135	85	101.667	37.9	110	8.4	199	90	70	7	15.9	N	N	0.28	N	0.4	0	32	N	N	15	N		0	0	0	0	0	0	0
BIT006	84	111	71	84.3333	36.2	79	9.8	472	81	74	3	15.4	N	N	0.21	N	N	0	15	N	N	15			0	0	0	0	0	0	0
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	N	N	15	Y		0	0	0	0	1	0	1
BIT008	110	85	60	68.3333	37.7	99	5.9	152	65	69	13	20.8	Y	N	0.21	58	2	0	23	N	Y	N	Y	empirical	1	0	0	0	0	0	1
BIT009	90	102	55	70.6667	37.9	108	22	232	81	101	15	16.3	N	N	0.28	N	N	0	6	N	N	14	Y		0	0	0	0	0	1	1
BIT010	116	99	35	56.3333	37.8	130	9.9	286	80	68	7	13.7	N	N	0.3	21.6	1.3	0	100	N	N	15	N		1	0	0	0	3	0	4
BIT011	90	103	71	81.6667	36.4	114	15.4	318	27	158	8	19.7	N	N	0.4	N	N	0	117	N	N	15	N		0	0	1	0	0	0	1
BIT014	112	102	56	71.3333	36.2	78	16.6	129	78	88		16.3	Y	N	0.4	29.2	3.4	0	8	N	Y	N	Y	empirical	0	1	0	0	2	0	3
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	N	N	15	Y		1	1	1	0	2	0	5
BIT017	127	94	63	73.3333	36.2	120	10.9	222	72	83	6	13.7	Y	N	0.6	71.9	1.7	0	6	N	Y	N	Y	empirical	0	0	0	0	0	0	0
BIT018	109	175	90	118.333	35.4	146	16.8	260	90	49	6	11.3	N	N	1	N	0.7	0	89	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT021	146	90	70	76.6667	35.2	109	33.5	235	50	146	10	14.2	Y	N	0.3	50	3.4	0	1	N	N	15	Y	empirical	0	0	1	0	1	0	2
BIT022	97	117	75	89	35.6	128	27.1	408	90	79	5	10.8	N	N	0.28	N	N	0	3	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT023	98	88	65	72.6667	35.7	112	22.1	156	83	91	18	12.3	N	N	0.32	43.6	1.1	0	17	N	N	14	Y	empirical	0	0	0	0	1	1	2
BIT024	115	74	40	51.3333	38.2	83	16.4	155	90	56	8	11.2	Y	N	0.6	62.3	3.8	0	170	N	Y	N	Y		1	0	0	0	0	0	1
BIT025	122	85	45	58.3333	33.9	97	16.1	130	31	241	20	11.9	N	Y	0.35	9.34	6.3	0.07	345	N	N	15	Y	empirical	3	1	2	1	4	0	11
BIT027	129	163	65	97.6667	38	100	28.8	203		111	61	10.7	Y	N	1	27.6	1.4	0	30	N	Y	N	Y	empirical	0	0	1	2	2	0	5
BIT028	130	89	50	63	35	87	16	191	43	166	4	11.2	N	N	0.4	N	N	0	38	N	N	15	N		1	0	1	0	0	0	2
BIT029	94	159	84	109	35.5	119	6.8	132	90	74	28	11.2	N	N	1	N	N	0	226	N	N	15	Y	empirical	0	1	0	1	0	0	2
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	N	N	15	N		1	0	2	0	3	0	6
BIT035	89	168	93	118	36.2	139	14	295	67	83	15	11.2	N	N	0.85	N	N	0	43	N	N	15	N		0	0	0	0	0	0	0
BIT037	NOT CONSENTED																														
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	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT042	99	135	67	89.6667	38.4	118	19.5	213	N	93	5	11.9	N	N	0.21	N	N	0	1	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT043	109	79	30	46.3333	37.7	117	19	194	81	101	9	12	N	N	0.35	33.4	3.3	0		N	N	15	N		1	0	0	0	2	0	3
BIT044	126	135	81	99	35.9	136	14	237	59	128	5	11.4	N	N	0.21	N	2.2	0	1	N	N	15	Y	empirical	0	0	1	0	0	0	1
BIT046	92	80	50	60	35.5	73	29.6	78	59	81	16	13.4	N	N	0.85	31.4	1.8	0	6	N	N	15	Y	empirical	1	2	0	0	2	0	5
BIT047	160	77	56	63	35	147	28.7	248	67	114	12	12.4	Y	N	0.7	41.9	4.8	1.01	75	N	Y	N	Y	empirical	4	0	1	0	1	0	6
BIT048				0		145	27.1	287	81	91	12	12.1	Y	N					71	N	Y		Y	empirical	1	0	0	4	4	0	9
BIT049	123	177	77		35.1	121	16.4	129	89	102	27	12	N	N	0.32	39.2	7.7	0	1	N	N	15	y	empirical	0	1	0	1	2	0	4
BIT050	97	123	59		35/6	149	8.7	359	64	116	10	11.5	Y	N	0.28	0	2	0	20	N	Y		y	empirical	0	0	1	0	4	4	9
BIT052	112	151	76		36.5	94	13.3	161	66	88	12	13	Y	N	0.35	40.3	4.1	0	2	N	Y		y	empirical	0	0	0	0	4	4	8
BIT053	85	103	52	69	34.6	117	8.1	154	62	77	7	11.5	N	N	0.85	30.6	1.7	0	32	N	N	15	y	empirical	1	0	0	0	2	0	3
BIT055	88	165	75	105	38.2	105	8.9	146	82	81	18	12.3	N	N	0.28	49.8	1.6	0	38	N	N	15	y	empirical	0	1	0	0	1	0	2
BIT060	130	70	40	50	35.7	80	13.9	101	45	146	25	12.7	Y	N	0.95	27.7	5.4	0.19		N	Y	N	Y	empirical	4	1	1	1	2	0	9
BIT061	116	180	105	130	38.1	128	14.4	220	89	63	10.9	11	Y	N	0.8	19.9	2	0	18	N	Y		y	empirical	0	0	0	0	3	4	7
BIT064	95	148	93	111.333	35.6	134	19.4	154	90	69	6	10.9	N	N	0.85	N	2.8	0	1	N	N	15	y	empirical	0	0	0	0	0	0	0
BIT065	100	106	61	76	35.6	123	29.1	338	90	47	19	11.2	N	N	0.24	44	0.7	0	41	N	N	13	N		0	0	0	0	1	1	2
BIT066	22?	162	85	110.667	35	138	13.2	178	81	83	8	11.9	N	N	0.28	N	N	0	3	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT067	82	146	81	102.667	36.9	124	12.5	166	99	84	15	11.4	N	N	0.35	N		0	13	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT068	109	109	55	73	35.8	114	21.4	230	90	88	4	12.1	Y	N	0.5	61.2	1.3	0	1	N	N		Y	empirical	0	0	0	0	4	0	4
BIT069	112	95	55	68.3333	35.5	104	19.7	257	79	104	15	11.9	Y	N	0.85			0	4	N	Y		Y	empirical	1	0	0	0	4	4	9
BIT070	110	178	117	137.333	35.8	143	26.9	339	89	88	11	11.8	Y	N				0	N	N	Y		Y	empirical	0	0	0	0	4	4	8
BIT071	36.5	86	42	56.6667	35.5	88	16	199	90	61	3	11.7	Y	N	0.3	N	0.6	0	1	N	Y		y	empirical	1	0	0	0	4	4	9
BIT072	53	138	72	94	35.6	130	6.8	242	n	76	20	12.6	N	N	0.21	N	N	0	1	N	N	14	y	empirical	0	0	0	1	0	1	2
BIT073	53	138	72	94	35.6	129	16	210	56	87	32	11.7	N	N	0.7	9.79	2	0	116		N	15	y	empirical	0	0	0	1	4	0	5

	Day 1	Sys BP	Dia BP	MAP	Temp	Hb	WCC	PLT	eGFR	Creat	Bili	PT	Intubated	NIV / CPAP	FiO2	P/F	Lactate	Norad	CRP	CVVH/HD	Sedated	GCS	Antibiotics	Septic source	MAP SOFA	PLT SOFA	CREAT SOFA	BILI SOFA	RESP SOFA	GCS SOFA	SOFA DAY 1
BIT074	90	130	90	103.333	36.2	110	11.3	238	90	65	13	11.4	N	N	0.21			0	29	N	N	15	y	empirical	0	0	0	0	4	0	4
BIT075				0																					1	4	0	0	4	4	13
BIT076	112	87	52	63.6667	35.7	105	17.3	381	46	93	3	11.1	Y	N	0.3	30.2	2.8	0.11	20	N	y	3	y	empirical	4	0	0	0	4	4	12
BIT030	71	70	48	55.3333	36.2	113	6	146	90	67	5	10	Y	N	0.85	N	0.8	0	4	N	Y		y	empirical	1	1	0	0	4	4	10
BIT078	88	102	51	68	35.7	118	16.4	174	83	92	11	11.2	y	y	0.85	32.4	2.6	0.11	9	n	y	3	y	empirical	4	0	0	0	2	4	10
BIT079	144	219	136	163.667	38.3	155	16	240	70	102	25	12.2	Y	N	0.35	N	7.6	0	272	N	y	15	y	empirical	0	0	0	1	0	0	1
BIT080	94	140		46.6667	35.1	132	5.4	253	59	97	5	12.3	Y	N	0.85	N	N	N	<1	N	Y	3	y	emp	0	0	0	0	4	4	8
BIT081	Declined																														
BIT082	Not consented																														
BIT083	Transferred to salford																														
BIT084	78	108	59	75.3333	36.1	80	11	168	70	102	11	12.5	Y	N	0.5	n/k	n/k	N	40	N	Y	15	Y	emp	0	0	0	0	4	4	8
BIT085	140	196	129	151.333	35	181	16.3	350	79	76	3	10.8	Y	N	65	25	3	0	18	N	Y	n/a	Y	emp	0	0	0	0	3	0	3
BIT086	Not consented																														
BIT087	50	140	87	104.667	35.9	138	17.7	199	90	82	9	11.7	N	N	0.21	n/a	n/d	0	1	N	N	15	N		0	0	0	0	0	0	0
BIT088	125	94	55	68	36.9	135	38.5	409	76	111	30	10.9	N	N	0.85	n/a	2.8	0	12	N	N	15	Y	empirical	1	0	1	1	0	0	3
BIT089	REFUSED																														
BIT090	WENT TO SALFORD																														
BIT091	49	185	81	115.667	35.2	81	9.1	235	30	152	10	10.9	Y	N	0.4	39.5	1	0.09	8	N	Y	n/a	Y	empirical	3	0	1	0	1	0	5
BIT092				0		136	143	274	90	89	12								61	N					1	0	0	0	0	0	1
BIT093				0		84	21.2	259	38	165	18													empirical	1	0	1	0	0	0	2
BIT094		60	25	36.6667	36.2	123	4.2	307	40	137		11.8								N		15	Y	unknown	1	0	1	0	0	0	2
BIT095	103	147	22	63.6667	36.6	119	28	200	28	230	7	11.6	N				0.7					15	Y	empirical	1	0	2	0	0	0	3
BIT096				0		146	20.7	141	90	74	13	10.6													1	1	0	0	0	0	2
BIT097	NON CONSENTED			0																											
BIT098	107	100	66	0	36.7	152	11	301				10.8	N				2.9					15			1	0	0	0	0	0	1
BIT099	95	130	90	103.333		144	5	187	90	50			N									15			0	0	0	0	0	0	0
BIT100				0		98	3.9	289	90	86		11.4	N	N			10.3						Y	Empirical	1	0	0	0	0	0	1
BIT101	REFUSED																														
BIT102	82	110	60	76.6667	37	165	12.8	233	46	136	11	11.2	Y	N	40%		0.8			N		10			0	0	1	0	0	2	3
BIT103	121	84	53	63.3333	33.1	85	3.2	142	61	120	3	10.4	Y	N	50	49	15		3	N				Empirical	1	1	1	0	1	0	4
BIT104	90	100	50	66.6667	37.4	89	11.6	195	90	43	6	10.3	N	N	21%		0.7		15	N		15		Empirical	1	0	0	0	0	0	1
BIT105	118	93	52	65.6667	37	102	11.6	171	90	99			N	N	60		2		5	N					1	0	0	0	0	0	1
BIT106	NON CONSENTED																														
BIT107	99	77	44	55	38	113	4.8	134	65	101	30	11	Y	N	70	11.25	12.6	0.1	7	N		3		Empirical	4	1	0	1	4	4	14
BIT108	NON CONSENTED																														
BIT109	100	94	54	67.3333	35.8	107	6.6	57	90	50	16	12.8	N	N	21		2			N				Empirical	1	2	0	0	0	0	3
BIT110	Not consented																														
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	N	N	15	N	NA	1	0	0	0	2	0	3
BIT112	Not consented																														
BIT113	133	160	108	125.333	37.9	102	19.1	195	36	193	51	11.2	Y	N	40%	31	3.5	NA	58	Y	Y	14	Y	Empirical	0	0	2	2	2	1	7
BIT114	Not consented																														
BIT115	129	164	94	117.333	35.2	153	14.8	267	69	100	4	10.9	N	Y	30%	23	3.6	n/a	1	N	N	14	Y	Empirical	0	0	0	0	3	1	4
BIT116	Not consented																														
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	N	Y	14	Y	Empirical	4	0	1	0	3	1	9
BIT118	129	97	53	67.6667	37.6	105	13.3	17.1	85	58	7	12	N	N	24	56	2.3	NA	6	N	N	15	N	NA	1	4	0	0	0	0	5
BIT119	124	146	73	97.3333	37.1	88	11.1	173	>90	63	12	11	N	N	60	38	3.1	NA	24	N	N	15	Y	Empirical	0	0	0	0	2	0	2
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	N	N	15	Y	empirical	0	0	0	0	0	0	0
BIT121	54	109	75	86.3333	36.8	104	11.2	135	90	64	NA	10	N	N	50%	NA	0.7	NA	32	N	N	3	Y	Emiprical	0	1	0	0	0	4	5
BIT122	Not consented																														
BIT123	135	61	44	49.6667	37	113	22.9	137	>90	86	16	10.9	Y	N	70%	17.9	1.9	3	7	N	Y	15	N		4	1	0	0	3	0	8
BIT124	109	177	76	109.667	35.5	110	13.3	211	69	70	7	9.8	N	N	35%	31.4	1.1	N	37	N	N	15	Y	Emiprical	0	0	0	0	2	0	2
BIT125	59	94	37	56	35.5	98	11.1	446	83	79	11	11	N	N	60	16	2.4	NA	N	N	N	15	N	n/a	1	0	0	0	3	0	4
BIT126	113	168	83	111.333	38.1	125	23	147	74	68	16	10.8	N	N	60	n/a	2.7	n/a	80	N	N	15	N	n/a	0	1	0	0	0	0	1
BIT127	91	118	69	85.3333	36.8	101	10.4	197	77	90	NA	11	N	N	40	NA	NA	NA	28	N	N	15	N	n/a	0	0	0	0	0	0	0
BIT128	108	140	90	106.667	35.4	121	22.1	198	61	108	6	n/a	N	Y	60	27	3.3	n/a	39	N	N	15	N	n/a	0	0	0	0	2	0	2
BIT129	59	93	88	89.6667	37.2	119	9.3	200	>90	58	15	12	N	N	32	34	2.9	0	37	N	N	15	Y	empirical	0	0	0	0	2	0	2
BIT130	47	101	90	93.6667	36.5	94	11.3	182	.>90	55	9	12	N	N	21	60	3	0	31	N	N	15	N	n/a	0	0	0	0	0	0	0
BIT131	45	91	41	57.6667	35.1	73	12.6	202	22	193	9	11	Y	N	50	29	2.1	0.516	337	N	Y	0.73333			4	0	2	0	2	4	12
BIT132	119	90	53	65.3333	34.8	100	10.2	165	33	175	14	12	Y	N	55	26	10.9	0.64	3	Y	Y	3	Y	empirical	4	0	2	0	3	4	13
BIT133				0		143	16.2	219	>90	79	6	11	N		35	28	4.2	0	12	N	N	15			0	0	0	0	2	0	2
BIT134	87	100	37	58	35.8	100	14.2	252		113	10	10	N	N	35	58.3	2.8	0	16	n	N	14	Y	empirical	1	0	1	0	0	1	3
BIT135	102	88	46	60	36	100	19.7	162	>90	83	6	1	N	N	32	46	2.7	0	2	N	N	15	Y	empirical	1	0	0	0	1	0	2
BIT136	117	110	56	74	36.5	122	23.7	131	55	129	6	10.6	N	N	45	32	1.8	0	12	N	N	15	N	n/a	0	1	1	0	2	0	4
BIT137	Not consented																														
BIT138	105	123	76	91.6667	35.9	119	13.9	216	>90	89	10	10.9	N	N	28	n/a	ND	0	100	N	N	15	N	n/a	0	0	0	0	0	0	0
BIT139	89	112	75	87.3333	37.1	121	11.2	220	>90	82	9	11	N	N	28	45	1.8	0	20	N	N	15	Y	empirical	0	0	0	0	1	0	1
BIT140	Discharged prior to day 3 - info			0																											
BIT141	94	94	55	68	37.5	90	10.9	173	>90	70	6	12	N	N	21	ND	2.9	0	3	N	N	15	N	n/a	1	0	0	0	0	0	1
BIT142	51	76	44	54.6667	35.5	104	22.4	203	58	100	42	10.8	N	N	32	38	2.6	0	6	N	N	15	N	n/a	1	0	0	2	2	0	5
BIT143	105	122	69	86.6667	37.9	110	13.9	265	>90	70	14	10.6	N	N	21	ND	3.5	0	17	N	N	15			0	0	0	0	0	0	0
BIT144	106	127	62	83.6667	37.5	131	15.1	254	>90	80	6	11	N	N	40	38	2.1	0	36	N	N	15	Y	empirical	0	0	0	0	2	0	2
BIT145	Declined consent			0																											
BIT146	110	86	40	55.3333	36.8	118	13.3	130		102	20	16	N	N		19	2.4	0	58	N	N	15	N	n/a	1	1	0	1	3	0	6
BIT147	150	83	53	63	38.5	110	12	236	78	109	11	13	Y	N	40	38	2.3	0.1333	44	N	Y	3	Y	empirical	4	0	0	0	2	4	10
BIT148	137	89	47	61	39.2	104	10.8	209	>90	107	36	12	Y	N	70	42	4	0.1932	3	N	Y	3	Y	empirical	4	0	0	2	1	4	11
BIT149	90	114	64	80.6667	37.6	132	14.9	216	>90	58	8	11	N	N	60	18	1.4	0	17	N	N	15	N	n/a	0	0	0	0	3	0	3
BIT150	113	118	73	88	37.5	148	7.1	276	>90	61	17	11.1	N	N	32	47	3.2	0	16	N	N	15	Y	empiracle	0	0	0	0	1	0	1
BIT151	113	159	133	141.667	33.1	97	26.6	132	69	125	6	17.7	N	N	85	ND	8.2	0.13	11	N	N	13	Y	arm	4	1	1	0	0	1	7
BIT152	52	209	143	165	35.1	101	9.3	246	30	137	7	10.6	N	N	36	34	1.2	0	54	N	N	15	N	n/a	0	0	1	0	2	0	3

7.4 Appendix 4: Pilot study -Clinical data for 200 patients from Central Manchester Foundation Trust – Day 5

	HR	Sys BP	Dia BP	MAP	Temp	Hb	WCC	PLT	eGFR	Creat	Bili	PT	Intubated	NIV / CPAP	FiO2	P/F	Lactate	Norad	CRP	CVVH/HD	Sedated	GCS	Antibiotics	Septic source	Steroids	CAM +ve	MAP SOFA	PLT SOFA	CREAT SOFA	BILI SOFA	RESP SOFA	GCS SOFA	SOFA DAY 5
BIT001	94	111	78	89	37.2	102	10.1	356	90	84	N	N	N	N	0.21	N	N	0	18	N	N	15	Y		N	N	0	0	0	0	0	0	0
BIT002	107	131	79	96.3333	36.6	91	7.5	191	90	65	18	16.9	N	N	0.28	45.2	0.8	0	244	N	N	15	Y		N	N	0	0	0	0	1	0	1
BIT003	110	85	65	71.6667	36.6	83	6.3	211	86	78	13	14	N	N	0.21	N	N	0	113	N	N	15	Y		N	N	0	0	0	0	0	0	0
BIT004	117	92	76	81.3333	37.3	102	12	221	90	45	17	15	N	N	0.21	53.6	0.5	0	329	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT005	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT006	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT007	85	115	85	95	36.5	122	7.7	217	90	61	10	13.1	N	N	0.21	N	N	0	55	N	N	15	N		N	N	0	0	0	0		0	0
BIT008	108	102	62	75.3333	35.7	85	20.3	199	90	48	22	12.5	Y	N	0.28		1.3	0	210	N	Y	N	Y	Abdomen	Y	N	0	0	0	1	4	4	9
BIT009	80	115	47	69.6667	37.1	83	7.6	233	90	91	15	14.7	N	N	0.21	N	N	0	95	N	N	15	Y		N	N	1	0	0	0	0	0	1
BIT010	124	126	94	104.667	36.2	114	6.7	270	90	38	7	13.6	N	N	0.4	N	N	0	181	N	N	15	Y	Chest	N	N	0	0	0	0	0	0	0
BIT011	98	115	60	78.3333	36.9	N	N	N	N	N	N	N	N	N	0.32	N	N	0		N	N	15	N		N	N	0	0	0	0	0	0	0
BIT012				0																							1	4	0	0	4	4	13
BIT013				0																							1	4	0	0	4	4	13
BIT014	103	130	64	86	35.9	91	10.6	357	90	78	N	N	N	N	N	N	N	0	194	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT015				0																							1	4	0	0	4	4	13
BIT016	100	113	70	84.3333	36.4	83	10	125	58	111	11	14.1	N	N	0.4	29.8	1.4	0	174	N	N	15	Y		N	N	0	1	1	0	2	0	4
BIT017	112	140	65	90	37.7	82	6.2	85	90	60	8	13.6	N	N	N	N	N	0	124	N	N	15	N		N	N	0	2	0	0	0	0	2
BIT018	113	176	96	122.667	37.6	111	8.5	228	90	53	13	10.7	Y	N	0.4	25.8	1.2	0	92	N	Y	N	Y	empirical	N	N	0	0	0	0	3	0	3
BIT019				0																							1	4	0	0	4	4	13
BIT020				0																							1	4	0	0	4	4	13
BIT021	98	133	78	96.3333	36	109	9.7	339	90	70	9	10.4	N	N	0.21	N	N	0	26	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT022	54	130	75	93.3333	36.1	N	N	N	N	N	N	N	N	N	N	N	N	0		N	N	15	N		N	N	0	0	0	0	0	0	0
BIT023	80	111	58	75.6667	37.1	N	N	N	N	N	N	N	N	N	0.21	N	N	0		N	N	15	N		N	N	0	0	0	0	0	0	0
BIT024	95	105	50	68.3333	36.7	82	9	198	90	52	6	9.9	Y	N	0.3	45.4	0.6	0		N	Y	N	Y		N	Y	1	0	0	0	1	0	2
BIT025	75	96	46	62.6667	34.7	92	14.9	166	83	79	16	11.1	N	N	0.6	22.1	1.4	0		N	N	15	N		N	N	1	0	0	0	3	0	4
BIT026				0																							1	4	0	0	4	4	13
BIT027	66	116	80	92	36.4	118	11.9	350	90	75	N	N	N	N	0.21	N	N	0	33	N	N	15	Y	POST OP	N	N	0	0	0	0	0	0	0
BIT028	60	132	65	87.3333	37.7	96	9	346	90	70	11		N	N	0.21	N	N	0	105	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT029	86	107	69	81.6667	35.4	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT030				0																							1	4	0	0	4	4	13
BIT031				0																							1	4	0	0	4	4	13
BIT032				0																							1	4	0	0	4	4	13
BIT033				0																							1	4	0	0	4	4	13
BIT034	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT035	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT036				0																							1	4	0	0	4	4	13
BIT037	Not consented																																
BIT038				0																							1	4	0	0	4	4	13
BIT039				0																							1	4	0	0	4	4	13
BIT040	117	134	82	99.3333	38.5	103	12.2	409	90	86	21	N	N	N	0.24	N	N	0	309	N	N	15	Y	empirical	N	N	0	0	0	1	0	0	1
BIT041				0																							1	4	0	0	4	4	13
BIT042	106	127	73	91	37.4	101	8.6	239	N	64	8	11.3	N	N	0.21	N	N	0	150	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT043	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT044	N	N	N	#VALUE!	N	N	N	N	N	N	N	N	N	N	N	N	N	Norad	N	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT045				0																							1	4	0	0	4	4	13
BIT046	110	86	37	53.3333	35.8	80	48.7	84	83	60	24	13.4	N	N	0.5	23.5	0.9	0	181	N	N	13	N	empirical	N	N	1	2	0	1	3	1	8
BIT047	112	139	65	89.6667	38.2	86	7.1	179	90	76	22	11.4	N	N	0.28	43.9	0.8	0	227	N	N	15	N		N	N	0	0	0	1	1	0	2
BIT048				0		74	12.7	245	19	321	12	11	y	N					350	n	y		y	empirical	N	N	1	0	3	0	4	4	12
BIT049	97	147	81	0	37.2	123	8.2	248	90	71	8	N	N	N	0.21	N	N	0	3	N	N	15	N		N	N	1	0	0	0	0	0	1
BIT050	98	115	74	87.6667	36.1	119	7.2	339	90	69	11	N	N	N	0.28	N	N	0	267	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT051				0																							1	4	0	0	4	4	13
BIT052	98	132	87	102	35.9	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT053	105	155	72	0	37.5	102	6.2	207	90	48	N	11	N	N	0.32	N	N	0	123	N	N	15	N		N	N	1	0	0	0	0	0	1
BIT054				0																							1	4	0	0	4	4	13
BIT055	90	98	63	74.6667	37.5	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT056				0																							1	4	0	0	4	4	13
BIT057				0																							1	4	0	0	4	4	13
BIT058				0																							1	4	0	0	4	4	13
BIT059				0																							1	4	0	0	4	4	13
BIT060	125	105	50	68.3333	37.7	77	6.5	165	75	94	15	10.8	Y	N	0.6	11.3	1.2	0		N	Y	N	Y	empirical	Y	Y	1	0	0	0	4	0	5
BIT061	97	140	55	83.3333	35.8	82	8.8	213	90	38	14	11.1	N	N	0.35	27.4	0.9	0	152	N	N	15	y	empirical	N	N	0	0	0	0	2	0	2
BIT062				0																							1	4	0	0	4	4	13
BIT063				0																							1	4	0	0	4	4	13
BIT064	DISCHARGED																																
BIT065	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT066	87	145	72		35.7	129	9.9	261	90	62	13	N	N	N	0.21	N	N	0	53	N	N	N	N		N	N	0	0	0	0	0	0	0
BIT067	Discharged																																
BIT068	Discharged																																
BIT069	110	149	88	108.333	37.1	125	8.4	242	90	67	7	11.1	N	N	0.28	63.7	1.1	0	N	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT070	56	145	63	90.3333	36	123	10.6	349	78	98	N	N	N	N	0.21	N	N	0	N	N	N	15	y	empirical	N	N	0	0	0	0	0	0	0
BIT071	DISCHARGED																																
BIT072	104	136	71	92.6667	35.7	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	15	y	eye	Y	N	0	0	0	0	0	0	0
BIT073	83	131	83	0	36.9	127	5.6	254	76	67	7	N	N	N	0.28	N	N	0	22	N	Y	15	Y	empirical	Y	N	1	0	0	0	0	0	1

	HR	Sys BP	Dia BP	MAP	Temp	Hb	WCC	PLT	eGFR	Creat	Bili	PT	Intubated	NIV / CPAP	FiO2	P/F	Lactate	Norad	CRP	CVVH/HD	Sedated	GCS	Antibiotics	Septic source	Steroids	CAM +ve	MAP SOFA	PLT SOFA	CREAT SOFA	BILI SOFA	RESP SOFA	GCS SOFA	SOFA DAY 5
BIT074	89	137	77	97	37.8	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT075				0																							1	4	0	0	4	4	13
BIT076	121	112	70	84	37.8	89	7.8	282	>90	48	4	n/d	N	N	0.24	N	N	N	N	N	N	15	Y	emp	N	N	4	0	0	0	0	0	4
BIT030	74	91	52	65	35.9	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	N		N	N	1	0	0	0	0	0	1
BIT078	105	141	70	93.6667	37.9	101	6.2	217	>90	80	N	N	N	N	0.21	N	N	N	127	N	N	15	Y	emp	N	N	4	0	0	0	0	0	4
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	Y	N	15	Y	emp	N	N	0	0	0	0	0	0	0
BIT080	Self discharged																																
BIT081	Declined																																
BIT082	Not consented																																
BIT083	Transferred to Salford																																
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	N	N	15	Y	abdo	N	N	0	0	0	0	0	0	0
BIT085	90	162	156	158	35.6	112	13.3	297	>90	63	6	11	Y	N	0.6	14	N	0	121	N	Y	15	Y	emp	Y	Y	0	0	0	0	3	0	3
BIT086	Not consented																																
BIT087	Discharged																																
BIT088	133	135	101	112.333	38.5	85	15.5	293	90	86	19	10.5	N	N	0.35	30.9	0.8	0	293	N	N	14	N		N	Y	0	0	0	0	2	1	3
BIT089	Refused																																
BIT090	Transferred to Salford																																
BIT091	89	160	71	100.667	37.2	79	10	262	17	128	7	10.8	Y	N	0.24	49.6	1.1	0.01	128	N	N	14	N		N	N	3	0	1	0	1	1	6
BIT092				0		100	11.6	233	90	71	7																1	0	0	0	0	0	1
BIT093				0		94	12.6	313	88	79	26								103				Y	spleen			0	0	0	1	0	0	1
BIT094				0		108	4.8	45	90	77										N		15	Y	CHEST			0	3	0	0	0	0	3
BIT095	DISCHARGED																																
BIT096																																	
BIT097	Not consented																																
BIT098	97	140	86	104	36.9	124	16.9	247	90	59	15	11.1	N	N									Y	empirical			0	0	0	0	0	0	0
BIT099				0		132	8.8	182	90	53	31		N	N					178	N	N	15					0	0	0	1	0	0	1
BIT100	105	88	64	72	37.3	84	1.6	155	84	98	8		N	N													0	0	0	0	0	0	0
BIT101	Refused			0																													
BIT102	91	100	70	80	36.6	126	11.1	185	67	98	15	10.9	Y	N	40%		0.8		251	N	N		Y	empirical	N		0	0	0	0	0	0	0
BIT103	109	115	63	80.3333	36.8	79	12.4	158	90	65	13	10.3	N	N	35		0.9		350	N	N						0	0	0	0	0	0	0
BIT104	80	102	56	71.3333	39.2	62	8.6	302	90	49	8	11	N	N	21				110	N	N	15					0	0	0	0	0	0	0
BIT105	Discharged																																
BIT106	Not consented																																
BIT107	113	94	50	64.6667	38	75	9.1	125	64	102	21	11	Y	N	60		1	0.4	372	N	Y	3					4	1	0	1	0	4	10
BIT108	Not consented																																
BIT109	101	140	79	99.3333	36.4	73	3.4	88	90	35	31		N	Y	21%		1.5			N	N	15					0	2	0	1	0	0	3
BIT110	Not consented																																
BIT111	72	100	52	68	37.2	NA	NA	NA	NA	NA	NA	NA	N	N	21%	NA	NA	NA	NA	N	N	15	N	NA	N	N	1	0	0	0	0	0	1
BIT112	Not consented																																
BIT113	123	158	96	116	37.3	108	14	124	67.78	108	23	10	Y	N	30%	38	0.7	NA	285	N	Y	14	Y	empirical	N	N	0	1	0	1	2	1	5
BIT114	Not consented																																
BIT115	96	130	72	91.3333	36.8	ND	ND	ND	ND	ND	ND	ND	N	N	N/A	ND	ND	N/A	ND	N	N	15	N	N/A	N	N	0	0	0	0	0	0	0
BIT116	Not consented																																
BIT117	106	138	74	95.3333	37.6	69	6.9	110	108	69	7	NA	N	N	24%	52	0.6	NA	NA	N	N	15	Y	empirical	N	N	0	1	0	0	1	0	2
BIT118	116	125	70	88.3333	37.4	NA	NA	NA	NA	NA	NA	NA	N	N	21%	NA	NA	NA	NA	N	N	15	N	NA	N	N	0	0	0	0	0	0	0
BIT119	99	143	81	101.667	37	NA	NA	NA	NA	NA	NA	NA	N	N	21%	NA	NA	NA	NA	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT120	60	142	82	102	36.2	127	5.1	266	>90	65	12	10.5	N	N	35%	n/a	n/a	n/a	n/a	N	N	15	N	n/a	N	N	0	0	0	0	0	0	0
BIT121	55	112	43	66	36.3	104	NA	185	90	60	NA	9.4	N	N	21%	NA	NA	N	NA	N	N	15	Y	empirical	N	N	1	0	0	0	0	0	1
BIT122	Not consented																																
BIT123	111	141	84	103	36.2	101	9.1	292	>90	54	16	NA	N	N	28%	NA	NA	NA	110	N	N	15	N	N	N	N	0	0	0	0	0	0	0
BIT124	87	171	70	104	35.7	122	7.2	190	90	47	10	9.9	N	N	35%	NA	NA	N	107	N	N	15	Y	Wound	N	N	0	0	0	0	0	0	0
BIT125	66	91	34	53	36.8	84	10.9	256	87	76	14	10	N	N	21	27	1.7	N	N	N	N	15	N	N	N	N	1	0	0	0	2	0	3
BIT126	Transferred to Salford																																
BIT127	78	103	64	77	37.1	95	5.3	217	90	79	NA	NA	N	N	28	NA	NA	0	186	N	N	15	N	N	N	N	0	0	0	0	0	0	0
BIT128	136	137	72	93.6667	37.9	71	31.1	391	41	152	56	10	Y	N	80	16	1	n/a	289	N	Y	3	Y	empirical	N	N	0	0	1	2	3	4	10
BIT129	64	116	68	84	37.2	123	7.6	217	90	58	NA	NA	N	N	21%	NA	N	n/a	NA	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT130	48	109	66	80.3333	37.6	92	5.8	263	>90	52	n/a	10	N	N	21	n/a	0.9	n/a	4	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT131	130	98	51	66.6667	35	80	8.3	139	41	110	23	12	Y	N	80	13	1.5	0.12	575	N	Y	3	Y	empirical		N	4	1	1	1	4	4	15
BIT132	120	96	57	70	37	91	21.3	141	57	108	141	12	Y	N	30	31	2.5	0.45	370	Y	Y	3	Y	empirical		N	4	1	0	3	2	4	14
BIT133	70	99	60	73	36.8	142	6.3	240	89	75	10		N	N	21	n/a	n/a	0	42	N	N	15				N	0	0	0	0	0	0	0
BIT134	89	120	45	70	35.5	106	6	167		69	18	10	N	N	21	55	0.8	0	131	n	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT135	86	115	60	78.3333	35.8	102	9.4	312	>90	90	11	ND	N	N	21	ND	ND	0	45	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT136	96	171	104	126.333	35.3	119	7.9	204	>90	66	17	ND	N	N	24	ND	ND	0	113	N	N	15	N	n/a	N	N	0	0	0	0	0	0	0
BIT137	Not consented																																
BIT138	Discharged																																
BIT139	96	119	71	87	36.9	104	9.7	238	>90	68	ND	11.3	N	N	28	ND	ND	0	303	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT140	Discharged																																
BIT141	89	101	50	67	37.5	94	5.1	184	>90	81	7	10	N	N	21	ND	ND	0	28	N	N	15	N	n/a	N	N	1	0	0	0	0	0	1
BIT142	89	119	58	78.3333	35.9	99	6.8	477	61	96	ND	ND	N	N	21	ND	ND	0	22	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT143	115	116	71	86	35.8	109	8.2	36.2	>90	64	8	10.4	N	N	21	ND	ND	0	24	N	N	15	N	n/a	N	N	0	3	0	0	0	0	3
BIT144	81	125	62	83	36.6	122	6.9	291	>90	64	4	10	N	N	21	ND	ND	0	19	N	N	15	N	n/a	N	N	0	0	0	0	0	0	0
BIT145	Not consented																																
BIT146	112	96	65	75.3333	37.8	101	4.8	75		58	13	10	N	N	24	ND	ND	0	ND	N	N	15	Y	empirical	N	N	0	2	0	0	0	0	2
BIT147	123	108	72	84	37.6	91	13	304	>90	64	25	13	N	N	50	22	1.1	0	169	N	N	14	Y	empirical	N	N	0	0	0	0	3	1	4
BIT148	110	99	65	76.3333	39.7	66	5.8	97	>90	86	55	11	Y	N	50	27	2.2	0	262	N	Y	7	Y	empirical	N	N	0	2	0	2	2	3	9
BIT149	95	107	94	98.3333	37.3	114	5.8	226	>90	55	12	11	N	N	32	ND	ND	0	128	N	N	15	N	n/a	N	N	0	0	0	0	0	0	0
BIT150	119	181	112	135	38.2	147	9.5	383	>90	58	ND	ND	N	N	21	ND	ND	0	44	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT151	81	136	62	86.6667	37.3	84	11.7	180	>90	125	ND	ND	N	N	21	ND	ND	0	48	N	N	15	Y	arm	N	N	0	0	1	0	0	0	1
BIT152	65	183	115	137.667	35.6	111	839	318	29	151	6	10.3	N	N	21	ND	ND	0	77	N	N	15	N	na	N	N	0	0	1	0	0	0	1

7.5 Appendix 5: Pilot study -Clinical data for 200 patients from Central Manchester Foundation Trust – Day 8

	HR	Sys BP	Dia BP	MAP	Temp	Hb	WCC	PLT	eGFR	Creat	Bili	PT	Intubated	NIV / CPAP	FiO2	P/F	Lactate	Norad	CRP	CVVH/HD	Sedated	GCS	Antibiotics	Septic source	Steroids	CAM +ve	Tranexamic acid	Transfusion	ISS	MAP SOFA	PLT SOFA	CREAT SOFA	BILI SOFA	RESP SOFA	GCS SOFA	SOFA DAY 8
BIT001	80	117	78	91	36.9	108	27.5	460	90	86	N	N	N	N	0.21	N	N	0	88	N	N	15	N	N	N	N	N	N	14	0	0	0	0	0	0	0
BIT002	111	134	95	108	36.6	91	12.5	184	90	64	29	16.9	N	N	0.35	N	N	0	219	N	N	15	Y	N	N	N	N	N	29	0	0	0	1	0	0	1
BIT003	89	93	53	66.33333333	37.1	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	N	N	N	N	N	Y	25	1	0	0	0	0	0	1
BIT004	92	114	64	80.66666667	36.6	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	N	N	N	N	Y	Y	25	0	0	0	0	0	0	0
BIT005	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	33	0	0	0	0	0	0	0
BIT006	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT007	75	134	65	88	36.3	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	N	N	N	N	N	N	20	0	0	0	0	0	0	0
BIT008	113	121	68	85.66666667	36	94	29.5	241	90	36	23	13.8	Y	N	0.4	N	1.3	0	52	N	Y	N	Y	Abdomen	Y	N	N	Y	8	0	0	0	1	0	0	1
BIT009	80	119	63	81.66666667	36.9	96	10.5	337	90	79	19	15.2	N	N	0.21	N	N	0		N	N	15	N	N	N	N	N	N	29	0	0	0	0	0	0	0
BIT010	125	134	100	111.3333333	37.3	121	9.6	546	90	44	6	14.2	N	N	0.35	N	N	0	45	N	N	15	Y	Chest	N	N	N	N	26	0	0	0	0	0	0	0
BIT011	91	121	68	85.66666667	36.9	73	15.8	123	59	79		15.1	N	N	0.21	N	N	0	74	N	N	15	N	N	N	N	N	Y	25	0	1	0	0	0	0	1
BIT012				0																									1	4	0	0	4	4	13
BIT013				0																									1	4	0	0	4	4	13
BIT014	100	112	72	85.33333333	36.2	101	15.2	481	88	90	N	N	N	N	N	N	N	0	158	N	N	15	N	N	N	N	Y	Y	16	0	0	0	0	0	0	0
BIT015				0																									1	4	0	0	4	4	13
BIT016	93	97	66	76.33333333	35.9	105	11.9	233	64	101	16	14.4	N	N	0.28	43.3	1.3	0	134	N	N	15	N	N	N	N	N	N	29	0	0	0	0	1	0	1
BIT017	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	4	0	0	0	0	0	0	0
BIT018	105	151	94	113	35.7	94	6.4	193	90	41	9	11.4	N	N	0.28	N	N	0	206	N	N	15	Y	empirical	N	N	N	N	41	0	0	0	0	0	0	0
BIT019				0																									1	4	0	0	4	4	13
BIT020				0																									1	4	0	0	4	4	13
BIT021	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	16	0	0	0	0	0	0	0
BIT022	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	25	0	0	0	0	0	0	0
BIT023	90	105	57	73	37.8	N	N	N	N	N	N	N	N	21	N	N	0			N	N	15	N	N	N	N	Y	N	9	0	0	0	0	0	0	0
BIT024	99	110	65	80	37.3	107	N	N	N	N	N	N	N	N	0.21	N	0.6	0		N	N	14	Y	N	N	N	N	Y	29	0	0	0	0	0	1	1
BIT025	87	83	45	57.66666667	35.3	80	14.2	329	75	86	9	10.7	N	N	8	19.2	1.1	0	1	N	N	15	Y	Chest	N	N	N	N		1	0	0	0	3	0	4
BIT026				0																									1	4	0	0	4	4	13
BIT027	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	30	0	0	0	0	0	0	0
BIT028	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	Y	Y	16	0	0	0	0	0	0	0
BIT029	N	104	68	80	36	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	N	N	N	N	N	N	20	0	0	0	0	0	0	0
BIT030				0																									1	4	0	0	4	4	13
BIT031				0																									1	4	0	0	4	4	13
BIT032				0																									1	4	0	0	4	4	13
BIT033				0																									1	4	0	0	4	4	13
BIT034	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	N	0	0	0	0	0	0	0
BIT035	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	17	0	0	0	0	0	0	0
BIT036				0																									1	4	0	0	4	4	13
BIT037	Not consented																																			
BIT038				0																									1	4	0	0	4	4	13
BIT039				0																									1	4	0	0	4	4	13
BIT040	112	149	73	98.33333333	39	100	17.3	708	90	82	26	N	N	N	0.28	N	N	0	329	N	N	15	Y	empirical	N	N	N	N	25	0	0	0	1	0	0	1
BIT041				0																									1	4	0	0	4	4	13
BIT042																											Y	N	14							
BIT043	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	19	0	0	0	0	0	0	0
BIT044	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Norad	N	N	N	N	N	N	N	N	Y	N	14	0	0	0	0	0	0	0
BIT045				0																									1	4	0	0	4	4	13
BIT046	154	86	55	65.33333333	35.9	77	44	96	90	54	96	15	N	N	0.45	20.7	1.6	0	73	N	N	14	Y	empirical	N	N	Y	Y	36	1	2	0	2	3	1	9
BIT047	120	150	62	91.33333333	38.8	72	8.7	302	90	68	40	11.5	N	N	0.28	52.1	0.8	0	N	N	N	15	N		Y	N	Y	Y	10	0	0	0	2	1	0	3
BIT048				0		84	20.4	270	12	470	18	10.9	y	n					563	y	y		y	empirical	y	n	y	y	Not coded	1	0	4	0	4	4	13
BIT049	76	126	63	84	36.2	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n	n	n	15	n		n	n	y	n	35	0	0	0	0	0	0	0
BIT050	86	101	57	71.66666667	36.3	125	7.1	351	90	64	13	n	n	n	0.21	n	n	0	166	n	n	15	n		n	n	y	n	9	0	0	0	0	0	0	0
BIT051				0																									1	4	0	0	4	4	13
BIT052	104	139	74	95.66666667	36.2	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n	n	n	15	n		n	n	y	y	36	0	0	0	0	0	0	0
BIT053	95	160	95	116.6666667	37	101	3.6	100	n	n	n	n	n	n	0.32	n	n	0	n	n	n	15	n		n	n	y	y	25	0	1	0	0	0	0	1
BIT054				0																									1	4	0	0	4	4	13
BIT055	71	80	51	60.66666667	37.4	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n	n	n	15	n		n	n	y	y	N	1	0	0	0	0	0	1
BIT056				0																									1	4	0	0	4	4	13
BIT057				0																									1	4	0	0	4	4	13
BIT058				0																									1	4	0	0	4	4	13
BIT059				0																									1	4	0	0	4	4	13
BIT060	118	106	46	66	37.3	92	14.6	317	90	80	17	10.6	Y	Y	0.65	12.7	1.3	0.07		N	Y	12	N		Y	Y	Y	Y	n	3	0	0	0	4	2	9
BIT061	99	140	77	98	35.5	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n	n	n	15	n		n	n	y	y	n	0	0	0	0	0	0	0
BIT062				0																									1	4	0	0	4	4	13
BIT063				0																									1	4	0	0	4	4	13
BIT064	Discharged																																			
BIT065	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	N	N	N	N	N	N	N	N	N	N							0
BIT066	61	140	65	90	35.6	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n	n	n	15	n		n	n	n	n	n	0	0	0	0	0	0	0
BIT067	Discharged																																			
BIT068	Discharged																																			
BIT069	56	120	69	0	36.5	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n	n	n	15	n		n	n	y	y	Noyt yet	1					0	1
BIT070	81	132	73	0	35.5	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n	n	n	15	y	empirical	n	n	y	n	Not yet	1					0	1
BIT071	Discharged																																			
BIT072	DISCHARGED																																			
BIT073	n	n	n	#VALUE!	n	126	5.9	271	68	74	n	11.4	n	n	0.21	n	n	0	15	n	n	15	y	empirical	y	n	y	n	Not yet		0	0			0	0

	HR	Sys BP	Dia BP	MAP	Temp	Hb	WCC	PLT	eGFR	Creat	Bili	PT	Intubated	NIV / CPAP	FiO2	P/F	Lactate	Norad	CRP	CVVH/HD	Sedated	GCS	Antibiotics	Septic source	Steroids	CAM +ve	Tranexamic acid	Transfusion	ISS	MAP SOFA	PLT SOFA	CREAT SOFA	BILI SOFA	RESP SOFA	GCS SOFA	SOFA DAY 8
BIT074	100	133	81	98.33333333	37.5	115	11.3	470	90	72	10	n	n	n	0.21	n	n	0	70	n	n	15	y	empirical	n	n	n	n	Not yet	0	0	0	0		0	0
BIT075				0																									1	4	0	0	4	4	13
BIT076	111	132	83	99.33333333	37.1	93	10	404	>90	46	n	11.3	n	n	n	n	n	n	302	n	n	15	n		n	n	y	y	Not yet	0	0	0			0	0
BIT030	88	127	58	81	36.2	n	n	n	n	n	n	n	n	n	0.21	n	n	0	n	n	n	15	n		n	n	y	y		0					0	0
BIT078	99	146	79	101.3333333	37.3	n	n	n	n	n	n	n	N	n	0.21	n	n	n	n	n	n	15	n		n	n	y	y		0					0	0
BIT079	n/k	n/k	n.k		n/k	87	11.1	198	36	179	7	12.2	n	n	n/k	n	n/k	n	170	y	n	15	n	n	n	n	n	n			0	2	0		0	2
BIT080	Self discharged																													1	4	0	0	4	4	
BIT081	Declined																													1	4	0	0	4	4	
BIT082	Not consented																													1	4	0	0	4	4	
BIT083	Transferred to Salford																													1	4	0	0	4	4	
BIT084	n/k	n/k	n/k		n/k	74	18	624	>90	54	9	n/d	n	n	n/k	n/k	n/k	0	295	n	n	15	y	abdomen	n	n	y	y			0	0	0		0	0
BIT085	121	138	81	100	37.8	115	11.4	540	>90	57	4	n/d	y	n	0.6	14	0.6	0	75	n	y	n.a	n		n	y	y	y		0	0	0	0	3		3
BIT086	Not consented																																			
BIT087	Discharged																																			
BIT088	100	133	71	91.66666667	37.6	101	15.6	644	90	78	19	10.5	n	n	0.35	n/a	n/a	0	142	n	n	15	n		n	n	y	y		0	0	0	0		0	0
BIT089	Refused																																			
BIT090	Transferred to Salford																																			
BIT091	132	95	43	60.33333333	37.3	66	12.7	386	17	179	5	11.4	y	n	0.4	31	0.9	0	69	n	n	14	n		n	y	y	y		1	0	2	0	2	1	6
BIT092						90	8.9	281	90	65	6																				0	0	0				0
BIT093						94	12.6	413	78	88	25	11							88												0	0	1				1
BIT094	DISCHARGED																																			
BIT095	Discharged																																			
BIT096																																				
BIT097	Not consented																																			
BIT098	93	123	77	92.33333333	36.4	122	11.1	389	90	63	15	10.9		N									Y	empirical	N					0	0	0	0			0
BIT099	99	104	78	86.66666667	36.5	113	7	219	90	43			N	N					225	N	N	15								0	0	0			0	0
BIT100	105	88	64	72	37.2	103	18.4	475	81	101	13		N	N									Y					Y		0	0	0	0			0
BIT101	Refused																																			
BIT102	75	104	53	70	36	133	9.1	274	82	83	11		N	N	40%												Y	Y		0	0	0	0			
BIT103	109	115	61	79	36.7	80	15	293	90	58	9		N				0.5		284	N	N				N		Y	Y		0	0	0	0			
BIT104	80	102	56	71.33333333	37	119	10.3	611	90	52	12	11	N		21%				82	N	N	15								0	0	0	0		0	
BIT105	Discharged																																			
BIT106	Not consented																																			
BIT107	121	87	47	60.33333333	38.1	77	18.7	553	75	89	11	11	y	N	40%	34.75	0.7		309	N	Y	3	Y	empirical			Y	Y		1	0	0	0	2	4	7
BIT108	Not consented																																			
BIT109	109	136	70	92	36	87	1.4	111	90	35	23		N	N	40%				67	N	N	15	Y	chest			Y			0	1	0	1		0	2
BIT110	Not consented																																			
BIT111	TRABSFERRED TO WRIGHTINGTON																																			
BIT112	Not consented																																			
BIT113	114	166	82	110	37.7	NA	NA	NA	NA	NA	NA	NA	N	N	21%	NA	NA	NA	NA	N	N	15	Y	Empirical	N	N	Y	Y	43	0					0	0
BIT114	Not consented																																			
BIT115	109	169	97	121	37.5	NA	NA	NA	NA	NA	NA	NA	N	N	21%	NA	NA	NA	NA	N	N	14	N	NA	N	N	Y	Y	13	0					1	1
BIT116	Not consented																																			
BIT117	92	138	62	87.33333333	36.9	NA	NA	NA	NA	NA	NA	NA	N	N	21%	NA	NA	NA	NA	N	N	14	N	NA	N	N	Y	Y	45	0					1	1
BIT118	DISCHARGED																												20							
BIT119	99	120	64	82.66666667	36.2	ND	ND	ND	ND	ND	ND	ND	N	N	21	ND	ND	NA	ND	N	N	15	N	NA	N	N	Y	N	26	0					0	0
BIT120	80	126	72	90	35.9	n/a	n/a	n/a	n/a	n/a	n/a	n/a	N	N	21%	n/a	n/a	n/a	n/a	N	N	15	N	n/a	N	N	N	N	21	0					0	0
BIT121	DISCHARGED																																			
BIT122	Not consented																																			
BIT123	109	126	74	91.33333333	35	103	9.5	323	>90	54	15	10.4	N	N	21%	N	NA	NA	108	N	N	15	N	N	N	N	N	N	17	0	0	0	0		0	0
BIT124	95	140	68	92	35.8	ND	ND	ND	ND	ND	ND	ND	N	N	21%	NA	NA	N	N	N	N	15	Y	wound	N	N	N	Y	32	0					0	0
BIT125	62	112	53	72.66666667	37.9	90	12.6	454	79	83	NA	11.3	N	N	21	NA	NA	N	45	N	N	15	N	N	N	N	N	N	not coded	0	0	0			0	0
BIT126	Transferred to Salford																																			
BIT127	94	102	62	75.33333333	36.6	103	5.4	348	90	79			N	N	21	NA	NA	NA	NA	N	N	15	N	N	N	N	y	y	not coded	0	0	0			0	0
BIT128	136	66	51	56	38.2	82	40	272	17	322	81	12	Y	N	100	11	3.9	1.54	130	Y	Y	3	Y	Empirical	Y	N	N	N	not coded	4	0	3	2	4	4	17
BIT129	63	111	57	75	37.8	ND	ND	ND	ND	ND	ND	ND	N	N	21%	NA	NA	NA	NA	N	N	15	Y	Empirical	N	N	Y	Y	9	0					0	0
BIT130	79	92	60	70	36.9	84	6.7	266	>90	58			N	N	21	n/a	nd	n/a	3	N	N	15	N		N	N	N	N		0	0	0			0	0
BIT131	52	84	57	66	38	73	15.1	142	59	81	27	11	Y	N	40	26	2.6	n/a	ND	N	N	N	Y	chest		N	N	N		1	1	0	1	3	4	10
BIT132	150	88	46	60	35.6	75	37.6	46	42	142	264	18	Y	N	100	16	19.3	1.06	129	Y	Y	3	Y	chest		N	N	Y		4	3	1	4	3	4	19
BIT133																																				
BIT134	93	91	52	65	34.9	86	7.2	180	ND	70	14	10	N	N	21	n/a	0.8	0	173	N	N	13	Y	empirical	N	N	N	N		1	0	0	0		1	2
BIT135	86	100	59	72.66666667	35.8	107	7.5	494	>90	73	ND	ND	N	N	21	n/a	ND	0	25	N	N	15	Y	empirical	N	N	N	N		0	0	0			0	0
BIT136	114	159	109	125.6666667	35.7	91	8.7	310	>90	64	ND	ND	N	N	40	n/a	ND	0	83	N	N	15	N	n/a	N	N	N	N		0	0	0			0	0
BIT137	Not consented																																			
BIT138	Discharged																																			
BIT139	89	133	79	97	37.5	105	9.5	350	>90	76	ND	ND	N	N	24	n/a	ND	0	192	N	N	15	Y	Empirical	N	N	N	N		0	0	0			0	0
BIT140	Discharged																																			
BIT141	Discharged																																			
BIT142	94	114	59		36.9	99	6.8	477	61	96	ND	ND	N	N	21	ND	ND	0	22	N	N	15	Y	Empirical	N	N	N	N			0	0			0	0
BIT143																																				
BIT144	90	129	60	83	36.7	ND	ND	ND	ND	ND	ND	ND	N	N	21	n/a	ND	0	ND	N	N	15	N	n/a	N	N	N	N		0					0	0
BIT145	Not consented																																			
BIT146																																				
BIT147	105	95	55		37.9	82	14.6	455	>90	50	25	12	N	N	28	45	0.7	0	110	N	N	15	Y	HAP/CAP	N	N	N	N			0	0	1	1	0	2
BIT148	81	76	51		37.5	78	7.8	135	>90	60	46	10	Y	N	40	43	1.1	0	85	N	Y	8	Y	Empirical	N	N	N	N			1	0	2	1	3	7
BIT149																																				
BIT150	100	134	91		37.9	ND	ND	ND	ND	ND	ND	ND	N	N	21	ND	ND	0	ND	N	N	15	N	NA	N	N	N	N							0	
BIT151																																				
BIT152	64	223	100		35.6	111	8.9	318	32	136	6	10.4	N	N	21	ND	ND	0	52	N	N	15	N	N	N	N	N	N			0	1	0		0	1

Appendix 6: Pilot study -Clinical data for 200 patients from Salford Royal Foundation Trust – Day 1

	HR	Sys BP	Dia BP	Noradrenaline	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
SR002	115	110	60	0	76.6667	0	356	0	132	1	34	2	38.2	290.32	2	15	0	5
SR003	84	149	59	5	167	4	161	0	47	0	7	0		0	4	3	4	12
SR004	126	138	80	30	206	4	186	0	127	1	12	0	22	167.2	4	N	4	13
SR005																		
SR006																		
SR007																		
SR008	110	158	60	0	172	0	205	0	58	0	13	0	11.32	86.032	4	11	2	6
SR009	132	119	80	0	93	0	169	0	53	0	11	0	n	N	0	13	1	1
SR010	108	209	139	0	162	0	307	0	84	0	13	0	n	N	0	6	3	3
SR011	101	147	78	0	101	0	250	0	79	0	10	0	n	N	4	3	4	8
SR012	84	125	50	5	75	4	193	0	84	0	3	0	n	N	4	N	4	12
SR013	97	180	101	0	127	0	124	1	75	0	14	0	N	N	4	3	4	9
SR014	95	105	74	0	84	0	235	0	59	0	6	0		N	4	3	4	8
SR015	58	158	61		93	0	246	0	54	0	8	0	375.05	2850.38	4	N	4	8
SR016	70	120	80	0	93.3	0	175	0	69	0	7	0	N	N	0	15	0	0
SR017																		
SR018																		
SR019	92	104	97	0	99.3	0	169	0	76	0	7	0	n	N	0	15	0	0
SR020	101	126	59	12	81.3	4	251	0	85	0	9	0	N	N	4	3	4	12
SR021	76	132	66	5.5	88	4	237	0	75	0	22	1	N	N	4	3	4	13
SR022	69	110	60	6	76.7	4	87	2	49	0	23	1	N	N	0	14	1	8
SR023	101	121	65	0	83.7	0	121	1	56	0	15	0	N	N	4	N	4	9
SR024	53	110	52	6	71.3	4	165	0	42	0	5	0	N	N	4	3	4	12
SR025	103	110	50	0	70	0	246	0	57	0	7	0	32.9	250.04	4	3	4	8
SR026	50	111	41	0	64.3	1	308	0	53	0	12	0	21.3	161.88	4	N	4	9
SR027	101	102	71	0	81.3	0	348	0	130	1	8	0	172	1307.2	4	3	4	9
SR028	86	130	68	0	88.7	0	78	2	67	0	28	1	N	N	4	6	3	10
SR029	58	91	49	0	63	1	242	0	79	0	21	1	N	N	0	14	1	3
SR030	100	114	70	0	84.67	0	310	0	51	0	9	0	N	N	0	14	1	1
SR031	117	113	64	2	93.67	4	276	0	120	1	11	0	N	N	4	3	4	13
SR032	80	123	53	6	76.33	4	162	0	47	0	5	0	N	N	4	3	4	12
SR033	95	115	60	0	78.33	0	208	0	62	0	21	1	N	N	0	15	0	1
SR034	90	136	62	0	86.67	0	185	0	102	0	12	0	N	N	0	15	0	0
SR035	104	121	73	4	89	4	109	1	148	1	15	0	N	N	4	3	4	14
SR036	60	97	48	0	64.33	1	198	0	68	0	13	0	N	N	0	14	1	2
SR037	60	136	72	7	93.33	4	202	0	61	0	5	0	N	N	4	3	4	12
SR038	54	118	95	0	102.67	0	212	0	66	0	12	0	N	N	4	3	4	8
SR039																		
SR040	63	114	58	0	76.67	0	82	2	97	0	16	0	N	N	0	15	0	2
SR041																		
SR042	126	110	80	0	90	0	207	0	82	0	21	1	N	N	4	N	4	9
SR043																		
SR044																		
SR045	106	60	98	2	75.33	4	116	1	97	0	28	1	50	380	1	15	0	7
SR046	66	98	50	0.1	65	3	193	0	192	2	10	0	N	N	4	3	4	13
SR047	102	161	69		99.7	0	248	0	71	0	12	0	2	15.2	4	15	0	4
SR048	95	150	72	N	94	0	214	0	81	0	19	0	23	174.8	3	15	0	3
SR049																		
SR050																		

	HR	Sys BP	Dia BP	Noradrenaline	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
SR051																		
SR052	85	123	55	0.03	78	3	270	0	67	0	14	0	56	425.6	4	ND	4	11
SR053	72	123	58	N	75	0	200	0	80	0	14	0	40	304	4	3	4	8
SR054	100	128	99	N	108.67	0	274	0	95	0	7	0	N	N	0	15	4	4
SR055	79	138	50	N	68	1	327	0	57	0	5	0	26	197.6	4	14	4	9
SR056	94	130	70	N	90	0	146	1	51	0	7	0	36	273.6	4	8	4	9
SR057	99	143	82	N	102	0	328	0	102	0	7	0	ND	N	0	15	0	0
SR060																		
SR061	100	130	67	N	88	0	307	0	86	0	17	0	ND	N	0	15	0	0
SR062	73	120	39	0.26	64	4	211	0	57	0	12	0	ND	N	4	3	4	12
SR063	106	102	56	N	72	0	174	0	62	0	29	1	ND	N	4	6	4	9
SR064	70	105	58	N	73.67	0	156	0	56	0	6	0	22	167.2	4	7	4	8
SR065	55	120	60	0.03	80	3	196	0	68	0	16	0	25	190	3	15	0	6
SR067	86	122	58	0.33	78	4	129	1	70	0	12	0	33	250.8	2	3	4	13
SR068	MRI					1		4		0		0		N	0		4	9
SR070	46	130	58	10	76	4	171	0	89	0	7	0	42	319.2	4	3	4	12
SR071	76	126	74		91.33	0	213	0	57	0	14	0	51	387.6	1	13	1	2
SR072	44	135	67		89.67	0	160	0	61	0	19	0	22	167.2	4	3	4	8
SR073																		
SR074	66	166	77	0.18	107	4	185	0	67	0	12	0	68	516.8	0	14	1	5
SR075	95	116	60		78.67	0	205	0	56	0	4	0	ND	N	0	13	1	1
SR076																		
SR077	72	178	60	0.133	94	4	235	0	66	0	24	1	57	433.2	4	3	4	13
SR078	72	129	76		93	0	233	0	75	0	4	0	58	440.8	4	5	4	8
SR079	94	140	70	0	93.3333	0	171	0	101	0	8	0	34	258.4	2	15	0	2
SR080	72	150	90	0	110	0	220	0	72	0	23	1	40	304	1	13	1	3
SR081	55	168	58	0.098	94.6667	3	229	0	62	0	10	0	40.5	307.8	4	3	4	11
SR082	100	110	52	0.288	71.3333	4	267	0	101	0	18	0	44.6	338.96	4	6	4	12
SR083	114	107	54	0.062	71.6667	3	267	0	101	0	18	0	43.2	328.32	4	3	4	11
SR084	88	125	68	0	87	0	259	0	68	0	16	0	39	296.4	2	15	0	2
SR085	90	182	94	0	123.333	0	327	0	78	0	4	0	23	174.8	3	14	1	4
SR086	84	105	55	No Weight	71.6667	0	188	0	67	0	30	1	62	471.2	0	15	0	1
SR087	75	120	77	0.17	91.3333	4	260	0	84	0	15	0	51	387.6	4	3	4	12
SR088	69	130	60	0.369	83.3333	4	309	0	82	0	17	0	63	478.8	4	3	4	12
SR089	75	126	65	0.122	85.3333	4	183	0	120	1	N	0	50.2	381.52	4	6	3	12
SR090	95	130	70	0	90	0	272	0	87	0	29	1	50	380	1	15	0	2
SR093	95	125	70	0	88.3333	0	231	0	90	0	7	0	49	372.4	1	15	0	1
SR094	58	130	55	0.09	80	3	199	0	65	0	24	1	55.5	421.8	0	3	4	8
SR095	78	80	52	0	61.3333	1	189	0	48	0	2	0	21	159.6	3	11	2	6
SR097	84	110	58	0	75.3333	0	277	0	72	0	7	0	N		#VALUE!	15	0	0
SR098	90	110	58	0	75.3333	0	253	0	95	0	6	0	60	456	4	7	3	7
SR099	72	120	60	0	80	0	213	0	66	0	15	0	69	524.4	4	10	2	6
SR101	90	110	57	0.031	74.6667	3	291	0	66	0	20	1	58.1	441.56	0	15	0	4
SR102	137	115	50	0.524	71.6667	4	349	0	59	0	4	0	44.7	339.72	4	3	4	12
SR103	74	118	65	0.4075	82.6667	4	214	0	66	0	6	0	40.7	309.32	4	6	3	11

7.6 Appendix 7: Pilot study -Clinical data for 200 patients from Salford Royal Foundation Trust – Day 5

	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	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	RESP SOFA	GCS	GCS SOFFA	SOFA DAY 5
SR002	37.5	77	18.1	90	16.7	N	N	0.21	2	145	N	N	Y	mpirical/unknow	N	N	94	111	78	0	89	0	356	0	84	0	N	0	40	304	1	15	0	1
SR003	39.3	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
SR004	39.6	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	34	258	4	N		8
SR005	withdrawn - no consent																																	
SR006	withdrawn - no consent																																	
SR007	withdrawn - no consent																																	
SR008	36.9	115	6.1	>90	13.6	N	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
SR009	39.2	93	10.6	>90	13.2	N	N	21	1.1	N	N	N	Y	1	N	N	74	112	50	0	70	0	207	0	207	2	N	0	N	N	0	15	0	2
SR010	37.1	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	4	9
SR011	36.3	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	4	12
SR012	37.2	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
SR013	36.9	88	14.5	>90	14.4	N	N	21	0.7	N	N	N	N	N	N	N	122	137	58	N	84	0	192	0	76	0	6	0	N	N	0	13	1	1
SR014	RIP																RIP																	
SR015	37.3	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	2242	0	N	4	8
SR016	36.8	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
SR017	withdrawn - no consent																																	
SR018	withdrawn - no consent																																	
SR019	36.8	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
SR020	37.5	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	55	0	10	0	N	N	4	3	4	8
SR021	37.2	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	4	12
SR022	36.8	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
SR023	37	102	10.3	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
SR024	36.4	105	9.3	N	12	N	N	21	1.2	N	N	N	Y	1	N	N	86	119	60	9	83	4	224	0	44	0	15	0	59	448	0	6	3	7
SR025	39.2	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
SR026	35.2	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
SR027	37.2	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
SR028	38.9	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	55	0	6	0	N	N	4	5	4	8
SR029	36.9	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
SR030	37.4	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
SR031	38.5	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	267	2	3	4	6
SR032	37.1	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
SR033	37.9	116	8.2	>90	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
SR034	38.4	92	8.1	>90	11.6	N	N	25	N	1.2	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
SR035	37.1	76	14.5	48	10.8	Y	N	25	1.1	N	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
SR036	36.1	116	6.9	>90	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
SR037	37.3	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
SR038	36.4	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
SR039	withdrawn - no consent																																	
SR040	37.5	95	7	>90	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
SR041	patient at manchester																																	
SR042	36.9	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	2614	0	11	2	6
SR043	sented but no lab capacity																																	
SR044	withdrawn - no consent																																	
SR045	37	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
SR046	37.2	N	N	N	N	N	N	40	N	N	N	N	Y	1	N	N	78	120	76	0	91	0	430	0	67	0	12	0	N	N	0	15	0	0
SR047	38	N	N	N	N	N	N	21%	N	ND	N	N	N	N	N	N	103	135	82	N	100	0	N	0	N	0	N	0	N	N	0	15	0	0
SR048	38.5	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
SR049	withdrawn - no consent																																	
SR050	withdrawn - no consent																																	

	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	FiO2	Lactate	CRP	CVVH/HD	Sedated	Antibiotics	septic sourc	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	RESP SOFA	GCS	GCS SOFFA	SOFA DAY 5
SR051																withdrawn - no consent																		
SR052	37.4	104	9.9	ND	12.4	N	N	21	ND	ND	N	N	N	N	N	N	68	122	78	ND	92.67	0	ND	0	ND	0	ND	0	ND	N	0	15	0	0
SR053	36.9	101	16.2	>90	11.5	N	N	21	N	ND	N	N	Y	6	Y	N	75	120	78	N	92	0	288	0	50	0	11	0	ND	N	0	15	0	0
SR054	37.6	89	6.7	>90	14.3	N	N	35	1.3	ND	N	N	Y	6	Y	N	81	122	76	N	91.33	0	274	0	64	0	12	0	ND	N	0	15	0	0
SR055	35.7	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	58	440.8	4	3	4	11
SR056	36.4	110	11.7	ND	16.9	N	N	25	1	ND	N	N	N	N	N	N	85	111	54	ND	73	0	176	0	140	1	8	0	ND	N	0	14	1	2
SR057	37.4	ND	ND	ND	ND	N	N	21	N	ND	N	N	N	N	N	N	98	120	70	ND	86.67	0	225	0	119	1	9	0	ND	N	0	15	0	1
SR058	36.6	ND	ND	ND	ND	N	N	21	N	ND	N	N	N	N	Y	N	84	132	82	N	50	1	ND	0	ND	0	ND	0	ND	N	0	15	0	1
SR059	37.2	95	6.9	ND	11.6	N	N	25	0.89	ND	N	N	N	N	Y	N	100	146	84	N	104.67	0	316	0	48	0	ND	0	ND	N	0	15	0	0
SR060																withdrawn - no consent																		
SR061	37	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	0	ND	0	85	0	4	0	ND	N	0	15	0	0
SR062	38	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	17	129.2	4	3	4	11
SR063	37	ND	ND	ND	ND	N	N	21	N	N	N	N	Y	6	N	N	70	120	70	ND	86.67	0	385	0	41	0	10	0	ND	N	0	14	1	1
SR064	37.4	83.1	8.9	ND	12.2	N	N	21	0.8	N	N	N	N	N	N	N																		
SR065																																		
																withdrawn - no consent																		
SR067	38.4	87	12.7	ND	12.9	Y	N	30	1.1	ND	N	Y	Y	6	N	N	136	138	70	ND	94	0	247	0	39	0	12	0	65	494	4	3	4	8
SR068	36.9	99	8.3	ND	11	N	Y	40	0.8	ND	N	N	Y	6	N	N	88	171	69	ND	103	0	188	0	51	0	15	0	ND		0	15	0	0
																withdrawn - no consent																		
SR070	36.9	130	9.1	>90	14	N	N	21	1	ND	N	N	N	N	N	N	62	122	70	ND	87	0	ND	0	ND	0	ND	0	ND		0	15	0	0
SR071	36.5	ND	ND	ND	ND	N	N	21	N	ND	N	N	N	N	N	N	52	120	50	ND	73.33	0	ND	0	ND	0	ND	0	ND		0	14	1	1
SR072	37.2	109	19.5	>90	14.5	N	N	30	0.9	ND	N	N	Y	6	N	N	75	136	64	ND	88	0	ND	0	49	0	16	0	ND		0	15	0	0
SR073																withdrawn - no consent																		
SR074	37.1	83	7.5	>90	12	N	N	60	1.2	ND	N	N	N	N	Y	N	76	113	46	ND	54	1	235	0	55	0	15	0	ND		0	14	1	2
SR075	37.2	ND	ND	ND	ND	N	N	21	ND	ND	N	N	Y	6	N	N	95	130	90	ND	103	0	ND	0	ND	0	ND	0	ND		0	14	1	1
SR076																withdrawn - no consent																		
SR077	36	116	7.7	>90	14.3	Y	Y	21	1.09	ND	N	Y	N	N	N	N	68	132	80	ND	98	0	ND	0	ND	0	ND	0	ND		0	14	1	1
SR078	36.9	121	7.8	>90	11.3	N	N	21	ND	ND	N	N	N	N	N	N	DISCHARGED																	
SR079	37.8	81	9.3	87	N	N	N	24	N	N	N	N	N		N	N	102	131	43	0	72.33333	0	320	0	45	0	15	0	N			15	0	0
SR080	36.8	128	14.5	90	N	N	N	21	N	N	N	N	N	N	N	N	85	149	70	0	96.33333	0	344	0	58	0	20	1	N			14	1	2
SR081	38	112	14	>90	12.8	Y	N	0.45	N	N	N	Y	N	N	N	N	98	185	70	0.368	108.3333	4	200	0	60	0	10	0	34	258.4	4	3	4	12
SR082	38.2	96	17.5	N	12.6	Y	N	0.35	0.9	N	N	N	Y	6	N	N	95	145	75	0	98.33333	0	547	0	46	0	25	1	42	319.2	1	10	2	4
SR083	39	107	11.8	N	N	Y	N	0.35	1	N	N	Y	Y	1	N	N	95	121	60	0.021	80.33333	3	247	0	45	0	12	0	44	334.4	4	3	4	11
SR084	37.2	104	13.5	>90	13.4	N	N	0.5	1	N	N	N	Y	1	N	N	75	135	55	0	81.66667	0	263	0	53	0	16	0	N			15	0	0
SR085	37.1	N	N	N	N	N	N	0.21	N	N	N	N	N	N	Y	N	98	130	62	0	84.66667	0	305	0	93	0	15	0	N			15	0	0
SR086	N	N		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	#VALUE!	0	N	0	N	0	N	0	N		N	N	N	0
SR087	38.2	110	15.7	N	12.9	Y	N	30	0.9	N	N	Y	Y	6	N	N	112	122	57	0	78.66667	0	302	0	45	0	16	0	34	258.4	4	3	4	8
SR088	37.4	98	10.5	N	14.8	Y	N	0.21	0.5	N	N	Y	Y	1	N	N	100	136	63	0.14	87.33333	4	346	0	52	0	8	0	60	456	4	5	4	12
SR089	37.6	91	10.6	73	11.7	Y	N	0.3	1.95	N	N	Y	Y	6	N	N	81	155	55	0	88.33333	0	194	0	52	0	56	2	35.3	268.28	2	15	0	4
SR090	37	N	N	N	N	N	N	0.21	N	N	N	N	Y	6	N	N	67	124	68	0	86.66667	0	359	0	82	0	11	0	N			15	0	0
SR093	37.6	85	13.2	N	N	N	N	0.21	N	N	N	N	Y	5	N	N	78	126	68	0	87.33333	0	N	0	N	0	N	0	N			15	0	0
SR094	37.4	122	16.3	>90	15.1	Y	N	0.25	1.5	N	N	Y	N	N	N	N	108	125	68	0.48	87	4	170	0	50	0	8	0	50.3	382.28	4	3	4	12
SR095	36.8	93	7.2	>90	13.1	N	N	0.21	3.3	N	N	N	Y	6	Y	N	90	124	60	0	81.33333	0	231	0	46	0	N	0	N			15	0	0
SR097	36.5	108	7.2	>90	N	N	N	0.21	N	N	N	N	Y	6	N	N	86	120	70	0	86.66667	0	351	0	68	0	7	0	N			15	0	0
SR098	37	90	8	>90	11.9	N	N	0.21	N	37	N	N	Y	6	N	N	92	132	70	0	90.66667	0	253	0	85	0	N	0	N			15	0	0
SR099	37	124	16	>90	13.3	N	N	0.21	1.6	N	N	N	N	N	N	N	77	138	80	0	99.33333	0	N	0	N	0	N	0	N			14	1	1
SR101	37.1	101	5.5	>90	11.6	N	N	0.21	N	120	N	N	N	N	N	N	91	132	80	0	97.33333	0	351	0	61	0	15	0	N			15	0	0
SR102	38.6	72	15.2	N	13.4	Y	N	0.3	1.2	N	N	N	N	N	N	N	75	140	68	0	92	0	325	0	48	0	6	0	54	410.4	4	4	4	8
SR103	36.9	118	8.2	>90	11.9	N	N	0.3	0.8	N	N	N	N	N	N	N	77	180	100	0	126.6667	0	263	0	59	0	7	0	N			11	2	2

7.7 Appendix 8: Pilot study -Clinical data for 200 patients from Salford Royal Foundation Trust – Day 8

	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	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	RESP SOFA	GCS	GCS SOFA	SOFA D8
SR003	37.2	102	10.1	90	N	N	N	0.21	N	18	N	N	Y	empirical/unknown	N	N	80	117	78	0	91	0	460	0	86	0	N	0	10	76	4	15	0	4
SR004	37.7	11.5	10.5	>90	N	Y	N	30%	0.9	N	N	N	N	N	N	N	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP
SR005	39.4	78	12.5	>90	13.2	Y	N	35%	1	N	N	N	N	N	N	N	95	120	60	N	160	0	453	0	63	0	9	0	50	380	4	4	4	8
SR006																	withdrawn																	
SR007																	withdrawn																	
SR008																	withdrawn																	
SR009	36.7	142	6.3	>90	N	N	N	N	N	N	N	N	N	N	N	N	76	142	82	0	211	0	387	0	52	0	N	0	N	N	0	15	0	0
SR010	37.9	74	8.3	>90	N	N	N	N	N	N	N	N	Y	1	N	N	70	122	54	0	73	0	325	0	49	0	N	0	N	N	0	15	0	0
SR011	38.2	69	2.6	44	10.7	Y	N	45%	2.3	N	N	Y	N	N	Y	N	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP	RIP
SR012	38.4	121	10.9	>90	16.8	Y	N	40%	1.3	N	N	Y	Y	1	N	N	97	125	74	14.5	91	4	212	0	59	0	5	0	N	N	4	N	4	12
SR013	37	118	7.1	N	12	N	N	21%	N	N	N	N	N	N	N	N	80	118	80	N	92	0	218	0	49	0	N	0	N	N	0	15	0	0
SR014	37.6	94	14.9	74	N	N	N	21%	N	N	N	N	Y	1	N	N	72	130	72	0	91	0	401	0	98	0	N	0	N	N	0	13	1	1
SR015																	RIP																	
SR016	37.7	137	13.6	>90	13.4	Y	N	30%	1.1	N	N	Y	N	N	N	N	88	137	63	7	87.7	4	N	0	40	0	7	0	N		0	13	1	5
SR017	36.6	134	6.6	>90	11.7	N	N	21%	N	N	N	N	N	N	N	N	59	105	70	0	81.67	0	N	0	N	0	N	0	N	N	0	15	0	0
SR018																	withdrawn																	
SR019																	withdrawn																	
SR020	37	N	N	N	N	N	N	21%	N	N	N	N	N	N	N	N	68	118	70	0	86	0	N	0	N	0	N	0	N	N	0	15	0	0
SR021	37.5	84	11.5	N	13.6	Y	N	35%	0.7	N	N	Y	N	N	N	N	85	130	52	0	78	0	422	0	54	0	7	0	N	N	4	3	4	8
SR022	27.5	108	9.1	N	13.7	Y	N	21%	1.2	N	N	Y	Y	6	N	N	98	118	63	6	81.3	4	406	0	47	0	26	1	N	N	4	3	4	13
SR023	37.2	70	8.7	N	11.7	N	N	21%	N	N	N	N	Y	6	N	N	72	104	70	0	81.3	0	211	0	27	0	18	0	N	N	0	14	1	1
SR024	37.9	N	N	N	N	N	N	21%	N	N	N	N	Y	6	Y	N	99	140	70	0	93.3	0	N	N	N	0	N	0	N	N	0	14	1	1
SR025	37.4	119	9.4	N	13.9	Y	N	21%	1.1	N	N	Y	Y	1	N	N	69	115	64	9	81	4	214	0	39	0	14	0	N	N	4		4	12
SR026	36.8	85.1	10.1	>90	14.8	N	N	30%	0.7	N	N	N	N	N	N	N	66	160	62	0	94.7	0	390	0	51	0	16	0	N	N	0	13	1	1
SR027	36.4	91	7.4	N	11.9	N	N	22%	1.4	N	N	N	N	N	N	N	83	160	80	0	106.7	0	242	0	45	0	7	0	N	N	0	14	1	1
SR028	36.2	N	N	N	N	N	N	21%	N	N	N	N	Y	6	N	N	105	115	60	0	78.33	0	211	0	56	0	N	0	N	N	0	15	0	0
SR029	38.4	63.7	10.3	>90	12.8	Y	N	21%	2.2	N	N	N	Y	6	N	N	77	147	78	0	101	0	426	0	43	0	11	0	N	N	4	6	3	7
SR030	37.6	92	90	N	11	N	N	30%	0.9	N	N	N	N	N	N	N	82	130	80	0	96.67	0	276	0	47	0	N	0	N	N	0	13	1	1
SR031	37	104	7.8	>90	N	N	N	21%	N	50	N	N	Y	6	Y	N	88	118	72	0	87.33	0	N	0	40	0	12	0	N	N	0	15	0	0
SR032	38.6	81.3	12.5	>90	13.2	Y	N	30%	1.6	N	N	Y	Y	1	N	N	74	118	44	0	68.6	1	347	0	48	0	9	0	N	N	4	12	2	7
SR033	36.8	N	N	N	N	N	N	21%	N	N	N	N	N	N	N	N	66	130	80	0	96.67	0	N	0	50	0	N	0	N	N	0	14	1	1
SR034	37.2	116	6.3	>90	12.3	N	N	21%	N	N	N	N	Y	6	N	N	74	128	68	0	88	0	N	0	N	0	N	0	N	N	0	15	0	0
SR035	37.4	90	7.5	>90	N	N	N	21%	N	N	N	N	Y	1	N	N	100	143	73	0	96.33	0	11.7	4	71	0	11	0	N	N	0	15	0	4
SR036	37.2	97	20.1	33	11.6	Y	N	35%	1.2	N	N	Y	N	N	N	N	79	148	79	0	102	0	377	0	295	2	21	1	N	N	4	3	4	11
SR037	37.1	N	N	N	N	N	N	21%	N	N	N	N	N	N	N	N	78	122	84	0	96.67	0	N	0	N	0	N	0	N	N	0	15	0	0
SR038	37.7	108.3	14.1	N	14.4	Y	N	21%	0.8	N	N	Y	Y	6	N	N	52	144	77	0	99.33	0	200	0	43	0	5	0	N	N	4	10	2	6
SR039	37.8	89	7.5	>90	13.6	Y	N	35%	1	N	N	Y	Y	1	N	N	RIP	RIP	RIP	RIP	RIP	RIP	RIP	0	RIP		RIP		RIP	RIP		RIP		RIP
SR040																	withdrawn																	
SR041	37	106	8.6	>90	N	N	N	21%	N	122	N	N	Y	1	N	N	60	104	66	0	78.67	0	426	0	39	0	23	1	N	N	0	15	0	1
SR042																	Patient at manchester																	
SR043	38.7	86	19.5	>90	12.3	Y	N	45%	2.1	N	N	N	Y	6	N	N	70	172	67	8.5	102	4	519	0	43	0	8	0	N	N	0	14	1	5
SR044																	consented but no lab capacity																	
SR045																	withdrawn																	
SR046	37.7	77	10.3	N	10.8	N	N	21%	1.2	N	N	N	Y	6	Y	N	91	158	74	0	102	0	283	0	46	0	24	1	N	N	0	15	0	1
SR047	36.9		12.1	81	14.4	N	N	21%	N	13	N	N	Y	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0	N	0	0
SR048	37	N	N	N	N	N	N	21%	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	0	N	0	0
SR049	37.6	77	10.8	>90	12.4	Y	N	55%	1.4	N	N	Y	Y	1	N	N	68	130	78	N	78	0	362	0	74		12	0	35	266	4	7	4	8
SR050																	withdrawn																	
																	withdrawn																	

	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	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	RESP SOFA	GCS	GCS SOFA	SOFA D8
SR051																	withdrawn																	
SR052	37	ND	ND	ND	ND	N	N	21	N	ND	N	N	Y	4	N	N	72	140	78	N	98.67	0	356	0	59	0	N	0	ND	N	0	15	0	0
SR053	37.1	109	11.4	>90	ND	N	N	21	N	ND	N	N	Y	6	Y	N	discharged																	
SR054	36.6	94	6.8	>90	12.3	N	N	21%	N	ND	N	N	N	N	Y	N	80	116	68	N	84.00	0	N	0	N	0	N	0	ND	N	0	15	0	0
SR055	35.6	77	9	ND	11	Y	N	21%	2	ND	N	N	N	N	N	N	69	155	58	0.34	97.67	4	301	0	30	0	7	0	ND	N	4	3	4	12
SR056	36.7	116	6	ND	ND	N	N	24%	N	222	N	N	Y	1	N	N	88	120	68	ND	85.33	0	ND	0	N	0	N	0	ND	NN	0	14	1	1
SR057	37.4	101	8.3	53	ND	N	N	21%	N	281	N	N	Y	1	N	N	94	140	78	N	98.67	0	ND	0	N	0	N	0	ND	N	0	15	0	0
	36.3	ND	ND	ND	ND	N	N	21%	N	ND	N	N	N	N	Y	N	83	135	85	N	101.67	0	ND	0	N	0	N	0	ND	N	0	15	0	0
	36.5	113	8.1	ND	ND	N	N	21%	N	ND	N	N	N	N	N	N	88	138	66	ND	90.00	0	ND	0	N	0	N	0	ND	N	0	15	0	0
SR060																	withdrawn																	
SR061	37.2	ND	ND	77	ND	N	N	21%	ND	ND	N	N	N	N	N	N	discharged																	
SR062	38	89.2	19.9	ND	15.3	Y	N	25%	1.2	ND	N	Y	N	N	N	N																	
SR063	37	89	5.4	ND	ND	N	N	21%	ND	100	N	N	Y	6	N	N																	
SR064																																	
SR065																																	
																	withdrawn																	
SR067	39.4	74	11.5	ND	14.5	Y	N	21%	1.4	ND	N	Y	Y	6	N	N	110	151	75	1.3	85.00	4	ND	0	37	0	13	0	55.2	419.52	4	3	4	12
SR068	37.5	82	8.4	>90	10.2	N	N	30%	ND	ND	N	N	Y	6	Y	N	86	144	83	ND	105.00	0	ND	0	N	0	N	0	ND		0	15	0	0
																	withdrawn													0				
SR070	36.6	ND	ND	ND	ND	ND	ND	0.21	ND	ND	N	N	N	N	N	N	68	130	80	N	96.67	0	ND	0	N	0	N	0	ND		0	15	0	0
SR071	36.1	ND	ND	ND	ND	N	N	0.21	ND	ND	N	N	N	N	N	N	49	110	64	N	79.00	0	ND	0	N	0	N	0	ND		0	14	1	1
SR072	36.5	ND	ND	ND	ND	N	N	0.21	ND	ND	N	N	Y	6	N	N	64	108	64	N	79.00	0	ND	0	N	0	N	0	ND		4	15	0	4
SR073																	withdrawn													0				
SR074	36.5	90	8.6	>90	ND	N	N	0.21	ND	ND	N	N	N	N	Y	N	67	133	61	N	84.33	0	ND	0	N	0	N	0	ND		0	15	0	0
SR075	36.8	ND	ND	ND	ND	N	N	0.21	ND	ND	N	N	Y	6	N	N	discharged													0				
SR076																													0				
SR077	36.8	ND	ND	ND	ND	N	N	0.21	ND	ND	N	N	N	N	N	N	80	125	83	N	97.00	0	ND	0	N	0	N	0	ND		0	14	1	1
SR078																	discharged																	
SR079	36.5	91	10	N	87	N	N	0.24	N	60	N	N	Y	1	N	N	87	139	58	0	85.00	0	424	0	50	0	11	0		0	4	15	0	4
SR080	37.3	129	11.3	90	N	N	N	0.21	N	135	N	N	N	N	N	N	77	136	88	0	104.00	0	N	0	N	0	N	0		0	4	15	0	4
SR081	38.9	98	16.5	>90	13.5	Y	N	0.45	N	309	N	Y	Y	1	N	N	75	168	65	0	99.33	0	401	0	44	0	5	0	38	288.8	4	4	4	8
SR082	38.2	88	25	N	13.7	N	N	0.24	1.1	N	N	N	Y	1+6	N	Y	103	133	67	0	89.00	0	763	0	54	0	17	0		0	4	12	2	6
SR083	38.6	94	10.4	N	12.4	Y	N	0.42	0.9	N	N	Y	Y	1	N	N	95	150	66	0.082	94.00	3	384	0	48	0	28	1	19	144.4	4	3	4	12
SR084	37.1	131	9.8	>90	N	N	N	0.28	N	N	N	N	Y	6	N	N	80	115	65	0	81.67	0	N	0	N	0	N	0		0	4	15	0	4
SR085	38.3	122	15.2	50	N	N	N	0.21	N	125	N	N	N	N	Y	N	103	148	65	0	92.67	0	N	0	N	0	N	0		0	4	15	0	4
SR086	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N	0	N	0	N	0		0	N	N	N	0
SR087	37.5	97	23.5	N	13.2	Y	N	0.3	1.9	N	N	Y	Y	6	N	N	80	160	97	N	118.00	0	520	0	39	0	12	0	51	387.6	4	5	4	8
SR088	37.3	97	9.4	N	14	Y	N	0.21	0.4	N	N	Y	Y	1	N	N	66	142	60	0.538	87.33	4	460	0	51	0	6	0	53.8	408.88	4	11	4	12
SR089	37.7	88.8	12.6	>90	11.1	N	N	0.3	1.36	N	N	N	N	N	N	N	90	160	80	0	106.67	0	513	0	41	0	35	2	43	326.8	1	15	0	3
SR090	36.6	124	9.3	83	N	N	N	0.21	N	15	N	N	N	N	N	N	67	132	78	0	96.00	0	N	0	N	0	N	0		0	4	15	0	4
SR093	36.2	N	N	N	N	N	N	0.21	N	N	N	N	Y	5	N	N	98	110	60	0	76.67	0	640	0	45	0	12	0		0	4	15	0	4
SR094	37.7	118	15.6	>90	15.3	Y	N	0.3	0.8	N	N	Y	Y	6	N	N	90	148	74	0.52	98.67	4	296	0	46	0	5	0	44	334.4	4	3	4	12
SR095	37.2	87	9.7	N	N	N	N	0.21	N	93	N	N	N	N	Y	N	80	140	76	0	97.33	0	N	0	N	0	N	0		0	4	15	0	4
SR097	37	108	7	>90	11.7	N	N	0.21	N	97	N	N	Y	5	N	N	82	110	60	0	76.67	0	N	0	N	0	N	0		0	4	15	0	4
SR098	36.2	98	10.7	82	N	N	N	0.21	N	N	N	N	Y	6	Y	N	100	120	58	0	78.67	0	N	0	N	0	N	0		0	4	15	0	4
SR099	37.3	N	N	N	N	N	N	0.21	N	N	N	N	N	N	N	N	70	116	68	0	84.00	0	N	0	N	0	N	0		0	4	15	0	4
SR101	37.3	113	7.8	>90	N	N	N	0.21	N	171	N	N	N	N	N	N	86	114	60	0	78.00	0	362	0	60	0	11	0		0	4	15	0	4
SR102	38.5	80	12.6	N	13.8	Y	N	0.3	1.1	N	N	N	Y	1	N	N	N	N	N	N		N	N	0	N	0	N	0		0	N	14	1	1
SR103	37.8	141	9.2	>90	N	N	N	25	N	N	N	N	N	N	N	N	61	158	6	N	56.67	4	N	0	N	0	N	0		0	N	12	2	6

7.8 Appendix 9: Mean Interleukin-13 and Interleukin-17 concentrations for 30 duplex patient serum samples along with SOFA scores, Δ-SOFA, CRP (mg/L) and lactate (mmol/L)

PATIENT	IL-17 D1	IL-17 D5	IL-13 D1	IL-13 D5	ISS	SOFA D1	SOFA D3	SOFE D5	SOFA D8	Δ-SOFA	CRP	LACTATE
MRI 010	2.83234	2.8600	2.7494	3.0811	26	4	2	0	0	-4	181	
MRI 011	2.68032	3.0396	2.7632	2.9982	25	1	1	0	1	-1		
MRI 014	4.71175	2.9843	2.9705	3.0258	26	3	0	0	0	-3	194	
MRI 016	2.97053	2.9429	2.7356	3.0120	29	5	5	4	1	-1	174	1.4
MRI 017	3.27455	3.0673	2.9429	3.1502	4	0	10	2	0	2	124	
MRI 021	2.92907	2.8738	2.9014	3.3160	16	2	0	0	0	-2	26	
MRI 078	3.77204	3.0949	2.9843	3.2193		10	4	4	0	-6	127	
MRI 084	2.99817	3.1640	3.0534	3.2746		8	1	0	0	-8	243	
MRI 088	3.15018	3.1778	4.6841	3.0811		3	6	3	0	0	293	0.8
MRI 091	3.02580	3.1364	2.9153	3.6891		5	7	6	6	1	128	1.1
MRI 092	3.20545	3.0534	2.9843	2.9705		1	0	1	0	0		
MRI 093	2.70796	3.0120	2.9153	2.8876		2	2	1	1	-1	103	
MRI 098	2.95671	2.9705	2.7771	2.9429		1	0	0	0	-1		
MRI 099	2.55595	3.0534	2.5145	3.0949		0	6	1	0	1	178	
MRI 100	3.15018	2.8738	2.8462	2.6803		1	1	0	0	-1		
MRI 102	2.99817	3.2607	3.0398	3.2331		3	0	0	0	-3	251	0.8.
MRI 103	3.21927	3.3851	2.9428	3.3022		4	5	0	0	-4	350	0.9
MRI 104	3.37129	3.0811	2.8184	2.9567		1	1	0	0	-1	110	
MRI 107	3.37129	3.3298	2.9568	3.1087		14	11	10	7	-4	372	1
SR 019	3.27455	3.1778	3.0398	3.0811		0	1	0	0	0		
SR0 23	3.34365	3.4404	2.9568	3.2884		9	2	1	1	-8		
SR 025	3.27455	3.1087	5.5687	5.8449		8	4	2	1	-6		0.7
SR 074	3.21927	3.4680	4.6981	5.0710	25	5	8	2	0	-3		
SR 078	2.94289	2.9843	2.9983	2.8047	13	8	4	0	0	-8		
SR 082	3.42656	2.8323	2.9428	3.0811	17	12	9	4	6	-8		
SR 083	3.56475	3.4680	2.9568	3.1364	38	11	11	11	12	0		
SR 087	4.42155	4.6288	3.0812	3.0396	9	12	12	8	8	-4	1.1	1.1
SR 088	2.85997	3.1364	2.8739	2.8738	13	12	8	12	12	0		
SR 089	2.90143	3.0673	2.7496	2.9291	41	12	13	4	3	-8		
SR 094	2.80470	3.0120	2.4594	2.9153	9	8	12	12	12	4		

7.9 Appendix 10: Readings obtained from the flow cytometry

Raw data for Interleukin-13										
Patient	PE-A pg/ml			Avg PE-A	D1-Conc	PE-A pg/ml			Average	D5 Conc
	D1 A	D1 B	D1 C			D5 A	D5 B	D5 C		
MRI 010	74	77	70	73.67	2.8323	69	71	83	74.33	2.8600
MRI 011	76	72	62	70	2.6803	83	70	83	78.67	3.0396
MRI 014	102	138	117	119	4.7117	71	76	85	77.33	2.9843
MRI 016	76	78	77	77	2.9705	77	69	83	76.33	2.9429
MRI 017	90	83	80	84.33	3.2746	75	86	77	79.33	3.0673
MRI 021	78	74	76	76	2.9291	67	78	79	74.67	2.8738
MRI 078	126	75	88	96.33	3.7720	71	75	94	80	3.0949
MRI 084	77	79	77	77.67	2.9982	77	78	90	81.67	3.1640
MRI 088	83	82	79	81.33	3.1502	77	81	88	82	3.1778
MRI 091	74	81	80	78.33	3.0258	77	78	88	81	3.1364
MRI 092	97	76	75	82.67	3.2055	73	76	88	79	3.0534
MRI 093	68	64	80	70.67	2.7080	67	78	89	78	3.0120
MRI 098	77	76	77	76.67	2.9567	69	78	84	77	2.9705
MRI 099	63	66	72	67	2.5560	80	67	90	79	3.0534
MRI 100	88	75	81	81.33	3.1502	66	66	92	74.67	2.8738
MRI 102	81	74	78	77.67	2.9982	75	82	95	84	3.2607
MRI 103	78	94	97	89.67	3.2193	83	82	96	87	3.3851
MRI 104	89	85	86	86.67	3.3713	84	66	89	79.67	3.0811
MRI 107	89	88	83	86.67	3.3713	84	83	90	85.67	3.3298
SR 019	88	86	79	84.33	3.2746	79	76	91	82	3.1778
SR0 23	89	90	79	86	3.3436	80	87	98	88.33	3.4404
SR 025	86	89	78	84.33	3.2746	79	73	89	80.33	3.1087
SR 074	84	70	83	79	3.2193	72	74	89	78.33	3.4680
SR 078	78	77	74	76.33	2.9429	77	66	89	77.33	2.9843
SR 082	85	89	90	88	3.4266	73	62	86	73.67	2.8323
SR 083	95	77	95	89	3.5648	65	74	89	76	3.4680
SR 087	124	107	112	114.33	4.4215	104	117	130	117	4.6288
SR 088	75	72	76	74.33	2.8600	75	74	94	81	3.1364
SR 089	74	81	71	75.33	2.9014	72	76	90	79.33	3.0673
SR 094	75	73	71	73	2.8047	71	74	89	78	3.0120

Raw data for Interleukin-17										
Patient	PE-A pg/ml			Avg PE-A	D1-Conc	PE-A pg/ml			Average	D5 Conc
	D1 A	D1 B	D1 C			D5 A	D5 B	D5 C		
MRI 010	71	77	67	71.67	2.7494	74	76	89	79.67	3.0811
MRI 011	73	71	72	72	2.7632	80	67	86	77.67	2.9982
MRI 014	80	73	78	77	2.9705	73	70	92	78.33	3.0258
MRI 016	74	70	70	71.33	2.7356	72	75	87	78	3.0120
MRI 017	73	76	80	76.33	2.9429	80	76	88	81.33	3.1502
MRI 021	76	73	77	75.33	2.9014	71	84	92	82.33	3.3160
MRI 078	76	80	76	77.33	2.9843	72	79	98	83	3.2193
MRI 084	77	78	82	79	3.0534	80	77	96	84.33	3.2746
MRI 088	119	113	123	118.33	4.6841	72	79	88	79.67	3.0811
MRI 091	77	74	76	75.67	2.9153	94	96	93	94.33	3.6891
MRI 092	87	64	81	77.33	2.9843	70	75	86	77	2.9705
MRI 093	84	69	74	75.67	2.9153	70	67	88	75	2.8876
MRI 098	68	77	72	72.33	2.7771	72	69	88	76.33	2.9429
MRI 099	61	65	72	66	2.5145	75	77	88	80	3.0949
MRI 100	77	73	72	74	2.8462	59	67	84	70	2.6803
MRI 102	80	79	77	78.67	3.0398	74	83	93	83.33	3.2331
MRI 103	77	74	78	76.33	2.9428	74	85	96	85	3.3022
MRI 104	79	69	72	73.33	2.8184	75	70	87	77.33	2.9567
MRI 107	78	68	84	76.67	2.9568	76	77	88	80.33	3.1087
SR 019	80	82	74	78.67	3.0398	75	72	92	79.67	3.0811
SR 023	77	78	75	76.67	2.9568	79	81	94	84.67	3.2884
SR 025	145	137	137	139.67	5.5687	141	135	163	146.33	5.8449
SR 074	79	76	82	79	4.6981	77	73	85	78.33	5.0710
SR 078	73	75	85	77.67	2.9983	71	69	79	73	2.8047
SR 082	67	82	80	76.33	2.9428	77	76	86	79.67	3.0811
SR 083	75	73	82	76.67	2.9568	76	86	81	81	3.1364
SR 087	77	84	78	79.67	3.0812	75	71	90	78.67	3.0396
SR 088	82	72	70	74.67	2.8739	70	73	81	74.67	2.8738
SR 089	72	74	69	71.67	2.7496	68	75	85	76	2.9291
SR 094	64	67	63	64.67	2.4594	67	70	90	75.67	2.9153

Phycoerythrin absorption (PE-A) is a fluorescence absorption used to identify the capture bead population of interleukins.

7.10 Appendix 11: Cross-sectional comparative analysis of cytokines panel

The following section contains a brief information about the cytokines used in cross-sectional comparative analysis in this study.

7.10.1 Interleukin-4 (IL-4)

IL-4 is a complex glycoprotein with pleotropic anti-inflammatory properties that promotes humoral response to combat extracellular pathogens. It provides protective immune response against extra-cellular parasites and helminths. IL-4 may be produced by mast cells, basophils, eosinophils, neutrophils, and some types of activated T cells (Chomarat and Banchereau, 1997). The specific behaviour of IL-4 depends on tissue distribution and its access to distinct target cells. IL-4 regulates the expression of the low affinity Fc receptor for IgE (CD23) on both lymphocytes and monocytes (Mak and Saunders, 2006). In macrophages, IL-4 regulates the expression of IL31RA and hinders the secretion of pro-inflammatory chemokines and cytokines such as TNF and IL-1β, thereby weakening the ability of these cells to produce reactive oxygen and nitrogen intermediates (Mak and Saunders, 2006). In B cells, IL-4 stimulates cell differentiation and induces up-regulation of MHC class II and FcεRII but the effect IL-4 derived from T cells and FcεRI^{+} has on immunological processes is very distinct (Brown and Hural, 2017).

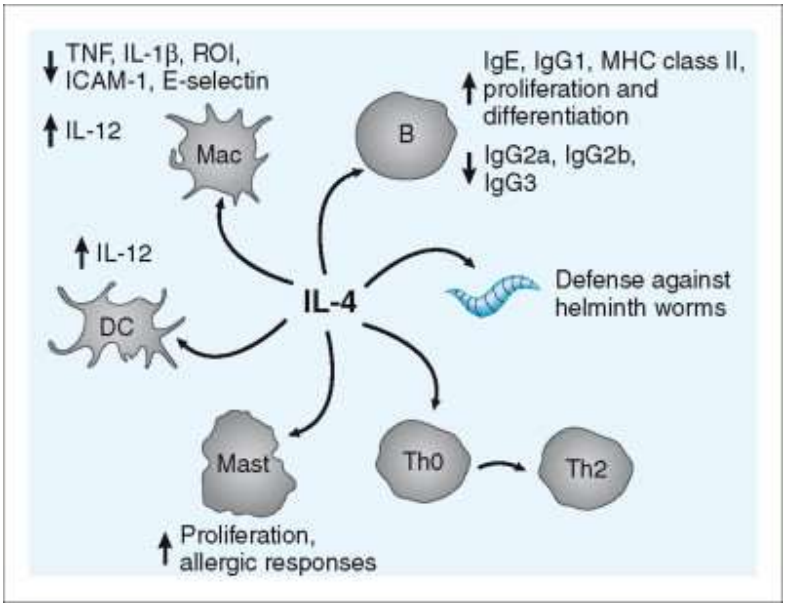

Figure 88: Major functions of IL-4 (Mak and Saunders, 2006).

In their murine study, (Mak and Saunders, 2006) showed that IL-4 supports isotype switching in murine B cells to IgG1, IgG4 and IgE but impedes isotype switching

to IgG2a, IgG2b and IgG3 (Ul-Haq et al., 2016). In allergic responses that involve IgE-mediated mast cell degranulation, IL-4 aids growth of mast cells. IL-4 supports IgE production, which allows FcεRIIB bearing eosinophils to promote antibody-dependent cellular cytotoxicity (ADCC), which is critical in the defence against helminth worms. IL-4 also blocks the expression of cellular adhesion molecules induced by IFN-γ. Because IL-4 stimulates Th2 differentiation in T cells and class switching in B cells, it has a role in type 2 inflammation (Izuhara et al., 2011). At the same time, IL-4 can also induce the up-regulation of IL-12 production by dendritic cells and macrophages. This may create a negative feedback mechanism in Th2 response regulation (Mak and Saunders, 2006). O'Garra et al. (1997) report in their clinical trials, IL-4 has seemingly been shown to be accountable for tissue damaging auto immunity effects.

IL-4 promotes differentiation of antigen-stimulated naïve Th cells (ThO) into Th2 effector cells and induces Th2 responses by binding to its receptor, IL-4Rα, and activates the signal transducer and activator of transcription signalling pathway - STAT6 (Chatterjee et al., 2014). IL-4 receptor (IL-4R) shows some similarities with IL-13 as discussed in section 1.14.7. IL-4R consists of two types. IL-4 signals through both type I and type II receptors, whereas IL-13 signals only through type II receptor (Seyfizadeh et al., 2015). In type 1, the IL-4Rα subunit combined with a common chain γc to form a ternary complex. Ligand binding by IL-4Rα activates the Janus family JAK1, JAK2 and JAK3 kinases. Type II IL-4R receptor, which is found in many non-hematopoietic cells, consists of IL-4Rα complexed with IL-13Rα1 chain (Andrews et al., 2002).

7.10.2 Interleukin-8 (IL-8)

IL-8 is a pro-inflammatory cytokine that has an important role in inflammation and wound healing. IL-8 is a prototype of the cysteine-X-cysteine (CXC) chemokines and is therefore also known as CXCL8. IL-8 has a distinct feature in that it shows target specificity for neutrophils and acts directly on neutrophil infiltration (Fujiwara et al., 2002). IL-8 recruits and activates neutrophils in inflammatory areas but has only minor effects on blood cells. IL-8 is a member of the Interleukin-8 supergene family that has many chemotactic peptides sharing structural homology. Waugh and Wilson (2008) showed that in vivo intracutaneous application of IL-8 induces local exudation and prolonged accumulation of neutrophils. IL-8's

major pathophysiological role lies in affecting neutrophils (Waugh and Wilson, 2008). The role that neutrophils play in periodontal disease is well understood (Rosales and Uribe-Querol, 2017, Hirschfeld 2020). Neutrophils are abundantly present in the oral cavity and neutrophil dysfunction leads to periodontitis and loss of periodontal tissue.

IL-8 activates neutrophils to recruit T and nonspecific inflammatory cells at the inflammation site (Feugate et al., 2002). Neutrophils respond to IL-8 with various extracellular changes and release granule enzymes that can degrade connective tissue. IL-8 is a chemoattractant cytokine produced by a variety of tissue and blood cells. As a pro-inflammatory cytokine, IL-8 carries out proangiogenic, proliferative, and promotility activities (Anton and Glod, 2016). IL-8 has also been found to play a major part in the development of cancer (Matsushima et al., 1992) and it also stimulates the production of α-smooth muscle actin antibodies in human fibroblasts (Feugate et al., 2002). The presence of leukoregulin improves the expression of IL-8 in human skin. Because IL-8 is chemotactic for fibroblasts, it accelerates their migration and it has an important role in wound healing because it stimulates the deposition of tenascin, fibronectin, and collagen (Qazi et al., 2011).

Human IL-8 genes are found between 4q13 and 4q21 in chromosome 4 (Long et al., 2016). IL-8 is secreted under inflammatory stimulus by multiple cell types (Matsushima and Oppenheim, 1989). Leukocytes and endothelial cells secrete IL-8 when there is exposure to IL-1 or TNF-α, whereas fibroblasts and malignant tumour cells secrete IL-8 when there is hypoxia or in the presence of chemotherapy agents. IL-8 can exist in monomer or dimer forms and it binds extracellularly with its two IL-8 surface cell receptors, CXCR1 or CXCR2 (Long et al., 2016, Xie, 2001). IL-8 binds at the membrane and triggers changes in Gα and Gβγ subunits of G protein, which then intracellularly couple with CXCR1 and CXCR2 receptors. The coupling triggers conformational change and activates downstream signalling when the G protein subunits dissociate from the receptor complex (Sharma et al., 2018)

The IL-8 cells that are secreted from cancer cells, through the autocrine signalling pathways boost the cancer proliferate and thrive in the tumour environment. IL-8 also boost angiogenesis and prompt chemotactic neutrophils infiltration into the site of tumour. Through their paracrine signalling, IL-8 promotes tumour epithelial-mesenchymal transition (EMT), increasing the invasiveness of the cancer.

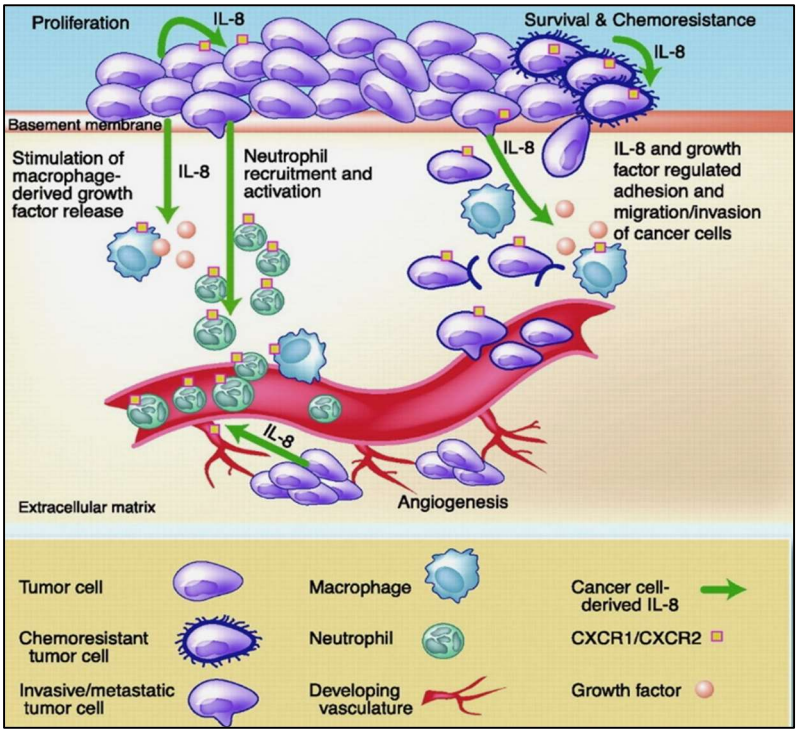

Figure 89: The role of IL-8 signalling in the tumour micro-environment (Waugh and Wilson, 2008).

Further, neutrophils and TAMs produce additional growth factors, chemokines and enzymes and stimulate EMT in the tumour microenvironment (Long et al., 2016). CXC-chemokine signalling plays an important role in seizing the cancer progression and helps increase their response to chemotherapy (Waugh and Wilson, 2008). This counter effect is due to the pleiotropy of IL-8 signalling in different tissue types.

7.10.3 Interleukin-12 (IL-12)

IL-12 is an immune cell stimulator that aids the production and propagation of T cells and IFN-γ. IL-12 promotes cellular differentiation and plays a critical role in the regulation of many immunologic processes such as CD4+ and CD8+ T-cell differentiation, and tumour cell recognition (Walter, 2006).

IL-12 is a disulphide-linked heterodimer containing p40 and p35 subunits. IL-12 is mainly produced by phagocytes such as monocytes, macrophages and neutrophils, and dendritic cells in response to different types of pathogens including bacterial, viral, fungal and parasitic. IL-12 signals through TLRs and other receptors, to membrane-bound and soluble

signals from activated T cells and natural killer (NK) cells, and to components of the inflammatory extracellular matrix (for example, low-molecular-weight hyaluronan) through CD44 and TLRs.

IL-12 is produced by phagocytes such as monocytes and neutrophils, macrophages, dendritic cells, and its action on T cells and NK cells, inducing differentiation towards Th1 type cells, IFN-γ production and increased cytotoxic activity of T and NK cells (Watford et al., 2003).

Cellular sources and responders of IL-12. Antigen-presenting cells and phagocytic cells, including monocytes and macrophages, dendritic cells, and neutrophils, are the primary producers of IL-12. The major actions of IL-12 are on T and NK cells. IL-12 induces proliferation, IFN-γ production and increased cytotoxic activity of these cells, and importantly, IL-12 induces the polarization of CD4+ T cells to the Th1 phenotype that mediates immunity against intracellular pathogens. IL-12, especially in combination with IL-18, also acts on macrophages and dendritic cells to induce IFN-γ production even in antigen presenting cells (Watford et al., 2003).

IL-12 was initially called natural killer cell stimulatory factor (NKSF) because of its capacity to stimulate interferon-gamma (IFN - γ) production from T and NK cells.

In vivo, IL-12 is produced very early during infections or immune response and exerts important pro-inflammatory functions and enhancement of innate resistance by activating natural killer cells and, through IFN-γ induction, phagocytic cells. The IL-12 produced during this inflammatory phase, both by direct action and, indirectly, by determining the composition of the cytokine milieu at the site of the murine response, induces differentiation of T helper type 1 (Th1) cells while inhibiting the generation of Th2 cells. Thus, because of its double function of a pro-inflammatory cytokine and an immunoregulatory factor, IL-12 plays a key role in the resistance to infections, particularly those mediated by bacteria or intracellular parasites, against which phagocytic cell activation and Th1-mediated responses are particularly effective. However, because of the same activities, IL-12 also plays a role in pathological situations, such as septic shock, tissue damage during inflammation and organ-specific autoimmune diseases (Trinchieri, 1998).

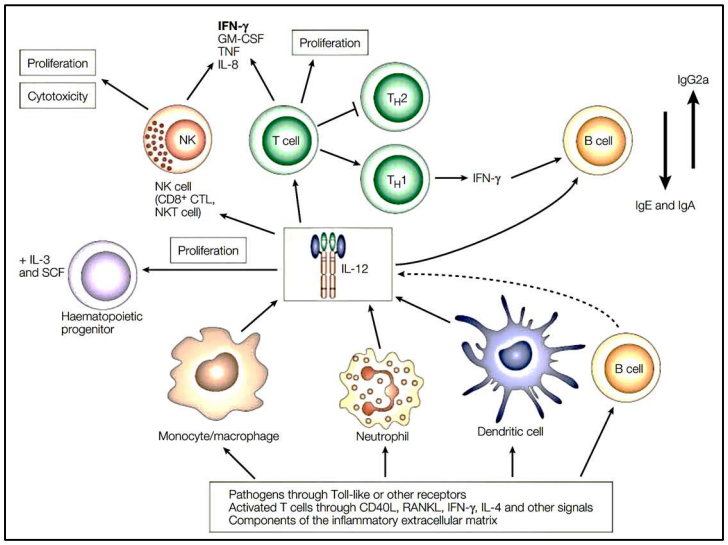

Figure 90: IL-12 production and diverse biological functions (Hamza et al., 2010).

The physiologically most important target cells of IL-12 are: haematopoietic progenitors, for which, in synergy with other colony-stimulating factors, IL-12 induces increased proliferation and colony formation; NK cells, NKT cells and T cells, for which IL-12 induces proliferation, enhancement of cytotoxicity and of the expression of cytotoxic mediators, and the production of cytokines, particularly interferon gamma (IFN-γ), as well as favouring differentiation to cells that produce type-1 cytokines (TH1, TC1 and NK1 cells); and B cells, for which IL-12, directly or through the effects of type-1 cytokines such as IFN-γ, enhances the activation and production of TH1-associated classes of immunoglobulin (for example, IgG2a in the mouse). CTL, cytotoxic T lymphocyte; GM-CSF, granulocyte–macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor-κB ligand; SCF, stem-cell factor; TC1, T cytotoxic 1; TH1, T helper 1; TNF, tumour-necrosis factor (Trinchieri, 1998)

IL-12 plays an important role in the activities of natural killer cells and T lymphocytes. IL-12 mediates enhancement of the cytotoxic activity of NK cells and CD8+ cytotoxic T lymphocytes. There also seems to be a link between IL-2 and the signal transduction of IL-12 in NK cells. IL-2 stimulates the expression of two IL-12 receptors, IL-12R-β1 and IL-12R-β2, maintaining the expression of a critical protein involved in IL-12 signalling in NK cells. Enhanced functional response is demonstrated by IFN-γ production and killing of target cells (Vignali and Kuchroo, 2012).