IMMUNE BIOMARKERS PREDICTING CLINICAL OUTCOMES FOR MAJOR TRAUMA PATIENTS

AMBER SALEEM

Master of Science by Research

School of Science, Engineering and Environment

University of Salford

2020

Table of Contents

Acknowledgments

I would firstly like to thank my supervisor Prof. Niroshini Nirmalan for the consistent support and guidance throughout my research, and academic career. Without her knowledge and patience I could not have achieved these results.

I would also like to acknowledge the contribution of Basmah Allarakia and Matthew Jones for the collection of data and laboratory procedures which allowed me to carry out my statistical research analysis.

Finally, my thanks go out to the teams at Central Manchester Foundation Trust and Salford Royal Foundation Trust for providing the clinical data required for this study.

List of Abbreviations

AIS	Abbreviated injury score
AMP	Adenosine monophosphate
APACHE	Acute physiology and chronic health evaluation
APC	Antigen presenting cell
ATLS	Advanced trauma life support
ATP	Adenosine triphosphate
BCR	B cell receptor
BP	Blood pressure
cAMP	Cyclic adenosine monophosphate
CARS	Counter anti-inflammatory response syndrome
CD	Cluster of differentiation
CMFT	Central Manchester Foundation Trust
CNS	Central nervous system
CRP	C-reactive protein
CT	Computed tomography
CTLA-4	Cytotoxic T-lymphocyte associated protein-4
CVVH/IHD	Continuous veno-venous hemofiltration/Intermittent haemodialysis
DAMPs	Damage associated molecular patterns
DMSO	Dimethyl-sulfoxide
DP	Double positive
eGFR	Estimated glomerular filtration rate
FACS	Fluorescence activated cell sorting
FAST	Focused assessment with sonography for trauma
FCS	Foetal calf serum
FiO2	Fraction of inspired oxygen
FOXP3	Forkhead box P3
FSC	Forward scatter
GCS	Glasgow coma score
GITR	Glucocorticoid induced tumour necrosis factor receptor related protein
GM-CSF	Granulocyte macrophage colony-stimulating factor
Hb	Haemoglobin
HMGB	High mobility group box
HR	Heart rate
ICOS	Inducible T-cell co-stimulator
IDO	Indoleamine 2.3-dioxygenase
IL	Interleukin
INF	Interferon
INF-γ	Interferon gamma
ISS	Injury severity score
JAK-STAT	Janus kinase signal transducer and activator of transcription pathway
LAG-3	Lymphocyte activating gene 3
MAP	Mean arterial pressure

MAPK	Mitogen-activated protein kinase
MHC II	Major histocompatibility complex 2
MODS	Multiple organ dysfunction syndrome
MOF	Multiple organ failure
mRNA	Messenger ribonucleic acid
NFAT	Nuclear factor of activated T cells
NF-$\kappa\beta$	Nuclear factor kappa-beta
NISS	New injury severity score
NIV/CPAP	Non-invasive ventilation/Continuous positive airway pressure
NK	Natural killer
OIS	Organ injury scale
PAMPs	Pattern associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PD-1	Programmed cell death protein 1
P/F	Ratio of arterial oxygen partial pressure to fractional inspired oxygen
PLT	Platelet
PT	Prothrombin time
RBC	Red blood cell
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment
SPSS	Statistical package for the social sciences
SRFT	Salford Royal Foundation Trust
SSC	Side scatter
TCR	T cell receptor
TGF	Transforming growth factor
TGF-β	Transforming growth factor beta
TH	T helper
TIM-3	T cell immunoglobulin domain and mucin domain
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor alpha
TNFR	Tumour necrosis factor receptor
TRAIL	Tumour necrosis factor related apoptosis inducing ligand
T reg	T regulatory cells
WCC	White blood cell count

Abstract

Major trauma continues to be the most common cause of death in the population under the age of 40 in England. Despite advances in trauma care in the UK, sepsis and multiple organ dysfunction syndrome (MODS) continue to be severe complications of trauma worldwide. Ongoing research exploring the immune response to trauma over the years has highlighted the systemic inflammatory response syndrome (SIRS) as an early immune response to trauma. Driven mainly via macrophages and innate immune cells, this response is followed by the counter anti-inflammatory response syndrome (CARS), mediated predominantly by T regulatory cells, in order to restore immune homeostasis.

The 'two-hit theory' proposes a link between MODS and an imbalance between proinflammatory and counter inflammatory responses, during which, an overexpression of SIRS results in early MODS and an overexpression of CARS results in late MODS, sepsis or septic shock. The suppressive abilities of T reg cells and the role they may play in this immune response is an area of great interest as early identification of T reg activity within the CARS response may act as a predictive biomarker of late MODS and poor clinical outcome.

This study aims to analyse the trends of 7 subsets of T lymphocytes; CD3+, CD4+, CD8+, CD25BRI, CD4+FOXP3+, CD25BRIFOXP3+, and CD127LOWFOXP3+ following major trauma, in the hopes of utilizing these as predictive biomarkers in the future of trauma care. Poor clinical outcome was represented in this study by a Sequential organ failure assessment (SOFA) score of greater than 1. In order to explore the relationship between CD markers, particularly T reg cells, and other mediators of inflammation, all CD markers used in this study were also tested against interleukin 6 (IL-6) and IL-10.

Prior to this study, blood samples from a cohort of 85 patients were gathered by Basmah Allarakia and Matthew Jones from the Central Manchester Foundation Trust (CMFT) and Salford Royal Foundation Trust (SRFT). Laboratory procedures were then carried out by Basmah Allarakia, during which, peripheral blood mononuclear cells were isolated, retrieved, and enriched. Immunofluorescent staining of T cell surface markers was done using fluorochrome labelled antibodies, before finally being analysed using FACS Verse flow cytometry.

During this study, T lymphocyte expression was analysed using the SPSS statistical software. A Spearman's rank correlation test highlighted a significant correlation between CD3+ expression on Day 1 and SOFA scores on Day 5 ($p= 0.007$). A significant correlation between CD3+, CD25BRIFOXP3+ and CD127LOWFOXP3+ on Day 3 against SOFA scores on Day 5 was also found ($p=0.02$, $p=0.049$ and $p=0.048$ respectively). Furthermore, a paired T test highlighted that CD25BRIFOXP3+ and CD127LOWFOXP3+ displayed higher expression on Day 1 in those patients who developed SOFA scores >1 on Day 5, in comparison to patients who developed SOFA scores <1 on Day 5. These differences were also found to be significant ($p= 0.05$ and $p= 0.03$ respectively). A second Spearman's rank correlation test also displayed a significant correlation between CD3+ and IL-10 on Day 3 ($p= 0.000$). furthermore, a significant correlation between both CD25BRIFOXP3+ and CD127LOWFOXP3+ and IL-6 on Day 3 was also found ($p= 0.026$ and $p= 0.029$ respectively).

Overall, significant findings were displayed regarding the role of T reg cells and their ability to be used as predictive biomarkers in the future of trauma care. Correlating to previous research, a higher expression of T regs was seen in patients who later developed a higher SOFA score, thus a poorer clinical outcome. The suppression mechanisms of T regs which may explain these results have been grouped into four main categories, these are; metabolic disruption, cytolysis, inhibitory cytokines such as interleukin-35 (IL-35), IL-10, and transforming growth factor-β (TGF-β), and finally, suppression by modulation of dendritic cell function or maturation.

The correlation between T regs and IL-6 also provide promising potential for the future of biomarker research, however a larger patient cohort and greater statistical analysis of these two immune markers must be carried out in order to confirm the validity of these findings.

CHAPTER 1: introduction

1.1 Background

Major trauma remains the most common cause of death in the population under the age of 40 in England (Forward *et al*., 2014), as well as one of the commonest causes of death and disability worldwide (Moran *et al*., 2018). This type of trauma is defined as a life altering injury which may involve a single system such as an isolated neck injury, or alternatively a severe multiple organ system trauma (Forward *et al*., 2014). The national audit office estimates that there are 5,400 major trauma deaths a year from 20,000 cases (Moran *et al*., 2018), with the most common cause being identified as road traffic accidents.

Over the years, much has been done to combat this problem and provide the best clinical outcomes, leading to the introduction of 27 designated major trauma centres in the UK: 11 for both adults and children, 10 for adults, 5 for children, and 1 collaborative centre (Moran *et al*., 2018). This new system allows patients to receive specialised care immediately, in comparison to the previous standards which meant they were sent to the nearest hospital irrespective of the severity of the injuries or the ability of the hospital to care for the injury at hand (Forward *et al*., 2014).

A major milestone in trauma care was the introduction of a complex trauma response system, which is now considered to be the standard adopted in the majority of countries worldwide. Using this system, severely injured patients do not pose a prognostic challenge during the early triage stage. However, this process does not work as effectively for patients who are initially stable, but whose conditions deteriorate quickly later on. Over the last decade, prognostic tools such as severity scores and biomarkers have been developed in order to estimate the burden of injuries for these patients in an attempt to predict poor clinical outcomes (Salvo *et al*., 2020).

1.2 The physiological response to trauma

In 1983 the distribution of trauma related mortality was described by Trunkey as trimodal, consisting of immediate, early and late phase deaths (Table 1.1) (Trunkey, 1983). Following many advances in trauma care, this distribution is now assumed to be largely bimodal, with a large majority of trauma deaths occurring immediately or during the late phase (Gunst *et al*., 2010).

Table 1.1: Classification of immediate, early and late deaths due to trauma.

Trunkey's 1983 classification of Immediate, early, and late trauma deaths				
Deaths	**Timing of death after sustaining injuries**	**Location**	**Cause**	**Interventions**
Immediate	Minutes	Scene	Nonsurvivable injuries	Injury prevention
Early	Hours	Hospital	Severe injuries, potentially survivable with optimal care.	Improved access to trauma care
Late	Weeks	Hospital	Multiple organ failure, sepsis	Improved resuscitation/critical care

Table displaying the timings, location, cause and interventions for trauma related deaths. Adopted from (Trunkey, 1983).

Stress responses induced by trauma include haemodynamic, metabolic and immunological changes, with greater stress resulting in increased reactions. Cytokines and hormones play a large role in these responses (Simsek *et al*., 2014). The haemodynamic response represents the patients initial ability to survive (Kirkman & Watts, 2014), whereas metabolic pathways are central to the bodies compensatory responses which occur later (Krepska *et al*., 2017).

Since the description of trimodality, several advancements in trauma prevention and treatment have been introduced such as advanced trauma life support (ATLS), implementations of CT scans and focused assessment with sonography for trauma (FAST), as well as developments in intensive care medicine. As a result, an overall reduction in trauma mortality has been observed (Rauf *et al*., 2019). Despite this, sepsis and multiple organ failure (MOF) are still severe complications following trauma. Recent studies analysing data from 78,310 severely injured patients found that the rate of sepsis and MOF among non survivors was 11.5% and 70.1% respectively (Rauf *et al*., 2019). The mean time to death in cases with sepsis was also found to be 23.3 days, highlighting the work done in previous studies which described sepsis and MOF as the leading causes of death (>1 week) (Baker *et al*., 1980).

The sequential organ failure assessment (SOFA) score is an objective score which allows for the calculation of the number and severity of organ dysfunction in six organ systems (liver, coagulation, respiratory, renal, cardiovascular, and neurologic) (Table 1.2), allowing measurement of individual or combined organ dysfunction (Grissom *et al*., 2010).

Table 1.2: The sequential organ failure assessment (SOFA) score

		SOFA SCORE			
Organ system		**1**	**2**	**3**	**4**
Respiration	PaO_2/FiO_2 (mmHg)	<400	<300	<220	<100
Coagulation	Platelets ($x10^3/mm^3$)	<150	<100	<50	<20
Liver	Bilirubin (mg/dL)	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular	Hypotension	MAP <70	Dopamine <5	Dopamine >5 or norepinephrine <0.1	Dopamine >15 or norepinephrine >0.1
CNS	GCS	13-14	10-12	6-9	<6
Renal	Creatine (mg/dL)	1.2-1.9	2.0-3.4	3.5-4.9	>5.0

MAP: Arterial pressure
CNS: Central Nervous system
GCS: Glasgow coma score
Adopted from (Grissom *et al*., 2010)

Trauma scores are used to assign numerical values to anatomical lesions and physiological changes after injury. Physiological scores describe changes in vital signs and consciousness and are often used during first contact with the patient. Physiological scoring systems include the revised trauma score, Glasgow trauma score, as well as APACHE scoring (acute physiology and chronic health evaluation). Anatomical scores describe all of the injuries recorded by imaging, clinical examination, surgery or autopsy. These scores are used following a complete diagnosis in order to stratify trauma patients. Anatomical scoring systems include the abbreviated injury scale (AIS), injury severity score (ISS), new injury severity score (NISS), organ injury scale (OIS) and anatomical profile. The ISS has been described as virtually the only anatomical scoring system in use which correlates linearly with morbidity, mortality, and hospital stay among other measures of severity. Each injury is assigned an AIS score allocated to one of six body regions. The sum of squares of the three highest regions will then produce the ISS value. Major trauma is considered when ISS > 15 (Javali *et al*., 2019).

<u>1.3 Effects of trauma on the Immune system</u>

Traumatic injuries are responsible for inducing a complex response which disrupts the homeostasis present within the immune system. Following the initial response to injury, cells of the innate and adaptive immune system produce proinflammatory and counter inflammatory responses, referred to as SIRS and CARS in order to restore immune homeostasis (Osuka *et al*., 2014).

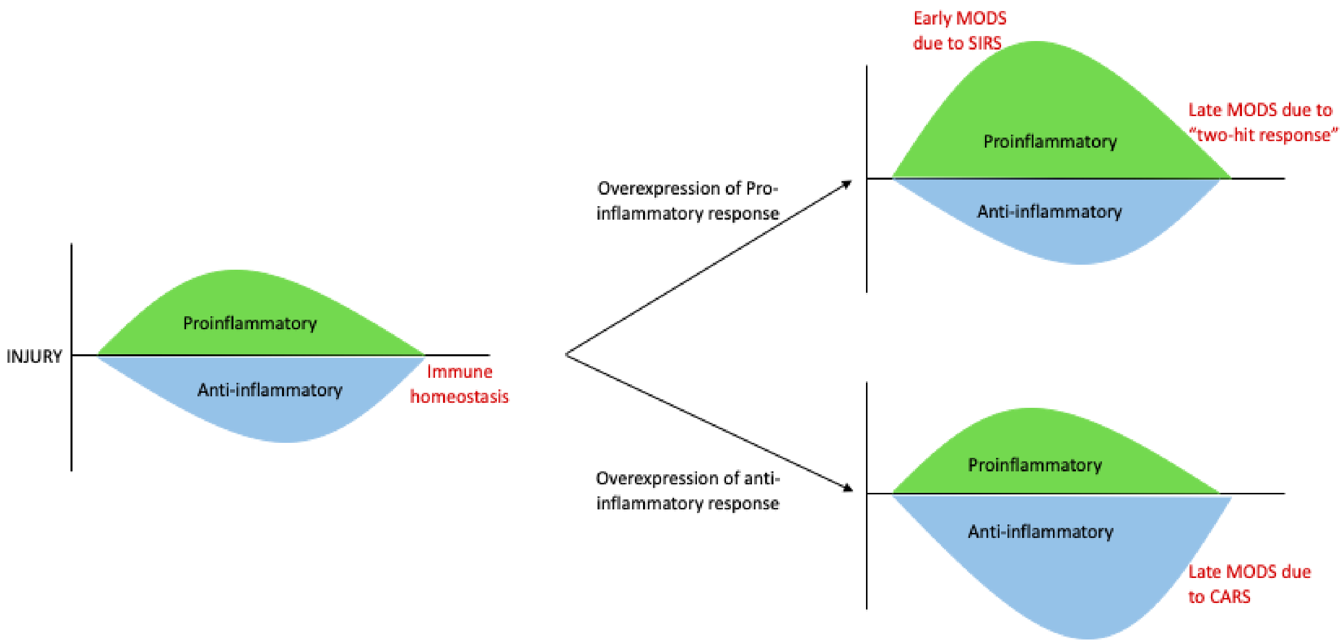

Figure 1.1: The 'two hit theory' proposes a link between multiple organ dysfunction syndrome (MODS) and an imbalance in proinflammatory and counter inflammatory immune responses.
The innate immune system driven systemic inflammatory response syndrome (SIRS) is achieved predominantly by macrophages which prime toll-like receptors (TLRs). However, the adaptive immune system driven compensatory anti-inflammatory response syndrome (CARS) is driven due to priming of regulatory T cells (T reg). If a pro inflammatory reaction is overexpressed, the patient may develop a 'two-hit' response, leading to multiple organ dysfunction (MODS). If the counter anti-inflammatory response is overexpressed, the patient will possess an increased risk of developing complications such as late MODS, sepsis, or septic shock (Osuka *et al*., 2014).

1.4 Innate immune response to trauma

The innate immune system is a non-specific system which acts as the first line of defence. Major trauma prompts a series of innate immune responses, in the hopes of restoring the cells of the body to their pre injury state (Huber-Lang *et al*., 2018).

Initially, trauma leads to the damage of internal and external barriers which exposes the immune system to damage associated molecular patterns (DAMPs) such as HMGB-1 and pattern associated molecular patterns (PAMPs). These danger signals are then sensed by inflammatory pathways containing lipids and proteins which participate in the first line of defence. In particular, the serine protease system, composed of the complement and coagulation cascades are activated after trauma and can detect DAMPs and PAMPs (Huber-Lang *et al*., 2018).

Either directly or via these systems, DAMPs and PAMPs can transmit their signals to leukocytes such as neutrophils and monocytes through pattern recognition receptors (PRRs) such as toll like receptors (TLR), complement receptors, or purinergic receptors. The activation of these inflammatory cells stimulates the production and release of inflammatory mediators such as interleukins, thus generating the response seen in the systemic inflammatory response syndrome pathway (Huber-Lang *et al*., 2018).

The reprioritizing of leukocytes following severe trauma have been described to be unique to each injury pattern. The release of micro vesicles from leukocytes also enhances adhesion, inflammation, and stimulates activation of the clotting system in order to contain haemorrhage. However, following a substantial injury which may be associated with extended surgical intervention or haemorrhage shock, the innate immune response may become imbalanced (Huber-Lang *et al*., 2018).

1.4.1 Pro inflammatory responses

Pro inflammatory responses develop following trauma in order to protect the host from infections via heightening the antimicrobial immunity. Inflammatory stimuli, including cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumour necrosis factor-α (TNF-α), mediate inflammation through interactions with the Toll like receptors (TLRs), IL-6 receptor (IL-6R), IL-1 receptor (IL-1R), and TNF receptor (TNFR) (Kaminska, 2005). The activation of these receptors triggers signalling pathways which include the nuclear factor kappa-B (NF-kB), mitogen-activated protein kinase (MAPK), and Janus kinase signal transducer and activator of transcription pathway (JAK-STAT) (Chen *et al*., 2017).

Following these pathways, cytokines are released from immune cells such as macrophages, monocytes and lymphocytes.

Table 1.3: Overview of pro-inflammatory cytokines and their functions.

Cytokine	Family	Main sources	Function
IL-1β	IL-1	Macrophages Monocytes	Proliferation Apoptosis Differentiation
IL-6	IL-6	Macrophages T cells	Differentiation Cytokine production
IL-8	CXC	Macrophages Epithelial cells Endothelial cells	Chemotaxis Angiogenesis
IL-12	IL-12	Dendritic cells Macrophages Neutrophils	Activates Natural killer (NK) cells
TNF-α	TNF	Macrophages NK cells CD4+ lymphocytes	Cytokine production Cell proliferation Apoptosis Anti-infection
INF-γ	INF	T-cells NK cells NKT cells	Innate and adaptive immunity
GM-CSF	IL-4	T cells Macrophages Fibroblasts	Macrophage activation Increase neutrophil and monocyte function

Adapted from (Chen *et al.*, 2017).

1.4.2 The inflammatory cascade

Trauma, inflammation or infection leads to the activation of the inflammatory cascade. Macrophages, monocytes, mast cells, endothelial cells, and platelets are responsible for producing cytokines. Firstly, inerleukin-1 (IL-1) and tissue necrosis factor-alpha (TNF-α) are released, and initiate several cascades. The release of TNF-α and IL-1 result in the cleavage of the nuclear factor-$\kappa\beta$ (NF-$\kappa\beta$) inhibitor. Following the removal of this inhibitor, NF-$\kappa\beta$ initiates the production of messenger ribonucleic acid (mRNA), further inducing the production of proinflammatory cytokines such as IL-8, IL-6 and interferon gamma (Kaplan, 2018).

These proinflammatory interleukins activate the complement and coagulation cascade as well as the release of prostaglandins, leukotrienes, and nitric oxide. Within the complement cascade, protein complements C3a and C5a are responsible for vasodilation, increased vascular permeability, and the release of additional cytokines (Kaplan, 2018).

1.4.3 Systemic inflammatory response syndrome

The two hit response is one of the serious complications of major trauma. Previous research findings by Paterson et al., 2003 have shown that TLR reactivity is enhanced following trauma. This suggests that the two hit response may be mediated through enhanced TLR reactivity by innate immune cells. These immune cells become increasingly reactive to toxins, resulting in high levels of cytokine production which may trigger a secondary SIRS response and MODS (Osuka *et al*., 2014). Excessive pro inflammatory cytokine production may lead to haemodynamic changes, tissue damage, organ failure, and death.

A counter inflammatory response acts to compensate for trauma induced inflammation. Previous studies have shown major trauma leads to a decreased resistance to infections due to CARS (Bone, 1996). Observations from numerous studies suggest that the adaptive immune system, particularly T cells, are responsible for mediating this response (Osuka *et al*., 2014).

1.5 Adaptive Immune response

Adaptive immunity is characterised on the basis of greater specificity than the innate immune response. Lymphocytes are the cells involved in the adaptive immune response and possess receptor sites on their cell surfaces. The receptors on each lymphocyte fits with a specific antigen on an invader, thus responding to a specific invader. Upon activation of these antigen specific cells, a population of cells is created with the same antigen specificity. As this process takes up to several days before a fully effective defence is achieved, the host must therefore rely on natural immunity during this delay (Segerstrom and Miller, 2004).

Adaptive immunity is composed of cellular and humoral responses. Cellular immunity responds against intracellular pathogens such as viruses and are coordinated by a subset of T helper (CD4+) lymphocytes called *Th1* cells and Cytotoxic T cells (CD8+). Humoral immunity responds against extracellular pathogens such as bacteria and parasites and are coordinated by a subset of T helper (CD4+) lymphocytes called *Th2* cells (Segerstrom & Miller, 2004).

1.5.1 Humoral immunity

During the humoral response antibodies are produced by B cells, causing the destruction of extracellular pathogens, therefore preventing the spread of intracellular infections. Helper T cells are required for the activation of B cells as well as their differentiation into antibody secreting plasma cells. During this response, the term helper T is used predominantly to describe a subset of CD4 T cells referred to as Th2 (Travers & Walport, 2001).

Interactions between antigen specific B cell receptors (BCR) on naïve mature B cells and protein antigens result in internalization of the antigen. Following this internalization, the antigen is then processed and presented with major histocompatibility complex II molecules (MHC II), which are found on antigen presenting cells such as dendritic cells. The presented antigen is then recognised by the T cell receptor of the T helper cells. As well as this, the CD4 molecule of the T cell interacts with the MHC II on the surface of the B cell.

Following this recognition, a subset of CD4+ T helper cells referred to as Th2 cells will produce and secrete cytokines such as IL-4, IL-5, and IL-13. These cytokines are responsible for several actions such as the activation of B cells, leading to proliferation into daughter cells. Cytokines released by Th2 cells are also responsible for the differentiation of B cell clones into memory B cells and plasma cells which will produce antibodies (Marshall *et al*., 2018).

1.5.2 T cell mediated immunity

Once T cells have developed in the thymus, they then enter the bloodstream. Here they circulate between peripheral lymphoid tissue and blood until they meet their specific antigen. During the cell mediated immune response, T cells differentiate into cytotoxic T cells (CD8+) or helper T cells (CD4+) (Marshall *et al*., 2018).

Helper T cells (CD4+) are activated following the interaction of their TCR with antigen bound to MHC II molecules. Unlike the Helper T cell response during humoral immunity, cell mediated immunity uses the Th1 subset. The Th1 response leads to the production of IFN-γ which is responsible for the activation of bactericidal activities of macrophages and enhances immunity to other intracellular pathogens. Th1 derived cytokines also contribute to the differentiation of B cells to produce antibodies responsible for enhancing the efficiency of phagocytosis.

Cytotoxic T cells (CD8+) are activated following the interaction of their TCR with peptide bound to MHC I molecules. Expansion of CD8+ cells produces effector cells which are responsible for the release of apoptosis inducing substances (Marshall *et al*., 2018).

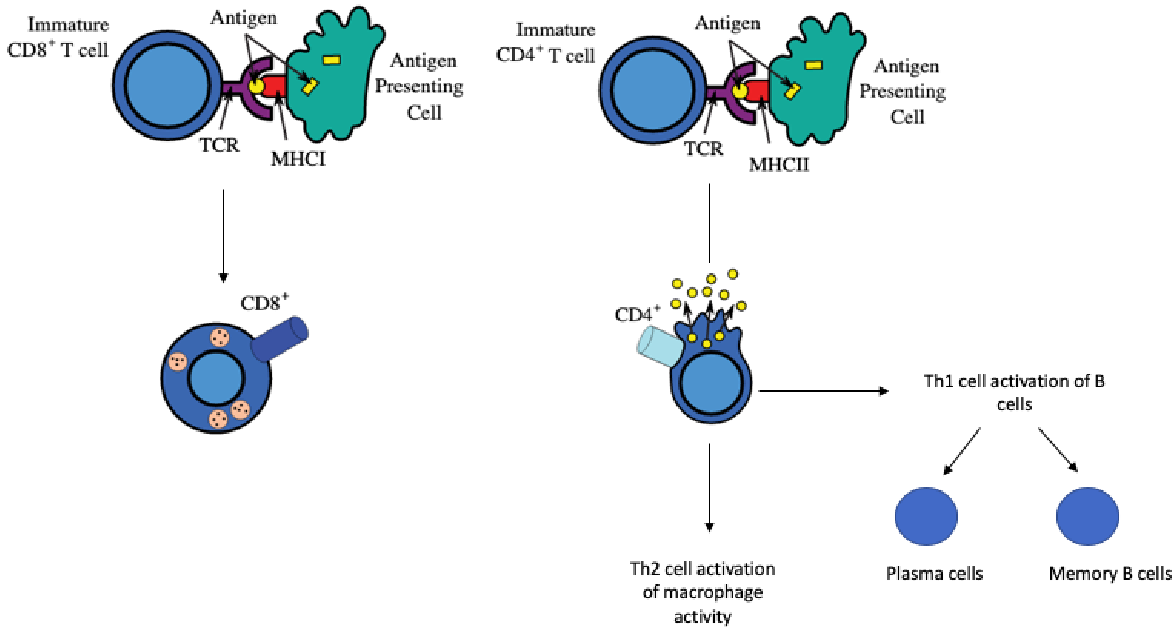

Figure 1.2: Overview of cells involved in the humoral and cell mediated immune responses.
Adapted from (Marshall *et al*., 2018).

1.6 Generation of T cells

T cells are derived from haematopoietic stem cells, which are found in the bone marrow. Following this they migrate and mature in the thymus. These cells express unique antigen binding receptors on the surface of their membrane, known as the T cell receptor (TCR). Each T cell expresses a specific type of TCR and will eventually proliferate and differentiate provided it receives the appropriate signal to do so. The majority of T cells develop as $\alpha\beta$ T cells, however 5% express the $\gamma\delta$ T cell receptor. Further on in development, the $\alpha\beta$ T cells will develop into the CD4 and CD8 subsets (Marshall *et al*., 2018).

During the earliest stage of development, the thymocytes will lack expression of both CD4 and CD8 receptors, and are therefore described as double negative (DN). This DN population is then further divided according to the expression of either an adhesion molecule termed CD44, or the interleukin-2 receptor a chain, termed CD25. Cells expressing CD25 and lacking CD44 will undergo beta selection, as they are able to produce a pre TCR which is able to form a complex with CD3 . This complex results in expression and up regulation of CD4 and CD8, creating double positive cells (DP).

DP cells will then arrange their TCR-α chain, producing an $\alpha\beta$-TCR. The cells will then undergo positive and negative selection. Cells which engage with antigen/MHC with an appropriate affinity undergo positive selection in the cortex and survive, whereas those that interact with a weaker affinity will die via apoptosis. During positive selection if cells were positively selected by MHC class II molecules, the thymocytes are then committed to becoming CD4+ cells. Similarly, if cells were positively selected by MHC class I molecules, the thymocytes are then committed to becoming CD8+ cells. Negative selection also takes place in the medulla, during which, self-antigens are presented to thymocytes on antigen presenting cells (APC) such as macrophages and dendritic cells. Thymocytes which interact too strongly with antigens during this process will undergo apoptosis. Following both positive and negative selection, down-regulation of either co-receptor will produce either naïve CD8+ or CD4+ cells which will exit the thymus and circulate to peripheral lymphoid tissues (Chaplin., 2010).

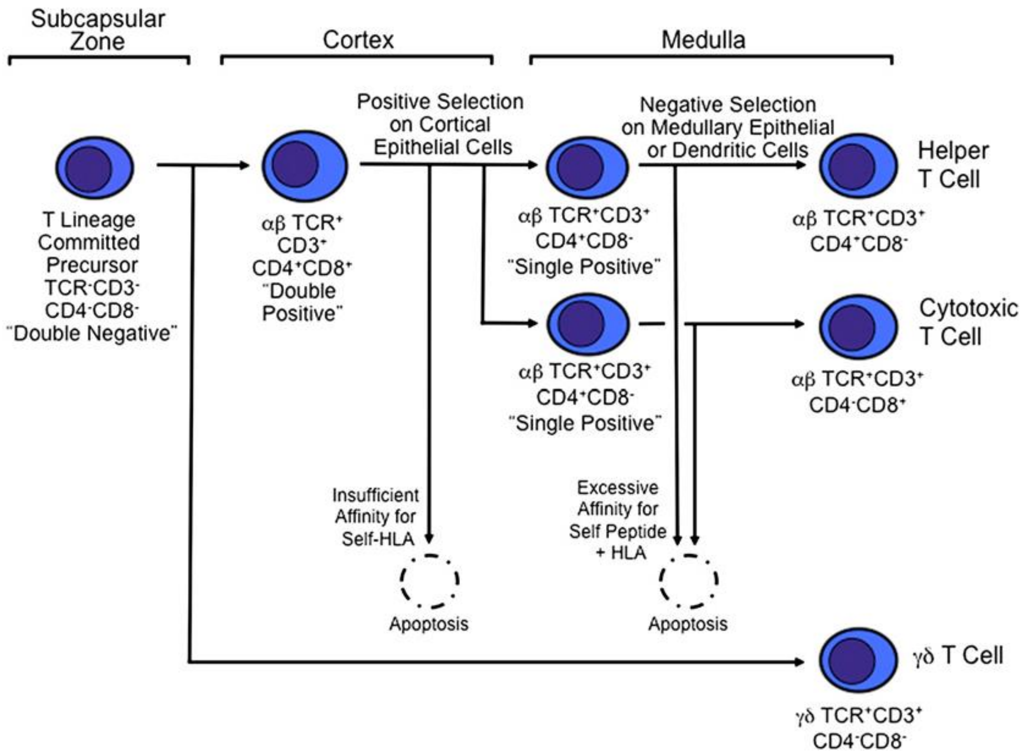

Figure 1.3: Overview of the pathways involved in the production of T helper cells, cytotoxic T cells, and gamma delta T cells. Source: (Chaplin, 2010).

1.7 T cell lineages

Several types of T cell lineages exist; T helper cells, cytotoxic T cells, memory T cells, suppressor T cells, natural killer and finally gamma delta T cells.

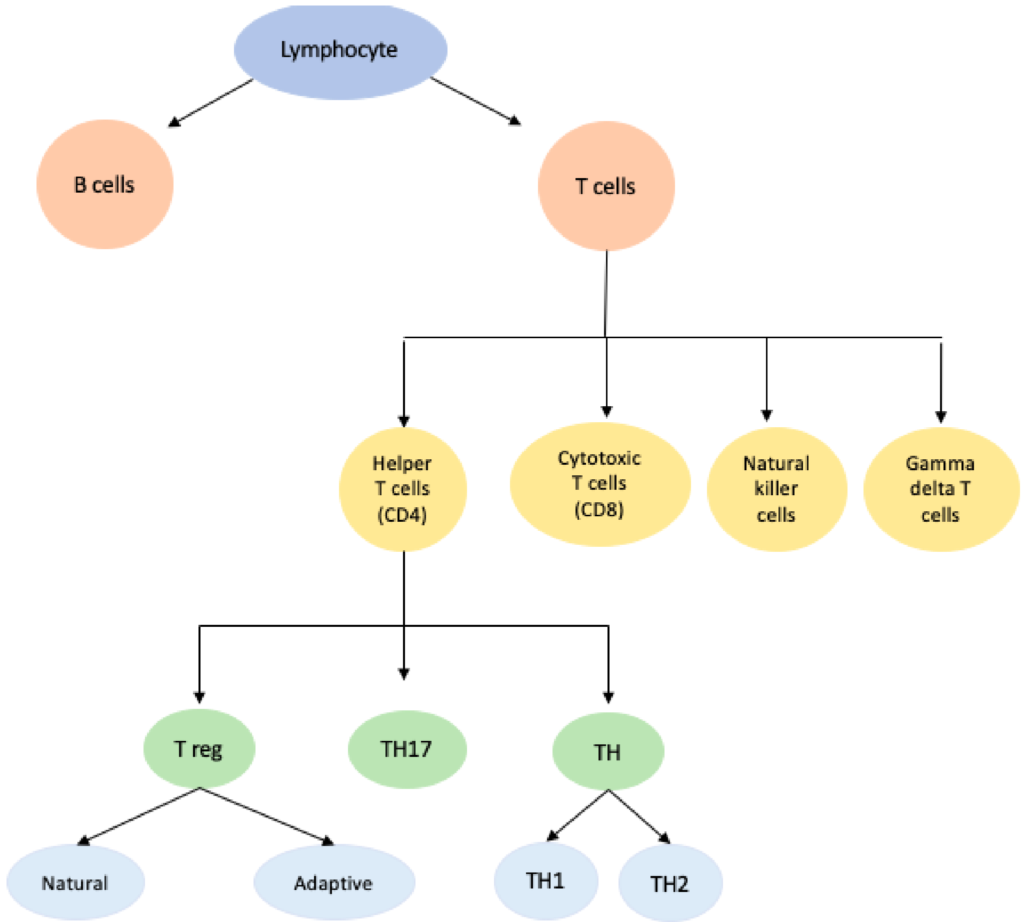

Figure 1.4: Overview of lymphocyte lineages. Adapted from (Alegre *et al., 2001).*

T helper cells (CD4+) have a vital role in maximizing the immune response. Although these cells cannot directly kill infected cells, they direct other cells to perform these tasks. Several types of T helper cells can be induced such as Th1, Th2, Th17 and T reg. The Th1 response produces IFN-γ, leading to the activation of bacterial activities of macrophages as well as an immunity to intracellular pathogens. The Th2 response produces cytokines IL-4. IL-5, and IL-13, which are responsible for the development of antibody producing B cells as well as the recruitment of eosinophils and mast cells which are essential for responses against parasites.
Th17 cells are responsible for the production of cytokines of the 1L-17 family which are predominantly descried as proinflammatory cells (Marshall *et al*., 2018).Regulatory T cells (T reg) also play a vital role in the immune response as they contribute to the counter inflammatory response to trauma (Hefele *et al*., 2019).

1.8 T lymphocyte response to trauma

As seen through both the humoral and cell mediated immune responses, T lymphocytes play an important role in the immune response to trauma. They are distinguished from other lymphocytes such as B cells and NK cells due to the presence of an antigen specific molecule on the surface, identified as a T cell receptor (TCR).

Following the initial response to traumatic injury, cells of the innate and adaptive immune system initiate the proinflammatory (SIRS) response as well as a counter inflammatory response referred to as CARS (Stoecklein *et al*., 2012). An imbalance between these inflammatory immune responses causes heightened susceptibility to infections and organ damage (Hefele *et al*., 2019). The CARS response is initiated in order to suppress trauma induced inflammation, control reactivity to damaged tissues, and to restore immune homeostasis (Stoecklein *et al*., 2012). This response is believed to be mediated by the adaptive immune system, in particular T cells (Osuka *et al*., 2014).

The counter inflammatory phase of the injury response is displayed at later time points following injury, suggesting it is a developed reaction to the molecular and cellular triggers which initiate the injury response. The CARS response has been labelled as an immune suppressive response. This is due to the anergic-like properties displayed by T mediated responses including: low proliferative responses to specific antigens and mitogens; suppressed Th1 type immune reactivity alongside increased Th2 type cytokine production; finally increased T reg activity (Stoecklein *et al*., 2012). A primary complication of the CARS response to traumatic injury is that it may actively suppress antimicrobial immunity, thus increasing the hosts susceptibility to opportunistic infections which occur during the counter inflammatory phase of injury (Osuka *et al*., 2014).

1.8.1 Regulatory T cells

Regulatory T cells are a subset of the thymic derived CD4+ T cells, and have been shown to be crucial for the maintenance of immune homeostasis and the regulation of the immune system (Okeke & Uzonna, 2019). This unique CD4+ T cell subset constitute approximately 5-10% of peripheral CD4+ T cells (Okeke & Uzonna, 2019) and are characterised by the expression of the surface interleukin-2 receptor α chain (IL-2Rα), also referred to as cell surface CD25 molecules (CD25) (Safina *et al*., 2015). The intracellular transcription factor, FOXP3 is also known to play a crucial role in the function and development of T regs (Safina *et al*., 2015). Studies have shown that the loss of FOXP3 expression in T reg cells may affect the competency of these cells acquiring effector T cell properties such as the production of cytokines IL-2, IL-4, IL-17, and IFN-γ (Wan & Flavell, 2007).

These natural regulatory T cells possess potent suppressive abilities and were initially thought to suppress CD4+CD25- T cells. Following T reg depletion, CD4+ Th cells have exhibited increased cellular activity, leading to the activation of immune cells, resulting in excessive inflammatory response, organ damage, and mortality (Okeke & Uzonna, 2014).

However, recently T regs have been recognised in the regulation of other cells within the adaptive immune system such as CD8+ T cells and B cells (Okeke & Uzonna, 2019).

The immunosuppressive qualities of T reg cells are expressed through a variety of mechanisms including, the production of anti-inflammatory cytokines, modulation of antigen presenting cell (APC) function and maturation, disruption of metabolic pathways, and the induction of apoptosis in target cells (Safina *et al*., 2015).

The ability of T reg cells to produce IL-10 and transforming growth factor-β1 also allow the suppression of pro inflammatory immune responses (Osuka *et al*., 2014).

Table 1.4 Overview of anti-inflammatory cytokines produced by T reg cells and their functions.

Cytokine	Family	Main sources	Function
IL-10	IL-10	Monocytes T cells B cells	Inhibition of the pro-inflammatory cytokines
TGF-β	TGF	Macrophages T cells	Inhibition of pro-inflammatory cytokine production

Adapted from (Chen *et al*., 2017)

The role of T reg cells in the maintenance of immunity during infection is controversial. Many studies indicate that the suppressive nature of regulatory T cells is detrimental to the host due to the limited immune response. In the case of sepsis, circulating T regs from trauma patients displayed increased T reg cell activity by 5-7 days following injury, in comparison with T reg cells from normal individuals or patients at day 1 following injury (MacConmara *et al*., 2006). Similarly, septic patients have displayed higher numbers of circulating T regs (Monneret *et al*., 2003), and an increased number of T reg cells has been associated with poor clinical outcome (Venet *et al*., 2009). However, alternative studies have reported that in vitro stimulated T reg cells caused an increase in bacterial clearance and improved survival in the murine model of sepsis (Heuer *et al*., 2005).

1.8.2 Markers of regulatory T cells

Reliable surface markers are essential for the quantification of viable T regulatory cells. These surface and intracellular markers differentiate these cells from other CD4+ T cells. The most widely used markers for T reg cells are CD25, CTLA-4, GITR, LAG-3, CD127, and Foxp3 (Corthay, 2009).

CD25 (IL-2Rα)

Upon activation all T cells express the α chain of the interleukin-2 receptor, known as CD25 (Corthay, 2009). The up-regulation of CD25 expression has been associated with increased T reg function upon tumour necrosis factor (TNF) stimulation (Chen *et al*., 2007), which may suggest that higher CD25 levels are indicative of an increase in suppressive T reg cells. The co-expression of CD4 and CD25 has also been found to define functional T reg cells within the peripheral blood. The utilization of CD25 as a definitive marker of T reg cells is problematic as it is also expressed on activated effector T cells, therefore a combination of $CD25^{+/HIGH}$ alongside other markers has been suggested in order to identify functional T regulatory cells (Chen & Oppenheim, 2011).

FOXP3

FOXP3 is an intracellular transcription factor and has been termed a 'master control gene' with regards to T reg development (Safina *et al*., 2015). Studies have shown that the expression of FOXP3 is restricted to $\alpha\beta$ T cells, whereas in $\gamma\delta$ T cells, B cells, natural killer (NK), dendritic cells, and macrophages, it is almost undetectable (Corthay, 2009). The loss of FOXP3 expression has been linked to the competency of T reg cells in acquiring certain properties including the production of IL-2, IL-17, IL-4, IFN-γ cytokines (Wan & Flavell, 2007). Based on studies which show that some effector cells may express FOXP3 with no associate regulatory activity, this marker alone cannot be deemed to be sufficient in identifying T reg cells (Corthay, 2009).

The inverse correlation between the expression of FOXP3 and CD127, on the α chain of the IL-7 receptor, with respect to the suppressive capabilities of T reg cells has been reported (Liu *et al*., 2006). Therefore, a combination of CD25, FOXP3, and CD127 are considered to be the most sufficient markers for defining T reg cells in a research setting (Safina *et al*., 2015). An increased proportion of CD4+CD25+CD127low has also been reported in thoracic trauma patients (Zhang *et al*., 2015).

1.9 Study aims and objectives

Currently there is little data available which assesses T lymphocyte cell surface markers and their role in the adaptive immune response to major trauma, which often leads to the onset of MODS and sepsis. As a variety of studies highlight that an imbalance between the SIRS and CARS responses result in an increased susceptibility to infections, analysing early changes in the components of these responses may be indicative of poor clinical outcomes.

T regulatory cells in particular, are a component of the adaptive immune response to trauma which has been linked to the counter inflammatory (CARS) reaction. These cells are known for their suppressive abilities against immune effector cells, and are most widely characterised by their expression of CD4+, CD25+, FOXP3+, and CD127Low marker profiles. Overexpression of T reg cells may therefore indicate overactivity of the CARS response and act as a predictive biomarker for late onset complications secondary to trauma such as multiple organ failure and sepsis.

In addition, although many studies have analysed cytokine expression in major trauma patients, few have characterised both the cytokine and T lymphocyte expression. It is postulated that the anergic like properties of T mediated responses result in suppressed Th1 type cytokine production and increased T reg activity, therefore a correlation between the expression of pro and anti-inflammatory cytokines with T reg cells may also be indicative of the CARS response and act as a predictive biomarker for poor clinical outcome.

Thus, the following objectives were outlined:

1) To correlate immune marker (CD3+, CD4+, CD25, CD25BRI, CD4+FOXP3+, CD25BRIFOXP3, CD127LOWFOXP3) changes with clinical outcomes (SOFA score), via full data analysis and grouped SOFA analysis.
2) To predict associations between T reg patterns and clinical outcomes.
3) To compare pro inflammatory (IL-6) and anti-inflammatory (IL-10) cytokine changes with T lymphocyte changes following traumatic injury.

CHAPTER 2: Materials and methods

2.1 Study designs

2.1.1 Patient selection criteria

A total study group of 85 major trauma patients were selected for inclusion into this project; 59 patients were from the Central Manchester Foundation Trust (CMFT), and the remaining 26 patients were from the Salford Royal Foundation Trust hospital (SRFT). All patients included in the study will have traumatic injuries severe enough to require intensive care unit admission, immediate surgery, or an injury severity score (ISS) greater than 15. As well, as this all patients under the age of 16 years old or on immunosuppressive medication were excluded from the study.

2.1.2 Data collection time points

Venous blood (20ml) was collected by medical staff working in the relevant hospitals within 24 hours of the injury. Subsequent blood samples were then collected on days 3 and 5 following the injury. A range of additional data was also collected on days 1, 3 and 5 (Table 2.1).

Table 2.1: Data collection sheet used for Day 1, 3, and 5.

DAY			DATE		
HR		BP		Temp	°C
Hb		WCC		PLT	
eGFR		Creatinine		Bilirubin	
PT		Intubated	Y/N	NIV/CPAP	Y/N
FiO2	%	P/F ratio	kPA	Lactate	mmol/L
Noradrenaline	μg/kg/min	CRP	Mg/L	CVVH/IHD	Y/N
Sedated		GCS			
Treated with antibiotics	Y/N	Source of sepsis			

HR: Heart rate, Hb: Haemoglobin, eGFR: estimated glomerular filtration rate, PT: Prothrombin time, FiO2: Fraction of inspired oxygen, BP: Blood pressure, WCC: White blood cell count, P/F ratio: Ratio of arterial oxygen partial pressure to fractional inspired oxygen, CRP: C-reactive protein, GCS: Glasgow coma scale, PLT: Platelet, NIV/CPAP: Non-invasive ventilation/Continuous positive airway pressure, CVVH/IHD: Continuous veno-venous hemofiltration/Intermittent haemodialysis.

2.2 Laboratory methods

The following laboratory procedures were carried out at the University of Salford (Cockcroft laboratory) prior to this study by PhD student Basmah Allarakia, 2018. The laboratory methods have been summarised below and the data collected from these procedures were used during this report for significant data analysis.

2.2.1 Peripheral blood mononuclear cells (PBMC) isolation and cryopreservation

Firstly, 20ml of blood was added to four 10ml centrifuge tubes. These were then diluted in an equal volume of PBS, before overlaid onto lymphoprep at a ratio of 2:1. All 4 tubes were then centrifuged at 1800 rpm for 20 minutes. PBMCs were found at an interface between lymphoprep and plasma due to the different densities of the component cells, therefore the PBMC rich interface was easily harvested and washed using 45ml of PBS, before being centrifuged at 1200 rpm for 10 minutes. Giemsa staining and microscope were then used to confirm cell pellets. Finally, the plasma layer was collected and divided between 1ml cryovials in order to be stored at -80°C for further analysis.

A cryopreservation media containing 10μl dimethyl-sulfoxide (DMSO) and 900μl fetal calf serum (FCS) was then used to re-suspend the PBMC pellet aliquoted in cryovials. These were placed into a container containing isopropanol and placed at -80°C. After 24 hours, all vials were stored in liquid nitrogen for future analysis.

2.2.2 Retrieval of PBMCs

In order to check the viability of retrieved PBMCs, 1 part of trypan blue dye was mixed with 1 part cells and applied to a disposable haemocytometer. Total cell numbers were calculated using the following formula:

PBMC/mL = Average number of PBMCs counted X dilution factor X 10^4

2.2.3 PBMC enrichment

200μl of anticoagulant treated whole blood was added to 2ml of diluted red blood cells (RBC) lysing buffer, containing 9 parts distilled water and 1 part 10X RBC lysis buffer. This was vortexed and intubated at room temperature, in the dark for 5-10 minutes. Following this the lysate was centrifuged at 1800 rpm for 10 minutes, cell pellets were also re-suspended in 2ml of diluted RBC lysis buffer, and a lysis was carried out a second time as mentioned above. Following centrifugation at 120 rpm for 10 minutes, cell pellets were finally re-suspended to 1ml of PBS and transferred into a 5ml tube for flow cytometry.

20ml of whole blood was centrifuged at 3000 rpm for 5 minutes in order to obtain the buffy coat layer. This buffy coat was removed from an interface between plasma and blood and washed with RBC BD lysis buffer. Finally, cell pellets were removed and aliquoted in 1ml cryovials and stored at -80°C. Similarly, the plasma layer was also aliquoted into 1ml cryovials and stored in the same manner.

2.2.4 Giemsa staining and microscopy

Smears of PBMC preparations were fixed in methanol. 1ml of concentrated giemsa stain was added to 9ml of giemsa buffer solution, and the slide stained for 20 minutes. An oil emersion objective lens (100x) from a light microscope was used for observation.

2.2.5 Immuno-fluorescent staining of T cell surface markers

A 100μl PBMC extract obtained in (section 2.2.3) was added to fluorochrome-labelled conjugated antibodies (Table 2.2). The selection of the antibody panels was dependant on the expression/density of antigens and the fluorochrome brightness (Table 2.3). The tubes were gently mixed and an additional 100μl of PBS was added. All tubes were incubated at 4°C for 30 minutes and shielded from light. Cells were then washed with 1ml of PBS, before being centrifuged at 1800 rpm for 10 minutes. The remaining cell-pellets were resuspended in 500μl of PBS and the supernatant was discarded.

Table 2.2: Cellular antibodies

Surface antibody	μl
CD3	1.5
CD4	1.5
CD8	1.5
CD25	1.5
CD127	10

(Allarakia, 2018).

Table 2.3: Antibody panel used for T reg cell staining

Antibodies panel	Clone	Fluorescence	Purpose
CD3	UCHT1	(PerCP-Cy5)	T cell subsets
CD4	RAP-T4 (RUO)	(APC-A)	T cell subsets
CD8	SK1	(APC-CY7-A)	T cell subsets
CD25	M-A251	(PE-Cy7-A)	T cell activation T reg identification
CD127	HIL-7R-M21 (RUO)	(FITC)	T reg marker
FoxP3	PCH101	(PE)	T reg identification

(Allarakia, 2018).

2.2.6 Staining for FOXP3+ transcription markers

The mixture of surface stained cells from (section 2.2.5) were resuspended in 1ml of Foxp3 fixation/permeabilization buffer, vortexed and incubated in the dark at 4°C for 30-60 minutes. 2ml of diluted permeabilization buffer was then added, followed by 5 minutes of centrifugation at 1800 rpm. Cell pellets were then resuspended in 2μl of 2% normal rat serum and 100μl diluted permeabilization buffer, vortexed and incubated in the dark for 15 minutes.

Next, 3μl of anti-human FoxP3-PE was added to each tube, vortexed and incubated for 60 minutes. Following this, the cells were resuspended in 2ml of diluted permeabilization buffer and centrifuged for 5 minutes at 1800 rpm, before being resuspended in 2ml flow cytometry staining buffer and centrifuged once again for 5 minutes at 1800 rpm. Finally, the cell pellet was resuspended in 1ml of flow cytometer staining buffer.

2.2.7 Flow cytometry analysis

FACS Verse flow cytometry was used to analyse the samples and FACS Suite software was used for data analysis. Forward (FSC) versus side scatter (SSC) displays were used to encircle the lymphocyte region and all fluorescent parameters were gated on this population in order to detect specific CD receptor subsets. The results were displayed as the percentage of cells which fell above the negative region for each of the antibodies.

2.2.8 Gating strategy

Gating strategy was created using the BD FAC Suite software. This allowed visualisation and differentiation of lymphocytes according to their granularity and cell size. A gate was created around the lymphocyte region and all data analysis was restricted to this cell population. Further gates according to specific antibody labelled sub populations of T cells was done for further analysis of these subsets. The percentage of positive cells of the gate was recorded.

2.3 Statistical analysis

All statistical analysis was done during this study using the SPSS statistical software.

2.3.1 Statistical analysis of early T cell expression and late SOFA scores

Firstly, in order to explore the relationship between T lymphocytes, specifically T reg cells, and poor clinical outcomes, the mean T lymphocyte (CD3+, CD4+, CD8+, CD25BRI, CD25BRIFOXP3+, CD4+FOXP3+, and CD127LOWFOXP3+) expression on Day 1 and 3 were tested against mean Day 5 SOFA scores using a Spearman’s rank correlation test.

A Spearman’s rank correlation test is used to analyse the relationship between two measurements which are not clearly unaffected by each other, when data is not normally distributed. Therefore, this was done in order to identify early expression of T reg cells which may correlate to poor outcome, represented by higher SOFA scores at a later date.

2.3.2 Statistical analysis of early T cell expression and late grouped SOFA scores

In order to test the hypothesis that poor clinical outcome is linked to higher T reg expression, patients were separated into two groups based on their Day 5 SOFA scores.

The mean T cell expression (CD3+, CD4+, CD8+, CD25BRI, CD25BRIFOXP3+, CD4+FOXP3+, and CD127LOWFOXP3+) on Day 1 was then tested against group 1 and then group 2. A paired T test was used as the statistical test as it effectively highlights significant differences between measurements which are in matched pairs, such as SOFA <1 and SOFA >1 in this case.

2.3.3 Statistical analysis of T cell and interleukin expression

In order to explore the relationship between T cell expression, particularly T reg cells, and other mediators of inflammation, all CD markers on Day 1 were tested against interleukin 6 and interleukin 10 also on Day 1, 3, and 5.

Similar to section (2.3.1), a Spearman's rank correlation test was the statistical test chosen for this analysis in order to explore relationships between two measurements which are not clearly unaffected by each other.

CHAPTER 3: Results

3.1 Trends in T cell subset expression on Day 1, 3, and 5 following traumatic injury.

A study group of 85 patients consented to provide T cell subset expression data for this study. On Day 1 the maximum data from all 85 patients was obtained. On Day 3, this reduced to 80, and on Day 5 reduced further to 76, due to deceased and discharged patients. The trends of T cell expression on all of these days were displayed by obtaining the average (mean) expression on each day for each subset, the results of which are displayed in a simple column graph (Figure 3.1).

As expected the CD4+/CD8+ ratio, an indicator of immune activity, continues to increase from day 1 to 5 (0.62, 0.65, and 0.68 respectively). Both CD125BRIFOXP3+ and CD127LOWFOXP3+, which are known to be efficient markers of T reg cells displayed similar trends as a decrease in expression is seen from Day 1 to 3 followed by a further increase on Day 5. Similar to CD4+ a steady increase was also seen in CD4+FOXP3+ expression.

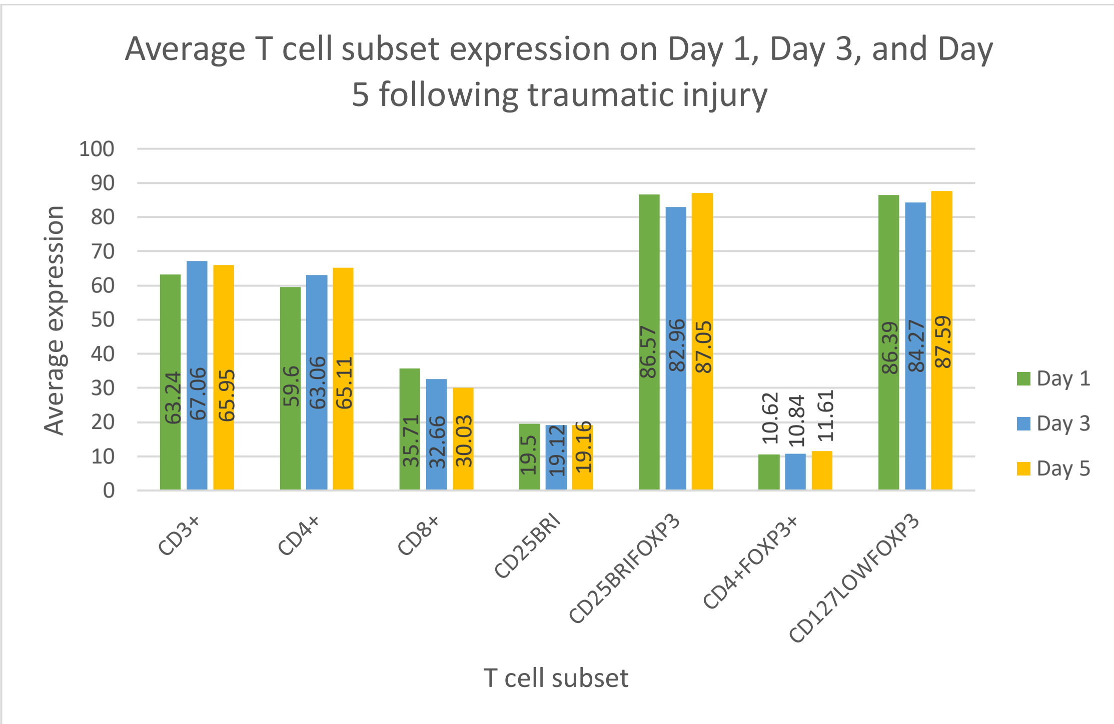

Figure 3.1: Trends in T cell subset expression on Day 1, 3, and 5 following traumatic injury. *(Day 1 n=85, Day 3 n=80, Day 5 n=76).*

3.2 Relationships between mean T cell data and SOFA scores.

A maximum study group of 85 major trauma patients were selected for inclusion into this project; 59 patients were from the Central Manchester Foundation Trust (CMFT), and the remaining 26 patients were from the Salford Royal Foundation Trust hospital (SRFT). On Day 1 patient cohort size for tests against CD3+, CD4+, CD8+, CD25BRI, CD25BRIFOXP3+, and CD4+FOXP3+ was 85. However, for CD127LOWFOXP3+ analysis, patient cohort was 74, consisting of 48 CMFT and 26 SRFT patients. This was due to incomplete CD127LOWFOXP3+ expression data from CMFT patients. On Day 3, due to death or discharge of additional patients, overall cohort size was 80, consisting of 56 CMFT patients and 24 SRFT patients. Once again, CD127LOWFOXP3+ patient cohort was lower at a total of 70; 46 from CMFT and 24 from SRFT.

As presented in (Table 1.2), the overall SOFA score was calculated for each patient on Day 1, 3, and 5 following injury, composing of SOFA scores from all six organs, with the maximum score possible being 24.

In order to test the hypothesis that T reg cells are involved in the immune response which may result in a poor clinical outcome, a Spearman's correlation test was done using SPSS Statistical software in order to identify relationships between average patient CD values (CD3+, CD4+, CD8+, CD25BRI, CD25BRIFOXP3+, CD4+FOXP3+, CD127LOWFOXP3+) on Day 1 and their SOFA scores on DAY 5 following injury. This was also done for each CD marker on DAY 3 against SOFA scores on DAY 5 (Figure 3.1, - 3.7). The results of this may allow identification of useful markers whose expression on Day 1 or 3 following injury may be indicative of clinical outcomes on Day 5, represented by SOFA scores.

A Spearman's correlation test showed that there is a significant relationship between patient CD3+ levels on Day 1 and their SOFA scores on Day 5 ($p=0.007$), as well as CD3+ on Day 3 and SOFA scores on Day 5 ($p=0.02$). All other CD markers on Day 1 failed to display any significant relationship to Day 5 SOFA scores. However, both CD25BRIFOXP3+ and CD127LOWFOXP3+ displayed significant relationships on Day 3 when tested against Day 5 SOFA scores ($p=0.049$ and $p=0.048$ respectively). (Table 3.1).

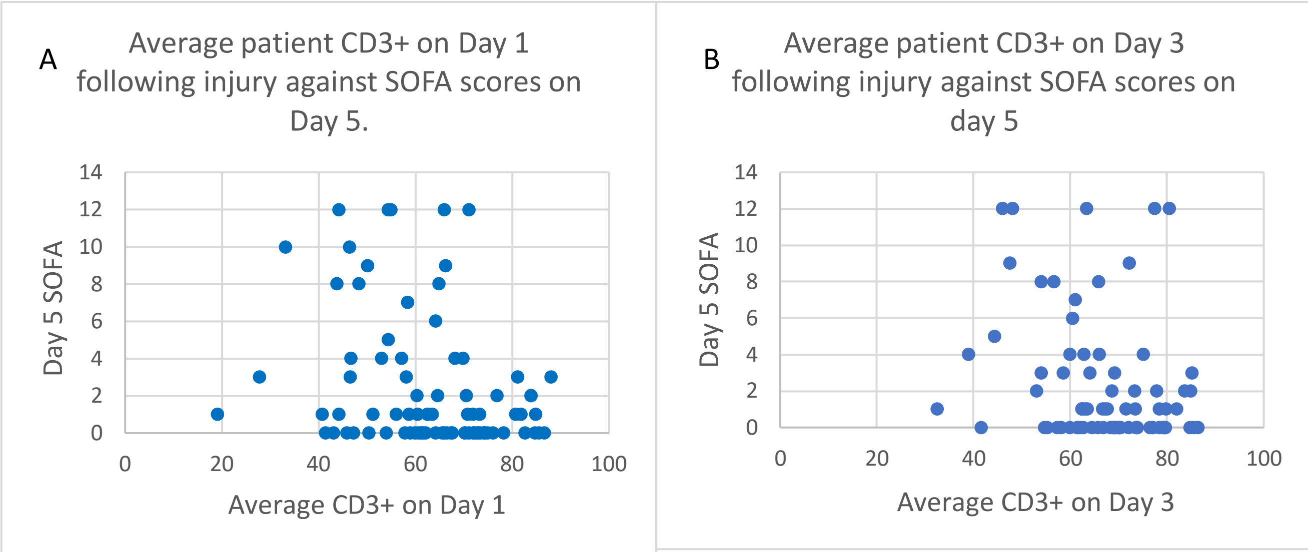

Figure 3.1: (A) Simple scatter displaying the average CD3+ values on Day 1 following traumatic injury against SOFA scores on Day 5 (n=85). (B) Simple scatter displaying the average CD3+ values on DAY 3 following injury against SOFA scores on DAY 5 (n=80). *(Spearman's correlation was carried out to find relationship between Day 1 CD3+ and Day 5 SOFA, as well as Day 3 CD3+ and Day 5 SOFA. (Part A; p=0.007) (Part B; p= 0.02)).*

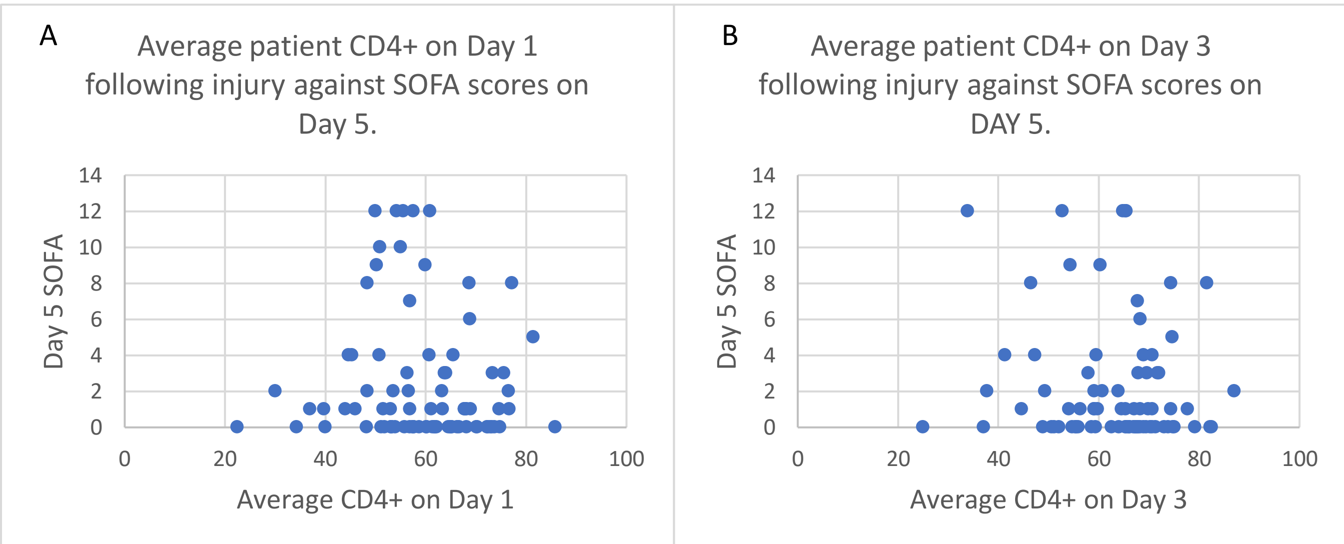

Figure 3.2: (A) Simple scatter displaying the average CD4+ values on Day 1 following traumatic injury against SOFA scores on Day 5 (n=85). (B) Simple scatter displaying the average CD4+ values on DAY 3 following injury against SOFA scores on DAY 5 (n=80). *(Spearman's correlation was carried out to find relationship between Day 1 CD4+ and Day 5 SOFA, as well as Day 3 CD4+ and Day 5 SOFA. (Part A; p=0.257) (Part B; p= 0.474)).*

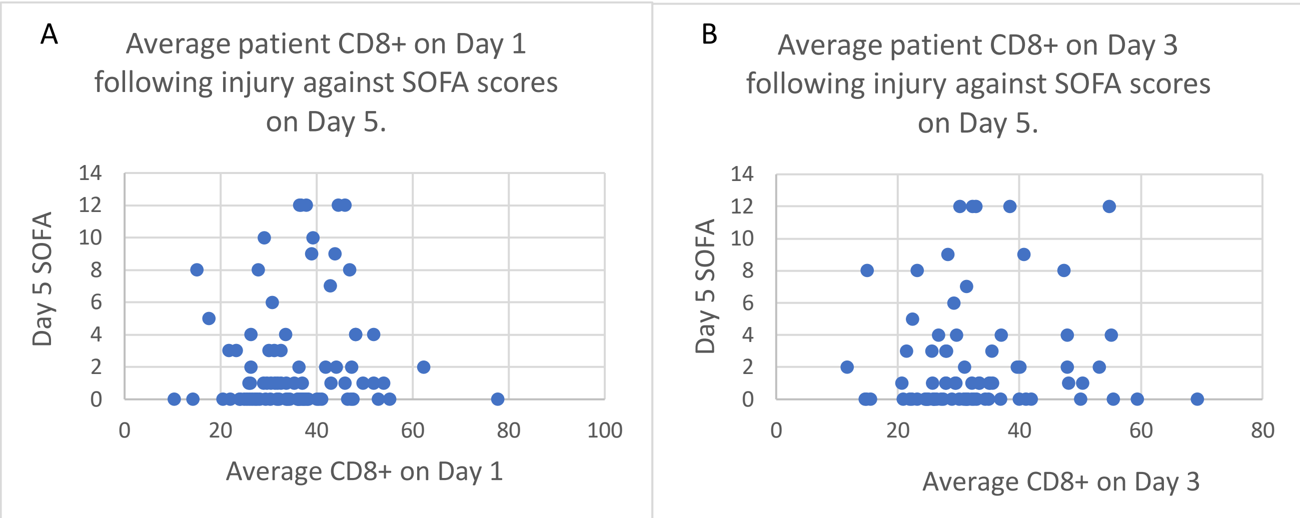

Figure 3.3: (A) Simple scatter displaying the average CD8+ values on Day 1 following traumatic injury against SOFA scores on Day 5 (n=85). (B) Simple scatter displaying the average CD8+ values on DAY 3 following injury against SOFA scores on DAY 5 (n=80). *(Spearman's correlation was carried out to find relationship between Day 1 CD8+ and Day 5 SOFA, as well as Day 3 CD8+ and Day 5 SOFA. (Part A; p=0.215) (Part B; p= 0.152)).*

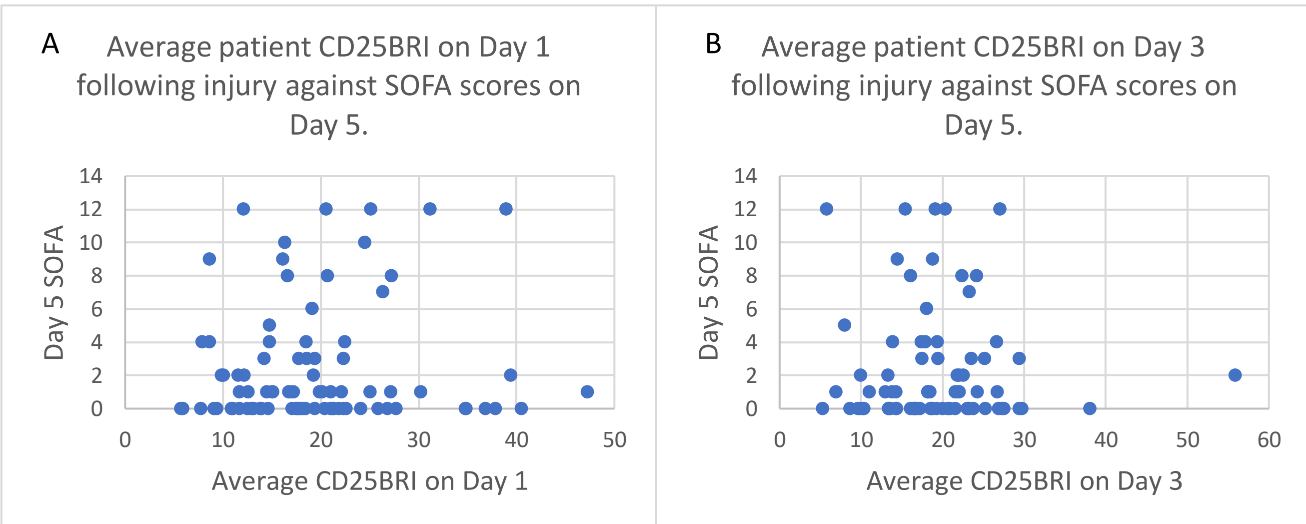

Figure 3.4: (A) Simple scatter displaying the average CD25BRI values on Day 1 following traumatic injury against SOFA scores on Day 5 (n=85). (B) Simple scatter displaying the average CD25BRI values on DAY 3 following injury against SOFA scores on DAY 5 (n=80). *(Spearman's correlation was carried out to find relationship between Day 1 CD25BRI and Day 5 SOFA, as well as Day 3 CD25BRI and Day 5 SOFA. (Part A; p=0.775) (Part B; p= 0.665)).*

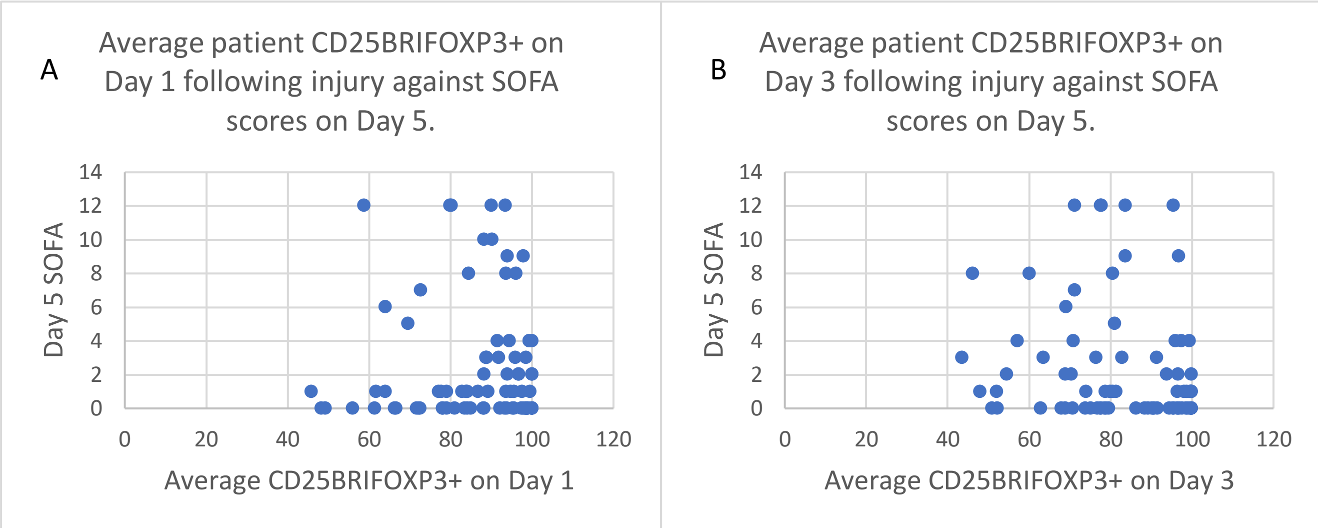

Figure 3.5: (A) Simple scatter displaying the average CD25BRIFOXP3+ values on Day 1 following traumatic injury against SOFA scores on Day 5 (n=85). (B) Simple scatter displaying the average CD25BRIFOXP3+ values on DAY 3 following injury against SOFA scores on DAY 5 (n=80).

(Spearman's correlation was carried out to find relationship between Day 1 CD25BRIFOXP3+ and Day 5 SOFA, as well as Day 3 CD25BRIFOXP3+ and Day 5 SOFA. (Part A; p=0.861) (Part B; p= 0.049)).

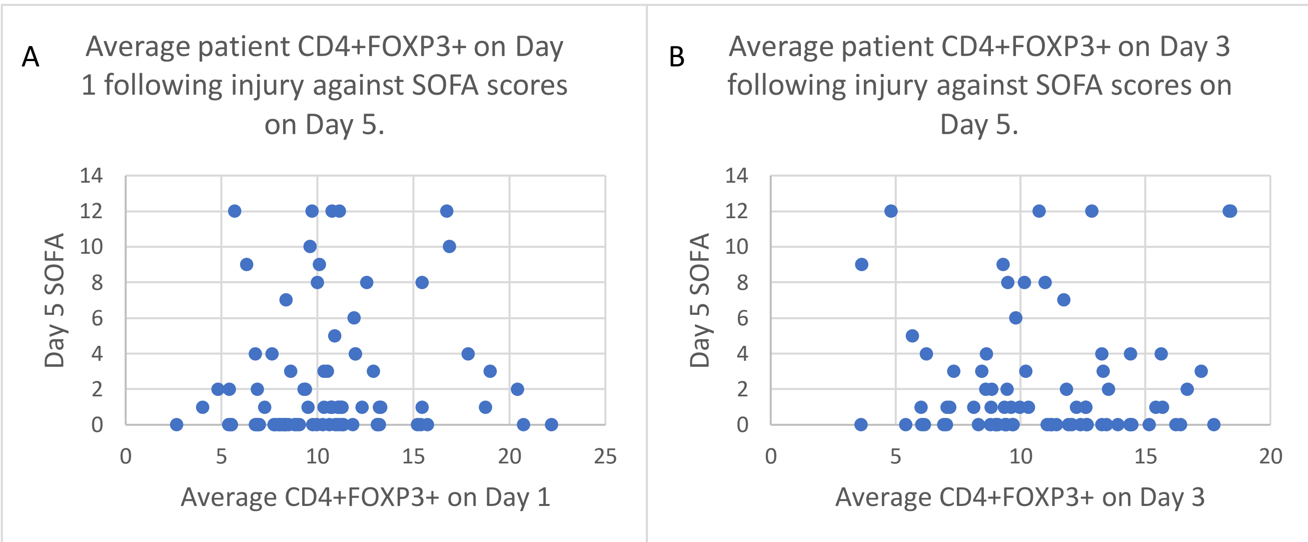

Figure 3.6: (A) Simple scatter displaying the average CD4+FOXP3+ values on Day 1 following traumatic injury against SOFA scores on Day 5 (n=85). (B) Simple scatter displaying the average CD4+FOXP3+ values on DAY 3 following injury against SOFA scores on DAY 5 (n=80).

(Spearman's correlation was carried out to find relationship between Day 1 CD4+FOXP3+ and Day 5 SOFA, as well as Day 3 CD4+FOXP3+ and Day 5 SOFA. (Part A; p=0.147) (Part B; p= 0.963)).

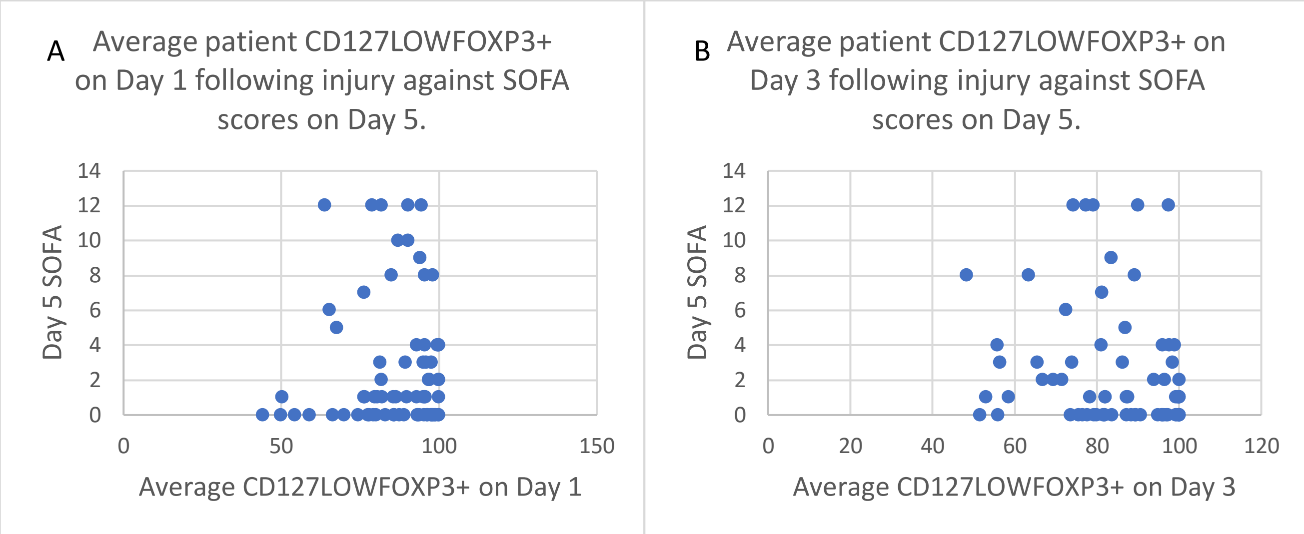

Figure 3.7: (A) Simple scatter displaying the average CD127LOWFOXP3+ values on Day 1 following traumatic injury against SOFA scores on Day 5 (n=74). (B) Simple scatter displaying the average CD127LOWFOXP3+ values on DAY 3 following injury against SOFA scores on DAY 5 (n=70).

(Spearman's correlation was carried out to find relationship between Day 1 CD127LOWFOXP3+ and Day 5 SOFA, as well as Day 3 CD127LOWFOXP3+ and Day 5 SOFA. (Part A; p=0.637) (Part B; p= 0.048)).

Table 3.1: Summary of p values generated from a Spearman's correlation test in order to test relationships between average CD values on day 1 and 3 against SOFA scores on Day 5.

	DAY 1 CD VS DAY 5 SOFA (P value)	Sig	DAY 3 CD VS DAY 5 SOFA (P value)	Sig
CD3+	0.007	**	0.02	*
CD4+	0.257		0.474	
CD8+	0.215		0.152	
CD25BRI	0.775		0.665	
CD4+FOXP3+	0.147		0.963	
CD25BRIFOXP3	0.861		0.049	*
CD127LOWFOXP3	0.637		0.048	*

Sig = Significance
**= Correlation is significant at the 0.05 level*
*** = Correlation is significant at the 0.01 level*

3.3 Relationship between mean T cell data and grouped SOFA scores

In order to test the hypothesis that higher expression of T reg cells are seen in patients with poorer clinical outcome, patients were separated into two groups; those with SOFA scores less than 1 on Day 5, and those with SOFA scores greater than 1 on Day 5. The mean CD marker expression on Day 1 of each patient were then tested against the two groups of Day 5 SOFA scores. Group 1 consisted of 38 patients; 34 from CMFT and 4 from SRFT, all with a Day 5 SOFA score of 0. Group 2 consisted of 43 patients; 23 from CMFT and 20 from SRFT, with Day 5 SOFA scores ranging from 1 to 12. A paired T test was used to highlight any significant differences.

In the case of the CD markers CD3+, CD4+, CD25BRI, and CD4+FOXP3+, a lower expression was seen on Day 1 for those patients which displayed SOFA scores greater than 1 on Day 5 (Figures 3.8 – 3.11). However, a paired T test highlighted that none of these differences were significant (p=0.052, p=0.550, p=0.173, and p=0.278 respectively). Alternatively, CD8+, CD25BRIFOXP3, and CD127LOWFOXP3 displayed higher expression on Day 1 for those patients which displayed SOFA scores greater than 1 on Day 5 (Figures 3.12 – 3.14). As anticipated, a paired T test highlighted that this was statistically significant for CD25BRIFOXP3 and CD127LOWFOXP3 (p=0.05, and p=0.03 respectively).

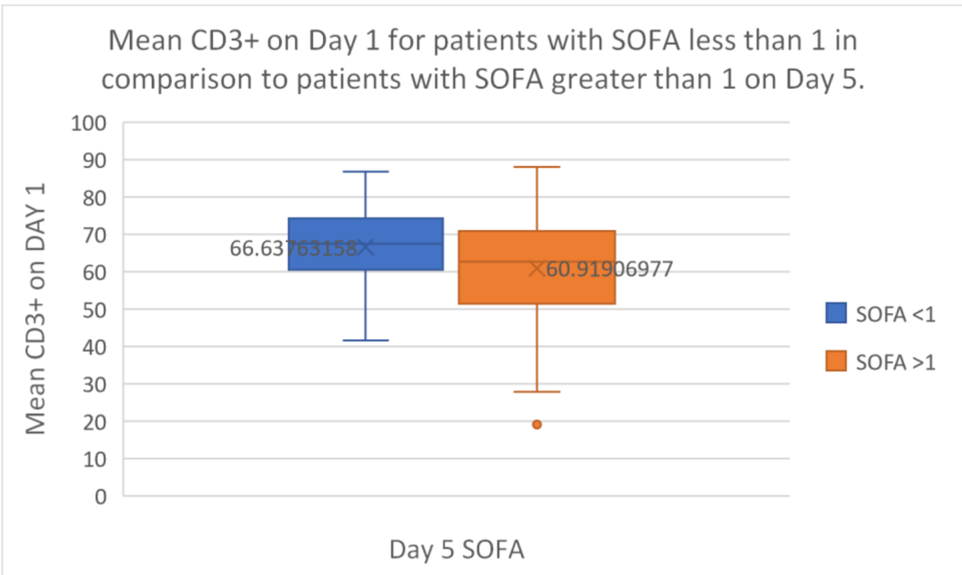

Figure 3.8: Simple histogram comparing mean CD3+ on Day 1 for patients with a SOFA score of less than 1 with patients with a SOFA score greater than 1 on Day 5.
(SOFA <1 N= 66.64, SOFA >1 N=60.92). *A paired T test found (p=0.052).*

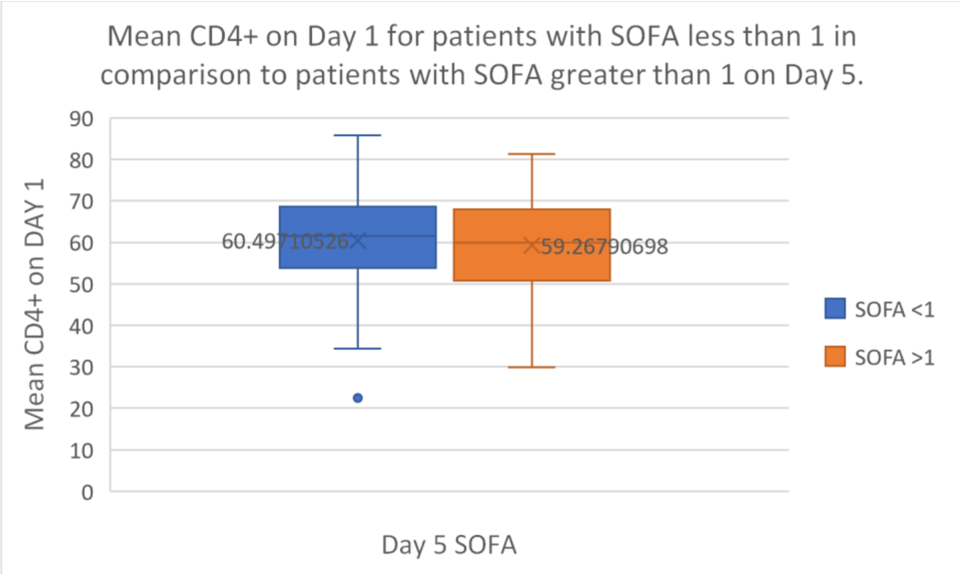

Figure 3.9: Simple histogram comparing mean CD4+ on Day 1 for patients with a SOFA score of less than 1 with patients with a SOFA score greater than 1 on Day 5.
(SOFA <1 N= 60.50, SOFA >1 N=59.27). *A paired T test found (p=0.550).*

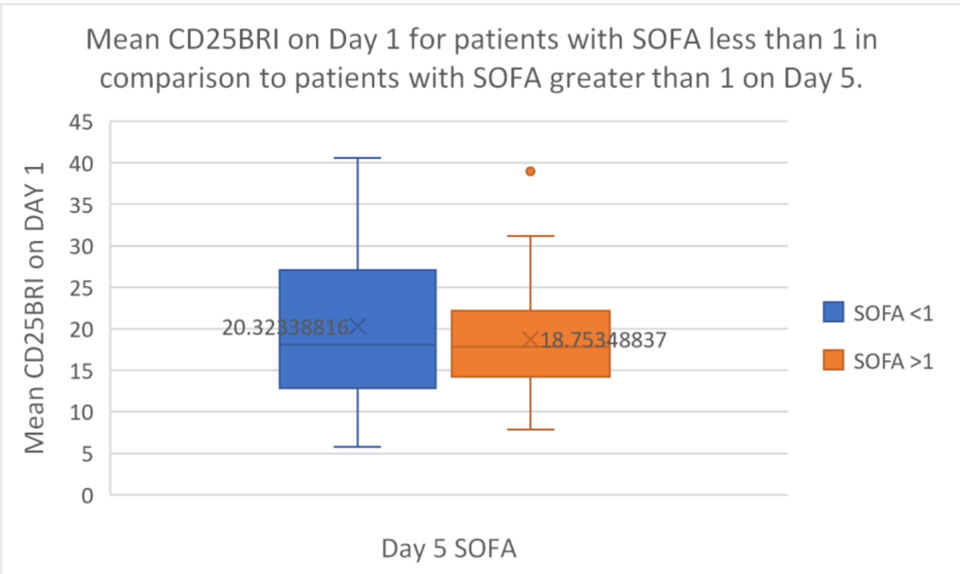

Figure 3.10: Simple histogram comparing mean CD25BRI on Day 1 for patients with a SOFA score of less than 1 with patients with a SOFA score greater than 1 on Day 5.
(SOFA <1 N= 20.32, SOFA >1 N=18.75). *A paired T test found (p=0.173).*

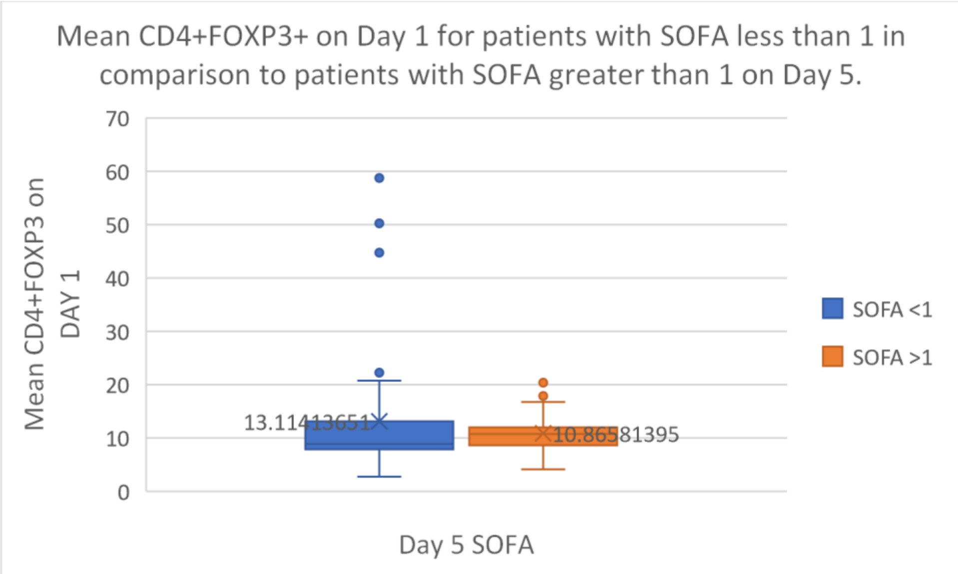

Figure 3.11: Simple histogram comparing mean CD4+FOXP3+ on Day 1 for patients with a SOFA score of less than 1 with patients with a SOFA score greater than 1 on Day 5.
(SOFA <1 N= 13.11, SOFA >1 N=10.87). *A paired T test found (p=0.278).*

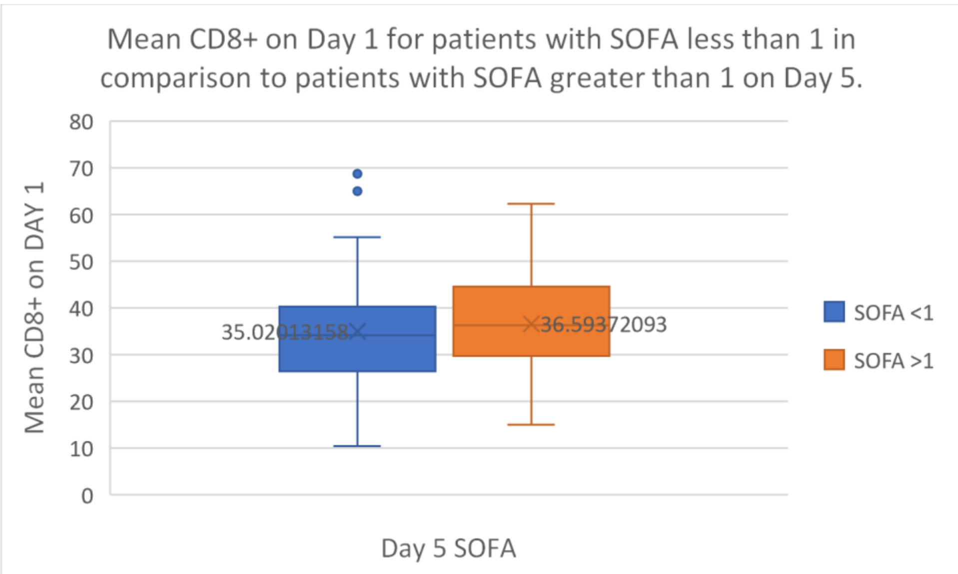

Figure 3.12: Simple histogram comparing mean CD8+ on Day 1 for patients with a SOFA score of less than 1 with patients with a SOFA score greater than 1 on Day 5.
(SOFA <1 N= 35.02, SOFA >1 N=36.59). *A paired T test found (p=0.332).*

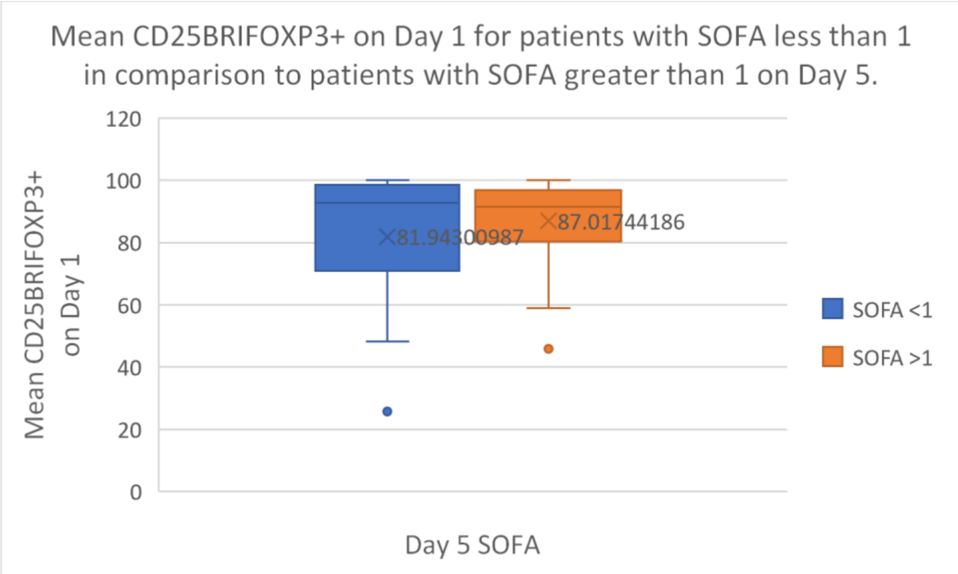

Figure 3.13: Simple histogram comparing mean CD25BRIFOXP3+ on Day 1 for patients with a SOFA score of less than 1 with patients with a SOFA score greater than 1 on Day 5. (SOFA <1 N= 81.94, SOFA >1 N=87.02). *A paired T test found (p=0.05).*

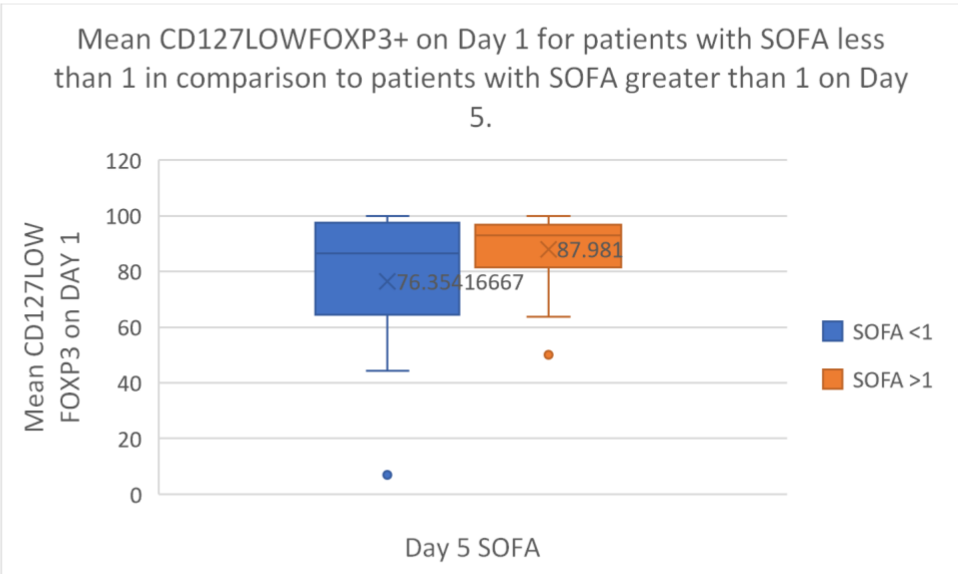

Figure 3.14: Simple histogram comparing mean CD127LOWFOXP3+ on Day 1 for patients with a SOFA score of less than 1 with patients with a SOFA score greater than 1 on Day 5. (SOFA <1 N= 76.35, SOFA >1 N=87.98). *A paired T test found (p=0.03).*

Table 3.2: Summary of difference in mean Day 1 T cell expression between patients with a Day 5 SOFA score less than 1 and patients with a Day 5 SOFA score greater than 1.

	Average expression on Day 1 when Day 5 SOFA is <1	Average expression on Day 1 when Day 5 SOFA is >1	Sig
CD3+	66.64	60.92	
CD4+	60.50	59.27	
CD25BRI	20.32	18.75	
CD4+FOXP3+	13.11	10.87	
CD8+	35.02	36.59	
CD25BRIFOXP3+	81.94	87.04	*
CD127LOWFOXP3+	76.35	87.38	**

Sig = Significance
**= Difference is significant at the 0.05 level*
*** = Difference is significant at the 0.01 level*

3.4 Relationship between T cell data and Interleukin 6 (IL-6) and interleukin 10 (IL-10).

In order to explore the relationship between CD markers, particularly T reg cells, and other mediators of inflammation, all CD markers used in this study (CD3+, CD4+, CD8+, CD25BRI, CD25BRIFOXP3+, CD4+FOXP3+, and CD127LOWFOXP3+), were tested against interleukin 6 (IL-6) and interleukin 10 (IL-10).

A study group of 60 patients was used and IL-6 and IL-10 expression was determined on Day 1, 3, and 5 following injury. A Spearman's rank correlation was done on Day 3 in order to identify any relationships between IL-6 and T reg cells , as well as IL-10 and T reg cells. For Day 1 analysis of CD markers against IL-6 and IL-10, patient cohort size was 60; 41 from CMFT and 19 from SRFT. This was reduced to 52 on Day 3; 40 from CMFT and 12 from SRFT due to incomplete patient records. Finally, it was increased on Day 5 again to 59; 41 from CMFT and 18 from SRFT due to one death.

Interestingly, significant correlations were only seen between T reg markers and interleukins on Day 3, similar to results seen in section (3.1) between Day 3 T regs and Day 5 SOFA scores. A Spearman's rank correlation test showed that there is a significant relationship between patient CD3+ and IL-10 levels on Day 3 (p-0.000) (Figure 3.15 (B)). All other CD markers failed to display any significant relationship to IL-10. However, as expected both CD25BRIFOXP3+ and CD127LOWFOXP3+ T reg cells displayed significant relationships on Day 3 when tested against IL-6 (p=0.026 and p=0.029 respectively) (Figure 3.19 (A) and 3.21 (A) respectively).

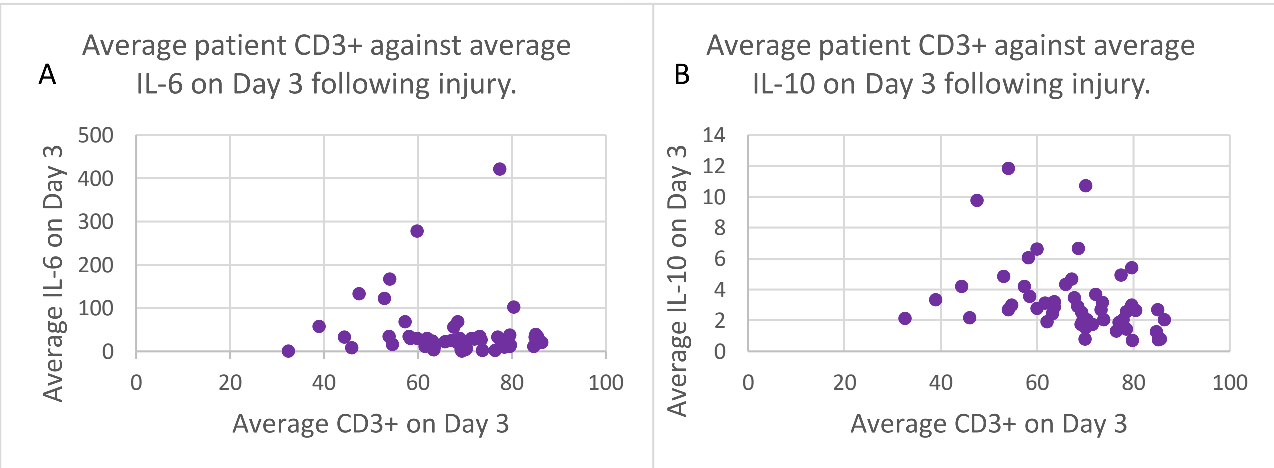

Figure 3.15: (A) Simple scatter displaying the relationship between average CD3+ expression against average IL-6 expression on Day 3 following injury (n=52). (B) Simple scatter displaying the relationship between average CD3+ expression against average IL-10 expression on Day 3 following injury (n=52).
Spearman's rank correlation found (Part A; p=0.308) (Part B; p=0.000).

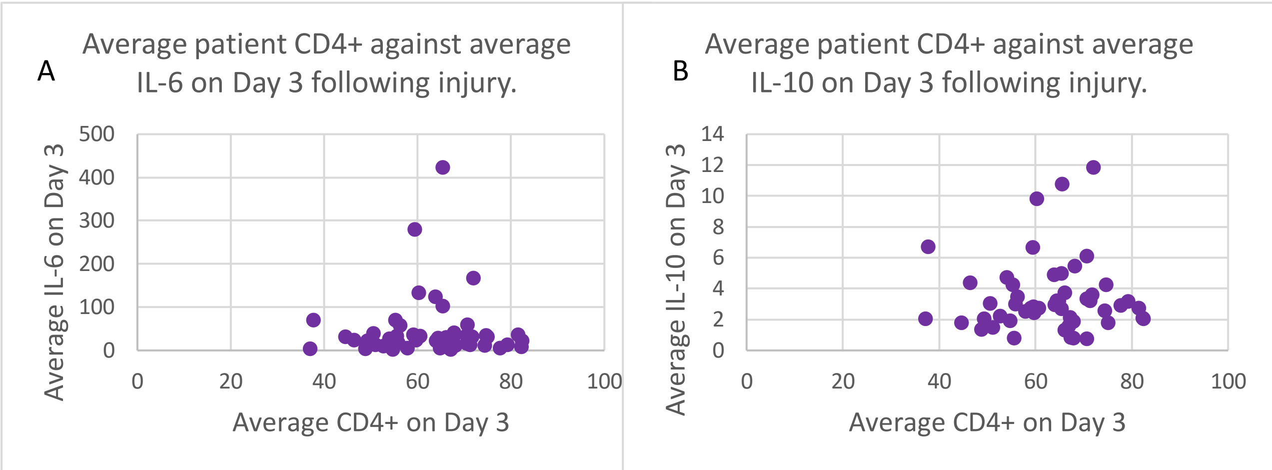

Figure 3.16: (A) Simple scatter displaying the relationship between average CD4+ expression against average IL-6 expression on Day 3 following injury (n=52). (B) Simple scatter displaying the relationship between average CD4+ expression against average IL-10 expression on Day 3 following injury (n=52).

Spearman's rank correlation found (Part A; p=0.933) (Part B; p=0.877).

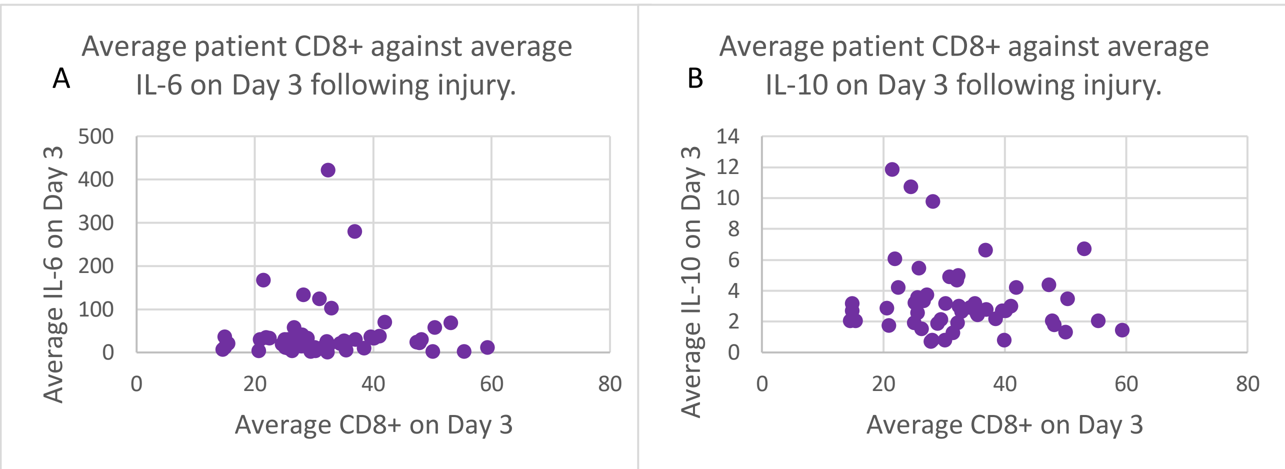

Figure 3.17: (A) Simple scatter displaying the relationship between average CD8+ expression against average IL-6 expression on Day 3 following injury (n=52). (B) Simple scatter displaying the relationship between average CD8+ expression against average IL-10 expression on Day 3 following injury (n=52).

Spearman's rank correlation found (Part A; p=0.581) (Part B; p=0.410).

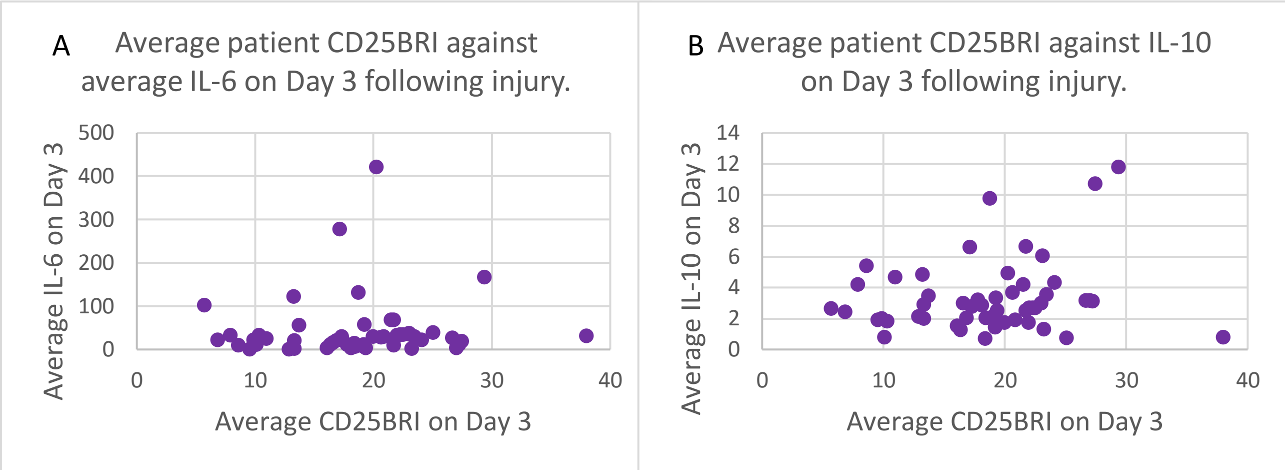

Figure 3.18: (A) Simple scatter displaying the relationship between average CD25BRI expression against average IL-6 expression on Day 3 following injury (n=52). (B) Simple scatter displaying the relationship between average CD25BRI expression against average IL-10 expression on Day 3 following injury (n=52).

Spearman's rank correlation found (Part A; p=0.200) (Part B; p=0.277).

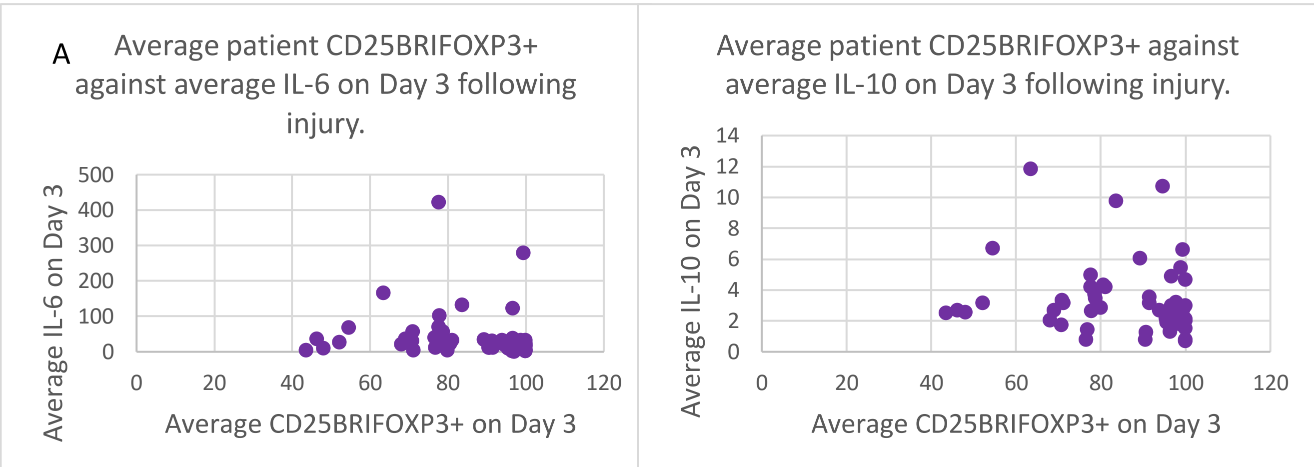

Figure 3.19: (A) Simple scatter displaying the relationship between average CD25BRIFOXP3+ expression against average IL-6 expression on Day 3 following injury (n=52). (B) Simple scatter displaying the relationship between average CD25BRIFOXP3+ expression against average IL-10 expression on Day 3 following injury (n=52).

Spearman's rank correlation found (Part A; p=0.026) (Part B; p=0.113).

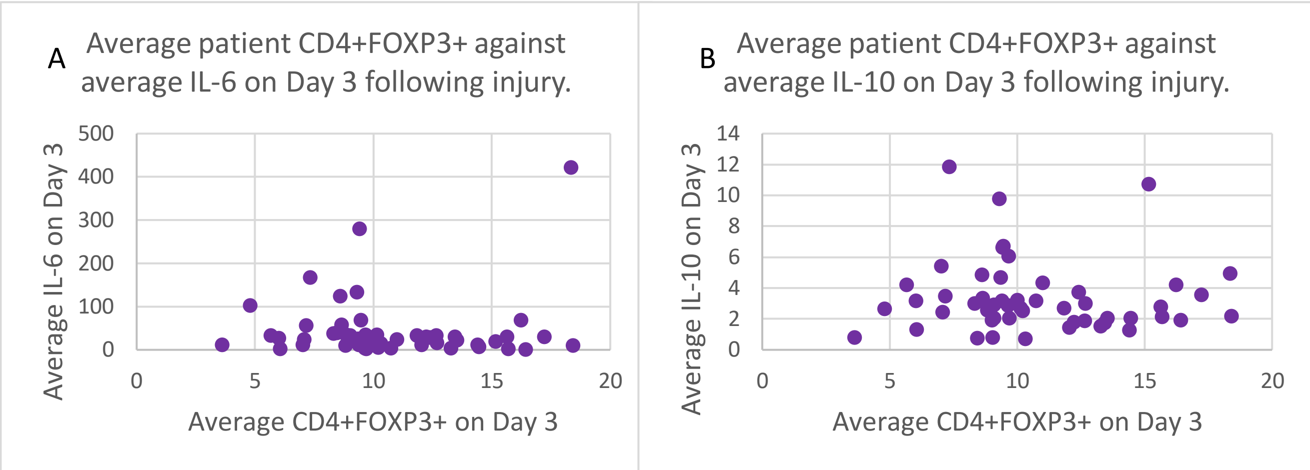

Figure 3.20: (A) Simple scatter displaying the relationship between average CD4+FOXP3+ expression against average IL-6 expression on Day 3 following injury (n=52). (B) Simple scatter displaying the relationship between average CD4+FOXP3+ expression against average IL-10 expression on Day 3 following injury (n=52).
Spearman's rank correlation found (Part A; p=0.083) (Part B; p=0.535).

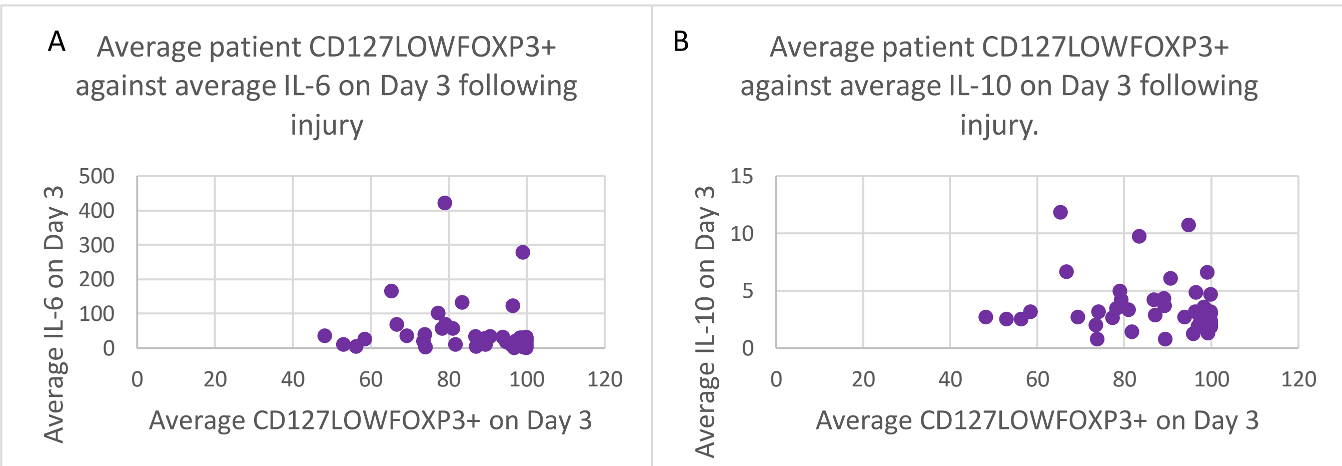

Figure 3.21: (A) Simple scatter displaying the relationship between average CD127LOWFOX3+ expression against average IL-6 expression on Day 3 following injury (n=45). (B) Simple scatter displaying the relationship between average CD127LOWFOXP3+ expression against average IL-10 expression on Day 3 following injury (n-45).
Spearman's rank correlation found (Part A; p=0.029) (Part B; p=0.155).

Table 3.2: Summary of p values generated from a Spearman's rank correlation test in order to test relationships between average CD expression with average IL-6 and IL-10 expression on Day 3.

	DAY 3 CD VS DAY 3 IL-6 (P value)	Sig	DAY 3 CD VS DAY 3 IL-10 (P value)	Sig
CD3+	0.308		0.000	**
CD4+	0.933		0.877	
CD8+	0.581		0.410	
CD25BRI	0.200		0.277	
CD4+FOXP3+	0.083		0.535	
CD25BRIFOXP3	0.026	*	0.113	
CD127LOWFOXP3	0.029	*	0.155	

Sig = Significance
**= Correlation is significant at the 0.05 level*
*** = Correlation is significant at the 0.01 level*

CHAPTER 4: Discussion

4.1 Trends in T lymphocyte expression following traumatic injury

Major traumatic injury is responsible for initiating a complex immunosuppressive response which is often associated with late multiple organ dysfunction syndrome (MODS) or opportunistic infections. Following the initial response to traumatic injury, cells of the innate and adaptive immune system initiate the proinflammatory (SIRS) response as well as a counter inflammatory response (CARS) in order to restore immune homeostasis (Stoecklein *et al*., 2012). Multiple organ dysfunction syndrome (MODS) accounts for a large fraction of the healthcare resources related to acute trauma care. It is often associated with poor clinical outcomes as it is common among critically injured patients who may survive the initial trauma (Cole *et al*., 2019). Early identification and prevention of MODS would therefore allow a large reduction in late phase mortality due to trauma. The first objective of this chapter aims to discuss the trends in T lymphocyte expression following traumatic injury.

One indicator of immune function is the CD4+/CD8+ ratio, with a ratio of less than 1 being associated with biomarkers of inflammation in studies assessing HIV therapies (Davy-Mendez *et al*., 2018). As shown in (Figure 3.1) the expression of CD4+ continued to increase from Day 1 to 5 following traumatic injury, whereas CD8+ expression continued to decrease. An elevated CD4+/CD8+ ratio was observed as a result of this (0.62, 0.65, 0.68), indicating elevated immunoactivity on each day following injury. The total lymphocyte count of CD4+ on Day 1, 3, and 5 following injury displayed an increasing trend. These findings are consistent with previous work which highlighted a rise of T cell populations in mice exposed to trauma (Rani & Schwacha, 2017). Alternatively, the decrease in CD8+ from day 1, 3, and 5, correlates to previous studies which proposed that the reduction in CD8+ T cells in major trauma patients may compromise their ability to establish an effective response to pathogens as well as enhancing their susceptibility to infectious disease (Sommershof *et al*., 2009).

Interestingly, trends in expression were also seen in CD4+FOXP3+, CD25BRIFOXP3, and CD127LOWFOXP3+, all of which have been recognised as effective markers of T reg cells. Studies have previously highlighted the role of T reg cells during traumatic injury due to their suppressive abilities. In the case of sepsis, circulating T regs from trauma patients displayed increased T reg cell activity by 5-7 days following injury, in comparison with T reg cells from normal individuals or patients at day 1 following injury (MacConmara *et al*., 2006). This is also highlighted in Figure 3.1 by CD4+FOXP3+, CD25BRIFOXP3+ and CD127LOWFOXP3+ as expression is elevated on Day 5 following injury.

The overall trend seen by CD25BRIFOXP3+ and CD127LOWFOXP3+ was an increase in expression from Day 1 to Day 5 following injury, however, a decrease was seen on Day 3 prior to the increase on Day 5. CD25BRIFOXP3+ expression initially decreased from Day 1 to Day 3 (N= 86.57, N= 82.96 respectively), as was the case for CD127LOWFOXP3+ (N= 86.39, N=84.27). This may correlate to studies done to analyse regulatory T cell dynamics following porcine polytrauma. In a study which analysed 20 peripheral blood samples with an injury severity score >16 on Day 1 and Day 3 following admittance, a comparable decrease in T reg cells was seen early after polytrauma (Serve *et al*., 2018).

4.2 Comparison of early T lymphocyte subsets in relation to late SOFA scores

The second objective of this study aims to display that the development of MODS and poor clinical outcome following major trauma results from the immunosuppressive CARS response, in particular T reg cells, causing increased patient susceptibility to opportunistic infections. As described in previous chapters, the Sequential Organ Failure Assessment (SOFA) is an approved scoring system which allows the identification of MODS. The collection of SOFA scores on Day 1, 3, and 5 following traumatic injury allowed for this study to use SOFA scores as an indicator of poor clinical outcome. Early trends in T lymphocyte subsets in relation to late SOFA scores were analysed in order to identify early T cell marker changes which may be indicative of late SOFA predictions. This may therefore be used as a diagnostic tool to identify poor clinical outcomes from a cohort of high risk patients.

As described in previous chapters, the counter anti-inflammatory response (CARS) to trauma acts in response to inflammation, in order to restore immune homeostasis. It has been labelled as an immune suppressive response due to the anergic like properties displayed by the T cell mediated response it entails. These include low proliferative responses to specific antigens; suppressed Th1 type immune reactivity alongside increased Th2 type cytokine production; finally increased T reg activity (Stoecklein *et al*., 2012). Therefore, a primary complication of the CARS response is that it may actively suppress antimicrobial immunity, thus increasing the hosts susceptibility to opportunistic infections (Osuka *et al*., 2014). The work of T reg cells in particular during this CARS response has been an area of debate over the years.

During this study, a relationship between CD25BRIFOXP3+ on Day 3 and SOFA scores on Day 5 was found to be significant (p= 0.049), as was also the case for CD127LOWFOXP3+ on Day 3 and SOFA on Day 5 (p= 0.048). Interestingly, all other T regulatory subsets failed to show any significant relationship to Day 5 SOFA scores. This may be explained by extensive studies which have highlighted that a combination of CD25, FOXP3, and CD127 are considered to be the most sufficient markers for defining T reg cells in a research setting (Safina *et al*., 2015). The up regulation of CD25 expression previously associated with increased T regulatory cell function upon TNF stimulation (Chen *et al*., 2007), may suggest that higher CD25 levels are indicative of an increase in suppressive T reg cells. However, CD25 alone has proven to be problematic, therefore a combination of CD25+ alongside other markers is preferred. This may explain the lack of significance seen when examining relationships between CD25BRI on Day 3 and SOFA on day 5 (p= 0.665). Similarly, CD4+FOXP3+ alone on Day 3 also displayed no significant relationship with Day 5 SOFA (p= 0.963), as predicted due to previous research highlighting that this marker alone cannot be deemed sufficient in identifying T regulatory cells (Corthay, 2009).

The only marker which displayed a significant relationship on both Day 1 and 3 to SOFA scores on Day 5 was CD3+ (p= 0.007 and p= 0.02 respectively). This correlates to the work of Rani and Schwacha who found an influx of CD3+ T cells into the wound site of a burn injury. Furthermore, in normal skin, only 6.8% of cells were found to be CD3+, whereas skin cells within a burn wound were found to be 24% CD3+ T cells (Rani & Schwacha, 2017).

The relationship seen between Day 3 CD25BRIFOXP3+ and CD127LOWFOXP3+ against Day 5 SOFA may be explained due to the known suppressive ability of T regulatory cells, which will ultimately affect clinical outcome, represented in this study by patient SOFA scores.

However, the extent of this relationship cannot be determined without clear categorisation of SOFA scores. In order to do this, the patient cohort was split into two groups. Group 1 consisted of 38 patients, all with a Day 5 SOFA score of 0, whereas group 2 consisted of 43 patients with Day 5 SOFA scores ranging from 1 to 12. The average (mean) expression of T lymphocytes on Day 1 between these two groups was then analysed using a paired T test.

The average (mean) expression of CD25BRIFOXP3+ on Day 1 for patients with a Day 5 SOFA score of less than 1 was found to be 81.94, however, this expression was 87.04 on Day 1 for patients with a Day 5 SOFA score greater than 1. As expected, this difference was found to be significant (p= 0.05). Similarly, the average expression of CD127LOWFOXP3+ on Day 1 for patients with a Day 5 SOFA score of less than 1 was found to be 76.35, which is seen to increase to 87.38 for patients with a Day 5 SOFA score greater than 1. Once again, this difference was found to be significant (p= 0.03). These results correlate with the previous studies which describe the role of T regulatory cells and their suppression of the immune responses. Some examples of this include; higher numbers of circulating T regs found in septic patients (Monneret *et al*., 2003), or the direct association of increased T reg numbers with poor clinical outcome (Venet *et al*., 2008). In addition, T reg cells have been found to inhibit the activation and proliferation of CD8+ and CD4+ T cells in TCR signals, as well as inhibit B lymphocytes, neutrophils, dendritic cells, and monocytes/macrophages (Cao *et al*., 2015). It is findings such as these which may explain the significant increase in CD25BRIFOXP3+ and CD127LOWFOXP3+ expression on Day 1 for patients who later developed higher SOFA scores (>1), and therefore a poorer clinical outcome.

The suppression mechanisms of T regs which may explain these results have been grouped into four main categories, these are; suppression by metabolic disruption, suppression by cytolysis, suppression by inhibitory cytokines such as interleukin-35 (IL-35), IL-10, and transforming growth factor-β (TGF-β), and finally, suppression by modulation of dendritic cell function or maturation (Cao *et al*., 2015).

Investigations into the effects of traumatic injury on T reg cells has displayed that an increased expression of costimulatory molecules such as CTLA-4, PD-1, ICOS, and CD28, is indicative of increased T reg survival or function. In particular an increase in CTLA-4 expression by T reg cells 7 days following traumatic injury may suppress APC function via an IDO mechanism, resulting in an increase in T reg potency. Alternatively, T cell activation may be suppressed by blocking CD80 and CD86 access to CD28 (Stoecklein *et al*., 2012).

During the disruption of metabolic pathways, enzymes expressed on T reg cells such as CD39 and CD73, cause the metabolism of ATP to AMP, resulting in the production of immunoregulatory adenosine. T regs also express high levels of intracellular cAMP which is transferred to T effector cells, resulting in the inhibition of nuclear factor of activated T cells (NFAT) and Il-2 transcription. This disruption leads to apoptosis due to Il-2 deprivation (Safina *et al*., 2015).

The final mechanism of T reg suppression is the induction of apoptosis. T reg cells are able to directly induce apoptosis via granzyme A/B and perforin, the Fas-ligand pathway, the falectin-9/TIM-3 pathway, and tumour necrosis factor related apoptosis inducing ligand (TRAIL) (Safina *et al*., 2015).

These mechanisms of suppression still remain poorly understood and are an area of ongoing research. However, several biological features of T reg cells may suggest their role in the control of the adaptive immune response to injury, specifically the CARS response. Their ability to suppress CD4+ T cell proliferation and activation firstly suggests that they may drive the suppression of Th1 type response (Stoecklein *et al*., 2012), which supports the significant correlation seen in this study between T regs and increased SOFA scores.

The average expression of CD8+ on Day 1 was also found to be higher in patients who went on to develop a SOFA score greater than 1 on Day 5 (Table 3.2), however, following statistical analysis this was not found to be significant (p= 0.332). All other T lymphocytes (CD3+, CD4+, CD25BRI, and CD4+FOXP3+) displayed lower average expression on Day 1 in those patients who went on to develop a SOFA score greater than 1 on Day 5, however these were also not significant (p=0.052, 0.550, 0.173, and 0.278 respectively).

4.3 Correlation between T reg cells and IL-6 and IL-10

The final objective of this study aims to highlight the role of T regulatory cells in the suppression of T cell activation and cytokine production following traumatic injury. Various clinical evidence displays that the increased susceptibility of trauma patients to infections can be attributed in part to the reduced Th1 type immune responses and an increased counter inflammatory Th2 response (MacConmara *et al*., 2006). T regulatory cells in particular are thought to control these inappropriate T cell responses via the suppression of CD4+ T cell reactivity to antigens. T reg cells have been found to suppress the Th1 type immune response and promote that shift to Th2 responses, increasing patient susceptibility to infection (MacConmara *et al*., 2006). In order to explore this role of T reg cells, T lymphocytes used in this study were tested against IL-6 and IL-10 in order to identify any relationships between T reg cells in particular and these key cytokines.

A Spearman's rank correlation found a significant correlation between CD25BRIFOXP3+ and IL-6 on Day 3 following injury (p= 0.026). Similarly, CD127LOWFOXP3+ and IL-6 on Day 3 also displayed a significant correlation (p= 0.029). IL-6 is described as a soluble mediator which has a pleiotropic effect on inflammation, haematopoiesis, and immune response (Tanaka *et al*., 2014). Previously, IL-6 was thought to be responsible for regulating the Th17/T reg balance. Specifically, it was found to support the development of Th17, and suppress the generation of T reg cells (Bettelli *et a*l., 2006). However, this was challenged when the T reg enhancing effects of IL-6 were described in both mice and humans (Nakagawa *et al*., 2010). In addition, IL-6 has been shown to be required for the induction of the anti-inflammatory cytokine IL-10 by T reg cells (Stumhofer *et al*., 2007) and overexpression of IL-6 was found to increase the number of T reg cells, rather than impairing their function (Fujimoto *et al*., 2011). Most recently, studies have found that contrary to previous research, IL-6 does not in fact inhibit the development or function of T reg cells. Alternatively, it enhances T effector cell activation, rendering them resistant to T regulatory mediated suppression (Nish *et al*., 2014).

These more recent findings correlate with the findings of this study and may explain why the average expression of both T reg CD25BRIFOXP3+ and CD127LOWFOXP3+ showed significant correlation to the expression of IL-6. Although previous studies may have suggested that this correlation is due to the suppression of T reg generation caused by IL-6, the work done since then suggests that this correlation may be due to the role of IL-6 in the resistance of T effector cells to T reg mediated suppression.

A Spearman's rank correlation also found a significant correlation between CD3+ and IL-10 on Day 3 following traumatic injury (p= 0.000). IL-10 is described as one of the vital anti-inflammatory cytokines due to its ability to decrease the production of inflammatory molecules such as IL-12, TNF-α, IFN-γ, major histocompatibility complex molecules, reactive nitric oxide metabolites, as well as its ability to inhibit antigen specific CD8+ T cells (Zeng *et al*., 2009). Elevated IL-10 has also been found in patients following trauma (Neidhardt *et al*., 1997) and has been correlated with the development of sepsis (Maier *et al*., 2007). The correlation between CD3+ T lymphocytes and IL-10 can therefore be explained due to the inhibitory effect of IL-10 on T cell proliferation and activation. This is shown to occur through the disruption of IL-2 production and IFN-γ action, resulting in the suppression of macrophage activation (Rowbottom *et al*., 1999).

4.4 Conclusions

In conclusion, this study confirmed a number of objectives which have previously been described regarding the role of T reg cells following major traumatic injury. Firstly, the increased expression of T reg cells seen from Day 1 to Day 5 following trauma confirm that the increase in T reg activity previously discovered in major trauma patients may be due to their involvement in the adaptive immune response.

Further findings such as the significant correlation between T regulatory cells on Day 3 and SOFA scores on Day 5, as well as a higher expression of T reg cells on Day 1 in those patients who developed a SOFA score of >1 on Day 5, highlighted the ability of T reg cells to be used as predictive biomarkers of poor clinical outcome. In particular, CD25BRIFOXP3+ and CD127LOWFOXP3+ displayed the most potential for this, correlating to previous research which states the inability of FOXP3 and CD25BRI alone to act as sufficient markers of T reg cells.

The roles of IL-6 and IL-10 in relation to T reg cells was also explored briefly and revisited the debate regarding IL-6 effects on T reg cells. A significant correlation between IL-6 and both CD25BRIFOXP3+ and CD127LOWFOXP3 on Day 3 agrees with recent research describing the ability of IL-6 to enhance T effector cell activation, rendering them resistant to T reg mediated suppression. Interestingly, no correlation was displayed between IL-10 and CD25BRIFOXP3+ or CD127LOWFOXP3+, despite the known suppressive abilities of all three. This may be explained due to the small cohort size of 52 patients involved in IL-10 analysis as well as the limited statistical analysis carried out on IL-10 during this study.

It must finally be noted that although the interpretation of data presented in this study is promising and may potentially lead to the development of an improved management and therefore improved outcomes among major trauma patients, it must be further validated using a larger cohort group. The present study, analysed a cohort of 85 patients from the Central Manchester Foundation Trust (CMFT) and Salford Royal Foundation Trust (SRFT), however a larger cohort of patients from these institutions may provide a clearer indication of the significance of the findings and potentially lead to further progress in the search for biomarkers which may predict clinical outcomes for major trauma patients.

CHAPTER 5: References

Alegre, M., Frauwirth, K. and Thompson, C., 2001. T-cell regulation by CD28 and CTLA-4. *Nature Reviews Immunology*, 1(3), pp.220-228.

Baker, C., Oppenheimer, L., Stephens, B., Lewis, F. and Trunkey, D., 1980. Epidemiology of trauma deaths. *The American Journal of Surgery*, 140(1), pp.144-150.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T., Oukka, M., Weiner, H. and Kuchroo, V., 2006. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*, 441(7090), pp.235-238.

Bone, R., 1996. Sir Isaac Newton, sepsis, SIRS, and CARS. *Critical Care Medicine*, 24(7), pp.1125-1128.

Cao, C., 2015. The role of regulatory T cells in immune dysfunction during sepsis. *World Journal of Emergency Medicine*, 6(1), p.5.

Chaplin, D., 2010. Overview of the immune response. *Journal of Allergy and Clinical Immunology*, 125(2), pp.S3-S23.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X. and Zhao, L., 2017. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9(6), pp.7204-7218.

Chen, X. and Oppenheim, J., 2011. Resolving the identity myth: Key markers of functional CD4+FoxP3+ regulatory T cells. *International Immunopharmacology*, 11(10), pp.1489-1496.

Cole, E., Gillespie, S., Vulliamy, P., Brohi, K., Akkad, H., Apostolidou, K., Ardley, R., Aylwin, C., Bassford, C., Bonner, S., Brooks, A., Cairns, T., Cecconi, M., Clark, F., Dempsey, G., Denison Davies, E., Docking, R., Eddlestone, J., Ellis, D., Evans, J., Galea, M., Healy, M., Horner, D., Howarth, R., Jansen, J., Jones, J., Kaye, C., Keep, J., Kerslake, D., Kilic, J., Leong, M., Martinson, V., McIldowie, B., Michael, S., Millo, J., Morgan, M., O'Leary, R., Oram, J., Ortiz-Ruiz De Gordoa, L., Porter, K., Raby, S., Service, J., Shaw, D., Smith, J., Smith, N., Stotz, M., Thomas, E., Thomas, M., Vincent, A., Ward, G. and Welters, I., 2019. Multiple organ dysfunction after trauma. *BJS*,.

Corthay, A., 2009. How do Regulatory T Cells Work?. *Scandinavian Journal of Immunology*, 70(4), pp.326-336.

Davy-Mendez, T., Napravnik, S., Zakharova, O., Kuruc, J., Gay, C., Hicks, C., Mcgee, K. and Eron, J., 2018. Acute HIV Infection and CD4/CD8 Ratio Normalization After Antiretroviral Therapy Initiation. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 79(4), pp.510-518.

Fujimoto, M., Nakano, M., Terabe, F., Kawahata, H., Ohkawara, T., Han, Y., Ripley, B., Serada, S., Nishikawa, T., Kimura, A., Nomura, S., Kishimoto, T. and Naka, T., 2011. The Influence of Excessive IL-6 Production In Vivo on the Development and Function of Foxp3+ Regulatory T Cells. *The Journal of Immunology*, 186(1), pp.32-40.

Grissom, C., Brown, S., Kuttler, K., Boltax, J., Jones, J., Jephson, A. and Orme, J., 2010. A Modified Sequential Organ Failure Assessment Score for Critical Care Triage. *Disaster Medicine and Public Health Preparedness*, 4(4), pp.277-284.

Gunst, M., Ghaemmaghami, V., Gruszecki, A., Urban, J., Frankel, H. and Shafi, S., 2010. Changing Epidemiology of Trauma Deaths Leads to a Bimodal Distribution. *Baylor University Medical Center Proceedings*, 23(4), pp.349-354.

Hefele, F., Ditsch, A., Krysiak, N., Caldwell, C., Biberthaler, P., van Griensven, M., Huber-Wagner, S. and Hanschen, M., 2019. Trauma Induces Interleukin-17A Expression on Th17 Cells and CD4+ Regulatory T Cells as Well as Platelet Dysfunction. *Frontiers in Immunology*, 10.

Heuer, J., Zhang, T., Zhao, J., Ding, C., Cramer, M., Justen, K., Vonderfecht, S. and Na, S., 2005. Adoptive Transfer of In Vitro-Stimulated CD4+CD25+ Regulatory T Cells Increases Bacterial Clearance and Improves Survival in Polymicrobial Sepsis. *The Journal of Immunology*, 174(11), pp.7141-7146.

Huber-Lang, M., Lambris, J. and Ward, P., 2018. Innate immune responses to trauma. *Nature Immunology*, 19(4), pp.327-341.

Javali, R., LNU, K., Patil, A., Srinivasarangan, M., LNU, S. and LNU, S., 2019. Comparison of Injury Severity Score, New Injury Severity Score, Revised Trauma Score and Trauma and Injury Severity Score for Mortality Prediction in Elderly Trauma Patients. *Indian Journal of Critical Care Medicine*, 23(2), pp.73-77.

Kaminska, B., 2005. MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1754(1-2), pp.253-262.

Kaplan, L., 2018. Systemic Inflammatory Response Syndrome (SIRS).

Kirkman, E. and Watts, S., 2014. Haemodynamic changes in trauma. *British Journal of Anaesthesia*, 113(2), pp.266-275.

Krepska, A., Hastings, J. and Roodenburg, O., 2017. The metabolic and endocrine response to trauma. *Anaesthesia & Intensive Care Medicine*, 18(8), pp.414-417.

Liu, W., Putnam, A., Xu-yu, Z., Szot, G., Lee, M., Zhu, S., Gottlieb, P., Kapranov, P., Gingeras, T., de St. Groth, B., Clayberger, C., Soper, D., Ziegler, S. and Bluestone, J., 2006. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. *Journal of Experimental Medicine*, 203(7), pp.1701-1711.

MacConmara, M., Maung, A., Fujimi, S., McKenna, A., Delisle, A., Lapchak, P., Rogers, S., Lederer, J. and Mannick, J., 2006. Increased CD4+ CD25+ T Regulatory Cell Activity in Trauma Patients Depresses Protective Th1 Immunity. *Transactions of the ... Meeting of the American Surgical Association*, 124, pp.179-188.

Marshall, J., Warrington, R., Watson, W. and Kim, H., 2018. An introduction to immunology and immunopathology. *Allergy, Asthma & Clinical Immunology*, 14(S2).

McCullough, A., Haycock, J., Forward, D. and Moran, C., 2014. II. Major trauma networks in England. *British Journal of Anaesthesia*, 113(2), pp.202-206.

Monneret, G., Debard, A., Venet, F., Bohe, J., Hequet, O., Bienvenu, J. and Lepape, A., 2003. Marked elevation of human circulating CD4+CD25+ regulatory T cells in sepsis-induced immunoparalysis. *Critical Care Medicine*, 31(7), pp.2068-2071.

Moran, C., Lecky, F., Bouamra, O., Lawrence, T., Edwards, A., Woodford, M., Willett, K. and Coats, T., 2018. Changing the System - Major Trauma Patients and Their Outcomes in the NHS (England) 2008–17. *EClinicalMedicine*, 2-3, pp.13-21.

Nakagawa, T., Tsuruoka, M., Ogura, H., Okuyama, Y., Arima, Y., Hirano, T. and Murakami, M., 2010. IL-6 positively regulates Foxp3+CD8+ T cells in vivo. *International Immunology*, 22(2), pp.129-139.

Neidhardt, R., Keel, M., Steckholzer, U., Safret, A., Ungethuem, U., Trentz, O. and Ertel, W., 1997. Relationship of Interleukin-10 Plasma Levels to Severity of Injury and Clinical Outcome in Injured Patients. *The Journal of Trauma: Injury, Infection, and Critical Care*, 42(5), pp.863-871.

Nish, S., Schenten, D., Wunderlich, F., Pope, S., Gao, Y., Hoshi, N., Yu, S., Yan, X., Lee, H., Pasman, L., Brodsky, I., Yordy, B., Zhao, H., Brüning, J. and Medzhitov, R., 2014. T cell-intrinsic role of IL-6 signaling in primary and memory responses. *eLife*, 3.

Okeke, E. and Uzonna, J., 2019. The Pivotal Role of Regulatory T Cells in the Regulation of Innate Immune Cells. *Frontiers in Immunology*, 10.

Osuka, A., Ogura, H., Ueyama, M., Shimazu, T. and Lederer, J., 2014. Immune response to traumatic injury: harmony and discordance of immune system homeostasis. *Acute Medicine & Surgery*, 1(2), pp.63-69.

Paterson, H., Murphy, T., Purcell, E., Shelley, O., Kriynovich, S., Lien, E., Mannick, J. and Lederer, J., 2003. Injury Primes the Innate Immune System for Enhanced Toll-Like Receptor Reactivity. *The Journal of Immunology*, 171(3), pp.1473-1483.

Rani, M. and Schwacha, M., 2017. The composition of T-cell subsets are altered in the burn wound early after injury. *PLOS ONE*, 12(6), p.e0179015.

Rauf, R., von Matthey, F., Croenlein, M., Zyskowski, M., van Griensven, M., Biberthaler, P., Lefering, R. and Huber-Wagner, S., 2019. Changes in the temporal distribution of in-hospital mortality in severely injured patients—An analysis of the TraumaRegister DGU. *PLOS ONE*, 14(2), p.e0212095.

Rowbottom, A., Lepper, M., Garland, R., Cox, C., Corley, E., Oakhill A. and Steward, C., 1999. Interleukin-10-induced CD8 cell proliferation. *Immunology*, 98(1), pp.80-89.

Safinia, N., Scotta, C., Vaikunthanathan, T., Lechler, R. and Lombardi, G., 2015. Regulatory T Cells: Serious Contenders in the Promise for Immunological Tolerance in Transplantation. *Frontiers in Immunology*, 6.

Salvo, F., Luppi, F., Lucchesi, D., Canovi, S., Franchini, S., Polese, A., Santi, F., Trabucco, L., Fasano, T. and Ferrari, A., 2020. Serum Copeptin levels in the emergency department predict major clinical outcomes in adult trauma patients. *BMC Emergency Medicine*, 20(1).

Segerstrom, S. and Miller, G., 2004. Psychological Stress and the Human Immune System: A Meta-Analytic Study of 30 Years of Inquiry. *Psychological Bulletin*, 130(4), pp.601-630.

Serve, R., Sturm, R., Schimunek, L., Störmann, P., Heftrig, D., Teuben, M., Oppermann, E., Horst, K., Pfeifer, R., Simon, T., Kalbas, Y., Pape, H., Hildebrand, F., Marzi, I. and Relja, B., 2018. Comparative Analysis of the Regulatory T Cells Dynamics in Peripheral Blood in Human and Porcine Polytrauma. *Frontiers in Immunology*, 9.

Simsek, T., Uzelli Simsek, H. and Canturk, N., 2014. Response to trauma and metabolic changes: posttraumatic metabolism. *Turkish Journal of Surgery*, 30(3), pp.153-159.

Stoecklein, V., Osuka, A. and Lederer, J., 2012. Trauma equals danger--damage control by the immune system. *Journal of Leukocyte Biology*, 92(3), pp.539-551.

Stumhofer, J., Silver, J., Laurence, A., Porrett, P., Harris, T., Turka, L., Ernst, M., Saris, C., O'Shea, J. and Hunter, C., 2007. Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nature Immunology*, 8(12), pp.1363-1371.

Sommershof, A., Aichinger, H., Engler, H., Adenauer, H., Catani, C., Boneberg, E., Elbert, T., Groettrup, M. and Kolassa, I., 2009. Substantial reduction of naïve and regulatory T cells following traumatic stress. *Brain, Behavior, and Immunity*, 23(8), pp.1117-1124.

Tanaka, T., Narazaki, M. and Kishimoto, T., 2014. IL-6 in Inflammation, Immunity, and Disease. *Cold Spring Harbor Perspectives in Biology*, 6(10), pp.a016295-a016295.

Travers, P. and Walport, M., 2001. *Immunobiology, 5Th Edition: The Immune System In Health And Disease*. Garland Publishing.

Trunkey, D., 1983. Trauma. *Scientific American*, 249(2), pp.28-35.

Venet, F., Chung, C., Kherouf, H., Geeraert, A., Malcus, C., Poitevin, F., Bohé, J., Lepape, A., Ayala, A. and Monneret, G., 2008. Increased circulating regulatory T cells (CD4+CD25+CD127−) contribute to lymphocyte anergy in septic shock patients. *Intensive Care Medicine*, 35(4), pp.678-686.

Venet, F., Chung, C., Monneret, G., Huang, X., Horner, B., Garber, M. and Ayala, A., 2008. Regulatory T cell populations in sepsis and trauma. *Journal of Leukocyte Biology*, 83(3), pp.523-535.

Wan, Y. and Flavell, R., 2007. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. *Nature*, 445(7129), pp.766-770.

Zeng, L., Gu, W., Chen, K., Jiang, D., Zhang, L., Du, D., Hu, P., Liu, Q., Huang, S. and Jiang, J., 2009. Clinical relevance of the interleukin 10 promoter polymorphisms in Chinese Han patients with major trauma: genetic association studies. *Critical Care*, 13(6), p.R188.

Zhang, Y., Li, X., Wu, W. and Chen, Y., 2015. Dynamic changes of circulating T-helper cell subsets following severe thoracic trauma. *International Journal of Clinical and Experimental Medicine*, 8(11), pp.21106-21113.

CHAPTER 6: Appendices

Appendix 1: Day 1 – Clinical data from Central Manchester Foundation Trust and calculated SOFA scores

																			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
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
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
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

	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
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
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
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
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
BIT098	107	100	66		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
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
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
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
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
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
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
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
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
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
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
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	empirade	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

Appendix 2: Day 3 – Clinical data from Central Manchester Foundation Trust and calculated SOFA scores

	Day 3																																
	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 3
BIT001	98	124	91	102	37.5	77	18.1	194	90	78	9	16.7	N	N	0.21	N		0	145	N	N	15	Y		N	N	0	0	0	0	0	0	0
BIT002	126	136	76	96	36.2	96	10.8	157	75	94	12	15.7	N	N	0.28	33.3	1.3	0	349	N	N	15	Y		N	N	0	0	0	0	2	0	2
BIT003	115	89	55	66.3333	36.4	70	5.4	135	64	101	7	15.2	N	N	0.21	N		0	120	N	N	15	Y		N	N	1	1	0	0	0	0	2
BIT004	123	92	80	84	38.1	79	15.8	152	90	54	9	15	N	N	0.21	51.2	0.5	0	279	N	N	15	N		N	N	0	0	0	0	1	0	1
BIT005	80	139	81	100.333	38.1	113	8	195	90	66	13		N	N	0.21	N		0	111	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT006	82	120	80	93.3333		82	5.1	420	90	66	3	16.1	N	N	0.21	N		0	58	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT007	87	125	70	88.3333	37.6	112	6.8	144	90	52	16	13.1	N	N	0.21	N		0	97	N	N	15	N		N	N	0	1	0	0	0	0	1
BIT008	130	140	90	106.667	36.9	96	20.6	147	89	65	26	14.4	Y	N	0.21	31	2.1	0	239	N	Y		Y		Y		0	1	0	0	4	4	9
BIT009	82	112	43	66	36.8	80	8.2	156	79	104	11	15.7	N	N	0.24	N	0.7	0	127	N	N	15	Y		N	N	1	0	0	0	0	0	1
BIT010	133	136	78	97.3333	38.2	109	8.1	236	90	57	6	14	N	N	0.5	32.4	0.9	0	317	N	N	15	N		N	N	0	0	0	0	2	0	2
BIT011	91	113	49	70.3333	38.2	78	13.5	190	36	137	11	17.9	N	N	0.4	N		0	147	N	N	15	N		N	N	0	0	1	0	0	0	1
BIT014	112	103	60	74.3333	36.4	112	21	163	77	101		15	N	N		N		0	250	N	N	15	Y	empirical	Y	N	0	0	0	0	0	0	0
BIT016	95	109	82	91	36.5	86	11.5	96	45	137	11	18	N	N	0.3	28.3	1.2	0	318	N	N	15	Y		N	N	0	2	1	0	2	0	5
BIT017	130	170	90	116.667	36.1	124	10.1	116	85	72	31	15.6	Y	N	1	55.4	1.3	0	41	N	Y		Y	empirical	N		0	1	0	1	4	4	10
BIT018	100	142	81	101.333	38	100	5.6	133	90	46	9	11.3	N	N	0.4	N		0	155	N	N	15	N		N	N	0	1	0	0	0	0	1
BIT021	109	141	86	104.333	36	94	13.1	154	90	80	16	10.9	N	N	0.28	N	0.8	0	178	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT022	87	136	82	100	37.2	97	8.6	278	90	62	6	11.4	N	N	0.21	N		0	53	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT023	90	100	56	70.6667	36.8	89	10	131	90	79	8	11.4	N	N	0.33	N		0	180	N	N	15	Y	empirical	N	N	0	1	0	0	0	0	1
BIT024	99	96	50	65.3333	37.3	69	15	166	90	147	3	10.2	Y	N	0.4	47.2	1.6	0	291	N	Y		Y		Y	N	1	0	1	0	4	4	10
BIT025	170	100	35	56.6667	36.9	97	17.2	142	63	100		10.4	N	Y	0.6	18.24	1.1	0	291	N	N	15	Y	empirical	N	N	1	1	0	0	3	0	5
BIT027	72	149	69	95.6667	37.5	94	13.3	138	90	73	6	10.2	N	N	0.21	31.2	1	0	60	N	N	15	Y	empirical	Y	N	0	1	0	0	2	0	3
BIT028	88	135	64	87.6667	37.6		5.7	223	90	84	4	9.7	N	N	0.21	N	N	0	46	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT029	98	107	69	81.6667	35.4	108	5.7	142	90	66	17	10.1	N	N	0.24	N	N	0	174	N	N	15	Y	empirical	N	N	0	1	0	0	0	0	1
BIT034	98	94	53	66.6667	37.4	114	8.8	203	63	107	13	11.9	N	N	0.6	24.2	1.4	0	N	N	N	15	N		N	N	1	0	0	0	3	0	4
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							0
BIT040	107	131	85	100.333	37.3	96	10.7	350	90	80	14	N	N	N	0.28	N	N	0	340	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT042	126	132	85	100.667	38.4	103	11.1	177	N	73	9	12.3	N	N	0.21	N	N	0	201	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	71	124	56	78.6667	36.8	144	10.1	254	62	122	8	10.9	N	N	0.21	N	N	0	20	N	N	15	N		N	N	0	0	1	0	0	0	1
BIT046	105	110	62	78	35.5	75	32.3	78	51	92	15	13	N	N	0.32	25.8	1.3	Norad	15	N	N	15	Y	empirical	N	N	0	2	0	0	3	0	5
BIT047	125	107	49	68.3333	38.4	83	6.8	97	90	89	20	11.5	N	N	0.28	40.6	0.9	0	291	N	N	15	Y	empirical	N	N	1	2	0	1	1	0	5
BIT048				0		74	6.9	101	14	427	8	11.2	y	N					273	y	y		y	empirical	y		1	1	3	0	4	4	13
BIT049	99	146	76	99.3333	37.4	111	7.9	111	90	63	12	11.1	N	N	0.32	N	N	0	55	N	N	15	N		N	N	0	1	0	0	0	0	1
BIT050	118	110	56	74	39	118	7	200	90	76	14	N	N	N	0.28	N	N	0	438	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT052	110	151	100	117	37.4	84	6.4	110	90	66	10	11.3	N	N	0.24	N	1.5	0	N	N	N	15	y	empirical	N	N	0	1	0	0	0	0	1
BIT053	103	160	88	112	37.6	107	6.4	116	90	47	17	11.9	N	N	0.55	21.9	0.8	0	197	N	N	15	N		N	N	0	1	0	0	3	0	4
BIT055	87	137	62	87	37.7	105	5.9	113	90	72	114	12.3	N	N	0.21	42.5	0.5	0	N	N	N	15	y	empirical	N	N	0	1	0	3	1	0	5
BIT060	87	100	50	66.6667	37.8	70	7.9	92	70	99	8	10.9	Y	N	0.5	18.4	1.9	0.05		N	Y	N	Y	empirical	N	N	3	2	0	0	3	0	8
BIT061	100	85	64	71	38.2	91	11	164	90	48	12	11.6	Y	N	0.4	22.6	1.2	0	223	N	y		y	empirical	N	N	0	0	0	0	3	4	7
BIT064	100	149	79	102.333	36	139	11.4	174	90	75	37	n	N	N	0.21	N	N	0	49	N	N	15	N		N	N	0	0	0	2	0	0	2
BIT065	125	95	50	65	38	98	18.2	258	90	50	9	11.9	Y	N	0.28	22.4	0.6	0	218	N	N	14	Y	Chest	N	Y	1	0	0	0	3	1	5
BIT066	87	147	77	100.333	36.1	132	8.8	233	90	66	N	N	N	N	0.21	N	N	0	N	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT067	76	135	76	95.6667	36.6	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
BIT068	DISCHARGED																																
BIT069	125	145	80	101.667	37.1	125	8.2	192	90	65	23	11	N	N	0.24	58.2	0.7	0	N	N	N	14	y	empirical	N	N	0	0	0	1	0	1	2
BIT070	54	139	63	88.3333	37.3	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
BIT071	92	106	57	73.3333	37.5	N	N	N	N	N	N	N	N	N	0.21	N	N	N	N	N	N	15	N		N	N	4	0	0	0	0	0	4
BIT072	98	153	70	97.6667	37	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	Y	empirical	Y	N	0	0	0	0	0	0	0
BIT073	98	153	70	0	37	122	4.5	208	75	68	N	11.9	N	N	0.21	N	N	0	59	N	N	15	Y	empirical	Y	N	1	0	0	0	0	0	1
BIT074	98	143	84	103.667	37.2	N	N	N	N	N	N	N	N	N	0.21	N	N	0	N	N	N	15	Y	e	N	N	0	0	0	0	0	0	0
BIT076	97	106	69	81.3333	37.1	92	7.3	212	>90	60	6	12.4	N	N	0.28	N	1.2	N	363	N	N	14	Y	empirical	N	Y	4	0	0	0	0	1	5
BIT030	77	131	80	97	37.2	84	5.1	132	90	73	26	10.6	N	N	0.21	N	N	0	N	N	N	15	N	empirical	N	N	0	1	0	1	0	0	2
BIT078	100	152	80	104	37.2	96	7.2	181	>90	75	8	10.8	N	N	0.21	N	N	N	242	N	N	15	N	emprilical	N	N	4	0	0	0	0	0	4

	Day 3																																
	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 3
BIT079	n/k	n/k	n/k	#VALUE!	n/k	96	7.9	159	64	109	N	11.4	N	N	n/k	n/k	N	N	309	N	N	15	Y	empirical	N	N	0	0	1	0	0	0	1
BIT080	Sel Discharged																																
BIT084	n/k	n/k	n/k	#VALUE!	n/k	72	16	242	>90	62	6	n/d	N	N	n/k	n/k	n.k	0	135	N	N	14	Y	abdo	N	Y	0	0	0	0	0	1	1
BIT085	89	161	77	105	35.8	108	13.4	251	0.9	70	5	10.8	y	N	0.5	24	0.8	0	203	N	Y	n/a	Y	emp	N	N	0	0	0	0	3	0	3
BIT087	50	140	87	104.667	35.9	138	8.5	183	90	82	9	n/d	N	N	0.21	n/d	n/d	0	n/d	N	N	15	N		N	N	0	0	0	0	0	0	0
BIT088	114	100	49	66	38	79	21.3	165	54	149	20	10.7	N	N	0.6	25.3	0.9	0	329	N	N	15	N		N	N	1	0	1	1	3	0	6
BIT091	86	181	66	104.333	37	82	10	239	21	206	5	10.7	Y	N	35	39	1.3	0.03	143	N	Y	n/a	y	emp	N	Y	3	0	2	0	2	0	7
BIT092						106	12.4	202	90	65	7																0	0	0	0	0	0	0
BIT093						71	21.2	259	36	172	15																0	0	2	0	0	0	2
BIT094	101	109	51	70.3333	36.5	111	6.7	108	90	74										N		15	Y	CHEST	Y		0	1	0	0	0	0	1
BIT095	85	110	80	90		91	8.7	100	83	90	5												Y	empirical			0	2	0	0	0	0	2
BIT096						140	14.9	135	90	73									203								0	1	0	0	0	0	1
BIT098	80	143	82	102.333	37.4	124	12.5	247	90	61	17	11.9	N									15	Y	empirical			0	0	0	0	0	0	0
BIT099						133	10.6	169	90	50	35		Y	N						N	N	15					0	0	0	2	4	0	6
BIT100	129	131	66	87.6667	36.7	99	11.4	149	90	86	7	12.7							7				Y	empirical			0	1	0	0	0	0	1
BIT102	92	121	80	93.6667	36.4	137	12.2	194	85	80			N	N	40%		0.8			N	N	15	Y	empirical	N		0	0	0	0	0	0	0
BIT103	118	104	52	69.3333	36.8	80	8.8	118	90	72	24	12	N	Y	40%	32.3	0.9		53	N	N	15	Y	empirical			1	1	0	1	2	0	5
BIT104	102	90	56	67.3333	36.4	74	11.9	223	90	43	8	10.6	N	N	21				110	N	N	15			N		1	0	0	0	0	0	1
BIT105	Discharegd																																
BIT107	138	86	52	63.3333	38.5	79	6.4	71	58	111	30	12	Y	N	50%	30	1.2	0.1	349	N	Y	14		empirical	Y		4	2	1	1	2	1	11
BIT109	96			0	36.8	73	2.1	86	90	46	20	12.1	N	N	21%				34	N	N	15		Chest			0	2	0	1	0	0	3
BIT111	75	85	79	81	36.8	91	7.7	199	NA	48	10	10	Y	N	50%	29	0.8	NA	114	N	Y	15	N	NA	N	N	0	0	0	0	2	0	2
BIT113	116	94	57	69.3333	37.7	84	10.4	61	55	132	26	10	Y	N	40%	47	1.2	NA	449	Y	Y	1	Y	EMPIRICAL	N	N	1	2	1	1	1	4	10
BIT115	121	162	82	108.667	36.5	104	8.3	146	90	77	6	10.8	N	Y	40%	23	1.7	N/A	438	N	N	15	Y	Emprical	N	N	0	1	0	0	1	0	2
BIT117	101	93	62	72.3333	35.9	69	6.9	110	108	69	7	10	Y	N	28%	51	1.3	0.06	73	N	Y	14	Y	Empirical	N	N	3	1	0	0	1	1	6
BIT118	95	96	54	68	35.7	101	4.4	154	ND	48	8	11	N	N	21%	53	0.5	NA	77	N	N	15	N	NA	N	N	1	0	0	0	0	0	1
BIT119	96	136	66	89.3333	36.7	86	5.6	169	>90	44	11	n/a	N	N	21	n/a	n/a	n/a	63	N	N	15	Y	Empirical	N	N	0	0	0	0	0	0	0
BIT120	60	154	78	103.333	37.8	126	6	153	>90	69	14	10.5	N	N	35%	n/a	n/a	n/a	n/a	N	N	15	Y	empirical	N	N	0	0	0	0	0	0	0
BIT121	49	122	76	91.3333	36.7	99	6.6	126	90	64	NA	11	N	N	21%	NA	0.6	N	N	N	N	15	Y	empirical	N	N	0	1	0	0	0	0	1
BIT123	110	164	91	115.333	37	93	9.5	154	>90	55	11	10.2	N	Y	47%	23.6	0.6	NA	239	N	N	15	N	NA	N	N	0	0	0	0	3	0	3
BIT124	115	192	79	116.667	36	74	7.1	170	84	59	7	9.8	N	N	35%	53	0.7	N	157	N	N	14	Y	WOUND	N	N	0	0	0	0	0	1	1
BIT125	38	64	57	59.3333	36.1	82	11.4	128	57	109	15	n/d	N	N	60	11	1.4	N	108	N	N	15	N	N	N	N	1	1	0	0	4	0	6
BIT126	Transferred to Salford																																
BIT127	84	90	54	66	36.6	101	6.2	152	90	79	NA	10	N	N	35	NA	NA	NA	235	N	N	15	N	N	N	N	1	0	0	0	0	0	1
BIT128	131	152	61	91.3333	35.5	95	32.6	176	50	128	22	11	N	Y	50	19	1.2	n/a	325	N	N	15	Y	empirical	N	N	0	0	1	1	3	0	5
BIT129	64	116	68	84	37.4	123	7.6	240	>90	58	6	na	N	N	21	NA	NA	0	38	N	N	14	Y	Empirical	N	N	0	0	0	0	0	1	1
BIT130	49	124	93	103.333	37.9	93	6.5	185	>90	58	4	11	N	N	21	n/a	1.3	0	14	N	N	15	Y	Empirical	N	N	0	0	0	0	0	0	0
BIT131	141	96	77	83.3333	37.4	91	9.1	148	27	161	20	12	Y	N	50	17	2	0.8	506	N	Y	4	Y	empirical		N	4	1	1	1	3	4	14
BIT132	125	100	45	63.3333	35.4	89	14.4	64	61	102	79	17	Y	N	30	36	8	0.74	327	Y	Y	3	Y	empirical		N	4	2	0	2	2	4	14
BIT133				0		137	8.4	219	88	76	7	9.6	N	N	35	16.4	1.2	0	94	N	N	15				N	1	0	0	0	3	0	4
BIT134	91	106	37	60	37.5	92	8.6	155	42	128	10	10	N	N	24	47	1.2	0	174	N	N	14	Y	empirical	N	N	1	0	1	0	1	1	4
BIT135	98	99	60	73	35.3	88	6.5	154	ND	78	5	9	N	N	24	44	0.5	0	60	N	N	15	Y	empirical	N	N	0	0	0	0	1	0	1
BIT136	104	102	57	72	35.4	108	8.2	122	>90	73	10	10.1	N	N	30	45	0.7	0	190	N	N	15	N	n/a	N	N	0	1	0	0	1	0	2
BIT138	84	135	69	91	37.1	100	8.4	210	>90	88	ND	ND	N	N	21	ND	ND	0	15	N	N	15	N	n/a	N	N	0	0	0	0	0	0	0
BIT139	92	115	64	81	37.6	94	8.4	167	>90	68	6	11	N	N	28	36	0.4	0	ND	N	N	15	Y	empirical	N	N	0	0	0	0	2	0	2
BIT141	103	86	49	61.3333	37.1	88	4.9	126	>90	73	10	11	N	N	21	54	1.3	0	50	N	N	15	N	n/a	N	N	1	1	0	0	0	0	2
BIT142	80	93	53	66.3333	35.5	96	9.5	149	60	98	23	11.3	N	N	28	32	0.7	0	104	N	N	15	N	n/a	N	N	1	1	0	1	2	0	5
BIT143	113	124	79	94	37.8	104	7.9	362	>90	64	ND	ND	N	N	21	ND	ND	0	142	N	N	15			N	N	0	0	0	0	0	0	0
BIT144	80	114	53	73.3333	37.1	125	8.7	264	>90	77	9	10.2	N	N	21	ND	ND	0	51	N	N	15	N	n/a	N	N	0	0	0	0	0	0	0
BIT146	140	87	55	65.6667	37.8	99	6.9	94		62	22	11	N	N	35	ND	1	0	106	N	N	8	Y	empiricle	N	Y	1	2	0	1	0	3	7
BIT147	120	89	55	66.3333	38.6	85	14.4	216	>90	86	10	15	Y	N	60	18	1.3	0.1	234	N	Y	7	Y	empiricle	N	N	4	0	0	0	3	3	10
BIT148	135	100	44	62.6667	38.3	77	9.7	125	>90	97	35	12	Y	N	35	18.5	2.2	0.052	335	N	Y	3	Y	empiracle	N	N	3	1	0	2	3	4	13
BIT149	98	115	76	89	37.5	125	7.1	177	>90	69	23	11	N	N	30	ND	ND	0	151	N	N	15	N	empiracle	N	N	0	0	0	1	0	0	1
BIT150	100	185	116	139	37.7	133	7.5	264	>90	61	ND	10.2	N	N	21	ND	ND	0	80	N	N	15	Y	empiracle	N	N	0	0	0	0	0	0	0
BIT151	100	96	65	75.3333	37	73	16.6	77	>90	83	7	12.7	N	N	21	25.2	0.9	0	80	N	N	15	Y	arm	N	N	0	2	0	0	3	0	5
BIT152	52	106	49	68	36.6	96	8.6	239	30	143	6	10.8	N	N	28	35	0.7	0	54	N	N	15	N	n/a	N	N	1	0	1	0	2	0	4

<u>Appendix 3: Day 5 – Clinical data from Central Manchester Foundation Trust and calculated SOFA scores</u>

	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
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
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
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
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
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
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
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
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
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
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
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
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
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	Y	N	0	0	0	0	0	0	0
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

	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
BIT079	n/k	n/k	n/k	#VALUE!	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																																
BIT084	n/k	n/k	n/k	#VALUE!	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
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
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																																	
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
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																																
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
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
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
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
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
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
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
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
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
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	empiracle	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

Appendix 4: Day 1 – Clinical data from Salford Royal Foundation Trust and calculated SOFA scores

	Day 1																																
	Date Of Admission	HR	Sys BP	Dia BP	Noradrenalin	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	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	FiO2	Lactate	CRP	CVVH/HD	Sedated	Antibiotics	Septic source
SR002	12/11/2016	115	110	60	0	76.667	0	356	0	132	1	34	2	38.2	290.32	2	15	0	5	37.8	134	31.6	42	15.6	N	N	0.21	1.2	19	N	N	Y	empirical/unknown
SR003	26/10/2016	84	149	59	5	167	4	161	0	47	0	7	0		0	4	3	4	12	38.7	108	10.9	N	11.7	Y	N	98%	3.6	N	N	Y	N	N
SR004	24/11/2016	126	138	80	30	206	4	186	0	127	1	12	0	22	167.2	4	N	4	13	39.1	136	28.5	62	12.1	Y	N	80%	2.3	N	N	Y	N	N
SR008	01/02/2017	110	158	60	0	172	0	205	0	58	0	13	0	11.32	86.032	4	11	2	6	36.7	132	7	>90	12.8	N	N	28%	0.9	N	N	N	N	N
SR009	21/02/2017	132	119	80	0	93	0	169	0	53	0	11	0	n	N	0	13	1	1	35	104	8.8	>90	11.9	N	N	21%	1	N	N	N	N	N
SR010	12/03/2017	108	209	139	0	162	0	307	0	84	0	13	0	n	N	0	6	3	3	37.1	97	14.4	60	9.9	N	N	100%	4.3	N	N	N	N	N
SR011	27/03/2017	101	147	78	0	101	0	250	0	79	0	10	0	n	N	4	3	4	8	37.5	134	18.3	>90	12.4	Y	N	25%	7.1	N	N	Y	N	N
SR012	13/04/2017	84	125	50	5	75	4	193	0	84	0	3	0	n	N	4	N	4	12	38.2	127.9	15.4	>90	10.7	Y	N	25%	2.9	N	N	Y	Y	N
SR013	09/05/2017	97	180	101	0	127	0	124	1	75	0	14	0	N	N	4	3	4	9	37	134	13.7	85	12.4	Y	N	21%	1	N	N	Y	N	N
SR014	11/05/2017	95	105	74	0	84	0	235	0	59	0	6	0		N	4	3	4	8	36.1	85	8.5	85	11.2	Y	N	40%	1.8	N	N	Y	N	N
SR015	11/06/2017	58	158	61		93	0	246	0	54	0	8	0	375.05	2850.38	4	N	4	8	37.7	145	15.6	>90	14.1	Y	N	21%	2.1	N	N	Y	Y	N
SR016	11/06/2017	70	120	80	0	93.3	0	175	0	69	0	7	0	N	N	0	15	0	0	36.8	115	11.4	73	11.7	N	N	30%	N	4.5	N	N	N	N
SR019	09/07/2017	92	104	97	0	99.3	0	169	0	76	0	7	0	n	N	0	15	0	0	36.8	110	7	>90	13.3	N	N	0%	2	N	N	N	Y	5
SR020	11/07/2017	101	126	59	12	81.3	4	251	0	85	0	9	0	N	N	4	3	4	12	37.6	89	15	87	13.1	Y	N	21%	1.6	N	N	Y	Y	6
SR021	13/07/2017	76	132	66	5.5	88	4	237	0	75	0	22	1	N	N	4	3	4	13	36.9	134	17.1	N	13.9	Y	N	21%	2.2	N	N	Y	N	N
SR022	17/07/2017	69	110	60	6	76.7	4	87	2	49	0	23	1	N	N	0	14	1	8	36.6	104	9.5	N	13.6	N	N	21%	2	N	N	N	Y	6
SR023	26/07/2017	101	121	65	0	83.7	0	121	1	56	0	15	0	N	N	4	N	4	9	38	104	10	>90	13.5	Y	N	21%	1.8	N	N	Y	Y	6
SR024	31/07/2017	53	110	52	6	71.3	4	165	0	42	0	5	0	N	N	4	3	4	12	36.7	130	15.2	>90	11.5	Y	N	30%	1.5	N	N	Y	Y	1
SR025	03/08/2017	103	110	50	0	70	0	246	0	57	0	7	0	32.9	250.04	4	3	4	8	36.7	135	10.2	>90	13.1	Y	N	40%	1.7	N		Y	Y	6
SR026	04/09/2017	50	111	41	0	64.3	1	308	0	53	0	12	0	21.3	161.88	4	N	4	9	33.1	95	6.7	>90	16.6	Y	N	40%	2.7	N	N	Y	N	N
SR027	06/09/2017	101	102	71	0	81.3	0	348	0	130	1	8	0	172	1307.2	4	3	4	9	36.4	155	30.8	N	12.4	Y	N	21%	1.7	N	N	Y	Y	
SR028	12/09/2017	86	130	68	0	88.7	0	78	2	67	0	28	1	N	N	4	6	3	10	37.9	103	9.1	>90	13.7	Y	N	21%	3.1	N	N	N	N	N
SR029	14/09/2017	58	91	49	0	63	1	242	0	79	0	21	1	N	N	0	14	1	3	37.8	134	16.6	89	11.6	N	N	21%	1.2	N	N	N	Y	6
SR030	18/09/2017	100	114	70	0	84.67	0	310	0	51	0	9	0	N	N	0	14	1	1	37	127	16.5	>90	11.9	N	N	21%	3.9	N	N	N	Y	6
SR031	19/09/2017	117	113	64	2	93.67	4	276	0	120	1	11	0	N	N	4	3	4	13	36.7	124.1	14.6	53	11.7	Y	N	30%	6.6	N	N	Y	N	N
SR032	26/09/2017	80	123	53	6	76.33	4	162	0	47	0	5	0	N	N	4	3	4	12	37.9	125.8	9.6	N	12.7	Y	N	21%	3.9	N	N	Y	N	N
SR033	26/09/2017	95	115	60	0	78.33	0	208	0	62	0	21	1	N	N	0	15	0	1	36.4	118	6.1	>90	11.4	N	N	21%	0.8	N	N	N	Y	6
SR034	01/10/2017	90	136	62	0	86.67	0	185	0	102	0	12	0	N	N	0	15	0	0	37.5	106	11.4	73	11.6	N	N	21%	1.7	N	N	N	Y	6
SR035	11/10/2017	104	121	73	4	89	4	109	1	148	1	15	0	N	N	4	3	4	14	38.7	94	16.8	32	12.6	Y	N	30%	4.8	N	N	Y	Y	6
SR036	11/10/2017	60	97	48	0	64.33	1	198	0	68	0	13	0	N	N	0	14	1	2	38.6	135	16.3	>90	11.6		Y	40%	1.3	N	N	N	Y	6
SR037	17/10/2017	60	136	72	7	93.33	4	202	0	61	0	5	0	N	N	4	3	4	12	36.6	132.9	8.4	>90	12.8	Y	N	40%	2.6	N	N	Y	Y	6
SR038	18/10/2017	54	118	95	0	102.67	0	212	0	66	0	12	0	N	N	4	3	4	8	37	116	9.2	89	12.2	Y	N	25%	0.25	N	N	Y	Y	1
SR040	06/11/2017	63	114	58	0	76.67	0	82	2	97	0	16	0	N	N	0	15	0	2	37.4	117	11.5	64	14.2	N	N	40%	2.6	N	N	N	N	N
SR042	13/11/2017	126	110	80	0	90	0	207	0	82	0	21	1	N	N	4	N	4	9	39.2	130.1	15.9	81	15.4	Y	N	35%	1.5	N	N	Y	Y	6
SR045	28/11/2017	106	60	98	2	75.33	4	116	1	97	0	28	1	50	380	1	15	0	7	36.5	85	10	N	11.6	N	N	21%	3	58	N	N	Y	6
SR046	22/01/2018	66	98	50	0.1	65	3	193	0	192	2	10	0	N	N	4	3	4	13	37	121	10.4	24	14.4	Y	N	40%	1.4	N	N	Y	Y	1
SR047	24/01/2018	102	161	69		99.7	0	248	0	71	0	12	0	2	15.2	4	15	0	4	38.2	157	12.9	>90	11.1	N	N	50%	3.4	N	N	N	N	N
SR048	31/01/2018	95	150	72	N	94	0	214	0	81	0	19	0	23	174.8	3	15	0	3	37.5	134	15.2	87	11.6	N	N	40%	1.6	N	N	N	N	N
SR052	14/03/2018	85	123	55	0.03	78	3	270	0	67	0	14	0	56	425.6	4	ND	4	11	37.2	121	11.6	79	12	Y	N	21%	2.4	N	N	Y	N	N
SR053	15/03/2018	72	123	58	N	75	0	200	0	80	0	14	0	40	304	4	3	4	8	38	118	8.9	>90	13.4	Y	N	21%	0.9	ND	N	Y	Y	6
SR054	06/04/2018	100	128	99	N	108.67	0	274	0	95	0	7	0	N	N	0	15	4	4	36.8	160	16.6	73	12	N	N	21%	1.9	ND	N	Y	Y	6
SR055	08/04/2018	79	138	50	N	68	1	327	0	57	0	5	0	26	197.6	4	14	4	9	37.7	113	10	ND	11.5	Y	N	21%	4.6	ND	N	Y	N	N
SR056	12/04/2018	94	130	70	N	90	0	146	1	51	0	7	0	36	273.6	4	8	4	9	36.4	135	14.3	>90	14.1	Y	N	30%	1.1	ND	N	Y	N	N
SR057	16/04/2018	99	143	82	N	102	0	328	0	102	0	7	0	ND	N	0	15	0	0	37.2	145	12.9	ND	12.4	N	N	21%	ND	ND	N	N	Y	6
SR061	01/05/2018	100	130	67	N	88	0	307	0	86	0	17	0	ND	N	0	15	0	0	36	128	12.7	76	11.4	N	N	21%	1.5	ND	N	N	Y	1

Day 1

	Date Of Admission	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**	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	FiO2	Lactate	CRP	CVVH/HD	Sedated	Antibiotics	Septic source
SR062	03/05/2018	73	120	39	0.26	64	4	211	0	57	0	12	0	ND	N	4	3	4	12	36.9	104	17.8	89	ND	Y	N	28%	5	ND	N	Y	N	N
SR063	03/05/2018	106	102	56	N	72	0	174	0	62	0	29	1	ND	N	4	6	4	9	36	96	18.8	>90	10.8	Y	N	30%	0.4	ND	N	Y	Y	6
SR064	06/05/2018	70	105	58	N	73.67	0	156	0	56	0	6	0	22	167.2	4	7	4	8	37.2	124	14.8	>90	12.2	Y	N	25%	2.77	ND	N	Y	N	N
SR065	06/05/2018	55	120	60	0.03	80	3	196	0	68	0	16	0	25	190	3	15	0	6	38	146	8.1	>90	11.4	N	N	21%	1.1	ND	N	N	N	N
SR067	04/06/2018	86	122	58	0.33	78	4	129	1	70	0	12	0	33	250.8	2	3	4	13	36.7	82	19	>90	12.8	Y	N	35%	3.8	ND	N	Y	Y	6
SR068	Admitted at MRI	MRI					1		4		0		0		N	0		4	9														
SR070	43282	46	130	58	10	76	4	171	0	89	0	7	0	42	319.2	4	3	4	12	37.8	134	13.1	78	171	Y	N	0.3	4.07	ND	N	Y	Y	6
SR071	43285	76	126	74		91.33	0	213	0	57	0	14	0	51	387.6	1	13	1	2	37	121	9.4	>90	12.7	N	N	0.21	1	ND	N	N	N	N
SR072	43292	44	135	67		89.67	0	160	0	61	0	19	0	22	167.2	4	3	4	8	35.5	134	19.4	>90	12.4	Y	N	0.5	1.2	ND	N	Y	Y	N
SR074	43305	66	166	77	0.18	107	4	185	0	67	0	12	0	68	516.8	0	14	1	5	36.6	104	14.4	>90	11.5	N	N	0.28	1.5	ND	N	N	N	N
SR075	43314	95	116	60		78.67	0	205	0	56	0	4	0	ND	N	0	13	1	1	35.9	113	7.7	>90	10.8	N	N	0.21	1.5	ND	N	N	Y	6
SR077	43318	72	178	60	0.133	94	4	235	0	66	0	24	1	57	433.2	4	3	4	13	36.4	139	18.2	>90	13	Y	Y	0.21	0.9	ND	N	Y	Y	6
SR078	43320	72	129	76		93	0	233	0	75	0	4	0	58	440.8	4	5	4	8	36.9	137	8.6	>90	10.2	Y	N	0.25	1.32	ND	N	N	N	N
SR079	8/27/2018	94	140	70	0	93.333	0	171	0	101	0	8	0	34	258.4	2	15	0	2	36.9	99	10.9	46	10.9	N	N	0.21	1.7	N	N	N	Y	6
SR080	09/06/2019	72	150	90	0	110	0	220	0	72	0	23	1	40	304	1	13	1	3	36.3	138	20.4	90	13.4	N	N	0.25	N	N	N	N	N	N
SR081	09/10/2018	55	168	58	0.098	94.667	3	229	0	62	0	10	0	40.5	307.8	4	3	4	11	37	138	16	>90	12	Y	N	0.3	N	32	N	Y	N	N
SR082	09/11/2018	100	110	52	0.288	71.333	4	267	0	101	0	18	0	44.6	338.96	4	6	4	12	36.8	136	20	N	12.3	Y	N	0.5	4.1	N	N	Y	Y	6
SR083	09/11/2018	114	107	54	0.062	71.667	3	267	0	101	0	18	0	43.2	328.32	4	3	4	11	38.3	136	20	N	12.3	Y	N	0.25	2.8	N	N	Y	N	N
SR084	09/12/2018	88	125	68	0	87	0	259	0	68	0	16	0	39	296.4	2	15	0	2	37.8	149	13.7	>90	12.4	N	N	0.24	0.83	N	N	N	N	N
SR085	9/13/2018	90	182	94	0	123.33	0	327	0	78	0	4	0	23	174.8	3	14	1	4	38.6	139	11.6	61	30	N	N	0.3	1.9	36	N	N	N	N
SR086	9/16/2018	84	105	55	No Weight	71.667	0	188	0	67	0	30	1	62	471.2	0	15	0	1	35.9	121	20.7	N	17	N	N	0.21	3.9	N	N	N	Y	6
SR087	9/20/2018	75	120	77	0.17	91.333	4	260	0	84	0	15	0	51	387.6	4	3	4	12	39.2	141	25.5	87	12.7	Y	N	0.21	6	N	N	Y	N	N
SR088	10/04/2018	69	130	60	0.369	83.333	4	309	0	82	0	17	0	63	478.8	4	3	4	12	36.4	115	16.4	N	20	Y	N	0.21	5.6	N	N	Y	Y	1
SR089	10/10/2018	75	126	65	0.122	85.333	4	183	0	120	1	N	0	50.2	381.52	4	6	3	12	37.1	123	11	54	12	Y	N	0.5	5.7	N	N	Y	Y	5
SR090	11/05/2018	95	130	70	0	90	0	272	0	87	0	29	1	50	380	1	15	0	2	37.3	114	13.5	77	11.7	N	N	0.21	1	N	N	N	N	N
SR093	11/05/2018	95	125	70	0	88.333	0	231	0	90	0	7	0	49	372.4	1	15	0	1	36.3	120	13.4	58	13	N	N	0.21	2.8	N	N	N	Y	5
SR094	11/07/2018	58	130	55	0.09	80	3	199	0	65	0	24	1	55.5	421.8	0	3	4	8	35.8	135	16.1	N	14.3	N	N	0.4	1.8	N	N	Y	N	N
SR095	11/26/2018	78	80	52	0	61.333	1	189	0	48	0	2	0	21	159.6	3	11	2	6	32.4	59	4.5	N	12.6	N	N	0.35	18.6	N	N	N	Y	6
SR097	11/26/2018	84	110	58	0	75.333	0	277	0	72	0	7	0	N		0	15	0	0	37.3	111	7.2	>90	11.2	N	N	0.21	N	34	N	N	Y	6
SR098	11/29/2018	90	110	58	0	75.333	0	253	0	95	0	6	0	60	456	4	7	3	7	37.3	137	11.3	72	13.9	Y	N	0.3	2.18	N	N	Y	Y	6
SR099	12/02/2018	72	120	60	0	80	0	213	0	66	0	15	0	69	524.4	4	10	2	6	37.2	134	16	>90	12.6	Y	N	0.25	2.7	N	N	Y	N	N
SR101	1/17/2019	90	110	57	0.031	74.667	3	291	0	66	0	20	1	58.1	441.56	0	15	0	4	37	128	11.5	>90	13	N	N	0.21	1	N	N	N	N	N
SR102	1/17/2019	137	115	50	0.524	71.667	4	349	0	59	0	4	0	44.7	339.72	4	3	4	12	38.6	124	23.4	>90	12.4	Y	N	0.3	3.9	N	N	Y	N	N
SR103	1/24/2019	74	118	65	0.4075	82.667	4	214	0	66	0	6	0	40.7	309.32	4	6	3	11	36.6	138	8.3	>90	11.1	Y	N	0.4	1.6	N	N	Y	N	N

Appendix 5: Day 3 – Clinical data from Salford Royal Foundation Trust and calculated SOFA scores

																		Day 3																
	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 D3	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	FiO2	Lactate	CRP	CVVH/HD	Sedated	Antibiotics	Septic source	Steroids	CAM +ve
SR002	98	124	91	0	102	0	194	0	78	0	9	0	40	304	1	15	0	1	37.5	77	18.1	90	16.7	N	N	0.21	2	145	N	N	Y	mpirical/unknow	N	N
SR003	108	169	111	0	278	0	128	1	53	0	9	0	N	N	4	4	4	9	39.3	116	11.3	>90	N	Y	N	30%	0.7	N	N	Y	N	N	N	N
SR004	101	134	58	9	160	4	176	0	97	0	15	0	42	319.2	1	N	4	9	39.6	87	14.2	85	14.5	Y	N	30%	0.9	N	N	Y	N	N	N	N
SR008	74	134	70	0	91	0	174	0	54	0	16	0	N	N	0	14	1	1	36.9	115	6.1	>90	13.6	N	N	N	N	N	N	N	N	N	N	N
SR009	93	101	54	0	68	1	132	1	48	0	12	0	N	N	0	13	1	3	39.2	93	10.6	>90	13.2	N	N	21	1.1	N	N	N	Y	1	N	N
SR010	87	177	67	0	103	0	259	0	162	1	6	0	N	N	4	8	3	8	37.1	74	16.9	28	9.9	Y	N	21	3.3	N	N	N	N	N	Y	N
SR011	73	149	75	10	99	4	183	0	57	0	13	0	N	N	4	N	4	12	36.3	126	20	>90	12.9	Y	N	45	1.9	N	N	Y	N	N	N	N
SR012	88	160	70	4.5	100	4	143	1	52	0	7	0	N	N	4	N	4	13	37.2	120	8.8	>90	12.2	Y	N	21	N	N	N	Y	Y	N	N	N
SR013	88	181	90	N	120	0	158	0	52	0	12	0	N	N	0	10	2	2	36.9	88	14.5	>90	14.4	N	N	21	0.7	N	N	N	N	N	N	N
SR014	RIP																																	
SR015	58	144	66		92	0	12.8	4	43	0	11	0	N	N	4	3	4	12	37.3	128	11.7	N	12.8	Y	N	30	0.9	N	N	Y	N	N	N	N
SR016	76	130	52	0	78	0	143	1	65	0	10	0	N	N	0	15	0	1	36.8	131	6.4	>90	12.3	N	N	21	N	N	N	N	N	N	N	N
SR019	78	145	55	0	85	0	149	1	49	0	5	0	N	N	0	15	0	1	36.8	95	8.1	N	15.1	N	N	21	2	184	N	N	Y	5	N	N
SR020	81	137	52	N	80.3	0	142	1	N	0	6	0	N	N	4	3	4	9	37.5	73.9	9.9	>90	11.6	Y	N	21	0.7	N	N	Y	Y	6	N	
SR021	59	145	65	6	91.7	4	151	0	52	0	11	0	N	N	4	N	4	12	37.2	11.1	12	N	14.5	Y	N	21	0.6	N	N	Y	Y	6	N	N
SR022	64	158	50	8	86	4	107	1	38	0	19	0	N	N	0	14	1	6	36.8	75	12.3	N	10.8	N	N	21	0.9	N	N	N	Y	6	Y	N
SR023	82	137	61	0	86.3	0	141	1	39	0	9	0	N	N	0	13	1	2	37	102	10.3	N	N	N	N	21	0.7	N	N	N	Y	6	N	N
SR024	60	128	60	4	82.7	4	166	0	35	0	8	0	N	N	0	11	2	6	36.4	105	9.3	N	12	N	N	21	1.2	N	N	N	Y	1	N	N
SR025	68	168	72	0	104	0	139	1	67	0	14	0	74	562.4	0	8	3	4	39.2	76.8	11	>90	13.7	Y	N	24	1	N	N	N	Y	6	N	N
SR026	75	108	70	0	82.7	0	181	0	39	0	10	0	N	N	0	14	1	1	35.2	82	6.2	N	15.4	N	N	80	0.8	N	N	N	N	N	N	N
SR027	86	115	95	0	101.67	0	191	0	82	0	10	0	N	N	0	14	1	1	37.2	100	14.2	>90	12.4	N	N	25	0.7	N	N	N	Y	6	N	N
SR028	109	99	88	0	91.7	0	79	2	49	0	6	0	N	N	4	4	4	10	38.9	83	10.2	N	11.2	Y	N	21	5.2	N	N	N	N	N	N	N
SR029	70	114	53	0	73.3	0	170	0	53	0	10	0	N	N	0	14	1	1	36.9	89	9.1	>90	11.4	N	N	60	1.4	N	N	N	N	N	Y	N
SR030	72	133	75	0	94.33	0	128	1	50	0	7	0	N	N	0	15	0	1	37.4	103.3	9.7	>90	12.2	N	N	21	1.1	N	N	N	Y	6	Y	N
SR031	85	172	48	0	89.33	0	229	0	49	0	11	0	37.3	283.48	2	3	4	6	38.5	81	12.4	N	12.4	Y	N	30	1.3	240	N	Y	Y	1	N	N
SR032	64	120	45	0	70	0	120	1	90	0	9	0	N	N	0	14	1	2	37.1	108	9	N	12.8	N	N	21	0.7	N	N	N	N	N	N	N
SR033	65	117	73	2	87.67	4	178	0	68	0	8	0	N	N	0	15	0	4	37.9	116	8.2	>90	N	N	N	21	N	N	N	N	Y	6	N	N
SR034	110	155	72	0	99.67	0	159	0	68	0	9	0	N	N	0	15	0	0	38.4	92	8.1	>90	11.6	N	N	25	N	1.2	N	N	Y	1	N	N
SR035	92	167	72	0	103.67	0	86	2	148	1	18	0	N	N	4	10	2	9	37.1	76	14.5	48	10.8	Y	N	25	1.1	N	Y	Y	Y	6	N	N
SR036	69	140	60	0	86.67	0	158	0	56	0	15	0	N	N	0	15	0	0	36.1	116	6.9	>90	N	N	N	21	N	N	N	N	Y	6	N	N
SR037	45	152	60	13	90.67	4	175	0	43	0	12	0	N	N	4	3	4	12	37.3	121	15.1	N	13	Y	N	21	1.4	N	N	Y	Y	6	N	N
SR038	96	160	62	7	94.67	4	148	1	56	0	16	0	N	N	4	3	4	13	36.4	103	8.5	>90	12.6	Y	N	35	1.9	N	N	Y	Y	1	N	N
SR040	104	114	72	N	86	0	82	2	55	0	N	0	N	N	0	15	0	2	37.5	95	7	>90	N	N	N	21	N	174	N	N	Y	1	N	N
SR042	44	148	62	5.5	90.67	4	310	0	176	2	8	0	N	N	4	N	4	14	36.9	90.2	22.5	34	11.4	Y	N	25	1.9	N	N	Y	Y	6	N	N
SR045	89	100	69	0	79.33	0	83	2	61	0	12	0	N	N	0	15	0	2	37	72	8	88	10.7	N	N	21	1.3	N	N	N	Y	6	Y	N
SR046	66	125	69	N	87.7	0	N	0	N	0	N	0	N	N	0	15	0	0	37.2	N	N	N	N	N	N	40	N	N	N	N	Y	1	N	N
SR047	105	130	85	N	100	0	N	0	N	0	N	0	N	N	0	15	0	0	38	N	N	N	N	N	N	21%	N	ND	N	N	N	N	N	N
SR048	96	98	57	0.19	70.67	4	215	0	118	1	27	1	12	91.2	4	3	4	14	38.5	104	8.5	56	12.5	Y	N	80%	1.4	ND	N	Y	Y	1	N	N
SR052	61	140	80	N	100	0	184	0	49	0	ND	0	ND	N	0	15	0	0	37.4	104	9.9	ND	12.4	N	N	21	ND	ND	N	N	N	N	N	N
SR053	70	145	78	N	100.33	0	204	0	56	0	9	0	ND	N	0	15	0	0	36.9	101	16.2	>90	11.5	N	N	21	N	ND	N	N	Y	6	Y	N
SR054	75	122	64	N	83.33	0	167	0	75	0	10	0	ND	N	0	15	0	0	37.6	89	6.7	>90	14.3	N	N	35	1.3	ND	N	N	Y	6	Y	N
SR055	79	171	66	N	109	0	224	0	23	0	8	0	61	463.6	4	10	4	8	35.7	86	11.8	ND	12.5	Y	N	21	1.1	ND	N	Y	N	N	N	N
SR056	70	128	54	N	78	0	139	1	29	0	13	0	41	311.6	1	14	1	3	36.4	110	11.7	ND	16.9	N	N	25	1	ND	N	N	N	N	N	N
SR057	99	124	60	N	81	0	ND	0	ND	0	ND	0	ND	N	0	15	0	0	37.4	ND	ND	ND	ND	N	N	21	N	ND	N	N	N	N	N	N

	Day 3																																	
	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 D3	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	FiO2	Lactate	CRP	CVVH/HD	Sedated	Antibiotics	Septic source	Steroids	CAM +ve
SR061	76	104	58	ND	75	0	304	0	74	0	7	0	ND	N	0	15	0	0	37	10.6	8.4	>90	11.2	N	N	21	1.1	ND	N	N	N	N	N	N
SR062	80	135	55	0.04	85	3	209	0	36	0	10	0	45	342	4	3	4	11	38	83	13.9	ND	13.3	Y	N	25	1.44	ND	N	Y	N	N	N	N
SR063	78	122	80	ND	94	0	ND	0	ND	0	ND	0	ND	N	0	15	0	0	37	ND	ND	ND	ND	N	N	21	N	N	N	N	Y	6	N	N
SR064	70	159	60	ND	93	0	101	1	40	0	7	0	26	197.6	3	14	1	5	37.4	83.1	8.9	ND	12.2	N	N	21	0.8	N	N	N	N	N	N	N
SR065																																		
SR067	96	130	60	14	83.33	4	89	2	39	0	14	0	33	250.8	2	3	4	12	38.4	87	12.7	ND	12.9	Y	N	30	1.1	ND	N	Y	Y	6	N	N
SR068	105	153	93	ND	95	0	115	1	49	0	13	0	25.5	193.8	3	15	0	4	36.9	99	8.3	ND	11	N	Y	40	0.8	ND	N	N	Y	6	N	N
SR070	120	170	90	ND	117	0	135	1	73	0	9	0	31	235.6	2	15	0	3	36.9	130	9.1	>90	14	N	N	21	1	ND	N	N	N	N	N	N
SR071	54	127	69		88.33	0	ND	0	ND	0	ND	0	ND	N	0	14	1	1	36.5	ND	ND	ND	ND	N	N	21	N	ND	N	N	N	N	N	N
SR072	85	136	64	ND	95	0	142	1	53	0	12	0	28	212.8	2	15	0	3	37.2	109	19.5	>90	14.5	N	N	30	0.9	ND	N	N	Y	6	N	N
SR074	62	154	60	0.17	87	4	165	0	56	0	13	0	18.2	138.32	3	14	1	8	37.1	83	7.5	>90	12	N	N	60	1.2	ND	N	N	N	N	Y	N
SR075	88	132	70	ND	91	0	ND	0	ND	0	ND	0	ND	N	0	14	1	1	37.2	ND	ND	ND	ND	N	N	21	ND	ND	N	N	Y	6	N	N
SR077	62	155	62	0.126	90	4	209	0	63	0	10	0	68	516.8	4	3	4	12	36	116	7.7	>90	14.3	Y	Y	21	1.09	ND	N	Y	N	N	N	N
SR078	50	138	81	ND	97	0	181	0	64	0	10	0	ND		4	15	0	4	36.9	121	7.8	>90	11.3	N	N	21	ND	ND	N	N	N	N	N	N
SR079	97	114	47	0	69.3333	1	130	1	58	0	8	0	N			15	0	2	37.8	81	9.3	87	N	N	N	24	N	N	N	N	N		N	N
SR080	102	140	80	0	100	0	344	0	56	0	N	0	N			14	1	1	36.8	128	14.5	90	N	N	N	21	N	N	N	N	N	N	N	N
SR081	104	147	55	0.331	85.6667	4	170	0	61	0	7	0	31.9	242.44	4	3	4	12	38	112	14	>90	12.8	Y	N	0.45	N	N	N	Y	N	N	N	N
SR082	96	137	72	0	93.6667	0	173	0	47	0	36	2	32	243.2	4	6	3	9	38.2	96	17.5	N	12.6	Y	N	0.35	0.9	N	N	N	Y	6	N	N
SR083	92	129	64	0.36	85.6667	4	195	0	52	0	12	0	36	273.6	4	6	3	11	39	107	11.8	N	N	Y	N	0.35	1	N	N	Y	Y	1	N	N
SR084	104	140	63	0	88.6667	0	212	0	66	0	20	1	36.13	274.59	2	15	0	3	37.2	104	13.5	>90	13.4	N	N	0.5	1	N	N	N	Y	1	N	N
SR085	92	131	75	0	93.6667	0	N	0	N	0	N	0	N		0	15	0	0	37.1	N	N	N	N	N	N	0.21	N	N	N	N	N	N	Y	N
SR086	N	N	N	0		N	N	0	N	0	N	0	N		0	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N	N	N	N
SR087	90	150	65	0.116	93.3333	4	239	0	49	0	18	0	45	342	4	3	4	12	38.2	110	15.7	N	12.9	Y	N	30	0.9	N	N	Y	Y	6	N	N
SR088	97	134	58	0	83.3333	0	292	0	54	0	12	0	51	387.6	4	3	4	8	37.4	98	10.5	N	14.8	Y	N	0.21	0.5	N	N	Y	Y	1	N	N
SR089	95	117	54	0.076	75	3	176	0	92	0	85	2	48	364.8	4	5	4	13	37.6	91	10.6	73	11.7	Y	N	0.3	1.95	N	N	Y	Y	6	N	N
SR090	55	156	80	0	105.333	0	N	0	N	0	N	0	N		0	15	0	0	37	N	N	N	N	N	N	0.21	N	N	N	N	Y	6	N	N
SR093	70	122	64	0	83.3333	0	252	0	43	0	7	0	N		0	15	0	0	37.6	85	13.2	N	N	N	N	0.21	N	N	N	N	Y	5	N	N
SR094	70	139	50	0.34	79.6667	4	161	0	61	0	15	0	47.2	358.72	4	3	4	12	37.4	122	16.3	>90	15.1	Y	N	0.25	1.5	N	N	Y	N	N	N	N
SR095	64	111	50	0	70.3333	0	169	0	55	0	15	0	65	494	0	15	0	0	36.8	93	7.2	>90	13.1	N	N	0.21	3.3	N	N	N	Y	6	Y	N
SR097	71	132	70	0	90.6667	0	285	0	65	0	N	0	N		0	15	0	0	36.5	108	7.2	>90	N	N	N	0.21	N	N	N	N	Y	6	N	N
SR098	94	132	68	0	89.3333	0	188	0	71	0	N	0	N		0	15	0	0	37	90	8	>90	11.9	N	N	0.21	N	37	N	N	Y	6	N	N
SR099	76	90	53	0	65.3333	1	191	0	35	0	10	0	60.5	459.8	0	14	1	2	37	124	16	>90	13.3	N	N	0.21	1.6	N	N	N	N	N	N	N
SR101	87	120	70	0	86.6667	0	202	0	61	0	11	0	N		0	15	0	0	37.1	101	5.5	>90	11.6	N	N	0.21	N	120	N	N	N	N	N	N
SR102	100	128	58	0	81.3333	0	251	0	46	0	6	0	54.7	415.72	4	4	4	8	38.6	72	15.2	N	13.4	Y	N	0.3	1.2	N	N	N	N	N	N	N
SR103	90	135	55	0	81.6667	0	179	0	53	0	10	0	32	243.2	2	14	1	3	36.9	118	8.2	>90	11.9	N	N	0.3	0.8	N	N	N	N	N	N	N

<u>Appendix 6: Day 5 – Clinical data from Salford Royal Foundation Trust and calculated SOFA scores</u>

	Day 5																																	
	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	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	FiO2	Lactate	CRP	CVVH/HD	Sedated	Antibiotics	Septic source	Steroids	CAM +ve
SR002	94	111	78	0	89	0	356	0	84	0	N	0	40	304	1	15	0	1	37.2	102	10.1	90	N	N	N	0.21	N	18	N	N	Y	mpirical/unknow	N	N
SR003	100	169	81	N		4	195	0	57	0	9	0	N	N	4	5	4	12	37.7	11.5	10.5	>90	N	Y	N	30%	0.9	N	N	N	N	N	N	N
SR004	134	172	82	9	112	4	176	0	85	0	10	0	34	258.4	4	N		8	39.4	78	12.5	>90	13.2	Y	N	35%	1	N	N	N	N	N	N	N
SR008	67	130	72	0	187	0	309	0	60	0	15	0	N	N	0	14	1	1	36.7	142	6.3	>90	N	N	N	N	N	N	N	N	N	N	N	N
SR009	74	112	50	0	70	0	207	0	207	2	N	0	N	N	0	15	0	2	37.9	74	8.3	>90	N	N	N	N	N	N	N	N	Y	1	N	N
SR010	123	162	52	0	88	0	188	0	110	1	3	0	N	N	4	N	4	9	38.2	69	2.6	44	10.7	Y	N	45%	2.3	N	N	Y	N	N	Y	N
SR011	96	150	70	10	96	4	194	0	59	0	5	0	N	N	4	N	4	12	38.4	121	10.9	>90	16.8	Y	N	40%	1.3	N	N	Y	Y	1	N	N
SR012	85	125	61	0	82	0	174	0	46	0	10	0	N	N	0	15	0	0	37	118	7.1	N	12	N	N	21%	N	N	N	N	N	N	N	N
SR013	122	137	58	N	84	0	192	0	76	0	6	0	N	N	0	13	1	1	37.6	94	14.9	74	N	N	N	21%	N	N	N	N	Y	1	N	N
SR014	RIP																																	
SR015	83	150	50	7	83.3	4	328	0	51	0	7	0	295.03	2242.2	0	N	4	8	37.7	137	13.6	>90	13.4	Y	N	30%	1.1	N	N	Y	N	N	N	N
SR016	68	120	80	0	93.3	0	163	0	58	0	9	0	N	N	0	15	0	0	36.6	134	6.6	>90	11.7	N	N	21%	N	N	N	N	N	N	N	N
SR019	71	124	64	0	84	0	N	0	N	0	N	0	N	N	0	15	0	0	37	N	N	N	N	N	N	21%	N	N	N	N	N	N	N	N
SR020	90	171	64	0	99.7	0	225	0	55	0	10	0	N	N	4	3	4	8	37.5	84	11.5	N	13.6	Y	N	35%	0.7	N	N	Y	N	N	N	N
SR021	76	152	59	4	90	4	228	0	48	0	6	0	N	N	4	N	4	12	27.5	108	9.1	N	13.7	Y	N	21%	1.2	N	N	Y	Y	6	N	N
SR022	67	111	43	0	65.7	1	104	1	35	0	19	0	N	N	0	14	1	3	37.2	70	8.7	N	11.7	N	N	21%	N	N	N	N	Y	6	N	N
SR023	90	188	80	0	116	0	N	0	N	0	N	0	N	N	0	14	1	1	37.9	N	N	N	N	N	N	21%	N	N	N	N	Y	6	Y	N
SR024	86	119	60	9	83	4	224	0	44	0	15	0	59	448.4	0	6	3	7	37.4	119	9.4	N	13.9	Y	N	21%	1.1	N	N	Y	Y	1	N	N
SR025	59	140	50	0	80	0	229	0	72	0	17	0	N	N	0	10	2	2	36.8	85.1	10.1	>90	14.8	N	N	30%	0.7	N	N	N	N	N	N	N
SR026	72	172	66	0	101.3	0	207	0	42	0	10	0	N	N	0	9	3	3	36.4	91	7.4	N	11.9	N	N	22%	1.4	N	N	N	N	N	N	N
SR027	66	160	90	0	113.33	0	N	0	N	0	N	0	N	N	0	15	0	0	36.2	N	N	N	N	N	N	21%	N	N	N	N	Y	6	N	N
SR028	88	142	70	0	94	0	222	0	55	0	6	0	N	N	4	5	4	8	38.4	63.7	10.3	>90	12.8	Y	N	21%	2.2	N	N	N	Y	6	N	N
SR029	65	117	63	0	80.33	0	208	0	43	0	14	0	N	N	0	13	1	1	37.6	92	90	N	11	N	N	30%	0.9	N	N	N	N	N	N	N
SR030	72	112	84	0	93.33	0	142	1	50	0	6	0	N	N	0	15	0	1	37	104	7.8	>90	N	N	N	21%	N	50	N	N	Y	6	Y	N
SR031	93	148	50	0	82.67	0	282	0	58	0	13	0	35.1	266.76	2	3	4	6	38.6	81.3	12.5	>90	13.2	Y	N	30%	1.6	N	N	Y	Y	1	N	N
SR032	61	150	80	0	103.33	0	N	0	N	0	N	0	N	N	0	14	1	1	36.8	N	N	N	N	N	N	21%	N	N	N	N	N	N	N	N
SR033	82	126	73	0.5	90.67	4	211	0	55	0	8	0	N	N	0	15	0	4	37.2	116	6.3	>90	12.3	N	N	21%	N	N	N	N	Y	6	N	N
SR034	88	142	69	0	93.33	0	261	0	74	0	13	0	N	N	0	15	0	0	37.4	90	7.5	>90	N	N	N	21%	N	N	N	N	Y	1	N	N
SR035	75	140	70	0	93.33	0	151	0	205	2	35	2	N	N	4	10	2	10	37.2	97	20.1	33	11.6	Y	N	35%	1.2	N	N	Y	N	N	N	N
SR036	74	112	62	0	78.67	0	N	0	N	0	N	0	N	N	0	15	0	0	37.1	N	N	N	N	N	N	21%	N	N	N	N	N	N	N	N
SR037	62	162	54	5.5	90	4	175	0	43	0	7	0	N	N	4	3	4	12	37.7	108.3	14.1	N	14.4	Y	N	21%	0.8	N	N	Y	Y	6	N	N
SR038	82	138	62	1	86	4	200	0	61	0	6	0	N	N	4	3	4	12	37.8	89	7.5	>90	13.6	Y	N	35%	1	N	N	Y	Y	1	N	N
SR040	64	118	72	0	87.33	0	175	0	40	0	33	2	N	N	0	15	0	2	37	106	8.6	>90	N	N	N	21%	N	122	N	N	Y	1	N	N
SR042	68	193	66	12	108.33	4	397	0	60	0	8	0	344	2614.4	0	11	2	6	38.7	86	19.5	>90	12.3	Y	N	45%	2.1	N	N	N	Y	6	N	N
SR045	97	172	56	0	94.67	0	173	0	44	0	19	0	N	N	0	15	0	0	37.7	77	10.3	N	10.8	N	N	21%	1.2	N	N	N	Y	6	Y	N
SR046	78	120	76	0	91	0	430	0	67	0	12	0	N	N	0	15	0	0	36.9		12.1	81	14.4	N	N	21%	N	13	N	N	Y	1	N	N
SR047	103	135	82	N	100	0	N	0	N	0	N	0	N	N	0	15	0	0	37	N	N	N	N	N	N	21%	N	N	N	N	N	N	N	N
SR048	81	110	51	0.05	70	3	298	0	74	0	11	0	N	N	4	3	4	11	37.6	77	10.8	>90	12.4	Y	N	55%	1.4	N	N	Y	Y	1	N	N
SR052	68	122	78	ND	92.67	0	ND	0	ND	0	ND	0	ND	N	0	15	0	0	37	ND	ND	ND	ND	N	N	21	N	ND	N	N	Y	4	N	N
SR053	75	120	78	N	92	0	288	0	50	0	11	0	ND	N	0	15	0	0	37.1	109	11.4	>90	ND	N	N	21	N	ND	N	N	Y	6	Y	N
SR054	81	122	76	N	91.33	0	274	0	64	0	12	0	ND	N	0	15	0	0	36.6	94	6.8	>90	12.3	N	N	21%	N	ND	N	N	N	N	Y	N
SR055	74	124	44	0.06	74	3	232	0	30	0	7	0	58	440.8	4	3	4	11	35.6	77	9	ND	11	Y	N	21%	2	ND	N	N	N	N	N	N
SR056	85	111	54	ND	73	0	176	0	140	1	8	0	ND	N	0	14	1	2	36.7	116	6	ND	ND	N	N	24%	N	222	N	N	Y	1	N	N
SR057	98	120	70	ND	86.67	0	225	0	119	1	9	0	ND	N	0	15	0	1	37.4	101	8.3	53	ND	N	N	21%	N	281	N	N	Y	1	N	N

	Day 5																																	
	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	Temp	Hb	WCC	eGFR	PT	Intubated	NIV / CPAP	FiO2	Lactate	CRP	CVVH/HD	Sedated	Antibiotics	Septic source	Steroids	CAM +ve
SR061	67	104	58	N	73.33	0	ND	0	85	0	4	0	ND	N	0	15	0	0	37.2	ND	ND	77	ND	N	N	21%	ND	ND	N	N	N	N	N	N
SR062	82	132	45	0.07	80	3	237	0	44	0	9	0	17	129.2	4	3	4	11	38	89.2	19.9	ND	15.3	Y	N	25%	1.2	ND	N	Y	N	N	N	N
SR063	70	120	70	ND	86.67	0	385	0	41	0	10	0	ND	N	0	14	1	1	37	89	5.4	ND	ND	N	N	21%	ND	100	N	N	Y	6	N	N
SR064																																		
SR065																																		
SR067	136	138	70	ND	94	0	247	0	39	0	12	0	65	494	4	3	4	8	39.4	74	11.5	ND	14.5	Y	N	21%	1.4	ND	N	Y	Y	6	N	N
SR068	88	171	69	ND	103	0	188	0	51	0	15	0	ND		0	15	0	0	37.5	82	8.4	>90	10.2	N	N	30%	ND	ND	N	N	Y	6	Y	N
SR070	62	122	70	ND	87	0	ND	0	ND	0	ND	0	ND		0	15	0	0	36.6	ND	ND	ND	ND	ND	ND	0.21	ND	ND	N	N	N	N	N	N
SR071	52	120	50	ND	73.33	0	ND	0	ND	0	ND	0	ND		0	14	1	1	36.1	ND	ND	ND	ND	N	N	0.21	ND	ND	N	N	N	N	N	N
SR072	75	136	64	ND	88	0	ND	0	49	0	16	0	ND		0	15	0	0	36.5	ND	ND	ND	ND	N	N	0.21	ND	ND	N	N	Y	6	N	N
SR074	76	113	46	ND	54	1	235	0	55	0	15	0	ND		0	14	1	2	36.5	90	8.6	>90	ND	N	N	0.21	ND	ND	N	N	N	N	Y	N
SR075	95	130	90	ND	103	0	ND	0	ND	0	ND	0	ND		0	14	1	1	36.8	ND	ND	ND	ND	N	N	0.21	ND	ND	N	N	Y	6	N	N
SR077	68	132	80	ND	98	0	ND	0	ND	0	ND	0	ND		0	14	1	1	36.8	ND	ND	ND	ND	N	N	0.21	ND	ND	N	N	N	N	N	N
SR078	DISCHARGED																																	
SR079	102	131	43	0	72.3333	0	320	0	45	0	15	0	N			15	0	0	36.5	91	10	N	87	N	N	0.24	N	60	N	N	Y	1	N	N
SR080	85	149	70	0	96.3333	0	344	0	58	0	20	1	N			14	1	2	37.3	129	11.3	90	N	N	N	0.21	N	135	N	N	N	N	N	N
SR081	98	185	70	0.368	108.333	4	200	0	60	0	10	0	34	258.4	4	3	4	12	38.9	98	16.5	>90	13.5	Y	N	0.45	N	309	N	Y	Y	1	N	N
SR082	95	145	75	0	98.3333	0	547	0	46	0	25	1	42	319.2	1	10	2	4	38.2	88	25	N	13.7	N	N	0.24	1.1	N	N	N	Y	1+6	N	Y
SR083	95	121	60	0.021	80.3333	3	247	0	45	0	12	0	44	334.4	4	3	4	11	38.6	94	10.4	N	12.4	Y	N	0.42	0.9	N	N	Y	Y	1	N	N
SR084	75	135	55	0	81.6667	0	263	0	53	0	16	0	N			15	0	0	37.1	131	9.8	>90	N	N	N	0.28	N	N	N	N	Y	6	N	N
SR085	98	130	62	0	84.6667	0	305	0	93	0	15	0	N			15	0	0	38.3	122	15.2	50	N	N	N	0.21	N	125	N	N	N	N	Y	N
SR086	N	N	N	0	#VALUE!	0	N	0	N	0	N	0	N		N	N	N	0	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SR087	112	122	57	0	78.6667	0	302	0	45	0	16	0	34	258.4	4	3	4	8	37.5	97	23.5	N	13.2	Y	N	0.3	1.9	N	N	Y	Y	6	N	N
SR088	100	136	63	0.14	87.3333	4	346	0	52	0	8	0	60	456	4	5	4	12	37.3	97	9.4	N	14	Y	N	0.21	0.4	N	N	Y	Y	1	N	N
SR089	81	155	55	0	88.3333	0	194	0	52	0	56	2	35.3	268.28	2	15	0	4	37.7	88.8	12.6	>90	11.1	N	N	0.3	1.36	N	N	N	N	N	N	N
SR090	67	124	68	0	86.6667	0	359	0	82	0	11	0	N			15	0	0	36.6	124	9.3	83	N	N	N	0.21	N	15	N	N	N	N	N	N
SR093	78	126	68	0	87.3333	0	N	0	N	0	N	0	N			15	0	0	36.2	N	N	N	N	N	N	0.21	N	N	N	N	Y	5	N	N
SR094	108	125	68	0.48	87	4	170	0	50	0	8	0	50.3	382.28	4	3	4	12	37.7	118	15.6	>90	15.3	Y	N	0.3	0.8	N	N	Y	Y	6	N	N
SR095	90	124	60	0	81.3333	0	231	0	46	0	N	0	N			15	0	0	37.2	87	9.7	N	N	N	N	0.21	N	93	N	N	N	N	Y	N
SR097	86	120	70	0	86.6667	0	351	0	68	0	7	0	N			15	0	0	37	108	7	>90	11.7	N	N	0.21	N	97	N	N	Y	5	N	N
SR098	92	132	70	0	90.6667	0	253	0	85	0	N	0	N			15	0	0	36.2	98	10.7	82	N	N	N	0.21	N	N	N	N	Y	6	Y	N
SR099	77	138	80	0	99.3333	0	N	0	N	0	N	0	N			14	1	1	37.3	N	N	N	N	N	N	0.21	N	N	N	N	N	N	N	N
SR101	91	132	80	0	97.3333	0	351	0	61	0	15	0	N			15	0	0	37.3	113	7.8	>90	N	N	N	0.21	N	171	N	N	N	N	N	N
SR102	75	140	68	0	92	0	325	0	48	0	6	0	54	410.4	4	4	4	8	38.5	80	12.6	N	13.8	Y	N	0.3	1.1	N	N	N	Y	1	N	N
SR103	77	180	100	0	126.667	0	263	0	59	0	7	0	N			11	2	2	37.8	141	9.2	>90	N	N	N	25	N	N	N	N	N	N	N	N

Appendix 7: Day 1 – T lymphocytes (CD3+, CD4+, CD8+, CD25BRI, CD25BRIFOXP3+, CD4+FOXP3+, CD127LOWFOXP3+) concentration and SOFA scores

DAY 1

	CD3+						CD4+						CD8+					
	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD
BIT001	47.36	47.00	47.39	47.37	47.28	0.19	72	72.23	72.75	72.33	72.33	0.31	27.93	27.5	27.38	27.67	27.62	0.24
BIT002	74.22	73.90	73.15	72.31	73.40	0.85	36.15	37.14	37.6	36.71	36.9	0.62	51.02	49.84	49.42	48.42	49.68	1.08
BIT003	66.72	66.08	66.69	66.83	66.58	0.34	63.13	66.76	65.18	64.62	64.92	1.5	27.59	26.73	26.55	27.52	27.1	0.53
BIT004	74.45	74.97	72.85	75.02	74.32	1.01	54.79	53.74	50.55	53.49	53.14	1.82	46.18	45.91	47.27	46.71	46.52	0.6
BIT005	73.28	75.54	72.06	71.61	73.12	1.76	68.33	65.54	66.45	66.26	66.65	1.19	31.97	30.53	33.55	32.72	32.19	1.28
BIT006	85.37	85.47	84.90	83.58	84.83	0.87	73.43	72.7	73	73.39	73.13	0.35	23.66	25.42	23.8	23.79	24.17	0.84
BIT007	60.73	60.90	61.60	61.74	61.24	0.50	60.22	60.53	59.34	60.39	60.12	0.54	33.75	34.72	33.18	32.98	33.66	0.78
BIT008	66.72	68.90	64.20	65.73	66.39	1.97	48.49	50.69	50.07	51.78	50.26	1.37	43.22	44.61	44.31	43.21	43.84	0.73
BIT009	71.94	69.25	70.67	71.48	70.84	1.18	67.45	68.21	68.61	68.46	68.18	0.52	32.5	29.95	31.69	31.06	31.3	1.08
BIT010	82.39	82.17	83.69	82.79	82.76	0.67	50.52	51.94	51.02	50.82	51.08	0.61	39.64	40.88	40.59	40.76	40.47	0.56
BIT011	73.88	75.00	73.92		74.27	0.64	75.09	74.99	74.27		74.78	0.45	22.47	20.75	22.67		21.96	1.06
BIT014	67.81	66.05	63.65	66.85	66.09	1.78	58.07	57.58	57.03	57.69	57.59	0.43	37.61	37.84	38.44	37.98	37.97	0.35
BIT016	57.03	58.15	57.01	56.84	57.26	0.60	44.29	44.76	44.6	44.88	44.63	0.26	53.25	51.31	51.69	51.3	51.89	0.93
BIT017	83.87	83.71	83.33	84.87	83.95	0.66	63.53	63.74	63.76	61.88	63.23	0.9	35.75	37.16	36.21	36.12	36.31	0.6
BIT018	88.23	87.59	87.47	88.98	88.07	0.69	75.95	75.59	75.29	75.73	75.64	0.28	21.75	24.84	22.41	23.82	23.21	1.39
BIT021	70.02	70.18	70.13	71.20	70.38	0.55	62.67	61.59	61.95	61.17	61.85	0.64	27	28.5	28.38	28.44	28.08	0.72
BIT022	73.00	73.07	71.30	71.04	72.10	1.08	60.44	60.98	59.94	59.33	60.17	0.7	37.97	36	36.06	37.86	36.97	1.09
BIT023	65.03	66.83	64.04	66.71	65.65	1.35	57.03	55	58.95	56.21	56.8	1.66	21.18	20.41	20.56	20	20.54	0.49
BIT024	77.72	76.38	77.11	76.63	76.96	0.59	48.59	48.14	48	48.61	48.34	0.31	47.28	47.96	47	47	47.31	0.45
BIT025	53.38	53.71	52.80	52.32	53.05	0.62	65	66.59	65.46	65.02	65.52	0.75	26.2	26.16	26.96	26.05	26.34	0.42
BIT027	67.46	67.63	67.80	67.70	67.65	0.14	68.76	68.25	67.08	68.45	68.14	0.73	30.46	30.91	30.2	30.07	30.41	0.37
BIT028	46.39	46.05	45.23	46.10	45.94	0.50	51.01	52.48	52.48	51.04	51.75	0.84	40.96	41.88	40.88	40.55	41.07	0.57
BIT029	61.25	62.93	60.41		61.53	1.28	57.97	56.77	57.87		57.54	0.67	36.39	36.49	36.8		36.56	0.21
BIT030	63.94	63.55	63.27	63.50	63.57	0.28	51	51.47	52.08	51.34	51.47	0.45	34	33.6	33.72	33.61	33.73	0.19
BIT034	61.28	59.12	61.06	58.33	59.95	1.45	53	52.13	53.78	53.88	53.2	0.81	38	38.74	38.03	38.48	38.31	0.36
BIT035	75.10	74.24	75.32	75.18	74.96	0.49	70.49	70.64	70.09	69.53	70.19	0.5	13.98	14.23	14.57	14.37	14.29	0.25
BIT040	71.84	72.30	72.46	71.10	71.93	0.61	47.33	45.73	45.76	44.78	45.9	1.06	44.57	45.92	45.54	47.62	45.91	1.27
BIT042	53.96	41.83	55.50		50.43	7.49	62.36	64.95	56.79		61.37	4.17	27.35	27.1	25.33		26.59	1.1
BIT043	71.00	70.87	70.48	72.28	71.16	0.78	73	72.73	71.71	74.21	72.91	1.03	26	25.48	27.87	24.71	26.02	1.35
BIT046	44.28	36.52	46.00	48.58	43.85	5.19	76.78	74.25	75.3	82.59	77.23	3.72	17.25	16.84	13.61	12.47	15.04	2.36
BIT047	65.06	65.06	65.02	63.42	64.64	0.81	30.03	29.7	30.02	30.05	29.95	0.17	60.71	61.88	63.43	63.04	62.27	1.23
BIT048	51.56	48.10	45.27	74.96	54.97	13.57	60.28	61.75	60.3	60.76	60.77	0.69	37.43	35.97	37.15	36.57	36.78	0.65
BIT049	81.00	81.59	82.30	82.64	81.88	0.73	74	74.72	75.53	74.13	74.6	0.7	26	26.1	25.61	26.18	25.97	0.25
BIT050	38.89	39.04	37.66	50.64	41.56	6.74	37.97	40.27	38.61	43.01	39.97	2.52	57.94	54.97	55.82	51.97	55.18	2.99
BIT052	75.26	77.52	75.56		76.11	1.23	56.92	53.59	56.69		55.73	1.86	46.8	50.69	45.28		47.59	2.79
BIT053	83.09	80.28	81.63	78.34	80.84	2.02	60	62.95	60.41	61.27	61.16	1.31	38.31	35.4	37.62	36.67	37	1.26
BIT055	79.36	78.76	78.05	77.36	78.38	0.87	45.56	49.35	49.92	48.06	48.22	1.94	47.13	47.73	46.88	46.87	47.15	0.4
BIT060	53.73	55.02	53.40	55.70	54.46	1.08	81.16	81.24	80.26	82.87	81.38	1.09	19.09	17.45	18.09	15.94	17.64	1.32
BIT061	71.46	72.92	70.02	67.84	70.56	2.17	53.76	54.03	52.81	53.6	53.55	0.52	44.58	43.12	45.02	43.57	44.07	0.88
BIT065	72.14	73.66	72.10	73.42	72.83	0.83	64.18	64.46	66.69	64.81	65.04	1.13	32.02	31.85	30.24	32.69	31.7	1.04
BIT066	66.22	68.42	65.84	69.22	67.43	1.65	69.81	69.75	71.23	70.23	70.26	0.68	26.61	25.23	23.39	24.24	24.87	1.38
BIT069	62.39	60.02	61.32	62.91	61.66	1.28	54.56	52.48	52.74	52.99	53.19	0.94	35.52	36.39	36.76	36.46	36.28	0.53

	CD25BRI						CD25BRI FOXP3+					CD4+ FOXP3					CD127 LOW FOXP3					
	FMO	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	DAY 1 SOFA
BIT001	11.11	11.11	17.14	16.67	14.01	3.35	100	100	100	100	0	8.64	9	9.16	8.93	0.27						3
BIT002		15.22	28.57	8.7	16.8	8.39	98.6	100	100	99.53	0.81	11.91	9.43	10.92	10.75	1.25						5
BIT003	24.19	4.55	17.14	24.24	17.53	9.27	100	100	100	100	0	8.91	9.43	8.87	9.07	0.31						2
BIT004	18.42	15.38	12.82	6.06	13.17	5.26	100	100	100	100	0	5.18	5.04	5.97	5.4	0.5						0
BIT005	5.56	5.77	5.99	6.45	5.94	0.38	100	100	100	100	0	9.4	9.61	10.94	9.98	0.84						0
BIT006	15.08	17.28	5.22	9.5	11.77	5.46	97.45	98.52	98.09	98.02	0.54	2.7	2.57	2.77	2.68	0.1						0
BIT007	5.34	6.86	20.97	3.29	9.12	8.04	100	98.02	98.5	98.84	1.03	8.23	6.16	8.83	7.74	1.4						1
BIT008	11	9.19	7.37	7.24	8.7	1.77	98.75	98	97	97.92	0.88	6.04	6.02	6.86	6.31	0.48						1
BIT009	19.31	14.4	20.43	14.79	17.23	3.08	78.38	77.23	76	77.2	1.19	3.25	4.85	4.03	4.04	0.8						1
BIT010	13.04	27.45	34.69	21.34	24.13	9.19	98.79	98.65	100	99.15	0.74	6.22	8.05	6.26	6.84	1.05						4
BIT011	30.77	24.63	27.73		27.71	3.07	98.51	98.51		98.51	0	5.87	5.17		5.52	0.49						1
BIT014	5.8	3.94	7.32	13.99	7.76	4.38	100	100	100	100	0	9.96	9.69	9.64	9.76	0.17	100	100	100	100	0	3
BIT016	11.63	20	14.91	12.71	14.81	3.72	98	100	100	99.33	1.15	6.58	6.89	6.83	6.77	0.16	98.31	100	100	99.44	0.98	5
BIT017	11.22	10.37	12	12.82	11.6	1.05	96.15	97	97.33	96.83	0.61	9.59	8.25	10.36	9.4	1.07	96.15	97	97.18	96.78	0.55	0
BIT018	15.19	12.64	10.31	36.21	18.59	11.92	88	89	89.4	88.8	0.72	19	19.09	18.95	19.01	0.07	80	80.5	83.65	81.38	1.98	0
BIT021	25.45	23.58	21.32	20.08	22.61	2.39	98.21	100	98	98.74	1.1	21.31	20.35	20.56	20.74	0.5	98.04	100	98.63	98.89	1.01	2
BIT022	30.28	14.83	12.93	19.69	19.43	7.77	96	95	96.08	95.69	0.6	15.43	15.04	15.22	15.23	0.2	95	96.01	97.67	96.23	1.35	0
BIT023	18.93	16.71	19.09	16.31	17.76	1.45	97.06	98.78	100	98.61	1.48	11.59	10.94	11.22	11.25	0.33	96.3	98	100	98.1	1.85	2
BIT024	8.86	20	4.11	7.5	10.12	6.88	100	100	100	100	0	5.3	5.66	5.33	5.43	0.2	100	100	100	100	0	1
BIT025	15.44	22.55	32.47	19.64	22.53	7.24	100	98.25	100	99.42	1.01	17.99	18	17.64	17.88	0.21	100	100	100	100	0	11
BIT027	27.34	22.86	7.58	13.04	17.71	9.01	66.67	66	66.3	66.32	0.34	8.49	7.44	7.5	7.81	0.59	54.2	54	54.44	54.21	0.22	5
BIT028	26.21	20.7	25.13	13.4	21.36	5.82	70	73.83	73	72.28	2.01	13.42	13.46	12.8	13.23	0.37	66	66.67	66	66.22	0.39	2
BIT029	8.93	9.78	22.78		13.83	7.76	48	48.57		48.29	0.4	6.13	7.84		6.99	1.21	44	44.44		44.22	0.31	2
BIT030	28.82	29.3	25.78	25	27.23	2.15	82	82.6	83.95	82.85	1	13	13.34	13.36	13.23	0.2	81	82	82.95	81.98	0.98	10
BIT034	22.55	19.63	27.71	19.5	22.35	3.84	93	93.52	93.55	93.36	0.31	8.71	8.04	8.78	8.51	0.41	93.17	93.33	93.55	93.35	0.19	6
BIT035	19.51	18.03	21.09	15.37	18.5	2.43	78	78.23	78	78.08	0.13	7.9	8	8.23	8.04	0.17	78	77.8	77.9	77.9	0.1	0
BIT040	16.47	9.09	11.18	10.14	11.72	3.28	91.67	100	92.86	94.84	4.51	8.89	12.36	10.87	10.71	1.74	91.67	100	93.75	95.14	4.34	0
BIT042	37.78	48.68	18.09		34.85	15.5	97.3	100		98.65	1.91	15.97	15.48		15.73	0.35	100	100		100	0	0
BIT043	51.76	40.95	34.25	24.53	37.87	11.45	90.7	86.67	87.13	88.17	2.21	12.16	10.88	11	11.35	0.71	91.67	87.67	87.5	88.95	2.36	3
BIT046	20.1	28.89	12.66	21.18	20.71	6.64	94.23	96.67	97.67	96.19	1.77	10.47	9.93	9.67	10.02	0.41	98.04	100	95.65	97.9	2.18	5
BIT047	21.27	21.63	13.24	21.1	19.31	4.05	100	100	100	100	0	5.38	4.92	4.2	4.83	0.59	100	100	100	100	0	6
BIT048	29.48	32.64	31.4	31.22	31.19	1.3	83.33	60.53	97.1	80.32	18.57	10.51	11.43	10.43	10.79	0.56	84.21	65.53	95.71	81.82	15.23	9
BIT049	15.56	14.2	14.23	14.23	14.56	0.67	43.24	51.43	42.96	45.88	4.81	9.12	10.17	9.23	9.51	0.58	44.92	54.69	50.89	50.17	4.93	4
BIT050	33.01	33.01	36.96	36.64	34.91	2.1	63.24	41.18	63.55	55.99	0.22	25.7	14.86	26.08	22.21	0.27	64.91	47.46	64.36	58.91	0.39	9
BIT052	27.09	18.36	18.6		21.35	4.97	86.51	81.97		84.24	3.21	13.37	12.91		13.14	0.33	89.43	85.48		87.46	2.79	8
BIT053	18.51	13.28	16.53	12.36	15.17	2.86	61.76	60	70.59	64.12	5.67	10.21	10.38	11.75	10.78	0.84	80	64.1	94.59	79.56	15.25	3
BIT055	19.69	9.23	5.93	9.35	11.05	5.97	91.67	85.71	60	79.13	16.83	5.52	5.78	5.22	5.51	0.28	100	77.78	71.43	83.07	15	2
BIT060	15.6	15.35	14.35	13.94	14.81	0.79	65.71	68.75	74.29	69.58	4.35	10.34	11.76	10.66	10.92	0.74	62.5	66.67	73.68	67.62	5.65	9
BIT061	8.93	12.14	10.71	7.94	9.93	1.87	64.71	100	100	88.24	20.37	5.43	9.63	5.61	6.89	2.37	60	92.86	92.31	81.72	18.81	7
BIT065	19.35	22.47	15.74	15.29	18.21	3.37	95.04	90.32	96.15	93.84	3.1	10.45	10.82	11.6	10.96	0.59	100	93.1	95.65	96.25	3.49	2
BIT066	5.69	5.69	3.65	8	5.76	1.78	78.57	100	56	78.19	22	8.72	8.61	7.69	8.34	0.57	85.71	100	47.06	77.59	27.39	0
BIT069	8.56	11.3	16.76	7.14	10.94	4.25	95	83.87	64.29	81.05	15.55	7.94	8.78	7.27	7.99	0.76	94.44	83.97	61.59	79.97	16.77	9

	DAY 1																																							
	CD3+						CD4+						CD8+						CD25BRI						CD25BRI FOXP3+					CD4+ FOXP3					CD127 LOW FOXP3					
	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	DAY 1 SOFA
BIT070	54.10	59.68	56.98	60.78	57.89	2.99	56.6	54.28	50.33	54.69	53.98	2.63	39.19	39.53	42.08	39.68	40.12	1.32	12.2	20.96	7.46	11.17	12.95	5.72	85.71	100	100	95.24	6.37	6.96	8.3	9.57	8.28	1.31	87.5	100	100	95.24	8.25	8
BIT072	70.00	71.34	71.00	69.00	70.34	1.06	30.56	33.76	35.02	37.95	34.32	3.06	59.01	55.72	48.28	48.64	52.91	5.32	19.27	17.89	20	14.71	17.97	2.34	100	75	80	85	13.23	8.39	8.14	8.43	8.32	0.16	94.44	100	100	98.15	3.21	2
BIT073	17.40	21.05	17.64	20.42	19.13	1.88	79.7	76	76.53	74.37	76.65	2.23	26.75	28.08	24.15	26	26.25	1.64	21.01	20.86	22	20.47	21.09	0.65	100	90.91	96.15	95.69	4.56	20.7	17.63	18	18.78	1.68	100	91.3	96	95.77	4.35	5
BIT074	61.59	60.66	59.68	60.98	60.73	0.96	60.63	58.62	57.77	57.92	58.74	1.32	36.13	37.26	36.96	34.34	36.17	1.31	9.17	11.35	7.69	9.49	9.43	1.5	100	100	93.33	97.78	3.85	9.45	8.7	6.91	8.35	1.31	100	100	92.86	97.62	4.12	4
BIT076	50.02	45.94	43.28	47.68	46.73	2.84	64.13	63	55.41	60.36	60.73	3.88	33.55	32.22	34.29	34.1	33.54	0.93	9.33	9.15	4.93	8.16	7.89	2.04	100	100	100	100	0	12.79	11.59	11.61	11.99	0.69	100	100	100	100	0	12
BIT078	69.44	68.7	62.74	72.25	68.28	3.99	45.92	44.83	45.74	44.63	45.28	0.64	51.87	40.83	47.47	52.26	48.11	5.32	7.44	3.6	10.35	13.24	8.66	4.12	100	83.33	100	94.44	9.62	7.36	7.11	8.42	7.63	0.69	100	78.75	100	92.92	12.27	10
BIT079	42.44	43	44.23	43	43.17	0.75	71.55	70.59	73.72	72.96	72.21	1.4	28.41	25.33	24.4	24.04	25.55	1.98	20.75	8.64	19.61	9.78	14.69	6.37	100	96.67	100	98.89	1.92	8.33	7.56	8.5	8.13	0.5	100	96.67	100	98.89	1.92	1
BIT084	76.02	72.2	72.36		73.53	2.16	67.19	64.96	66.5		66.22	1.14	23.44	23.21	25.35		24	1.17	20.71	13.89	16.71		17.1	3.43	80	87.04		83.52	4.98	12.67	13.63		13.15	0.68	90.77	67.8		79.29	16.24	8
BIT085	60	60.93	52.38	59.32	58.18	3.91	64.03	76.25	76.07	76.94	73.32	6.21	19.8	22.69	22.72	22.02	21.81	1.38	21.49	11.4	8.39	15.69	14.24	5.68	100	100	95.83	98.61	2.41	10.01	11.1	9.9	10.34	0.66	96.77	100	95.92	97.56	2.15	3
BIT088	30.47	30.49	26.5	23.9	27.84	3.23	52.52	57.65	58.57	56.51	56.31	2.67	31.16	29.55	30.26	29.08	30.01	0.91	17.65	21.88	25.61	12.48	19.41	5.65	82.14	100	84.62	88.92	9.68	8.25	8.2	8.32	8.61	0.06	81.48	100	86.67	89.38	9.55	3
BIT099	40.62	39.99	42.88	39.56	40.76	1.48	57.10	56.53	58.87	54.95	56.86	1.62	36.40	34.65	35.19	35.39	35.41	0.73	34.62	20.38	37.69	28.65	30.24	7.62	71.88	96.00	83.67	83.85	12.06	10.89	11.10	11.68	11.22	0.41	84.86	94.67	77.27	85.60	8.72	0
BIT100	63.4	69.88	57.25	66.29	64.21	5.34	67.48	63.58	65.3	64.6	65.24	1.65	32.74	34.28	35.84	34.57	34.36	1.27	35.71	20.35		24.53	26.86	7.94	65.72		57.09	61.41	6.1	10.72	11.61	10.98	11.1	0.46	87.72		83.67	85.69	2.86	1
BIT102	87.02	88.17	87.50	84.32	86.75	1.69	75.45	73.22	73.04	73.35	73.77	1.13	37.74	39.63	39.48	33.52	37.59	2.85	26.79	17.07	17.70	22.77	21.08	4.58	81.32	87.23	49.12	72.56	20.51	14.33	17.92	13.90	15.38	2.21	89.29	93.48	74.43	85.73	10.01	3
BIT103	76.00	72.73	77.46	71.28	74.37	2.85	63.00	61.87	61.86	62.09	62.21	0.54	33.74	37.39	36.69	36.31	36.03	1.59	12.18	23.65	24.35	21.66	20.46	5.64	88.57	90.16	86.27	88.33	1.96	10.94	10.89	10.03	10.62	0.51	92.38	96.77	92.08	93.74	2.63	4
BIT104	87.34	85.12	84.00	86.00	85.62	1.41	21.25	22.64	23.98	22.00	22.47	1.16	81.84	78.07	76.05	75.00	77.74	3.02	28.40	18.88	20.37	20.00	21.91	4.37	76.60	63.64	75.00	71.75	7.07	9.87	10.96	10.00	10.28	0.60	76.60	71.74	75.00	74.45	2.48	1
BIT107	24.96	35.30	32.90	39.84	33.25	6.23	60.02	55.98	52.10	52.01	55.03	3.81	37.66	39.26	41.76	38.30	39.25	1.80	17.00	10.93	15.34	22.28	16.39	4.68	90.00	92.00	82.93	88.31	4.77	17.61	15.87	17.18	16.88	0.91	84.47	92.59	83.72	86.93	4.92	14
BIT111	84.96	84.60	86.91	83.47	84.98	1.43	45.33	42.74	43.49	44.38	43.98	1.12	53.17	55.17	53.15	54.16	53.91	0.96	21.94	29.18	25.91	23.33	25.09	3.18	84.00	71.88	81.63	79.17	6.42	12.37	11.87	12.77	12.34	0.45	84.13	75.81	81.63	80.52	4.27	3
BIT SR002	56.4	54.04	57.46	56.73	56.16	1.48	54.5	52.81	52.49	52.09	52.97	1.06	50.67	52.22	52	52.85	51.94	0.92	19.66	10.87	17.42	19.86	16.95	4.2	80	91.3	89.29	86.86	6.03	6.94	6.83	8.01	7.26	0.65	80	90.91	88	86.3	5.65	5
BIT SR003	43.37	42.9	44.1	46.57	44.24	1.63	50.27	49.28	49.71	50.75	50	0.64	45.89	46.9	45.58	54.04	45.85	0.78	9.91	11.34	12.07	15.2	12.13	2.23	90.91	85.71	63.16	79.93	14.75	6.24	7.12	3.75	5.7	1.75	91.67	86.67	57.69	78.68	18.35	12
BIT SR008	60	60.24	62.4	59.28	60.48	1.34	66.67	63.28	60.77	62.42	63.29	2.49	31.24	32.04	34.04	33.03	32.59	1.21	22.22	19.63	20.49	17.22	19.89	2.08	97.62	95.24	100	97.62	2.38	11.33	10.23	9.46	10.34	0.94	100	100	100	100	0	6
BIT SR009	83	83.06	82.88	87.1	84.01	2.06	57.46	56.22	56.35	56.49	56.63	0.56	40.05	42.56	42.4	42.66	41.92	1.25	15.12	9.94	8.63	15.28	12.24	3.46	96.97	100	93.48	96.82	3.26	9.67	9.38	8.94	9.33	0.37	97.22	100	93.62	96.95	3.2	1
BIT SR010	50.81	50.02	50.12	50.06	50.25	0.37	61.38	59.22	58.42	60.53	59.89	1.32	38.06	39.54	40.54	37.87	39	1.27	28.07	9.36	18.07	9.17	16.17	8.96	81.82	100	100	93.94	10.49	9.25	10.52	10.56	10.11	0.75	81.82	100	100	93.94	10.5	3
BIT SR011	65.59	67.4	63.41	68.01	66.1	2.07	59.25	56.32	57.07	57.5	57.54	1.24	37.01	39.26	37.62	37.73	37.91	0.96	22.66	18.42	29.07	12.18	20.58	7.11	85.71	87.88	96.77	90.12	5.86	16.86	16.8	16.6	16.75	0.14	87.88	88.71	93.75	90.11	3.18	8
BIT SR012	60	57.34	57.82	60.84	59	1.69	85.53	87.62	84.75	85.29	85.8	1.26	10.76	9.34	11.48	9.87	10.36	0.95	10.75	12.75	18.71	12.09	12.58	3.52	97.37	90.1	89.19	92.22	4.48	12.44	10.78	12.33	11.85	0.93	100	91.8	87.8	93.2	6.22	12
BIT SR013	59.47	56.42	61.3	57.7	58.72	2.13	69.89	68.95	69.13	67.9	68.97	0.82	30.5	30.52	30.03	31.16	30.55	0.46	58.83	45.63	41.89	42.69	47.26	7.88	90.76	94.37	95.89	93.67	2.64	15.48	15.82	15.11	15.47	0.36	92.5	95.58	98.47	95.52	2.99	9
BIT SR019	54.5	52.8	56.81	52.14	54.06	2.08	61.8	59.51	64.15	61.57	61.76	1.89	28.4	30.53	28.8	29.57	29.33	0.94	38	42.86	36.44	45.03	40.58	4.03	47.44	47.29	53.25	49.33	3.4	6.43	7.24	6.6	6.76	0.43	47.95	49.18	52.7	49.94	2.47	0
BIT SR020	64.44	66.36	65.44	63.6	64.96	1.2	47.89	47.54	48.08	49.75	48.32	0.98	48.45	48.75	44.74	45.68	46.91	1.99	29.41	30	27.4	22.34	27.29	3.48	90.76	94.37	95.89	93.67	2.64	15.48	15.82	15.11	15.47	0.36	92.5	95.58	98.47	95.52	2.99	12
BIT SR021	53.74	50.74	54.06	59.48	54.51	3.64	54.11	58.81	53.82	55.14	55.47	2.3	38.1	34.1	37.49	35.98	36.42	1.78	40.71	33.33	40.89	40.92	38.96	3.76	94.74	97.66	88.24	93.55	4.82	10.98	11.15	11.36	11.16	0.19	94.12	98.25	90.72	94.36	3.77	13
BIT SR022	80.00	82.23	81.71	81.14	81.27	0.96	65	65.05	62.23	62.58	63.72	1.52	31	31.02	33.77	34.56	32.59	1.85	20	13.91	17.23	17.26	17.83	2.49	85.99	96.3	93.51	91.8	5.56	10.27	10.46	10.87	10.53	0.31	88.24	100	96.88	95.04	6.09	8
BIT SR023	40.39	43.05	46.3	47.21	44.24	3.13	67.93	66.63	69.06	67.36	67.75	1.03	30.13	29.85	28.37	30.19	29.64	0.86	30.3	14.74	19.39	16.27	20.18	7.02	72.92	48	64.58	61.83	12.69	13.41	10.25	10.28	11.31	1.82	82.61	72.22	73.91	76.25	5.57	9
BIT SR024	58.40	56.05	59.48	59.88	58.45	1.72	54.66	53.58	58.78	60.22	56.81	3.19	45.07	46.1	40.74	39.78	42.92	3.13	32.71	26.48	19.86		26.35	6.43	85.6	60		72.8	18.1	9.08	7.28	8.76	8.37	0.96	86.92	65.45		76.19	15.18	12
BIT SR025	58.52	60.00	61.00	62.14	60.42	1.54	76.52	75.54	77.8	76.05	76.48	0.97	26.73	26.99	24.95	26.46	26.28	0.91	48.07	37.2	29.89	42.8	39.49	7.79	92.94	96	93.27	94.07	1.68	20.26	20.69	20.31	20.42	0.24	98.17	97.54	94.84	96.85	1.77	8
BIT SR026	45.79	45.93	46.09	48.48	46.57	1.28	61.65	63.93	64.23	66.05	63.97	1.81	30	30.78	32.54	31.11	31.12	1.06	38.1	20.11	16.73	14.58	22.38	10.72	97.3	95.55	95.24	96.03	1.11	13.81	12.17	12.8	12.93	0.83	96.1	93.75	97.62	95.82	1.95	9
BIT SR027	60.00	62.16	60.78	65.90	62.21	2.62	63	63.64	64.53	67.01	64.55	1.76	35	35.3	33.53	32.25	34.02	1.41	35.48	37.28	37.63	36.95	36.84	0.95	98.41	97.14	96.97	97.51	0.79	8.2	8.86	9.38	8.81	0.59	98.41	97.14	96.97	97.51	0.79	9
BIT SR028	50.24	49.32	45.84	47.96	48.34	1.91	68.43	69.38	67.32	69.45	68.65	0.99	27.95	26.85	29.45	26.89	27.79	1.22	22.75	12.13	12.68	19.09	16.66	5.14	82.76	92.59	78.26	84.54	7.33	12.22	12.12	13.39	12.58	0.71	82.86	92.59	79.17	84.87	6.93	10
BIT SR029	64.02	61.93	62.17	62.59	62.68	0.94	41.27	41.6	36.62	39.2	39.67	2.29	44.91	43.53	38.41	44.96	42.95	3.09	24.6	13.76	10.42	11.62	15.1	6.48	80.77	100	86.96	89.24	9.82	13.24	13.91	12.76	13.3	0.58	88	100	90.91	92.97	6.26	3
BIT SR030	53.54	52.04	48.56	51.24	51.35	2.09	70.3	67.91	67.3	66.63	68.04	1.6	30	31.98	31.88	33.76	31.91	1.54	17.79	22.5	20.96	27.47	22.18	4.04	84.13	77.08	72	77.74	6.09	10.7	10.65	12.01	11.12	0.77	84.48	72.09	72.6	76.39	7.01	1
BIT SR031	62.94	67.69	64.07	62.33	64.26	2.4	69.36	69.12	67.79	68.76	68.76	0.69	31.19	30.38	29.88	31.58	30.76	0.77	29.7	20.12	6.8	20.13	19.19	9.41	62.12	75	55	64.04	10.14	11.34	13.54	10.87	11.92	1.43	64.52	75	56.14	65.22	9.45	13
BIT SR032	68.27	53.62	67.44	61.14	62.62	6.79	68.83	66.8	66.96	68.21	67.7	0.98	28.1	29.5	29.63	28.44	28.92	0.76	17.35	9.43	15.58	7.99	12.59	4.57	75	90.7	86.96	84.22	8.2	12.12	11.82	9.3	11.08	1.55	84.21	90	95.24	89.82	5.52	12
BIT SR033	71.46	71.00	70.00	67.40	69.97	1.82	50.93	50.16	50.73	51.22	50.76	0.45	49.18	47.25	46.84	49.46	48.18	1.33	15.57	21.05	14.84	22.66	18.53	3.91	86.11	94.74	93.65	91.5	4.7	11.21	12.66	12.09	11.99	0.73	91.43	94.74	100	95.39	4.32	1
BIT SR034	73.16	68.16	72.44	74.80	72.14	2.83	59.1	57.07	56.35	57.7	57.55	1.17	36.11	38.23	37.16	37.75	37.31	0.91	26.09	26.23	22.75	28.64	25.93	2.42	81.25	51.16	67.8	66.74	15.07	6.74	6.96	7	6.9	0.14	80.43	55.81	73.21	69.82	12.66	0
BIT SR035	43.92	47.64	47.20	47.00	46.44	1.7	50.91	49.75	49.66	52.97	50.82	1.54	29.66	29.47	29.15	28.35	29.16	0.58	22.22	28.13	23.53	24.24	24.53	2.54	83.33	100	87.5	90.28	8.67	9.87	9.39	9.66	9.64	0.24	82.87	100	87.5	90.12	8.86	14
BIT SR037	71.70	75.01	65.90	71.79	71.1	3.79	52.91	58.06	52.56	53.41	54.24	2.57	46.33	43.86	45.01	42.84	44.51	1.5	30.03	32.71	24.66	13.27	25.17	8.61	61.79	68.49	46.34	58.87	11.36	9.6	9.73	9.82	9.72	0.11	63.87	74.29	53.33	63.83	10.48	12

Appendix 8: Day 3 – T lymphocytes (CD3+, CD4+, CD8+, CD25BRI, CD25BRIFOXP3+, CD4+FOXP3+, CD127LOWFOXP3+) concentration and SOFA scores

DAY 3

	CD3+						CD4+						CD8+						CD25BRI						CD25BRI FOXP3+					CD4+ FOXP3					CD127 LOW FOXP3					
	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	DAY 3 SOFA
BIT001	69.88	69.33	71.35	70.14	70.18	0.85	66.44	67.7	66.29	67.45	66.97	0.71	26.65	26.51	26.3	26.05	26.38	0.26	19.57	20.21	7.50	17.09	16.09	5.88	100.00	100.00	100.00	100	0	13.48	13.28	13.09	13.28	0.19						0
BIT002	71.37	73.08	71.26	70.53	71.56	1.08	44.99	46.62	44.09	42.99	44.67	1.53	47.43	46.71	48.55	50.04	48.18	1.45	15.31	26.55	22.58	23.56	22	4.77	98.00	98.43	100.00	98.81	1.05	11.85	12.30	12.60	12.25	0.38						2
BIT003	64.86	69.97	68.17	73.41	69.10	3.57	76.46	70.77	78.48	75.93	75.1	2.93	17.47	20.19	29.11	18.22	20.93	4.7	13.46	33.33	17.20	17.53	20	7.69	72.22	70.02	71.03	70.75	1.13	13.77	13.11	13.22	13.45	0.33						2
BIT004	79.18	79.86	79.51	80.38	79.73	0.51	51.6	50.53	51.04	49.14	50.58	1.05	40.79	41.97	40.6	41.1	41.12	0.61	19.51	27.59	25.00	20.00	23.03	3.93	90.00	100.00	100.00	96.67	5.77	8.38	8.19	8.45	8.34	0.13						1
BIT005	73.21	74.37	73.96	73.25	73.70	0.57	70.01	69.69	69.12	60.06	69.47	0.46	31.91	30.31	31.13	31.74	31.27	0.72	12.96	14.75	17.02	21.43	16.54	3.65	100.00	100.00	100.00	100	0	13.18	14.69	13.85	13.91	0.76						0
BIT006	79.51	78.48	79.16	80.00	79.29	0.64	71.97	69.8	69.32	69.93	70.26	1.17	24.75	24.05	24.24	25.09	24.53	0.47	29.64	3.48	3.43	2.69	9.81	13.22	86.52	86.07	86.23	86.27	0.23	6.44	6.07	6.01	6.17	0.23						0
BIT007	78.33	81.20	80.00	79.23	79.69	1.22	68.22	68.67	67.98	68.12	68.25	0.3	26.57	25.36	25.3	26.66	25.97	0.74	8.04	5.02	13.33	8.14	8.63	3.45	100.00	98.00	98.78	98.93	1.01	7.12	7.01	6.99	7.04	0.07						1
BIT008	72.38	72.48	71.90		72.25	0.31	54.48	54.4	54.2		54.36	0.14	40.91	40.88	40.61		40.8	0.17	15.02	17.85	10.46		14.44	3.72	96.79	96.75		96.77	0.03	3.85	3.47		3.66	0.27						9
BIT009	79.00	79.77	79.01	81.73	79.88	1.29	70.45	71.35	70.38	70.37	70.64	0.48	28.2	27.73	28.2	27.67	27.95	0.29	29.63	12.28	20.00	11.71	18.41	8.38	100.00	100.00	100.00	100	0	10.71	10.18	10.09	10.33	0.34						1
BIT010	85.17	85.99	86.20	85.35	85.68	0.50	55.38	54.26	57.4	55.22	55.57	1.32	42.27	39.9	38.45	39.5	40.03	1.61	45.00	22.73	44.44	40.00	38.04	10.45	100.00	100.00	100.00	100	0	8.98	9.15	9.00	9.04	0.09						2
BIT011																																								1
BIT014	60.49	59.93	59.16	60.24	59.96	0.58	59.62	59.31	59.16	60.24	59.46	0.49	38.06	37.97	35.4	36.66	36.95	1.1	18.79	13.82	21.50	20.47	17.17	4.42	98.90	98.60	100.00	99.38	0.73	8.36	9.47	10.88	9.43	1.07	98.00	98.70	100.00	99.18	0.99	0
BIT016	60.49	59.93	59.16	60.24	59.96	0.58	59.62	59.31	59.16	60.24	59.58	0.48	38.06	37.97	35.40	36.66	37.02	1.26	25.00	17.71	13.66	13.00	17.34	5.51	98.55	100.00	100.00	99.52	0.84	16.41	15.51	15.00	15.64	0.71	96.77	100.00	100.00	98.92	1.86	5
BIT017	85.24	83.46	85.10	86.34	85.04	1.19	62.53	60.62	58.87	60.71	60.68	1.49	38.66	40.75	40.00	41.28	40.17	1.14	25.06	24.37	20.78	18.00	22.05	3.29	94.34	94.05	93.33	93.91	0.52	12.39	12.02	11.14	11.85	0.64	93.64	94.00	94.06	93.9	0.23	10
BIT018	85.80	85.60	85.00	84.38	85.20	0.64	67.51	67.25	68.73	68.25	67.94	0.68	28.22	29.18	27.13	27.76	28.07	0.86	25.76	24.90	24.53	25.22	25.1	0.52	75.60	76.23	77.86	76.56	1.16	9.85	8.39	7.13	8.46	1.36	74.23	73.33	74.07	73.88	0.48	1
BIT021	71.42	69.51	69.86	69.89	70.17	0.85	66.49	65.59	65.41	64.84	65.58	0.68	24.80	25.91	24.42	23.46	24.65	1.01	23.14	32.50	31.46	22.85	27.49	5.21	95.32	94.50	94.00	94.61	0.67	14.44	15.75	15.32	15.17	0.67	95.27	94.25	95.00	94.84	0.53	0
BIT022	84.90	85.00	83.43	85.83	84.79	0.99	66	65.62	66.67	66.02	66.08	0.44	31.50	32.16	31.00	31.45	31.53	0.48	20.72	15.50	16.12	13.22	16.39	3.14	90.35	90.91	90.81	90.69	0.29	14.03	14.61	14.61	14.42	0.33	96.15	95.89	96.02	96.02	0.13	0
BIT023	58.97	57.41	57.59	58.95	58.23	0.85	69.12	70.97	72.97	69.53	70.65	1.74	22.07	22.12	21.66	22.12	21.99	0.22	21.72	30.94	24.69	15.19	23.14	6.54	89.02	89.83	88.89	89.25	0.51	9.11	10.30	9.65	9.69	0.59	90.59	90.00	91.43	90.67	0.72	1
BIT024	78.34	76.79	78.02	78.39	77.89	0.75	48.81	50.23	49.28	48.96	49.32	0.64	48.48	47.50	47.53	48.13	47.91	0.48	10.69	9.48	4.67	14.89	9.93	4.2	100.00	100.00	100.00	100	0	12.95	13.55	14.11	13.54	0.58	100.00	100.00	100.00	100	0	10
BIT025	38.87	38.00	40.70	38.46	39.01	1.18	70.22	71.37	69.64	71.61	70.71	0.94	26.00	26.81	27.14	26.91	26.72	0.5	23.50	27.40	21.19	5.07	19.29	9.82	70.45	71.00	71.43	70.96	0.49	8.68	8.55	8.74	8.66	0.09	80.00	81.06	82.33	81.13	1.17	5
BIT027	69.26	70.60	69.96	69.94	69.94	0.55	67.05	67.93	67.55	67.17	67.43	0.4	30.16	29.81	30.19	30.52	30.17	0.29	9.43	9.13	11.57	10.37	10.13	1.1	91.30	90.29	90.00	90.53	0.68	3.48	3.77	3.61	3.62	0.15	89.67	89.29	89.47	89.48	0.19	3
BIT028	69.78	69.00	68.90		69.23	0.48	68.2	67.27	68		67.82	0.49	26.17	26.47	25.66		26.1	0.41	19.81	22.91	13.21		18.64	4.95	90.00	90.48		90.24	0.34	6.89	7.00		6.95	0.08	80.00	80.00		80	0	0
BIT029	54.10	54.25	55.02	55.64	54.75	0.72	56.93	55.2	55.83	55.8	55.94	0.72	32.87	32.45	32.76	32.11	32.55	0.34	12.61	7.59	19.32	26.92	16.61	8.39	100.00	100.00	100.00	100	0	13.45	12.09	12.53	12.69	0.69	100.00	100.00	100.00	100	0	1
BIT030	82.00	82.80	81.00	82.52	82.08	0.79	70.02	69.56	69.45	70.22	69.9	0.43	28.37	28.43	28.28	27.51	27.96	0.52	32.59	25.25	30.59	34.42	24.21	10.56	98.80	99.20	100.00	99.6	0.61	8.00	8.21	8.20	8.13	0.08	100.00	100.00	100.00	99.78	0.49	2
BIT034	64.93	64.00	64.84		64.59	0.51	62.23	62	63.6		62.61	0.87	22.55	22.46	21.99		22.33	0.3	11.64	12.94	18.26		14.28	3.51	52.00	52.60		52.3	0.42	10.70	11.69		11.2	0.7	52.00	51.28		51.64	0.51	4
BIT035	70.62	70.00	70.62	70.62	70.47	0.31	82.94	82	82.2	82	82.29	0.45	13.85	14.50	15.13	15.00	14.62	0.58	21.61	21.45	15.35	15.48	18.47	3.53	100.00	100.00	100.00	100	0	14.00	14.64	14.77	14.47	0.41	100.00	100.00	100.00	100	0	0
BIT040	72.94	75.62	59.98	60.60	67.29	8.15	51.58	51.81	56.34	56.57	54.08	2.75	38.2	37.42	26.42	26.8	32.21	6.48	12	11.32	7.69	13.04	11.01	2.32	100	100	100	100	0	9.6	9.08	9.39	9.36	0.26	100	100	100	100	0	0
BIT042	67.02	67.02	57.24		62.13	6.92		66.1	68.56		67.33	1.74		24.74	25.53		25.14	0.56		29.07	12.69		20.88	11.58	96.97	94.12		95.55	2.02	9.07	8.96		9.02	0.08	100	100		100	0	0
BIT043	87.18	85.48	86.14	87.32	86.53	0.88	82.66	82.08	83.65	81.7	82.52	0.85	15.78	16.14	14.77	15.32	15.5	0.59	24.71	13.86	15.51	13.51	16.89	5.28	74.47	58.62	71.15	68.08	8.36	9.06	9.27	8.94	9.09	0.17	82.61	63.64	74.47	73.57	9.52	0
BIT046	53.00	53.62	54.18	55.38	54.05	1.01	80	81.24	82.83	82.05	81.53	1.21	14.8	15.4	14.73	14.95	14.97	0.3	22.66	15.79	22.83	27.95	22.31	4.99	58.97	41.27	38.55	46.26	11.09	10.51	9.76	10.21	10.16	0.38	64.1	45.31	35.44	48.28	14.56	5
BIT047	68.00	69.02	69.00		68.67	0.58	39.44	36	38		37.81	1.73	50.7	55.83	53		53.18	2.57	33.33	20	12		21.78	10.78	54.17	55		54.58	0.59	9.56	9.4		9.48	0.56	66.67	67		66.84	0.23	5
BIT048	77.64	78.98	76.14	77.28	77.51	1.17	65.77	65.51	65.69	65	65.49	0.35	32.77	32.39	32.28	32	32.36	0.32	22.13	19.31	19.58	20.09	20.28	1.28	82.22	66.67	83.87	77.59	9.49	18.25	18.19	18.65	18.36	0.25	86.36	67.03	83.87	79.09	10.51	13
BIT049	81.44	77.52	78.54	76.68	78.55	2.07	75.49	73.86	74.74	73.58	74.42	0.87	24.83	26.52	25.59	25.9	25.71	0.7	20.58	23.05	22.87	20.56	21.77	1.38	56.25	50	37.84	48.03	9.36	9.1	7.8	9.61	8.84	0.93	56.63	56.79	45.88	53.1	6.25	1
BIT050	53.92	58.18	58.66	58.78	57.39	2.32	55.61	54.72	55.2	55.91	55.36	0.52	42.08	42.73	42.21	41.01	42	0.72	30.9	18.13	21.3	15.79	21.53	6.64	75	82.35	75.44	77.59	4.12	15.94	16.43	16.35	16.24	0.26	75.71	84.52	77.78	79.34	4.61	0
BIT052	78.86	77.74	77.50	79.98	78.52	1.14	50.78	51.63	50.7	51.31	51.11	0.44	60.12	59.74	58.23	59.49	59.4	0.82	17.78	17.48	17.51	24.11	19.22	3.26	76.74	80	73.77	76.84	3.12	12.31	11.65	12.23	12.06	0.36	80.95	87.5	77.05	81.83	5.28	1
BIT053	77.98	69.86	74.16	72.36	73.59	3.42	64.38	63.61	65.08	65.04	64.53	0.69	34.78	36.3	33.6	35.79	35.12	1.19	21.52	23.33	22.37	39.58	26.7	8.62	48.57	50	57.89	52.15	5.02	6.48	5.18	6.42	6.03	0.73	56.25	59.38	60	58.54	2.01	4
BIT055	77.00	77.24	76.00	76.00	76.56	0.65	48.53	48.32	49.68	49	48.88	0.6	50.19	50.93	49.45	50	50.14	0.61	27.19	23.28	24	18.52	23.25	3.58	96.3	96	97	96.43	0.51	6.22	5.99	6	6.07	0.13	98	100	100	99.33	1.15	5
BIT060	44.42	44.86	44.24	44.06	44.4	0.34	75.01	75.93	73.92	74.13	74.75	0.92	21.88	21.49	23.19	23.47	22.51	0.97	6.86	9.64	7.73	7.51	7.94	1.19	87.5	71.43	84.62	81.18	8.57	6.34	4.77	5.94	5.68	0.82	87.5	78.95	94.44	86.96	7.76	8
BIT061	71.02	76.07	74.94	71.34	73.34	2.54	59.34	59.16	60.26	57.75	59.13	1.04	40.41	39.93	37.31	41.07	39.68	1.65	22.22	23.86	32.32	11.76	22.54	8.44	59.52	75.44	72.22	69.06	8.41	8.79	9.57	8.25	8.87	0.66	60	75.93	72.22	69.38	8.34	7
BIT065	62.62	81.76	81.86	81.98	77.06	9.62	64.39	65.24	66.24	65.63	65.38	0.77	33.66	33.02	31.54	33.64	32.97	0.99	18.56	22.37	9.13	4.24	13.58	8.35	53.03	73.91	80	68.98	14.14	5.81	5.35	5.06	5.41	0.38	79.03	70.83	80	76.62	5.04	5
BIT066	63.17	62.74	68.28	69.22	65.85	3.37	68.82	73.83	71.56	71.45	71.42	2.05	25.56	21.16	23.23	22.85	23.2	1.81	6.54	3.76	5.88	5.02	5.3	1.2	80	93.75	91.67	88.47	7.41	6.48	11.09	9.5	9.02	2.34	82.35	92.86	86.67	87.29	5.28	0
BIT069	67.20	68.18	75.14	67.35	69.47	3.81	56.85	54.06	52.54	55.4	54.71	1.84	32.41	34.09	30.08	32.5	32.27	1.65	9.93	6.15	9.09	13.16	9.58	2.88	90.91	100	100	96.97	5.25	16.39	19.4	13.49	16.43	2.95	90.91	100	100	96.97	5.24	2

DAY 3

	CD3+						CD4+						CD8+					
	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD
BIT070	62.62	81.76	81.86	81.98	77.06	9.62	68.01	69	67.9	66.57	67.87	0.99	29.06	27.96	28.99	29.88	28.97	0.79
BIT072	73.38	75.10	73.28		73.92	1.02	36.92	38.4	36.08		37.13	1.17	55.18	54.81	56.39		55.46	0.83
BIT073	31.92	33.28	29.88	35.25	32.58	2.26	68.4	64.84	66.43	69.02	67.17	1.91	29.58	26.74	30.86	30.86	29.51	1.94
BIT074	66.48	70.85	66.30	70.04	68.42	2.37	62.18	62.37	67.12	64.48	64.04	2.3	36.66	35.57	31.61	33.81	34.41	2.21
BIT076	63.81	66.66	66.7	67.22	66.09	1.55	68.54	70.51	68.34	68.6	68.99	1.01	30.16	27.9	30.52	30.15	29.68	1.2
BIT078	77.62	71.76	76.00		75.13	3.03	41.18	41.88	40.88		41.31	0.51	55.6	54.97	54.78		55.12	0.43
BIT079	37.15	38.62	47.02	43.63	41.61	4.55	75.12	74.58	74.44	75.4	74.88	0.45	24.72	25.72	24.63	24.32	24.85	0.61
BIT084	60.38	60.08	62.03	63.57	61.52	1.62	53.69	52.76	49.45	52.41	52.08	1.83	27.72	28.36	27.08	27	27.54	0.63
BIT085	59.90	58.60	54.04	61.72	58.57	3.28	72.26	72.53	69.58	72.72	71.77	1.47	26.49	25.19	26.05	24.89	25.65	0.74
BIT088	56.36	54.45	52.26	53.20	54.07	1.77	72.14	72.88	71.07	72.18	72.07	0.75	21.72	20.82	22.16	21.2	21.48	0.59
BIT099	72.46	64.89	60.43	69.30	66.77		67.26	68.24	69.73	68.23	68.37	1.02	37.90	35.09	33.13	34.60	35.18	1.99
BIT100	52.60	57.05	59.66	52.20	55.38	3.6	58.25	60.09	57.36	58.98	58.67	1.16	35.52	34.33	36.57	33.6	35	1.3
BIT102	69.50	68.00	70.00	68.19	68.92	0.98	74	74	74.37	73.15	73.88	0.52	25	27.5	25.71	25.05	25.82	1.17
BIT103	74.96	74.28	72.68	73.48	73.85	0.99	68.33	70.27	68.99	68.15	68.94	0.96	32.22	29.97	30.30	31.22	30.93	1.01
BIT104	77.83	79.32	78.13	81.59	79.22	1.71	21.34	26.75	28.93	22.94	24.99	3.47	69.82	68.15	66.39	72.96	69.33	2.8
BIT107																		
BIT111	DISCHARGED						DISCHARGED						DISCHARGED					
BIT SR002	68.38	66.96	68.3	67.36	67.75	0.7	56.71	56	56.16	56.47	56.34	0.32	50.19	49.28	50.88	51.31	50.42	0.89
BIT SR003	81.24	80.04	80.7	80.3	80.57	0.52	64.16	65.87	66.37	65.38	65.45	0.95	34.22	32.62	32.04	32.85	32.93	0.92
BIT SR008	62.67	62.24	62.82	65.34	63.27	1.4	59.99	59.25	60.11	59.66	59.75	0.39	35.87	35.44	34.96	36.12	35.6	0.51
BIT SR009	42.26	55.8	56.57	57.56	53.05	7.23	63.18	65.73	61.96	64.77	63.91	1.67	24.09	32.47	35.79	31.62	30.99	4.94
BIT SR010	48.21	46.36	47.25	48.54	47.59	0.98	53.97	65.49	58.83	63.16	60.36	5.08	27.49	31.38	25.82	28.27	28.24	2.33
BIT SR011	45.62	43.28	51.66	43.78	46.09	3.85	53.27	49.91	54.78	52.99	52.74	2.04	38.05	38.22	39.29	38.6	38.54	0.55
BIT SR012	60.81	60.68	63.72	61.40	61.65	1.41	79.43	80.69	78.03	78.84	79.25	1.12	15.08	13.38	16.07	15.17	14.93	1.12
BIT SR013																		
BIT SR019	69.66	74.44	72.69	71.97	72.19	1.98	65.13	64.72	67.68	67.23	66.19	1.48	27.75	28.51	26.28	26.38	27.23	1.09
BIT SR020	60	59.96	61.5	82.26	65.93	10.91	46	46.46	48.42	45.1	46.5	1.4	46.53	48.8	46.47	47.74	47.39	1.11
BIT SR021	60	62.3	64.64	66.86	63.45	2.96	64.54	64.3	64.71	65.83	64.85	0.68	27.59	32.13	31.09	30.34	30.29	1.94
BIT SR022	63.74	74.99	68.45	70.00	69.3	4.64	58.08	59.08	57.77	56.88	57.95	0.91	32.44	35.42	37.21	36.89	35.49	2.18
BIT SR023	64.86	65.28	63.42	60.66	63.56	2.09	78.19	76.05	77.86	78.96	77.77	1.23	21.05	20.94	20.8	19.88	20.67	0.53
BIT SR024	61.00	62.84	60.62	60.00	61.12	1.22	67.00	69.29	66.57	68.05	67.73	1.21		30.87	32.26	31.08	31.4	0.75
BIT SR025	83.00	81.74	84.78	85.22	83.69	1.61	84.48	87.54	88.39	87.61	87.01	1.73	12.00	12.03	11.11	11.85	11.75	0.43
BIT SR026	66.20	61.30	68.14	60.98	64.16	3.57	68.94	73.28	67.99	68.48	69.67	2.44	28.82	25.02	28.65	29.12	27.9	1.93
BIT SR027	55.05	65.64	68.55	61.66	62.73	5.84	63.71	68.04	66.95	65.45	66.04	1.88	28.62	31.41	31.96	33.21	31.3	1.94
BIT SR028	52.02	58.28	60.72	55.52	56.64	3.74	74.98	74.57	74.01	74.17	74.43	0.43	22.43	23.61	23.19	23.70	23.23	0.58
BIT SR029	62.62	59.62	65.08	62.44	62.44	2.23	60.08	60.38	58.04	58.04	59.14	1.27	32.08	33.01	34.69	34.14	33.48	1.17
BIT SR030	62.62	59.62	65.08	62.44	62.44	2.23	60.08	60.38	58.04	58.04	59.14	1.27	32.08	33.01	34.69	34.14	33.48	1.17
BIT SR031	60.00	64.42	60.67	56.93	60.51	3.08	66.00	70.96	67.45	68.67	68.27	2.1	29.00	28.03	30.97	29.18	29.3	1.23
BIT SR032	66.50	54.81	69.50	60.29	62.78	6.55	67.25	64.00	65.17	65.09	65.38	1.36	29.63	29.61	29.89	29.48	29.65	0.17
BIT SR033	63.76	61.82	56.28	69.97	62.96	5.65	47.96	45.79	46.01	49.26	47.26	1.65	45.64	49.63	48.51	47.85	47.91	1.68
BIT SR034	69.29	64.91	63.91	64.33	66.86	2.6	72.40	73.72	72.40	74.00	73.13	0.85	26.69	23.92	25.36	23.68	24.91	1.4
BIT SR035	REFUSED TO GIVE BLOOD						REFUSED TO GIVE BLOOD						REFUSED TO GIVE BLOOD					
BIT SR037	52.73	50.00	49.32	40.49	48.14	5.31	36.71	34.00	33.05	31.64	33.85	2.14	59.57	57.00	55.45	47.35	54.84	5.28

	CD25BRI						CD25BRI FOXP3+					CD4+ FOXP3					CD127 LOW FOXP3					
	FMO	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	DAY 3 SOFA
BIT070	12.98	7.6	13.75	7.06	10.35	3.5	100	97.3		98.65	1.91	12.85	12.48		12.66	0.26	100	100		100	0	0
BIT072	20	10.74	9.7	13.04	13.37	4.64	100	100		100	0	9.51	9.91		9.71	0.28	100	100		100	0	0
BIT073	22.89	2.94	13.45	12.35	12.91	8.15	100	100	100	100	0	16.98	15.32	14.8	15.7	1.14	100	100	100	100	0	1
BIT074	20.51	9.43	10.55	13.01	13.38	4.98	96	100	97.14	97.71	2.06	9.85	8.67	8.81	9.11	0.64	94.74	100	97.14	97.29	2.63	0
BIT076	24.63	13.92	11.47	5.35	13.84	8.04	100	100	92.31	97.44	4.43	11.19	14.52	14.08	13.26	1.81	100	100	92.86	97.62	4.12	5
BIT078	18.39	16.35	18.84		17.86	1.33	52.94	61.54		57.24	6.08	6.16	6.33		6.25	0.12	50	61.54		55.77	8.16	4
BIT079	13.57	15.13	15.38	13.14	14.31	1.11	66.67	88.1	78.38	77.72	10.73	9.24	8.51	8.65	8.8	0.39	64.86	88.1	80	77.65	11.79	1
BIT084	19.67	14.32	14.49	17.73	16.55	2.6	63.16	69.56	56	62.91	6.78	17.1	18.84	17.3	17.75	0.95	82.46	88.64	73.58	81.56	7.57	1
BIT085	22.26	27.59	23.59	20.55	23.49	2.99	86.25	94.03	94.03	91.44	4.49	17.55	17.18	17	17.24	0.28	97.47	100	97.62	98.36	1.42	3
BIT088	39.42	32.16	23.5	22.52	29.4	7.96	57.98	61.11	71.43	63.51	7.04	7.1	7.11	7.81	7.34	0.41	62.67	62.22	71.43	65.44	5.19	6
BIT099	18.95	21.92	25.97	19.20	21.51	3.26	77.68	63.92	80.49	74.03	8.87	9.83	10.24	9.90	9.99	0.22	88.60	86.67	87.65	87.64	0.97	6
BIT100	39.87	29.01	31.93	18.18	29.74	8.97	69.41	81.32	86.11	78.95	8.59	12.32	10.11	10.85	11.09	1.12	82.76	91.3	90.91	88.32	4.82	1
BIT102	20	24.16	13.8	16.94	18.73	4.42	68.21	83.08	70.59	73.96	7.99	11.13	13.39	11.27	11.93	1.27	76.28	93.15	76.36	81.93	9.72	0
BIT103	26.80	25.53	25.88	22.47	25.17	1.88	96.97	91.45	98.04	95.49	3.54	14.47	14.92	13.78	14.39	0.57	96.83	92.73	97.89	95.82	2.73	5
BIT104	35.88	28.89	24.44	18.14	26.84	7.47	75.38	85.45	78.18	79.67	5.2	10.84	11.42	11.53	11.26	0.37	84.09	85.42	81.48	83.66	2	1
BIT107																						11
BIT111	DISCHARGED						DISCHARGED					DISCHARGED					DISCHARGED					DISCHARGED
BIT SR002	13.89	15.63	9.77	15.75	13.76	2.79	80	82.35	73.91	78.75	4.36	8.69	5.33	7.52	7.18	1.71	77.28	79.17	78.57	78.34	0.97	1
BIT SR003	6.46	4.51	3.64	8.3	5.73	2.08	54.55	100	78.95	77.83	22.74	4.74	5.15	4.57	4.82	0.29	52.94	100	79.17	77.37	23.58	9
BIT SR008	5.56	4.03	6.72	11.25	6.89	3.1	100	100	94.44	98.14	3.21	7	7.1	7.13	7.08	0.07	100	100	100	100	0	1
BIT SR009	17.39	13.02	10.39	12.33	13.28	2.96	90	100	100	96.67	5.77	9.76	9.28	6.81	8.62	1.58	89.47	100	100	96.49	6.08	3
BIT SR010	19.67	19.31	18.02	18.12	18.78	0.83	64.81	93.55	92.59	83.65	16.32	7.86	9.78	10.31	9.32	1.29	64.29	93.55	92.59	83.48	16.62	8
BIT SR011	20.34	18.67	21.43	15.98	19.11	2.37	100	95	91.43	95.48	4.3	18.61	18.59	18.1	18.43	0.29	100	98.25	94.29	97.51	2.93	12
BIT SR012	27.74	28.21	25.47	27.61	27.26	1.22	95.45	96.84	82.26	91.52	8.05	10.86	8.81	8.55	9.41	1.27	100.00	100.00	89.19	96.4	6.24	12
BIT SR013																						2
BIT SR019	15.19	22.54	21.8	23.11	20.66	3.69	80.85	92.5	62.38	78.58	15.19	13.78	12.84	10.65	12.42	1.61	93.15	94.59	80.52	89.42	7.74	1
BIT SR020	29.06	24.63	22.26	20.58	24.13	3.68	71.64	84.13	85.96	80.58	7.79	12.2	9.94	10.9	11.01	1.13	85.25	90.32	91.91	89.16	3.48	9
BIT SR021	22.86	40.42	21.77	23.12	27.04	8.94	65.93	79.07	68.6	71.2	6.95	10.28	11.96	10.05	10.76	1.04	74.07	77.42	71.08	74.19	3.17	12
BIT SR022	27.89	19.46	14.24	16.03	19.41	6.06	56.00	32.65	42.11	43.59	11.74	11.44	8.55	10.67	10.22	1.5	64.58	49.02	55.56	56.39	7.81	6
BIT SR023	26.83	15.57	10.1	20.17	18.17	7.09	92.42	84.62	62.89	79.98	15.3	9.75	9.49	9.68	9.64	0.13	96.97	90.38	74.23	87.19	11.7	2
BIT SR024	20.00	28.41	21.16	23.50	23.27	3.72	75.49	70.31	67.95	71.25	3.86	13.23	9.88	12.15	11.75	1.71	85.29	79.03	79.22	81.18	3.56	6
BIT SR025	57.98	57.02	54.70	53.88	55.9	1.92	81.30	62.00	67.91	70.4	9.89	16.45	16.54	17.08	16.69	0.34	82.03	64.00	68.36	71.46	9.41	4
BIT SR026	29.84	10.00	12.53		17.46	10.8	74.29	91.67		82.98	12.29	14.17	12.47		13.32	1.2	78.57	93.88		86.23	10.83	1
BIT SR027	18.95	32.18	26.37	17.36	23.72	6.87	45.45	41.86	65.82	51.04	12.92	9.58	9.36	9.42	9.45	0.11	52.50	41.67	73.47	55.88	16.17	1
BIT SR028	10.36	13.21	20.19	20.55	16.08	5.09	50.79	79.69	50.00	60.16	16.92	9.30	9.88	9.32	9.5	0.33	56.90	82.81	50.33	63.35	17.17	10
BIT SR029	19.92	17.46	16.61	18.67	18.17	1.44	81.36	82.69	80.00	81.35	1.35	12.44	13.23	12.25	12.64	0.52	81.36	82.00	82.93	82.1	0.79	1
BIT SR030	19.92	17.46	16.61	18.67	18.17	1.44	79.03	82.69	80.00	80.57	1.9	12.44	13.23	12.25	12.64	0.52	81.36	82.00	82.93	82.1	0.79	1
BIT SR031	20.00	22.07	14.94	14.90	17.98	3.63	80.30	62.50	64.44	69.08	9.77	9.77	9.67	10.05	9.83	0.2	85.71	61.96	69.77	72.48	12.1	6
BIT SR032	21.54	11.34	13.47	10.64	14.25	5.01	100.00	94.33	95.00	96.44	3.1	15.57	15.00	15.68	15.42	0.37	100.00	97.50	100.00	99.17	1.44	2
BIT SR033	19.37	22.60	30.06	34.31	26.59	6.82	95.74	96.15	96.34	96.08	0.31	14.03	14.47	14.78	14.43	0.38	97.83	95.24	95.12	96.06	1.53	4
BIT SR034	20.42	32.17	33.00	31.83	29.36	5.98	66.34	82.44	76.77	75.18	8.17	9.86	13.23	11.29	11.46	1.69	66.67	84.80	75.00	75.49	9.07	0
BIT SR035	REFUSED TO GIVE BLOOD						REFUSED TO GIVE BLOOD					REFUSED TO GIVE BLOOD					REFUSED TO GIVE BLOOD					9
BIT SR037		18.84	12.03		15.44	4.82	76.92	90.63		83.78	9.69	12.68	13.19	12.77	12.88	0.27	88.98	90.91		89.95	1.36	12

Appendix 9: Day 5 – T lymphocytes (CD3+, CD4+, CD8+, CD25BRI, CD25BRIFOXP3+, CD4+FOXP3+, CD127LOWFOXP3+) concentration and SOFA scores

DAY 5

	CD3+						CD4+						CD8+						CD25BRI						CD25BRI FOXP3+					CD4+ FOXP3					CD127LOW FOXP3					
	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	DAY 5 SOFA
BIT001	69.88	69.33	71.35	70.14	70.18	0.85	66.44	68.70	68.29	68.45	67.97	1.03	25.04	25.85	25.00	25.20	25.27	0.39	3.51	8.45	18.18	14.63	11.19	6.51	100.00	100.00	100.00	100	0	9.03	8.74	8.82	8.86	0.15						0
BIT002	72.94	70.38	71.67	71.21	71.55	1.07	47.95	49.65	50.68	47.97	49.07	1.16	45.81	47.46	46.22	46.50	46.82	0.94	43.97	16.67	10.89	8.25	19.77	14.21	100.00	97.10	97.50	98.28	1.29	16.40	17.30	16.08	16.42	0.63						1
BIT003	72.26	73.44	73.30	74.50	73.38	0.92	76.30	77.09	77.83	75.60	76.71	0.97	19.91	18.88	22.76	29.02	22.64	4.56	25.71	23.38	19.44	23.39	22.98	2.6	94.44	92.86	93.33	93.54	0.81	14.77	14.15	13.78	14.23	0.5						0
BIT004	78.76	79.80	79.89	81.96	80.10	1.34	49.89	50.02	48.57	50.56	49.76	0.84	34.83	35.51	35.47	35.00	35.2	0.34	18.42	10.42	28.57	13.79	17.8	7.89	100.00	100.00	100.00	100	0	14.02	15.25	14.47	14.58	0.62						0
BIT005																																								0
BIT006																																								0
BIT007	82.91	81.05	80.96	80.08	81.25	1.19	76.83	76.69	76.95	75.50	76.46	0.58	20.30	20.70	20.94	20.72	20.67	0.23	8.54	9.39	11.13	11.25	11.09	2.54	95.70	95.00	95.45	95.44	0.31	10.00	10.48	9.93	10.04	0.31						0
BIT008	47.45	46.75	48.72	48.57	47.87	0.94	55.36	54.54	54.81	55.58	54.67	0.99	41.46	42.35	41.61	41.42	41.45	0.7	12.00	18.44	22.08	17.24	18.73	4.62	95.89	96.00	94.00	95.36	0.93	12.23	13.38	13.04	12.51	0.89						9
BIT009	80.02	79.35	82.28	79.51	80.29	1.36	68.62	67.12	69.26	69.17	68.85	0.95	27.30	28.56	27.06	29.44	28.13	0.86	11.32	11.84	5.11	19.83	10.93	5	100.00	100.00	100.00	100	0	7.69	7.76	7.40	7.62	0.18						1
BIT010	84.97	86.56	85.72	84.97	85.56	0.76	55.30	56.15	54.58	55.30	55.33	0.64	39.35	38.00	40.07	39.35	39.19	0.86	17.84	18.50	28.00	25.52	22.47	5.07	100.00	100.00	100.00	100	0	4.96	5.53	4.71	5.07	0.42						0
BIT011	61.00	62.19	63.83	62.40	62.36	1.16	72.94	70.64	71.03	70.32	71.23	1.17	21.00	21.38	22.12	21.76	21.57	0.48	30.77	42.07	36.07	42.64	37.89	5.59	97.10	98.00	98.81	97.97	0.86	9.60	8.50	8.57	8.89	0.62						0
BIT014	60.49	59.93	59.16	60.24	59.96	0.58	67.35	67.00	65.16	66.17	66.69	1.04	32.90	31.23	31.00	32.44	31.71	0.89	13.20	18.36	18.69	27.68	20.98	6.19	98.00	100.00	100.00	99	1.15	13.16	12.46	13.90	13.1	0.6	98.65	100.00	100.00	99.2	0.95	0
BIT016	59.00	59.73	60.00	60.23	59.74	0.53	71.00	72.23	71.02	71.33	71.4	0.58	32.90	31.23	31.00	32.44	31.89	0.92	14.00	15.68	19.39	21.77	17.71	3.52	94.59	92.11	93.00	93.23	1.25	12.99	12.18	11.97	12.38	0.54	94.12	94.12	94.00	94.08	0.07	4
BIT017	87.96	88.74	86.51	86.21	87.36	1.20	61.32	61.85	65.33	63.25	62.94	1.79	35.00	36.93	35.72	34.11	35.44	1.19	15.91	13.55	11.28	12.84	13.4	1.93	100.00	100.00	100.00	100	0	11.90	10.52	11.14	11.19	0.69	100.00	100.00	97.37	99.12	1.52	2
BIT018	79.01	83.56	82.00	82.37	81.74	1.93	67.37	64.36	63.55	63.05	64.58	1.94	30.11	29.45	29.68	30.91	30.04	0.64	8.72	12.96	19.23	6.84	11.94	5.49	96.00	97.00	95.45	96.15	0.79	15.15	16.63	16.11	15.96	0.75	100.00	100.00	100.00	100	0	3
BIT021	71.42	69.51	69.86	69.89	70.17	0.85	65.39	67.99	69.68	68.34	67.85	1.79	24.80	24.01	23.04	23.71	23.89	0.73	15.60	46.22	29.20	25.74	29.19	12.74	90.00	89.99	90.75	90.25	0.44	12.84	11.54	12.81	12.4	0.74	92.00	93.75	92.89	92.88	0.88	0
BIT022	84.90	85.00	83.43	85.83	84.79	0.99	70.11	64.07	65.58	63.68	65.86	2.95	23.21	29.36	30.07	30.40	28.26	3.39	20.03	10.79	24.04	10.26	16.28	6.85	98.23	100.00	100.00	99.41	1.02	9.31	7.85	9.62	8.93	0.95	98.00	97.50	97.92	97.81	0.27	0
BIT023	58.97	57.41	57.59	58.95	58.23	0.85	78.11	79.10	78.80	79.43	78.86	0.56	17.65	17.43	18.90	17.62	17.9	0.67	17.97	20.50	23.46	18.35	20.07	2.52	98.12	97.62	100.00	98.58	1.25	13.60	13.89	13.16	13.55	0.37	98.00	100.00	100.00	99.33	1.15	0
BIT024	78.34	76.79	78.02	78.39	77.89	0.75	43.45	43.00	43.04	43.06	43.35	0.5	54.25	55.92	54.90	54.85	54.98	0.6	12.17	7.06	10.48	12.50	11.3	2.73	98.12	100.00	98.00	98.57	0.96	10.60	10.06	10.08	10.19	0.28	100.00	100.00	100.00	100	0	2
BIT025	62.26	60.16	61.00	62.00	61.36	0.96	66.18	64.60	64.89	65.68	65.34	0.72	29.00	29.09	29.89	29.29	29.32	0.4	11.23	13.56	4.82	13.07	10.67	4.03	98.50	100.00	100.00	99.5	0.87	8.50	8.95	8.30	8.58	0.33	100.00	100.00	98.28	99.43	0.99	4
BIT027	71.23	73.95	72.92		72.70	1.37	71.75	70.63	71.00		71.13	0.57	26.80	27.96	28.32		27.69	0.79	10.80	8.33	22.58		13.9	7.62	88.24	88.90		88.57	0.47	8.09	9.50		8.8	1	93.75	93.00		93.38	0.53	0
BIT028	62.46	62.32	65.04	64.97	63.70	1.51	54.57	54.21	54.50	55.50	54.7	0.56	23.41	24.00	23.57	24.00	23.75	0.3	16.00	23.08	16.07	13.43	17.15	4.14	85.02	85.19	85.00	85.07	0.1	11.58	11.12	11.00	11.23	0.31	96.00	96.30	95.20	95.83	0.57	0
BIT029	55.41	55.00	54.55	55.00	54.99	0.35	40.00	40.07	39.03	40.14	39.81	0.52	40.84	39.52	38.52	40.00	39.72	0.97	20.30	14.78	8.11	12.24	13.86	5.09	50.96	50.12	50.00	50.36	0.52	7.23	8.60	7.74	7.86	0.69	49.90	50.00	50.00	49.97	0.06	0
BIT030	81.92	81.80	81.06	81.60	81.60	0.38	69.64	68.90	68.52	68.32	68.85	0.58	27.49	27.90	27.55	27.00	27.49	0.37	28.14	30.70	22.02	20.49	25.34	4.87	96.50	97.30	97.00	96.93	0.4	12.96	12.33	13.22	12.84	0.46	96.72	97.27	97.00	97	0.28	1
BIT034																																								0
BIT035	76.70	75.78	74.04	75.40	75.48	1.10	67.00	66.80	67.42	67.59	67.2	0.37	13.02	12.65	12.25	12.47	12.6	0.33	24.20	22.81	23.75	28.00	24.69	2.28	100.00	100.00	97.00	99	1.73	11.71	11.59	11.30	11.53	0.21	100.00	100.00	98.66	99.55	0.77	0
BIT040	74.92	74.54	85.16		70.41	16.36	56.41	58.25	66.04		60.23	5.11	35.93	31.8	26.88		31.54	4..53	32.75	20.17	16.95		23.29	8.35	91.49	90	83.33	88.27	4.35	12.21	10.26	11	11.16	0.98	90.91	86.36	83.33	86.87	3.82	1
BIT042	65.00	68.90	63.63		66.27	3.73		59.41	57.37		58.39	1.44		28.76	27.62		28.19	0.81		21.95	23.94		22.95	1.41	84.44	100		92.22	11	11.99	11.78		11.88	0.15	81.4	100		90.7	13.15	0
BIT043	87.40	88.67	87.76	88.87	88.18	0.71	84.92	84.4	84.48	84.61	84.6	0.23	13.3	14.45	14.56	13.61	13.98	0.62	26.35	28.68	23.92	22.37	25.33	2.77	65.38	44.44	43.94	51.25	12.24	7.85	7.07	7.44	7.45	0.39	67.11	53.52	47.69	56.11	9.97	0
BIT046	32.47	50.42	41.83		41.57	8.98	84.82	85	84.9		84.91	0.09	6.39	6.35	6.5		6.41	0.08	19.61	12.26	29.35		20.41	8.57	84.62	100		92.31	10.88	5.44	5.3		5.37	0.09	100	100		100	0	8
BIT047	58.70	71.79	35.81		55.43	18.21	36.57	43.999	52.04		44.2	7.73	58.72	51.43	19.73		43.29	20.73	24.64	22.39	28.57		25.2	3.13	86.67	100		93.34	9.43	6	6.54		6.27	0.38	85.71	100		92.85	10.1	2
BIT048	68.52	65.10	57.58	65.49	64.17	4.65	78.42	80.06	79.61	80.49	79.65	0.89	20.88	19.29	19.59	20.4	20.04	0.73	24.2	25.5	20.51	20.37	11.65	2.6	71.3	75	70.42	72.24	2.43	14.58	13.06	14.8	14.15	0.95	72.65	73.61	71.25	72.5	1.19	12
BIT049	66.56	77.44	78.32	77.30	74.91	5.58	76.36	77.22	77.07	77.02	76.92	0.38	21.73	22.93	23.24	22.82	22.68	0.66	30.52	27.19	22.98	16.08	24.19	6.23	81.1	75.24	85.51	80.62	5.15	15.58	14.38	15.72	15.23	0.74	83.78	78.3	87.69	83.26	4.72	1
BIT050	70.86	68.63	71.63		70.37	1.56	55.85	56.26	56.6		56.24	0.38	37.31	40.64	39.55		39.17	1.69	30	16.14	17.11		21.08	7.74	85.11	76.92		81.02	5.79	11.93	11.2		11.56	0.52	84.74	77.78		81.26	4.92	0
BIT052	75.86	75.88	75.19	76.43	75.84	37.82	50.51	53	50.9	51.11	51.38	1.11	59.41	55.67	57.48	56.97	57.38	1.55	24.42	8.13	7.09	11.03	12.67	8.01	60	66.67	26.67	51.11	21.43	7.66	7.28	7.01	7.32	0.33	60	60	35.29	51.76	14.27	0
BIT053	65.00	65.06	64.72	55.42	62.55	4.76	74	75.68	75	73.69	74.59	0.92	23.5	23.3	24.41	24.97	24.04	0.78	20	22.84	18.18	16.2	19.31	2.82	70.73	58.93	67.39	65.68	6.08	13.73	13.02	13.57	13.44	0.37	66.2	58.62	69.09	64.64	5.41	1
BIT055	67.00	67.61	77.64	63.95	69.05	5.95	41.63	40.16	41.16	41.78	41.18	0.73	50.27	57.62	56.9	54.21	54.75	3.33	13.33	17.17	14.39	17.65	15.64	2.1	88.24	90	94.44	90.89	3.19	8.14	7.82	8.58	8.18	0.38	88.24	89.74	100	92.66	6.4	0
BIT060	44.46	33.93	44.54	44.68	41.9	5.32	79.17	78.61	77.37	78.83	78.49	0.78	19.68	19.45	21.33	19.43	19.97	0.91	10.63	3.92	9.66	18.99	10.8	6.21	100	47.06	73.33	73.46	26.47	6.92	5.63	7.84	6.79	1.11	80	48.15	78.57	68.91	17.99	5
BIT061	65.13	69.66	69.02	64.98	67.2	2.49	58.79	58.74	57.67	57.03	58.06	0.86	40.02	41.17	41.08	42.11	41.09	0.85	8.52	17.67	16.73	12.14	13.77	4.25	87.23	92.68	92	90.64	2.97	9.38	13.49	10.85	11.24	2.08	87.5	92.86	87.5	89.29	3.09	2
BIT065	DISCHARGED						DISCHARGED						DISCHARGED						DISCHARGED						DISCHARGED					DISCHARGED					DISCHARGED					0
BIT066	67.94	72.11	72.28	67.58	69.98	2.57	69.36	69.85	71.72	71.83	70.69	1.27	25.67	25.07	23.77	22.91	24.35	1.25	10.25	8.56	9.85	6.3	8.74	1.78	86.36	85.19	73.33	81.63	7.21	10.74	9.89	9.22	9.95	0.76	82.14	83.33	63.16	76.21	11.32	0
BIT069	69.18	67.34	61.94	67.74	66.55	3.17	59.03	57.8	55.75	57.45	57.51	1.35	31.19	29.64	31.62	30.09	30.64	0.92	5.76	7.97	5.68	6.61	6.51	1.06	100	100	94.12	98.04	3.39	14.65	12.76	13.12	13.51	1	100	100	95.65	98.55	2.51	0

DAY 5

	CD3+						CD4+						CD8+						CD25BRI						CD25BRI FOXP3+					CD4+ FOXP3					CD127LOW FOXP3					
	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	FMO	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	R1	R2	R3	Average	SD	DAY 5 SOFA
BIT070	70.28	66.72	70.20	65.86	68.27	2.31	64.14	61.22	64.49	66.41	64.07	2.14	24.26	26.28	28.3	28.65	26.87	2.03	16.22	5	11.52	12.62	11.34	4.68	100	100	80.77	93.59	11.1	8.97	9.33	9.2	9.17	0.18	100	100	85.19	95.06	8.55	0
BIT072	67.32	70.90	60.26	72.52	67.75	5.45	40.58	35.66	35.15	38.25	37.41	2.51	47.53	48.01	48.36	48.43	48.08	0.41	7.27	2.7	13.33	8.64	7.99	4.38	100	100	100	100	0	5	5.54	6.42	5.65	0.72	100	100	100	100	0	0
BIT073	33.49	39.58	42.00	42.12	39.3	4.04	69.05	62.96	67.86	57.03	64.22	5.47	28.25	24.51	29.24	28.3	27.58	2.09	8.15	14.29	8.38	14.69	11.38	3.59	91.67	93.75	100	95.14	4.34	16.29	16.9	17.9	17.03	0.81	92.86	94.12	100	95.66	3.81	1
BIT074	76.50	75.48	75.95	75.82	75.94	0.42	68.21	66.4	66.21	67.63	67.11	0.97	30.91	31.9	31.24	29.31	30.84	1.09	8.91	15.64	18.68	16.84	15.02	4.26	96.43	94.12	96.97	95.84	1.51	6.54	8.07	8.46	7.69	1.01	96.43	96.97	100	97.8	1.92	0
BIT076																																								4
BIT078																																								4
BIT079																																								0
BIT084	62	63.67	66.22	61.7	63.39	2.07	60.42	60.84	61.43	59.83	60.63	0.67	28.46	28.73	27.67	28.64	28.37	0.48	22.14	17.47	15.53	17.34	18.12	2.82	92.16	84	90.63	88.93	4.34	13.09	13.37	12.55	13	0.42	96.08	90	98.44	94.84	4.35	0
BIT085	65.42	67.7	66.99	64	66.03	1.65	70.56	70.37	69.41	70	70.09	0.51	28.71	29.13	28.38	28	28.55	0.48	28.41	19.5	22.1	23	23.25	3.74	95.16	63.22	94	84.13	18.11	17.88	15.36	15.23	16.16	1.49	93.65	95.45	94	94.37	0.95	3
BIT088	59.6	60.72	62.32	59.08	60.43	1.43	76.54	74.54	74.52	74.14	74.94	1.08	20.81	21.38	21.21	21.87	21.32	0.43	17.65	21.88	25.61	12.48	19.41	5.65	82.14	100	84.62	88.92	9.67	8.57	8.48	8.22	8.42	0.18	81.48	100	86.67	89.38	9.55	3
BIT099	42.10	57.42	64.34	61.62	56.37	9.93	60.67	66.11	60.55	59.86	61.8	2.89	37.62	38.80	44.73	44.97	41.53	3.86	33.44	15.19	20.22	14.71	20.89	8.73	80.60	62.64	92.86	78.7	15.19	15.65	15.66	15.23	15.51	0.25	84.13	64.84	97.47	82.15	16.4	1
BIT100	49.81	54.9	50.93	55.8	52.86	2.93	60.07	60.66	60.11	59.25	60.02	0.58	35.44	34.94	36.07	36.72	35.79	0.77	33.93	27.63	23.89	18.21	25.92	6.59	87.32	51.22	92.73	77.09	22.57	9.13	7.94	9.51	8.86	0.82	88.24	65.41	93.55	82.4	14.95	0
BIT102	82.00	81.53	86.69	85.00	83.81	2.46	71.88	72.93	72.49	73.00	72.58	0.52	27.00	26.16	27.22	27.00	26.85	0.47	23.24	16.12	18.49	20.00	19.46	2.98	92.93	85.96	92.00	90.3	3.78	12.28	14.04	13.55	13.29	0.91	94.44	85.96	94.00	91.47	4.77	0
BIT103	73.49	71.17	73.64	70.40	72.18	1.64	70.20	71.96	70.23	71.33	70.93	0.86	29.80	26.76	29.33	26.88	28.19	1.6	28.33	20.00	24.08	24.21	24.16	3.4	91.84	96.35	92.61	93.6	2.41	18.56	17.59	15.16	17.1	1.75	93.14	98.54	92.40	94.69	3.35	0
BIT104	87.44	83.90	87.51	85.53	86.1	1.73	43.73	45.20	42.31	41.53	43.19	1.62	55.19	54.09	56.70	58.76	56.19	2.02	28.89	20.76	28.71	21.32	24.92	4.49	80.00	89.89	82.35	84.08	5.17	14.14	15.08	15.09	14.77	0.55	87.72	87.80	85.92	87.15	1.06	0
BIT107																																								10
BIT111	DISCHARGED						DISCHARGED						DISCHARGED						DISCHARGED						DISCHARGED					DISCHARGED					DISCHARGED					1
BIT SR002	48.74	54.74	59.54		54.34	5.41	58.76	56.3	54.87		56.64	1.97	48.14	48.23	48.94		48.44	0.44	19	18	23		20	2.65	75	76		75.5	0.71	9.21	9.49		9.35	0.2	79.17	77.42		78.3	1.24	1
BIT SR003	71.91	71.91	73.62	72.28	72.43	0.81	63.26	63.26	64.09	63.45	63.52	0.39	33.68	33.68	32.36	33.67	33.35	0.66	16.2	16.2	13.34	13.55	14.82	1.59	100	100	100	100	0	8.66	7.97	8.33	8.32	0.35	100	100	100	100	0	12
BIT SR008	69.43	69.16	68.44	65.9	68.23	1.61	65.9	62.78	63	62.88	63.64	1.51	32.19	32.04	32.03	32.08	32.09	0.07	13.52	14.8	14.85	14.66	14.46	0.63	90.91	93.33	82.35	88.86	5.77	8.2	9.51	8.64	8.78	0.67	93.55	100	88.24	93.93	5.89	1
BIT SR009	49	48	47		48	1	69	66	68		67.67	1.53	25	24	23		24	1	15	14	13.5		14.17	0.76	100	100		100	0	11	11.5		11.25	0.35	100	100		100	0	2
BIT SR010	49.69	46.88	47.5	55.28	49.84	3.82	67.3	66.51	68.59	68.02	67.61	0.9	31.92	31.19	29.01	30.35	30.62	1.25	28.29	17.16	18.72	17.7	20.47	5.25	100	95.12	91.3	95.47	4.36	12.25	14.12	15.27	13.88	1.52	100	95.12	97.3	97.47	2.44	9
BIT SR011	50.82	55.32	50.98	51.64	52.19	2.12	56.33	56.65	58.45	56.93	57.09	0.94	36.03	34.06	31.89	35.24	34.31	1.8	25.85	22	24.05	18.99	22.72	2.94	97.4	92.68	92.65	94.24	2.73	27.95	25.7	26.73	26.79	1.13	96.1	97.56	96.83	96.83	0.73	12
BIT SR012	63.34	66.73	65		65.02	1.7	79.89	83.83	80.11		81.28	2.21	13.29	11.17	12.44		12.3	1.07	16.67	20.6	19.48		18.92	2.02	52	82.09		67.05	21.28	5.54	8.93		7.24	2.4	56.16	88.89		72.53	23.14	0
BIT SR013																																								1
BIT SR019	59.86	63.2	71.98	69.54	66.15	5.59	69.64	69.27	68.19	67.54	68.66	0.97	25.26	25.16	24.92	26.49	25.46	0.7	36.25	20.91	20.73	19.03	24.23	8.06	49.48	67.59	74.42	63.83	12.88	10.51	9.25	9.48	9.75	0.67	60	71.68	88.04	73.24	14.08	0
BIT SR020	62.83	68.16	67.94	64.6	65.88	2.61	56.37	51.23	50.34	53.73	52.92	2.71	45.41	46.51	46.1	43.52	45.39	1.32	24.32	21.71	24.51	23.04	23.39	1.29	89.29	96	91.79	92.36	3.39	12	12.72	11.78	12.17	0.49	95.06	98.15	88.37	93.86	4.99	8
BIT SR021	71.47	70.7	70	71.02	70.79	0.62	69.68	74.44	75.74	69.67	72.38	3.17	25.34	24.64	23.42	27.03	25.11	1.51	42.2	22	27.42	24.08	28.92	9.13	56	57.98	44.35	52.78	7.36	13	13.23	12.89	13.04	0.17	62	63.03	50.39	58.47	7.02	12
BIT SR022	55.16	56.02	60.26	64.74	59.05	4.4	67.43	65.81	65.02	66.40	66.17	1.02	28.46	29.07	29.31	29.23	29.02	0.38	30.26	21.72	16.16	14.51	20.66	7.1		63.37	89.87	76.62	18.74	9.86	14.34	12.21	12.14	2.24		65.31	95.96	80.64	21.67	3
BIT SR023	66.63	71.74	65.17	62.08	66.41	4.03	85.14	83.05	81.04	82	82.81	1.76	13.91	15.64	13.36	14	14.23	0.98	20.48	16.51	17.86	20	18.71	1.86	84.91	93.81	90	89.57	4.47	13.49	14.98	14	14.16	0.76	84.76	95.06	92	90.61	5.29	1
BIT SR024	76.41	71.24	71.76	64.49	70.98	4.91	54.33	53.93	53.50	52.42	53.55	0.82	46.35	45.45	46.37	47.06	46.31	0.66	33.28	20.25	22.28	20.00	23.95	6.3	85.00	95.12	95.00	91.71	5.81	12.39	13.73	12.29	12.8	0.8	96.20	97.56	97.00	96.92	0.68	7
BIT SR025	75.26	78.26	82.80	78.94	78.82	3.1	87.26	87.27	87.72	87.61	87.47	0.24	15.98	14.67	14.13	12.03	14.2	1.64	48.40	55.17	48.73	38.78	47.77	6.76	75.08	88.40	86.04	83.17	7.11	23.81	19.98	18.97	20.92	2.55	80.52	92.02	90.02	87.52	6.14	2
BIT SR026	66.00	67.00	67.10	62.40	66.63	2.21	63.93	69.07	69.12	69.06	67.8	2.58	26.41	28.21	28.44	28.40	27.87	0.98	22.92	19.18	19.04	18.33	19.87	2.07	80.00	92.00	75.32	82.44	8.6	14.00	17.81	15.60	15.8	1.91	83.57	91.67	82.28	85.84	5.09	3
BIT SR027	65.00	65.14	66.40	68.00	66.14	1.39	73.00	73.99	73.53	73.85	73.59	0.44	24.80	24.84	25.51	25.00	25.04	0.33	20.00	18.41	18.83	21.39	19.66	1.34	66.90	70.49	61.04	66.14	4.77	8.55	6.82	8.36	7.91	0.95	71.93	75.00	65.28	70.74	4.97	0
BIT SR028	56.68	58.42	61.58		58.89	2.48	75.19	76.28	74.89		75.45	0.73	23.36	22.46	23.68		23.17	0.63	16.28	12.58	23.08		17.31	5.33	75.61	70.83		73.22	3.38	11.89	11.19		11.54	0.49	87.60	72.73		80.17	10.51	8
BIT SR029	67.83	64.36	66.02	68.02	66.56	1.72	77.79	77.59	77.34	78.17	77.72	0.35	19.23	19.20	19.99	18.92	19.34	0.46	21.13	19.16	23.66		21.32	2.26	96.36	80.30		88.33	11.36	13.78	11.91	11.01	12.23	1.41	96.55	80.52		88.54	11.33	1
BIT SR030	62.52	60.70	59.00	63.46	61.42	1.98	68.52	70.15	67.93	69.93	69.13	1.08	30.77	29.88	32.07	29.06	30.45	1.29	26.44	16.71	17.06	16.75	19.24	4.8	78.33	93.85	80.28	84.15	8.45	10.85	12.82	11.90	11.86	0.99	80.33	92.31	87.88	86.84	6.06	1
BIT SR031																																								6
BIT SR032	52.89	53.38	46.92	39.61	48.2	6.44	66.46	65.30	64.03	64.72	65.13	1.03	29.40	29.48	31.29	27.51	29.42	1.54	26.21	17.50	16.51	24.86	21.27	4.97	88.10	94.29	91.11	91.17	3.1	11.42	11.53	11.58	11.51	0.08	94.33	91.18	93.02	92.84	1.58	1
BIT SR033	39.01	54.01	30.92		41.31	11.72	49.01	46.70	47.00		47.57	1.26	52.01	52.01	52.00		52.01	0.01	11.11	24.90	29.60		21.87	9.61	81.67	86.49		84.08	3.41	11.00	11.84		11.42	0.59	88.68	91.89		90.29	2.27	4
BIT SR034	73.08	75.41	81.22	67.45	74.29	5.7	73.20	72.35	71.03	69.69	71.57	1.54	23.68	23.30	24.96	25.59	24.38	1.07	32.47	31.14	43.86	34.43	35.48	5.75	74.83	69.83	59.59	68.08	7.77	11.99	12.68	11.41	12.03	0.64	74.17	65.77	56.59	65.51	8.79	0
BIT SR035	REFUSED TO GIVE BLOOD						REFUSED TO GIVE BLOOD						REFUSED TO GIVE BLOOD						REFUSED TO GIVE BLOOD						REFUSED TO GIVE BLOOD					REFUSED TO GIVE BLOOD					REFUSED TO GIVE BLOOD					10
BIT SR037																																								12

Appendix 10: Day 1 and Day 3 – Average IL-6 and IL-10 concentration

	IL-6		IL-10	
	DAY 1	DAY 3	DAY 1	DAY 3
BIT001	80.026482	3.177816	16.000697	1.5192
BIT002	75.410863	29.710713	11.812779	1.7576
BIT003	23.174223	29.835086	1.477746	1.7265
BIT004	6.2595014	37.435655	3.4991417	3.0016
BIT007	25.302382	10.51582	5.8729859	5.4169
BIT009	25.951886	14.454297	2.265572	0.7003
BIT010	60.389374	32.170533	6.1736037	0.7832
BIT011	32.350183	N	2.1515446	N
BIT014	255.48908	278.63627	19.390422	6.609
BIT016	44.276167	29.738351	20.0091	2.7745
BIT017	136.07723	32.22581	14.103694	2.6906
BIT018	54.28128	39.46708	4.5357549	0.7625
BIT021	55.068976	18.517147	15.202504	10.7243
BIT022	25.979524	10.985674	1.4881121	1.2704
BIT023	62.006223	33.911755	5.5827342	6.0596
BIT024	296.98819	21.474459	7.5004685	2.0168
BIT025	1188.8666	57.252412	18.488568	3.3229
BIT027	82.18228	10.87512	4.9607663	0.7936
BIT029	88.442385	16.223157	10.237127	2.9808
BIT035	23.312415	6.743174	3.5095078	2.0375
BIT040	77.331734	25.371479	6.6815441	4.6809
BIT042	85.388338	29.144125	8.1431687	1.9028
BIT043	21.322448	20.797318	4.5046565	2.0272
BIT046	7.3926772	34.934377	2.8564415	2.6802
BIT047	46.1694	68.197232	8.1224365	6.6815
BIT048	41.512324	421.36114	20.437401	4.9504
BIT049	46.556338	9.7419441	7.5419331	2.5351
BIT050	56.174513	68.819097	5.8315213	4.204
BIT052	19.000819	11.372612	23.692366	1.4363
BIT053	30.830069	26.822496	3.9241531	3.1674

	IL-6		IL-10	
	DAY 1	DAY 3	DAY 1	DAY 3
BIT055	3.6338502	2.1690132	1.4984783	1.3015
BIT060	117.57329	32.875313	49.390007	4.2144
BIT061	12.450511	34.934377	2.8564415	2.6802
BIT069	88.124543	0.248142	31.176714	1.9028
BIT070	18.254582	32.654206	2.4003317	1.8509
BIT072	0.8285491	1.9617249	3.0119335	2.0168
BIT073	55.566468	1.077295	3.8826886	2.1101
BIT074	10.33617	20.631487	2.6698511	2.9083
BIT077	12.422872	11.386431	3.9656176	3.1882
BIT085	31.949426	29.613978	5.5620019	3.551
BIT088	361.33046	166.42423	155.54956	11.8231
SR002	57.376785	56.506174	4.7119791	3.4473
SR003	807.12458	101.73647	39.241564	2.6388
SR008	110.86406	22.828743	7.6818759	2.4211
SR009	146.08234	122.86605	4.5564871	4.8571
SR010	28.093864	132.34604	5.9766472	9.7603
SR011	53.908162	8.8298758	3.447311	2.1826
SR012	28.314972	11.068589	25.195455	3.1363
SR013	57.224774	RIP	5.572368	RIP
SR019	47.012372	28.577537	5.3961438	3.6961
SR020	50.854115	22.787285	7.5833976	4.3388
SR021	17.287237	3.6753078	10.568844	3.1571
SR022	32.972048	4.2695342	5.5516358	2.504
SR023	70.767606	3.8273192	11.014587	2.8564
SR028	119.57947		48.159728	
SR029	9.1750356		6.5623339	
SR030	122.48946		12.533901	
SR032	17.34202		1.6199572	
SR033	47.64897		2.2080661	
SR034	70.217516		35.470148	