

Persisting Inflammation after Critical
Illness: Prevalence, Risk Factors, and
Association with Physical Recovery

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Declaration

This thesis was written entirely by me. It has not been submitted for any other degree or professional qualification. The work described is all my own work with a number of exceptions:

The systematic review reported in Chapter 2 was conceived, designed, analysed, and reported by me. For purposes of methodological rigor, a second investigator was required to assist with article selection, both at the abstract, and full text stages. Dr Matthew Vale provided this assistance.

Chapters 3-5 report the results of several secondary studies built in to the RECOVER trial designed and conducted by Professor Timothy Walsh and his team. I designed these secondary studies and all analyses were carried out by me, in some cases using data provided to me from the RECOVER trial database.

Members of the Edinburgh Critical Care Research Group assisted me in the recruitment of patients, collection of and immediate processing and storage of blood samples, and the follow up of patients.

I performed many of the biomarker assays described in the Chapters 3-5. Miss Jillian Rennie (appointed as part of the CSO grant supporting this work) contributed significantly to this very large task.

The CMV IgG and PCR assays described in chapter 4 were coordinated by Dr Kate Templeton and carried out by staff at the Virology laboratory at the Royal Infirmary of Edinburgh.

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Abstract

Introduction Survivors of critical illness suffer physical, psychological, and social problems. The factors hindering recovery and the best rehabilitation interventions remain illusive. Critical illness is associated with inflammation that persists in some individuals and this might affect recovery. The hypotheses for the thesis are 1. Persistent inflammation is common after critical illness. 2. Persistent inflammation is associated with functional recovery. 3. Persistent inflammation is associated with critical illness-induced viral reactivation. 4. Biomarkers may help predict functional disability. The main part of the project was based on 197 of the 240 patients enrolled in the RECOVER trial who also consented to take part in an inflammation sub study.

Systematic Review Aims: Prevalence of systemic inflammation in ICU survivors; association of inflammation with physical recovery. 7433 references identified. 208 full text articles were reviewed. 57 were eligible. 22 studies included the relevant data. CRP at ICU discharge was elevated (>10mg/L) in most cases (70% of mixed medical / surgical patients and 100% of severe sepsis survivors). Lower CRP observed in trauma patients (23mg/L), VAP (46mg/L), > 6 days in ICU (45mg/L), and medical ICU patients (36mg/L). CRP was higher in sepsis (107mg/L) and surgical ICU patients (99mg/L). IL-6, TNF- α , and PCT were elevated in most patients at ICU discharge. Ninety percent of acute COPD exacerbations admitted to ICU had elevated CRP at hospital discharge and 43% general adult ICU patients fulfilled SIRS criteria 3 days after ICU discharge. Anaemic ICU survivors had elevated CRP and IL-6 at 6 months. There were no studies that measured both inflammation and physical function after ICU discharge.

Association of inflammation with functional outcome after critical illness

Aims: Prevalence of inflammation in heterogeneous ICU cohort, association of CRP with Rivermead Mobility Index (RMI) and other outcome measures at 3 months. At ICU discharge, 173 patients (94%) had elevated serum CRP with a median concentration of 27 (11-60) mg/L. At hospital discharge 169 patients (90%) had elevated CRP with a median concentration of 21 (8-42) mg/L, At 3 months 72

patients (59%) had elevated CRP with a median concentration of 4 (1-12) mg/L. CRP was associated with RMI ($p<0.01$), and percentage of predicted handgrip strength (HGS) ($p=0.03$) at 3 months.

CMV infection, systemic inflammation and the post ICU syndrome Aims: Prevalence of active and latent CMV at ICU discharge; association between CMV, inflammation, and recovery. 115 patients (62.8%) had latent CMV. 13 (11.4%) had active CMV (11.4% of those with prior CMV and 7.2% of ICU survivors). Active CMV associated with longer hospital length of stay (57 days v 28 days $p=0.016$), poorer baseline physical function (HGS 12 v 16 $p=0.032$; RMI 1 v 2 $p=0.018$). At 3 months, patients with latent CMV infection had higher CRP (5.4 v 2.8mg/L $p=0.06$), higher HNE (118 v 91.3 pg/mL $p<0.00$), lower TGF β (9.2 v 11.4 ng/mL $p=0.01$), and were slower on 2 min timed up and go test ($p=0.03$). Active CMV infection at ICU discharge was not associated with inflammation or physical function at 3 months.

Prediction of physical disability after ICU discharge Aims: To identify risk factors for poor physical function; to derive a prognostic index to identify for poor functional outcome. Age, Functional Comorbidity Index, Scottish Index of Multiple Deprivation quintile, CMV IgG status, ventilator days, baseline RMI, physical component of SGA, CRP, and SLPI met statistical criteria for consideration in the multivariable models. 2 linear multivariable models with reasonable fit ($R^2=0.175$; 0.193) were constructed. AUCs were 0.759 for the clinical model and 0.725 for the model incorporating biochemical markers. The models did not perform any better than a baseline assessment of mobility (RMI).

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Chapter 1 - Introduction

1.1 Critical Care

1.1.1 History and definitions

The origins of the modern specialty of Critical Care are debated but most accounts celebrate the contributions of a handful of individuals who recognised the importance of concentrating resources within specialised units to deliver high quality care (Vincent, 2013) (Ristagno & Weil, 2009). During the Crimean War, Florence Nightingale highlighted the importance of good postoperative care, and the development of special wards for managing these patients.

The development of the first intensive care unit (ICU) is credited to the Danish anaesthetist Bjorn Ibsen, who in 1953 at the height of an epidemic provided positive pressure ventilation to patients suffering from paralysis caused by polio. A shortage of negative pressure ventilation chambers (iron lungs) led to the establishment of units where positive pressure ventilation could be delivered – initially by anaesthetists.

Since then, units providing advanced respiratory and multiple organ support (ICU), and other units providing increased levels of monitoring and therapy (High Dependency Units (HDU)), have developed sporadically – responding to the demands of evolving patterns of human disease, and to surgical advancement.

The units within which advanced monitoring and organ support could be provided therefore defined critical illness. These units differed widely in terms of provision of organ support, expertise, and specialisation.

In the year 2000, in a document entitled “Comprehensive Critical Care – A Review of Adult Critical Services”, the UK government developed a strategy for improvement of critical care services in England and Wales (Day, 2009). A key part of this strategy was to move away from defining critical care by the location of the patient – i.e. within an ICU, or HDU, but on the requirements of the patient as

determined by the severity of their illness. Within this document, definitions of critical care were established and are summarized below (Table 1-1).

Level of Care	Description
0	Patients whose needs can be met through normal ward care in an acute hospital.
1	Patients at risk of their condition deteriorating, or those recently relocated from higher levels of care, whose needs can be met on an acute ward with additional advice and support from the critical care team
2	Patients requiring more detailed observation or intervention including support for a single failing organ system or post-operative care and those 'stepping down' from higher levels of care
3	Patients requiring advanced respiratory support alone or basic respiratory support together with support of at least two organ systems. This level includes all complex patients requiring support for multi-organ failure.

Table 1-1 Levels of Care

In Scotland, critical care is delivered in 3 main types of unit – HDUs (providing level 1-2 care), ICUs (providing level 3 care) and combined units containing a mixture.

1.1.2 Epidemiology

In Scotland, a well established national audit programme administered by the Scottish Intensive Care Society Audit Group (SICSAG) provides high quality data relating to the provision of critical care across the whole country (<http://www.sicsag.scot.nhs.uk>). In 2010, 9802 patients were admitted to the combined ICU/HDUs and ICUs and 25000 patients were admitted to HDUs. The majority of patients were post-operative, with the remainder admitted from accident and emergency departments and hospital wards.

Of those admitted to ICUs or combined units, the average length of stay was 5 days and 70% of the patients required mechanical ventilation. Twelve per cent of these patients required renal support and 80% of patients admitted were level 3.

ICU mortality was 18% whilst hospital mortality for ICU treated patients 30% (Cole, 2010).

1.2 Long-term complications of critical illness

As more patients survive critical illness, the problems facing survivors become ever more evident. Most patients suffer a combination of physical, psychological, and social problems that impact significantly on their physical and mental wellbeing (Desai, Law, & Needham, 2011).

Increasingly these issues are recognised not just as a major burden on individual survivors. As the number of patients surviving critical illness grows, so too does the socioeconomic effects of their disabilities and comorbidities.

1.2.1 Pulmonary complications

Most of the data relating to pulmonary function after critical care relates to ARDS patients. Patients suffer a variety of abnormalities including restrictive and obstructive deficits, diffusion limitation, and dyspnoea. They are generally mild or moderate, and improve significantly over the first 6 months, with improvement continuing at a reduced rate up to a year after ICU discharge. Defects are still seen after 5 years of follow up (Herridge et al., 2003; Herridge et al., 2011; Heyland, Groll, & Caeser, 2005; Neff, Stocker, Frey, Stein, & Russi, 2003; Orme et al., 2003; Schelling et al., 2000).

1.2.2 Neuromuscular dysfunction

The most prevalent symptoms for ICU survivors are fatigue and muscle weakness. Muscle weakness is severe in about a quarter of patients who require prolonged mechanical ventilation (De Jonghe et al., 2002). Muscle biopsy studies reveal skeletal muscle abnormalities in virtually all patients recovering from critical illness (Coakley, Nagendran, Honavar, & Hinds, 1993) and include axonal neuropathy, denervation, fibre atrophy, non-specific neuropathy and necrotising myopathy.

Acute respiratory distress syndrome (ARDS) survivors experience clinically important functional impairment after ICU discharge that is significantly associated with muscle wasting, weakness, and fatigue (Herridge et al., 2003). Most of the physical recovery observed in these patients happens close to ICU discharge, after which it flattens out and often does not return to pre-illness levels (Herridge et al., 2011). Interestingly, these patients have normal pulmonary function tests (Herridge, 2009).

Although functional limitation seems to be worst in ARDS survivors (T. A. Davidson, Caldwell, Curtis, Hudson, & Steinberg, 1999), other groups are affected. Trauma patients have poorer physical function than other ICU patients (Hilde Myhren, Ekeberg, & Stokland, 2010).

ICU-acquired weakness (ICU-AW), the term currently used to describe the spectrum of neuromuscular lesions acquired during a critical care admission occurs in 25% to 58% of ventilated patients and up to 100% of severely septic patients (R. D. Griffiths & Hall, 2010). Its pathophysiology is debated but the risk factors of corticosteroid treatment, severe illness, poor glycaemic control, and immobility are generally accepted (R. D. Griffiths & Hall, 2010).

Recovery from ICU-AW is not guaranteed. Many patients have on-going electrophysiological or clinical neuromuscular dysfunction at 12 months following ICU discharge (Guarneri, Bertolini, & Latronico, 2008).

1.2.3 Depression and anxiety

Symptoms of depressive illness are often evident at hospital discharge. These findings are associated with depression in the months following hospital discharge. The prevalence of depression ranges from 17-43% and varies according to study cohort and sampling time point (Davydow, Desai, Needham, & Bienvenu, 2008). Prevalence decreases in the 12 months following discharge. Age, gender and severity of illness do not seem to be associated with an increased incidence of depression. Presence of cognitive impairment, and poor health-related quality of life (HRQOL) may be associated - relationships that are probably confounded by

other factors (Davydow, Gifford, Desai, Bienvenu, & Needham, 2009) (Davydow, Desai, et al., 2008).

Anxiety is also frequently observed in ICU survivors. Prevalence depends on the assessment tool used and the chosen cut-offs, however rates are probably around 10% at 1 year and decline over time. Female gender, younger age, and pre-existing anxiety are predictors of post-ICU anxiety disorders (Eddleston, White, & Guthrie, 2000; Rattray & Hull, 2008; Rattray, Johnston, & Wildsmith, 2005).

1.2.4 Post-traumatic stress disorder

Post-traumatic stress disorder (PTSD) affects between 10 and 39% of ICU survivors of ICU survivors. Risk factors appear to be prior psychiatric illness, the use of benzodiazepine drugs for sedation, and recollection of frightening or alarming physical experiences during the ICU stay (Davydow, Gifford, Desai, Needham, & Bienvenu, 2008).

1.2.5 Neurocognitive impairment

Long term neurocognitive dysfunction is extremely common in ICU survivors and is present in up to 91% of general ICU survivors and 78% of ARDS survivors at ICU discharge (Hopkins & Jackson, 2006). It is related to duration of acute brain dysfunction (delirium) during critical illness (Girard et al., 2010; Pandharipande et al., 2013). Improvement after ICU discharge is gradual at best and problems are still seen up to 6 years after ICU discharge. Memory is the most common domain effected, followed by executive function, and attention deficits. In a highly selective cohort that excluded patients with pre-existing cognitive impairment, 80% of general ICU patients ventilated for more than 12 hours had cognitive impairment at ICU discharge (60% had severe impairment). Seventy percent had persistent cognitive impairment at 1 year (36% had severe impairment). Acquired cognitive impairment is thus common and long lasting (Girard et al., 2010).

1.2.6 Health-related Quality of life

Quality of life has been defined by WHO as *“the individuals perception of their position in life in the context of culture and value systems in which they live and in relation to their goals, expectations, standards and concerns”*(WHO, 1998). Health-related quality of life focuses on those aspects of quality of life that impact negatively on health (CDC, 2012). A number of assessment tools have been developed, and are used to measure HRQOL in survivors of critical illness of which the medical outcomes study forms (SF-12 and SF-36) are the most common.

ARDS is the most frequently studied cohort of critical care survivors. Perhaps the first cohort studied was by Hughes and colleagues who examined performance on the Sickness Impact Profile Score (SIP) and found decreased physical function in ICU survivors that dramatically improved over the first 3-6 months but remained low at 1 year. In other studies, scores on the SF-36 are well below age and gender-matched controls at ICU discharge, and persist for up to 5 years with the most dramatic improvement seen over the first 12 months. There seems to be a lesser impact on the mental component scores and these seem to change less with time (A. M. Cheung et al., 2006; Herridge et al., 2003; Herridge et al., 2011; Orme et al., 2003; Schelling et al., 2000).

1.2.7 Importance of a conceptual model for patient outcomes

HRQOL is commonly used as an outcome measure in interventional studies due to the demonstrable validity and ease-of-use of the newer scoring systems. Furthermore, the available HRQOL scoring systems are amenable to clinical intervention with drug and physical therapies. The challenge faced by clinical researchers is relating clinical variables to health-related quality of life measures. Of paramount importance to this process is that derivation of conceptual models to help explain how clinical variables interact with social and environmental influences to affect patients' quality of life.

One such conceptual model is reproduced in diagram-form below (Figure 1-1). This model illustrates five increasing levels of complexity ranging from

biological and physical factors through to perceived quality of life. The model helps explain the contributions of environmental and patient factors that add this complexity.

Better understanding of this complexity helps place in context the findings of outcomes research. It is necessary to inform researchers the potential pitfalls of focusing just on the biological and physical variables.

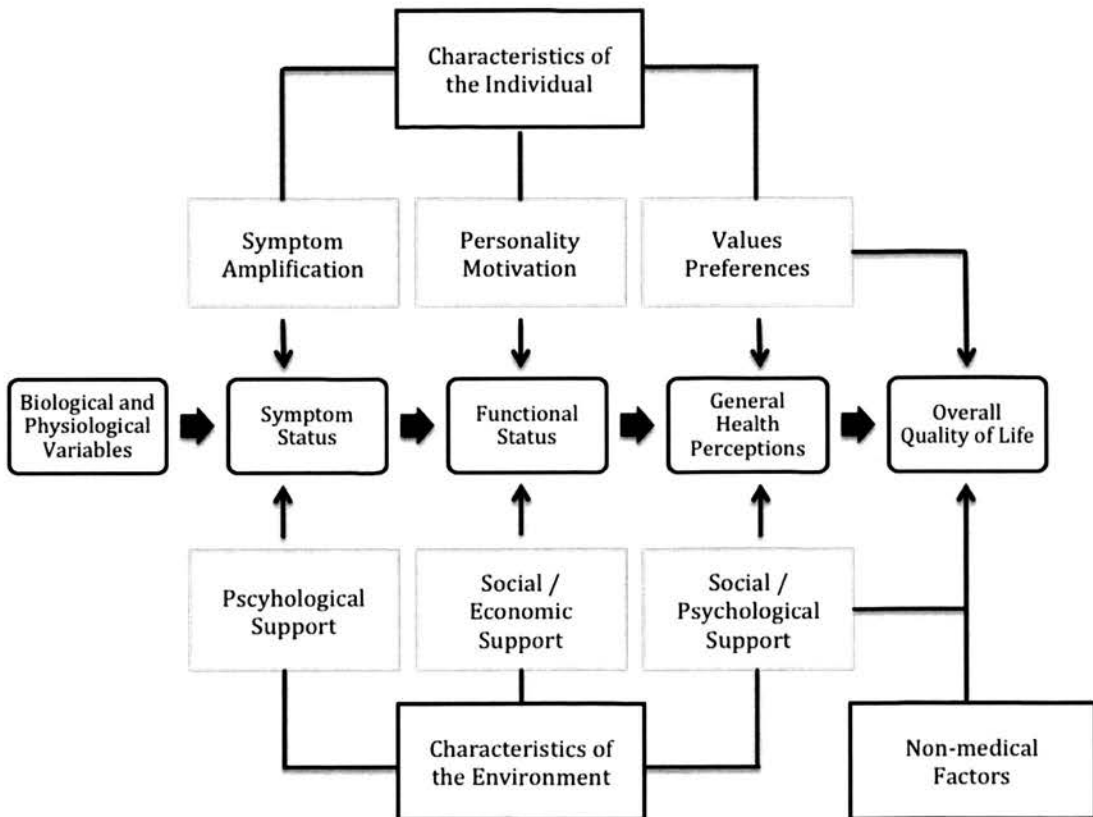


Figure 1-1 Conceptual Model of Patient Outcome adapted from Wilson et al (Wilson & Cleary, 1995).

1.2.8 Economic implications of critical illness survivorship

The economic implications of critical illness should not be overlooked. A large proportion of patients don't return to work for many years after critical illness, and consume a huge quantity of health care resource in the years following ICU discharge (J. Griffiths et al., 2013; Herridge et al., 2003; Lone et al., 2013). Although quantification has so far been difficult, health care costs attributable to hospital

readmissions, outpatient appointments, primary care, mental health services, and rehabilitation services are likely to run to many tens of thousands of US dollars per year. In some contexts these costs may run to hundreds of thousands of dollars (Lone et al., 2013).

1.2.9 Post-ICU disability – a major public health concern

Whilst persistent ICU acquired disability is now recognised, it is still not clear how to prevent or treat it (Herridge, 2009). Rehabilitation for critically ill patients who have illnesses that fall out with the remit of established disease specific rehabilitation pathways (stroke, myocardial infarction, head injury) is not routinely provided.

Reflecting the importance of this issue, a National Institute of Clinical Evidence (NICE) group was recently charged with creating a short guideline to inform rehabilitation practice in patients whose illness necessitated a critical care admission ("NICE Guideline 83 - Rehabilitation after critical illness," 2009) (T. Tan, Brett, Stokes, & Guideline Development, 2009). The striking finding of the review board was the lack of evidence pertaining to timing, composition, and effectiveness of rehabilitation interventions in this group of patients.

It is also clear that there is sparse data linking the critical illness event, to the pathologies demonstrated in the post-ICU syndrome. In order to develop effective treatments, this area needs more rigorous investigation.

1.3 Post-ICU Inflammation

1.3.1 Definition of inflammation

Inflammation is the term used to describe activation of the innate immune system of the human organism in response to tissue injury. Tissue injury may be the result of infection, mechanical trauma, extremes of temperature, or radiation. It is characterized by increased blood supply, increased capillary permeability, and influx of immune cells to the site of injury. The aim of inflammation is the

elimination of pathogen, removal of dead tissue and waste products of the inflammatory process, and return to normal function.

In the most severe cases, the localized response spills out into the systemic circulation and causes more global activation of the immune system and manifests clinically as the systemic inflammatory response syndrome (SIRS). SIRS is seen in virtually all critically ill patients and when it is overwhelming it is associated with failure of organ systems producing acute kidney injury, lung injury, and cardiovascular failure, amongst others.

1.3.2 The key events of inflammatory initiation and resolution

Inflammation is characterised by the infiltration of the inflammatory site by granulocytes (e.g. neutrophils, eosinophils and basophils). This process begins with pattern recognition of pathogen associated molecular patterns (PAMPS) and the endogenous equivalent damage associated molecular patterns (DAMPS) by pattern recognising receptors (PRRs) on tissue resident macrophages and mast cells (Barton, 2008) (Medzhitov, 2008a). PRR ligation results in the release of a myriad of inflammatory mediators (chemokines, cytokines, vasoactive amines, and eicosanoids). These mediators work in synergy to produce a localised inflammatory exudate consisting of plasma proteins and granulocytes (Medzhitov, 2008b).

Following their recruitment into the inflammatory environment, granulocytes and particularly neutrophils become activated, a process that is caused directly by pathogens and indirectly via stimulation by inflammatory cytokines. Activated neutrophils undergo degranulation – releasing proteases, reactive oxygen species (ROS), reactive nitrogen species, human neutrophil elastase (HNE) and cathepsin G that act to destroy the invading pathogen (Medzhitov, 2008b). The products of neutrophil degranulation are useful for pathogen killing, however their effects are non-specific and therefore cause collateral damage to host tissues (Segel, Halterman, & Lichtman, 2011).

The success of the inflammatory response therefore requires a programme of resolution and repair that can only be achieved by the successful and safe removal

of inflammatory cells from the site of inflammation (Segel et al., 2011). The first and most important part of this programme, is the removal of the inflammatory stimulus – the pathogen or damaged tissue (Serhan et al., 2007). By eliminating this inflammatory trigger, the production of pro-inflammatory mediators is halted, and further neutrophil recruitment and oedema formation is prevented (Serhan et al., 2007).

The next stage of inflammatory resolution is inflammatory cell clearance. Granulocytes that undergo apoptosis (programmed cell death) maintain their cell membranes and die without spilling their damaging contents. This contrasts with necrosis, which results in perpetuation of the immune response. Apoptosis is therefore the preferred mode of cell death. Apoptotic cells also express cell surface proteins that are recognized by macrophages and promote phagocytosis. Failure of phagocytosis results in apoptotic neutrophils that eventually undergo necrosis, known as 'secondary' necrosis, with the same consequences as 'primary' necrosis in terms of pro-inflammatory consequences. Finally, macrophages must be removed from the site of injury and some of these undergo apoptosis, whilst the rest are removed via the lymphatic system (Serhan et al., 2007).

Whilst many of the regulatory steps involved in the activation of acute inflammation are well established, those involved in inflammatory resolution are in the early stages of investigation. It is becoming clear, however, that the steps involved are complex, and depend on events that occur early during inflammatory activation (Serhan & Savill, 2005).

Chemokines are small proteins that attract cells and control cell migration during inflammation. A shift in chemokine expression is thought to be one of the earliest events in inflammatory resolution and results in the preferential attraction of monocytes into the inflamed region (Lawrence & Gilroy, 2007).

Early in the inflammatory process, arachidonic acid is oxygenated into inflammatory prostaglandins and leukotrienes. Later on, synthesis is diverted to producing lipoxins that suppress chemotaxis, vascular dilation, permeability, fibrosis and pain and promote ingestion of apoptotic neutrophils. Pro-resolution

lipids – resolvins, protectins and maresins are also produced during the resolution phase whilst their production is dependent on the pro-inflammatory activation of prostaglandin E2 (PGE2) and prostaglandin D2 (PGD2) early in the inflammatory reaction (Schwab & Serhan, 2006).

1.3.3 Potential causes of post-ICU inflammation

Persistence of inflammation beyond critical illness may be the result of a number of pathophysiological possibilities.

It is possible that there is a failure of some aspect of the inflammatory resolution pathway discussed above. This may be due to ineffective neutralisation of the inciting pathogen, or a failure to adequately clear inflammatory cells.

It is also possible that inflammation detected in the post-ICU period is not a failure to resolve acute inflammation, but a return to a chronic inflammatory baseline. Given the age, frailty, and pre-existing comorbidity of many ICU patients, it may be that many patients will have chronic low-grade activation of their immune system prior to their episode of critical illness. Chronic inflammation resulting from diseases such as rheumatoid arthritis, Type 2 diabetes (Donath & Shoelson, 2011), COPD (Walter et al., 2008), advanced age (Bruunsgaard & Pedersen, 2003), are often present and may co-exist in the same patient.

Another possibility is activation of the innate immune system caused by the reactivation of latent viruses (see 1.3.7).

There are likely to be other potential explanations for chronic inflammation after critical illness. Most obviously perhaps, is the possibility that survivors of critical illness are more likely to be affected by recurrent infections in the post ICU period. This certainly seems to be the case with sepsis survivors (T. Wang et al., 2012).

1.3.4 Effects of inflammation on muscle mass and function

Chemical products of the inflammatory response have an established role in regulating muscle mass. Tumour necrosis factor alpha (TNF- α), interleukin 1 (IL-1),

interleukin 6 (IL-6), and endotoxin infusions cause muscle wasting syndromes (Fong et al., 1989; Hoshino et al., 1991) due to increased protein catabolism (Baracos, Rodemann, Dinarello, & Goldberg, 1983; Flores et al., 1989; Goodman, 1991, 1994; Llovera, Lopez-Soriano, & Argiles, 1993), inhibition of protein synthesis (Charters & Grimble, 1989), inhibition of muscle cell differentiation (Miller, Ito, Blau, & Torti, 1988) and reduced amino acid uptake (Zamir, Hasselgren, James, Higashiguchi, & Fischer, 1993).

Numerous mechanisms have been proposed. Ubiquitin and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa B) mechanisms have been most extensively studied. Muscle cells increase their transcription of ubiquitin messenger ribonucleic acid (mRNA) in response to TNF- α , leading to muscle protein catabolism via the ubiquitin protease pathway (Garcia-Martinez, Agell, Llovera, Lopez-Soriano, & Argiles, 1993; Garcia-Martinez, Llovera, Agell, Lopez-Soriano, & Argiles, 1994, 1995; Raj et al., 2003). NF-kappa B activation by TNF- α leads to disturbance of the muscle regulating factor myogenic differentiation 1 (MyoD). MyoD regulates muscle specific transcription and inhibition results in reduced myogenesis (Costelli et al., 2005; DeJong et al., 2005; Langen, Schols, Kelders, Wouters, & Janssen-Heininger, 2001; Y. P. Li & Reid, 2000).

Chronic diseases such as cancer, chronic obstructive pulmonary disease (COPD), heart failure and end stage renal disease as well as normal aging are associated with loss of muscle mass and function. Numerous studies have observed associations between markers of inflammation and muscle function in these groups (Barbieri et al., 2003; DeJong et al., 2005; Eid et al., 2001; Raj et al., 2003; Roubenoff et al., 2003; Schaap et al., 2009; Schaap, Pluijm, Deeg, & Visser, 2006; Toth, Ades, Tischler, Tracy, & LeWinter, 2006; Visser et al., 2002; Yende et al., 2006).

1.3.5 Inflammation and the central nervous system

Systemic inflammation is also implicated in diseases of the central nervous system (Warnberg, Gomez-Martinez, Romeo, Diaz, & Marcos, 2009). A relevant example is the role of inflammation in the pathophysiology of dementia and in

particular Alzheimer's disease (Wyss-Coray, 2006). In 779 patients enrolled in the MacArthur study of successful aging, those patients with highest levels of interleukin-6 had the greatest risk of cognitive decline 7 years later (Weaver et al., 2002). In 691 cognitively intact elderly patients in the Framingham Heart Study, higher levels of IL-1 and TNF alpha production were associated with subsequent onset of dementia at a mean follow up time of 7 years (Z. S. Tan et al., 2007).

It is also plausible that inflammation plays a role in the pathophysiology of depressive disorders. The observation that components sickness behavior such as nausea, anorexia, lack of interest, sleep disturbance, depression, irritability, and cognitive disturbance broadly resemble some of the features of clinical depression, has led some investigators to believe that inflammation has a causative role. In support of this, many diseases characterised by chronic inflammation (COPD, type 2 diabetes, rheumatoid arthritis) are associated with a greater incidence of depression. In addition, infusion of recombinant cytokines for the treatment of cancer has been associated with onset of major depressive disorders (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008).

PTSD is also commonly associated with elevated markers of inflammation (Gill, Saligan, Woods, & Page, 2009) and levels of cytokines in the period following myocardial infarction are associated with subsequent development of PTSD (von Kanel et al., 2010).

Hence it is plausible that persistent inflammation in ICU survivors could play a causative role in the onset of cognitive dysfunction, depressive symptoms, and PTSD or indeed prevent recovery from the depressive symptoms of sickness behaviour or acute illness related cognitive dysfunction.

1.3.6 Persistent inflammation after intensive care

Whilst the early initial inflammatory response has been well investigated in relation to both short and long term outcomes, inflammation that persists into the recovery phase has been relatively neglected. This may be due in part to the logistical difficulties in collecting and processing community samples, but may also

reflect the lack of understanding of the pathological processes at play during this phase of critical illness.

Several investigators have recognised that inflammation at the time of ICU discharge may be significant (Al-Subaie et al., 2010; Grander et al., 2010; Grander & Dunser, 2010; K. M. Ho, G. J. Dobb, K. Y. Lee, S. C. Towler, & S. A. R. Webb, 2006; Ho, Lee, Dobb, & Webb, 2008; Axel Kaben et al., 2008; Litton, Ho, Chamberlain, Dobb, & Webb, 2007; Memis et al., 2007). Local practice and clinician preference ultimately determines when patients are discharged from the intensive care unit. Despite this variation, many patients have evidence of inflammation at this time. Serum CRP levels at ICU discharge are elevated (Grander et al., 2010; Kwok M. Ho et al., 2006; Ho et al., 2008; Axel Kaben et al., 2008; Litton et al., 2007) and are associated with increased mortality (Grander et al., 2010; Ho et al., 2008; Litton et al., 2007; Memis et al., 2007), and ICU readmission rates (Kwok M. Ho et al., 2006; Axel Kaben et al., 2008).

1.3.7 Cytomegalovirus and inflammation in ICU survivors

Cytomegalovirus (CMV) is one of 9 viruses of the Herpesviridae family that cause disease in humans (Pellett & Roizman, 2007). Owing to common biological characteristics such as a restricted number of hosts, long reproductive cycle, slow culture times, site of latency, and its tendency to cause cytomegaly, CMV is classified in the betaherpesvirinae subfamily. CMV is transmitted vertically from mother to foetus, or by contact with the body fluids of an infected individual. CMV infection is highly prevalent. In the developed world, more than a third of primary school age children have serological evidence of prior CMV exposure and more than 80% of people are infected by 60 years (Staras et al., 2006).

Acute CMV infection is usually subclinical in the immunocompetent host. Occasionally, patients suffer an episode of mononucleosis and in very rare cases, patients suffer a severe multi-system disease. In cases of immunodeficiency, reactivation of latency CMV infection or primary CMV infection can result in

pneumonitis, hepatitis, retinitis, colitis, and encephalitis. CMV pneumonitis following bone marrow transplantation has a mortality of up to 80%.

Traditionally, CMV infection is associated with several well described contexts: following bone marrow transplantation, following solid organ transplantation, and in patients with HIV infection when CD4 count falls below 50,000. Increasingly, critical illness is recognised as a context in which up to 40% of previously exposed adults will experience infection or reactivation (Chiche et al., 2009; Cook, Yenchar, Kraner, Davies, & Ferguson, 1998; Heininger et al., 2011; Heininger et al., 2001; Kutza, Muhl, Hackstein, Kirchner, & Bein, 1998; Limaye et al., 2008; Stephan et al., 1996). In addition, CMV reactivation is associated with increased mortality, longer duration of mechanical ventilation, longer duration of ICU and hospital length of stay, and a greater number of nosocomial infections. CMV reactivation is therefore common and is not benign in critically ill patients.

CMV infection is also known to have effects on the immune system. Acute infection is associated with elevated levels of inflammatory cytokines that persist after the immune system has effectively controlled viral replication (van de Berg et al., 2010). As a frequent occurrence in critically ill patients, and a potential mediator of systemic inflammation, it is therefore hypothesised that CMV reactivation might be a risk factor for persisting inflammation and prolonged recovery after critical illness.

1.3.8 Inflammation and functional outcome

Through whatever pathophysiological process, it seems likely that inflammation in survivors of critical illness persists beyond the period of ICU discharge and into the recovery phase. In addition, there is sufficient experimental and observational data to suggest that the presence of circulating inflammatory mediators during this period might be sufficient to retard physical recovery, and contribute in a negative manner to psychological outcomes. Further study is required to understand whether these relationships are indeed causal. Inflammation may therefore be of crucial importance in the pathophysiology of the

post-ICU syndrome. Given the recent intense interest in unravelling the complex events of inflammatory resolution, and the pursuit of new drug treatments in this field, a better understanding of its contribution to post-illness recovery is long overdue.

1.4 Main hypotheses and outline of thesis

The over-riding hypothesis of this thesis is that acute inflammation does not resolve adequately in some individuals after critical illness and that this hampers physical recovery. A further hypothesis is that potentially modifiable factors such as critical illness-induced CMV reactivation may contribute to a sustained innate immune response and might therefore be implicated in poor functional recovery. Finally, the identification of early evidence of dysfunctional inflammatory resolution may assist in the identification of those who are likely to have a poor functional outcome after critical illness and therefore might gain greatest benefit from rehabilitation outcomes.

Chapter 2 describes a systematic review that was conducted in order to identify any previous studies of the inflammatory response after discharge from critical care. The aim of this work was to present all the available evidence of the prevalence of systematic inflammation at 3 distinct time points. These were ICU discharge, between ICU discharge and hospital discharge, and after hospital discharge. Furthermore, the review was designed to identify studies that in addition to measuring the inflammatory response had also measured some characteristic of physical recovery.

Chapter 3 reports an observational study of 197 survivors of critical illness. These patients made up a representative sample of the 240 patients that participated in the RECOVER study (a randomised controlled trial of a complex in-hospital rehabilitation package) (T. S. Walsh et al., 2012). These patients donated weekly blood samples starting at the point of ICU discharge until they were discharged from hospital. A further sample was donated at a follow up appointment, usually in the patient's home. Analysis was carried out on the blood samples to determine

circulating concentrations of inflammatory mediators in the recovery phase of critical illness. These results were correlated with measures of recovery (primary and secondary outcome measures from the RECOVER study) in order to explore the associations between inflammation and physical recovery.

Chapter 4 builds on the work described in Chapter 3 to provide a rationale for the role of CMV latency and reactivation as a possible mechanism for persistent inflammation after ICU discharge and hence a possible contributor to poor functional outcome after critical illness. CMV immunoglobulin G (IgG) was measured in the plasma of ICU survivors at ICU discharge to identify those with prior CMV infection. Quantitative CMV assays were carried out on IgG positive samples to identify those with evidence of active CMV infection. The characteristics of CMV infected patients, their inflammatory profiles, and their physical outcomes were explored.

Chapter 5 describes derivation of a prognostic model to distinguish at an early stage patients that are likely to struggle to recover (those likely to benefit from intensive rehabilitation) from those who will do well, regardless of input. Statistical modelling was carried out using a linear regression technique. A selection of models is presented accompanied by a critique of their relative merits, and limitations. These models were differentiated by their inclusion, or exclusion of biomarkers of inflammation, and also their adherence to the assumptions of linear regression modelling.

Chapter 6 summarises the major findings of the thesis drawing from all 4 elements of the research programme in the context of the literature to address each of the aims of the thesis in turn. Conclusions are stated and areas for future study are proposed.

Chapter 2 - Prevalence of Systemic Inflammation in Survivors of Critical Illness and Relationship with Physical Recovery: A Systematic Review of the Literature

2.1 Introduction

Systemic inflammation in critical illness has been extensively studied in the acute phase of critical illness (usually defined as during ICU admission), but it is unclear how many patients have evidence of on-going inflammation in the recovery phase (defined as the period starting at the point of ICU discharge). During this phase of illness, organ failures have for the most part resolved and patients are starting a long battle to return their bodies to pre-illness levels of function. It is a hypothesis of this thesis that inflammation and recovery of muscle function are inter-related during this period but the extent to which this hypothesis has been previously investigated has not been explored.

In order to investigate the effects of inflammation in this target population, it is necessary to clarify what is meant by the term “inflammation”. Inflammation is the normal response of the human body to external invasion or injury. Initiation of inflammation is an active process triggered by host recognition of pathogens or damaged tissue. Traditionally resolution of inflammation was considered a passive process. The theory was that inflammation subsides spontaneously when pro-inflammatory signals cease. It is now considered an active process, orchestrated by circulating mediators created during the early inflammatory response and their effects on the apoptotic and phagocytic pathways of immune cells.

Inflammation is a physiological response. It is not a diagnosis. The classic signs of heat, pain, swelling, and loss of function are the local manifestations of a systemic process. The cytokine release, signalling, and cell recruitment occurring on a systemic level often occurs in the absence of overt clinical signs. When the response is greater in magnitude, certain systemic features of inflammation emerge.

Thermoregulatory changes resulting in fever or hypothermia, sympathetic stimulation results in increased heart rate and inotropy, and changes in endothelial function result in vasodilation and lowered blood pressure. Some of these features form the basis of a consensus definition of a “systemic inflammatory response syndrome” or SIRS (Bone et al., 1992).

Clinical signs are therefore useful in the localisation of an inflammatory trigger, and the identification of patients with particularly severe illness, but they lack sensitivity in diagnosing the presence or absence of an inflammatory response. To detect subclinical inflammation, it becomes necessary to measure characteristics of the inflammatory process that are present at the start of the process and persist as long as the inflammatory process continues.

An early event in inflammation is the release of pro-inflammatory molecules such as IL-1, IL-6 and TNF- α in response to binding of damage-associated molecular patterns (DAMPs) to pathogen recognition receptors (PRRs) such as the Toll-like receptors (TLRs). These events trigger a wide variety of responses including the expression of adhesion molecules on vascular endothelium, the release of acute phase proteins, and the induction of a number of plasma enzyme systems. Relevantly, these events are accompanied by changes in the expression and activity of a plethora of inflammatory mediators often accompanied by release of substances into the blood that normally undetectable or are detectable only at a very low concentration.

Nearly 180 of these substances have been studied as potential biomarkers of sepsis (Marshall & Reinhart, 2009; Pierrakos & Vincent, 2010). Whilst many of them are sensitive markers of systemic inflammation, their use in the diagnosis of sepsis is limited due to their lack of specificity. When the focus of interest is on the consequences of the inflammatory process and the aetiology is not of key importance, the sensitivity of these biomarkers is valuable. Detection of increased concentrations of these molecules above the normal range will signify activation of inflammatory pathways even when there are no clinical signs of inflammation.

This systematic review was designed to identify studies that included measurements of the clinical or molecular manifestations of the innate immune response specifically focussing on the period after ICU discharge. The aims were to describe the proportion of patients that have clinical manifestations of inflammation, or that have elevations in systemic biomarkers of inflammation and to explore the relationship between inflammation and measures of physical recovery in these studies. This part of the thesis was designed to use rigorous systematic review methodology to unearth and appraise all available data relating to these aims in previously published studies.

2.2 Hypothesis

Acute inflammation persists after ICU discharge and this is associated with physical recovery after critical illness.

2.3 Aims and research questions

To perform a systematic search of previous literatures and collate and interpret the available data pertaining to the following 2 questions:

- What is the evidence that systemic inflammation persists into the recovery phase of critical illness?
- Is there any association between markers of systemic inflammation and physical function after ICU discharge?

2.4 Methods

2.4.1 Sources of articles

Electronic databases EMBASE and MEDLINE databases were systematically searched using the OVID user interface. CINAHL was searched using the EBSCO host interface. In addition grey literature sources were searched for conference citations (CPCISSH and CPCIC) using the Web of Science interface.

2.4.2 Study characteristics

Inclusion	Exclusion
Participants are human AND Adults >16 years old AND ICU patients or survivors AND Included measurements of the clinical or molecular manifestations of the systemic inflammation at ICU discharge or any time point after ICU discharge	Studies of patients in Cardiothoracic intensive care units, coronary care units, or neurosurgical intensive care units. Studies including participants <16 years old.

Table 2-1 Inclusion and exclusion criteria

Inclusion and exclusion criteria are summarised in Table 2-1. A search was carried out to identify reports of studies of adult ICU patients published between January 1981 and December 2011 in whom clinical or biochemical markers of systemic inflammation were measured. Studies carried out in medical, surgical, or mixed intensive care units were considered. To maximise generalisability to a general intensive care population, studies including children, neonates, neurosurgical, or post-operative cardiothoracic patients were not considered. The search strategy used for the MEDLINE and EMBASE searches is shown in Table 2-2. Search strategies for CINAHL, CPCISSH and CPCIC can be found in Appendix A.

1	interleukin*.ti,ab.
2	(CRP or TNF* or "C-reactive").ti,ab.
3	inflammation/
4	acute phase reaction/
5	systemic inflammatory response syndrome/
6	C-reactive protein/
7	interleukin-1 alpha/
8	interleukin-1 beta/
9	interleukin-6/

10	interleukin-8/
11	tumor necrosis factor/
12	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11
13	(critical adj3 care).ti,ab.
14	(intensive adj3 care).ti,ab.
15	(intensive adj3 therapy).ti,ab.
16	(ICU or ITU).ti,ab.
17	(critical\$ adj3 ill\$).ti,ab.
18	Critical illness/
19	Critical care/
20	Intensive care/
21	intensive care units/
22	respiratory care units/
23	intensive care unit/
24	13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25	humans/ not animals/
26	12 and 24 and 225
27	limit 28 to yr="1982 -Current"
28	remove duplicates from 29

Table 2-2 Search strategy for MEDLINE and EMBASE database using the OVID interface

A measurement of the clinical manifestations of systemic inflammation was defined by the presence one of the following:

1. Measurement of all of the elements making up the systemic inflammatory response syndrome (SIRS) as defined by the Bone et al (Body temperature, heart rate, respiratory rate (or PaCO₂), and leukocyte count) to allow calculation of a SIRS score (Bone et al., 1992) (see also table 2-3).
2. A diagnosis of SIRS according to the definition by Bone et al (Bone et al., 1992).

Criteria	Limit
Body temperature	>38C or <36C
Heart rate	>90 bpm

Respiratory rate	>20/min or PaCO ₂ <32mmHg
White blood cell count	>12,000/mL

Table 2-3 American College of Chest Physicians / Society of Critical Care Medicine consensus conference definition of SIRS.

A measurement of the molecular manifestations of systemic inflammation was defined as the measurement of a molecule in the serum or plasma considered by the authors to be a component of the inflammatory response such that an elevated concentration of that molecule would be likely to indicate activation of the innate immune system.

For a study to be considered, the marker of systemic inflammation had to be measured at one of 3 pre-specified time points: within 24 hours of ICU discharge, between ICU discharge and hospital discharge, and after hospital discharge.

In many studies, markers were measured whilst the patient was in ICU for periods of follow up which were not clearly linked with these 3 time points. For this reason the review adhered to the following principles: if a study reported a measurement of systemic inflammation whilst the patient was in ICU it was included if sampling continued until ICU discharge. If there was no reference to ICU discharge, the study was only considered if the last sample taken was at a time point greater than 14 days after ICU admission on the basis that there was reasonable probability that the majority of patients being sampled at this time point would have been discharged from ICU (Rosenberg, Zimmerman, Alzola, Draper, & Knaus, 2000). For these studies, the authors were contacted for further information.

No language restrictions were placed on the search. Where an English abstract was available, the study remained in the review provided there was sufficient information in the abstract. Where no English abstract was available, foreign language publications were excluded.

2.4.3 Selection of studies

Following retrieval of the search results and removal of duplicates, the title list was searched to remove clearly irrelevant studies (e.g. studies of paediatric,

neonatal, cardiothoracic, or neurosurgical patients, review articles, editorials, case reports and commentaries). Two authors independently screened the abstracts of the remaining studies and excluded studies that did not meet inclusion criteria. Disagreements about eligibility were resolved by discussion between the 2 screening authors. An inclusive approach was adopted. Where there was insufficient information in the abstract to allow outright rejection, the article remained in the review list.

Full text versions of the remaining articles were obtained whenever possible using the resources of the NHS, University of Edinburgh, and the British Library. Where an article could not be retrieved in full text and there was insufficient information in the abstract to determine eligibility, it was excluded from the review.

Two authors reviewed the full-text articles independently. This resulted in a final short list for further evaluation and data extraction.

2.4.4 Data extraction

A single author extracted data into a data table (see appendix C). Parameters extracted were: author, publication title, publication journal, publication year, country, number of patients, number of patients lost to follow up, inflammation mediator, time point, prevalence estimate, and precision of estimate (95% confidence interval). Where a prevalence estimate was not available, a summary measure was extracted (mean +/- standard deviation or median +/- interquartile range or range). Where a relationship between a measure of inflammation and physical outcome was documented, a relevant summary statistic for the relationship was extracted along with a 95% confidence interval where applicable.

2.4.5 Contact with study authors

When relevant data was not provided in the text, attempts were made to contact authors for raw data to allow calculation of prevalence estimates. Acknowledging that raw data may not be available in older studies, authors were

asked if they could provide summary measures with distributions as a minimum. Authors were contacted by email and post on 2 occasions 1 month apart allowing 2 months in total to respond after the initial contact. An example data request letter and form can be found in Appendix B.

2.4.6 Data synthesis

It was anticipated that a variety of different inflammatory markers would have been measured depending on the primary objective of each included study. In order to calculate the proportion of patients considered to have molecular evidence of systemic inflammation, cut-off values for each of the markers had to be determined. In order to do this, each included biomarker was noted, and literature searches were carried out to identify the range of values that could be expected in a normal healthy population. It was intended that a cut-off of the 97.5th centile would be identified as the upper limit of normal for each chosen biomarker. Subjects with biomarker concentrations greater than this cut-off were considered inflamed. In order to allow comparability between cut offs, summary estimates (mean (SD), or median (IQR)) were quoted.

A narrative account of the prevalence and summary data extracted from each study is provided in addition to a summary account in the main data table. No attempt was made to combine estimates in a meta-analysis due to the heterogeneity of the included studies in terms of study population, stage of illness, inflammatory mediators measured, and the methods used to measure them.

An assessment of study validity was carried out (details of the process of validity assessment can be found in section 2.4.7). The results of each study were discussed with reference to their internal and external validity.

2.4.7 Validity assessment

Although 2 questions were asked in the current review, the first relating to prevalence of inflammation, and the second relating to inflammation as a risk factor for poor recovery, the literature search only identified studies including data that

could answer the first. Therefore, the following discussion is limited to validity assessment of prevalence estimates in this review.

2.4.7.1 Definition of validity assessment

Validity assessment (often termed quality assessment) is an essential component of a systematic review. It is an assessment of the degree to which each reported finding accurately reflects what is happening in the real world (internal validity), and the extent to which that finding can be generalised to the population of interest (external validity). For a study to have internal validity, it must answer the specific question being asked by the researcher, and the populations and methods employed must be capable of answering this question. For a study to have external validity, the sample population must be true reflection of the population of interest. This is often a major challenge to study design.

Bias, the 'systematic error introduced into sampling or testing by selecting or encouraging one outcome or answer over others' (Pannucci & Wilkins, 2010) is the biggest threat to study validity and can creep into the research process at any stage from conception and design, right through to analysis and publication (Bhopal, 2008). Presentation of information about the risk of bias of included studies allows readers to decide how much confidence to place in any data presented. This is particularly important for systematic reviews of health care interventions where clinical decision-making might be influenced. Since it is virtually impossible to eliminate the influence of bias on the results of a clinical study, even in the most rigorous randomised controlled trials, validity assessment can be considered an assessment of the risk of bias – an approach supported by the Cochrane collaboration.

Assessment of validity is not the same as assessment of methodological quality. The traditional approach to validity assessment has been to assume a lower risk of bias if certain characteristics of methodological rigor are satisfied (such as by giving evidence of a power calculation). The issue with this approach is that some of the factors considered to represent methodological excellence, do not actually

negatively impact on bias. Additionally, lack of evidence of certain methodological features in a study report, may represent poor reporting rather than poor design or conduct.

2.4.7.2 Sources of bias in prevalence studies

Bias can affect clinical studies at any stage from the framing of the research question, right through to the analysis and publication of results (Bhopal, 2008). Most prevalence studies are epidemiological studies of diseases, and within them a number of study design features have been identified as sources of bias (Loney, Chambers, Bennett, Roberts, & Stratford, 1998). Prevalence is defined as 'the total number of individuals who have an attribute or disease at a particular time divided by the population at risk' (Porta, 2008). With this in mind, for a prevalence estimate to be valid, 3 general requirements need to be met:

1. The method used to identify or diagnose the disease or attribute is a valid and reliable method of doing.
2. The design and conduct of the study results in a sample that is representative of the target population
3. The target population of the study reflects the population of interest.

Where 1 and 2 reflect internal validity and 3 reflects external validity.

2.4.7.3 Validity assessment instruments

A vast number of tools have been designed to assist authors of systematic reviews in the process of validity assessment. Sanderson and colleagues (Sanderson, Tatt, & Higgins, 2007) identified 86 tools for assessing the quality and susceptibility to bias in observational studies and Shamliyan and colleagues identified 97 tools that could be used to evaluate studies of prevalence or risk factors of disease (T. Shamliyan, Kane, & Dickinson, 2010). Despite this choice of instruments, most systematic reviews of epidemiological outcomes fail to make adequate assessments of validity. This is partly due to a lack of consensus about how this should be optimally carried out (T. Shamliyan, Kane, & Jansen, 2012). Choosing an optimal

tool to assess validity in observational studies is therefore challenging. Selection of an appropriate method warrants careful consideration of a number of key issues. A summary of these is provided at the end of this section (Table 2-4).

Most validity assessment tools are scales or checklists. Scales prompt authors to score several aspects of study design or conduct that are then combined to create a summary score. Checklists prompt authors to look for the presence or absence of various features deemed important for study quality. These tools can often be administered rapidly and are reliable (agreement between individual assessors can be high). However their validity is questionable (Sanderson et al., 2007).

The use of summary scores to quantify overall risk of bias is considered to be misleading because it makes unfounded assumptions about the relative importance each assessed validity criterion. Indeed the use of validity assessment scales is 'explicitly discouraged' by the Cochrane collaboration (Higgins & Green, 2011). In their critique of quality scales, the Cochrane draw attention to one such scale developed by Jadad and colleagues. This scale has been widely used to assess the 'quality' of randomised controlled trials. In addition to the problems associated with weighting, this score is also criticised for its focus on reporting of trials rather than steps taken to reduce risk of bias.

Instead a 'domain-based' approach is recommended, allowing a subjective assessment of risk of bias (high, unclear or low) in specific domains. This approach makes allowances for the subjective nature of validity assessment, and gives the reader the information they need to interpret the findings presented to their own satisfaction.

The development process for a validity assessment tool is extremely important. This should include a rigorous method of criteria selection that is based on the best evidence or the opinion of research methodology experts. Reliability is the degree to which different independent assessors obtain the same result using the same measurement instrument and is discussed further in section 3.10.1. The

reliability of any new tool should be assessed to ensure that the assessments carried out by different researchers using the same tool give the same answer.

For the majority of scales and checklists, the development process is not described or there are issues relating to the inclusion of features not relevant to study validity. Similarly, very few authors are able to demonstrate reliability data (Higgins & Green, 2011; Sanderson et al., 2007).

A pre-requisite to reliability is ease of use. Instruments that are detailed and lengthy may allow a very precise analysis of all relevant sources of bias but the added complexity may make them cumbersome to administer. This may jeopardise reliability, especially when assessing a large number of studies.

Systematic reviews of healthcare interventions are likely to source data from interventional studies. Validity tools therefore focus on sources of bias in interventional studies (such as the Cochrane domain based evaluation tool) (Higgins & Green, 2011). For epidemiological outcomes, (e.g. incidence, prevalence, or association), depending on how inclusive the authors wish to be; data may potentially be drawn from a variety of study designs (e.g. cohort studies, cross-sectional studies, or interventional studies). Thus the ability to apply a particular tool to a variety of study designs is a relevant consideration.

Most validity tools focus on the features of single study designs. This limits their applicability to reviews that consult multiple designs. One approach might be to use several different tools to allow the assessment of data in each study design included. However, this precludes the comparison of validity between studies. The ideal solution would be to use tools that assess elements of bias relevant to the research question regardless of study design.

Feature	Considerations
Addresses sources of bias relevant to research question	Effect of an intervention Risk factors and associations Prevalence or incidence

Underlying design	Scale Checklist Domain-based evaluation
Validity	Rigorous development process Expert consensus Evidence based criteria
Reliability	Evidence of reliability testing
Complexity	Balances comprehensiveness with ease of use
Applicability	Can be applied to single study only Can be applied to multiple study designs

Table 2-4 Considerations when choosing a validity assessment tool

2.4.7.4 Criteria for validity assessment tools for this review

- Based on the considerations described in Table 2-4, a number of criteria were defined to allow selection of potentially useful validity assessment tools. These were:
 - Research question: The tool can be used to assess important biases in prevalence estimation.
 - Underlying design: Scales should be avoided. Domain based approaches should be favoured over checklists.
 - Validity: The tool has undergone a rigorous development process based on the available evidence base or expert opinion
 - Reliability: The tool has been demonstrated to be reliable.
 - Complexity: The tool is simple to use but includes criteria for assessing all relevant bias
 - Applicability: The tool can assess risk of bias relating to prevalence estimates in multiple study designs.

2.4.7.5 Available tools for prevalence estimates

A recent systematic review identified and assessed validity assessment tools suitable for evaluating studies of prevalence estimates (T. Shamliyan et al., 2010). Of the 97 instruments identified by the authors, 22 were considered potentially suitable for assessing the validity of prevalence estimates. Based on their assessment:

Underlying design: Fourteen (64%) were scales and 8 (36%) were checklists. None of the identified instruments employed a domain-based approach to validity assessment.

Validity: In the case of eleven (50%) instruments, the authors described the development process. Nine of these described adaptations or modifications to previously described tools originally designed for another purpose.

Reliability: Thirteen (59%) quoted reliability data. In those assessed, agreement was considered to be good to excellent with agreement between assessors ranging from 70% to 95%.

Applicability: Six of the instruments were considered to be applicable across the full range of observational study designs. Eight of the studies were only applicable to single study designs. The remaining six were applicable to a limited number of study designs.

Considering each criterion in turn, of the 22 instruments identified, 14 were excluded because they were scales. Of the 8 checklists, only 3 described any form of development process and of these, 1 was a modification of another tool. Both of the remaining checklists were designed for risk factor estimates and of limited use for prevalence estimates.

In keeping with the findings of the authors, none of the tools identified in the review could be considered evidenced based, reliable, instruments for assessing the bias in prevalence studies. In addition, none of the tools could be considered to be domain-based evaluations of risk of bias in prevalence estimates.

Since the publication of this review, researchers have continued to pursue improved instruments for assessing risk of bias in prevalence studies by the

pragmatic adaptation of previous instruments (Hoy et al., 2012), and by literature review and expert consultation (T. A. Shamliyan et al., 2011). An example of the later approach, published by Shamliyan and colleagues in the *Journal of Clinical Epidemiology*, demonstrates the rigorous development process of a checklist specifically developed to help appraise the quality of prevalence studies and represents the current most credible instrument for this purpose. The elements in the checklist are described below (Table 2-5).

Element	Criteria
General	Funding Conflicts of Interest Ethical approval Aim of study Design / target population
External Validity	Sampling source / method Assessment of sampling bias Response rate Exclusion rate from analysis Addressing sampling bias in analysis Study flow / recruitment rates
Internal Validity	Source of measure Reference period Disease definitions e.g. frequency / severity of symptoms Validation of measurement technique Reliability of measurement technique Reporting of estimates e.g. precision, subgroups of interest

Table 2-5 Validity assessment criteria by Shamliyan et al. Criteria highlighted in bold font were considered unlikely sources of bias in the current review.

Whilst this tool is likely to be the best available approach for assessing validity in prevalence studies, it has limitations in the current review.

First, the presence or absence of ethical approval might be an indicator of methodological quality but it does not assist in detection of bias.

Second, some of the criteria in the checklist make the assumption that the study authors set out to measure prevalence and that this was a predefined part of the analysis if not the primary analysis. In the current review, measurements of inflammation were performed as a supplementary analysis and not the primary analysis. Indeed the derivation of prevalence estimates was carried out by the author of this review based on raw data provided by the study authors.

As a result, external sources such as funders and their involvement in the study analysis would be unlikely sources of bias. Similarly, it seems unlikely that declared conflicts of interest of authors would be relevant if raw data is being used to perform the analysis. In addition, reporting of prevalence estimates cannot be considered an assessment of bias of the primary study and is therefore a redundant component of the checklist.

2.4.7.6 A review-specific validity assessment tool

Modifying an existing domain-based risk of bias instrument produced by Roy and colleagues which is itself a modification of preceding quality assessment scales, the sources of bias described in 2.4.7.2 and the relevant criteria identified by Shamliyan et al, were incorporated in to a review specific risk of bias instrument (Table 2-6).

Title		
Author		
Journal		
Year		
Risk of Bias Item	Criteria (circle)	Notes
External Validity		
1. Was the study's target population a close representation of adult ICU survivors	<p>Yes (LOW RISK): The study's target population was a close representation of adult ICU survivors.</p> <p>No (HIGH RISK): the study's target population was clearly NOT representative of adult ICU survivors (e.g. described a single disease or patients managed in a trauma ICU)</p>	

	Not reported	
2. Was the sampling frame (setting from which the sample was drawn) a true or close representation of the target population?	<p>Yes (LOW RISK): The sampling frame was a <u>true or close</u> representation of the target population</p> <p>No (HIGH RISK): The sampling frame was NOT a true or close representation of the target population</p> <p>Not reported</p>	
3. Was some form of random selection used to select the sample?	<p>Yes (LOW RISK): A random selection method was used.</p> <p>No (HIGH RISK): A random selection method was NOT used.</p> <p>Not reported</p>	
4. Was the likelihood of non-response bias low? (Were the number of subjects originally sampled that did not have a measure of systemic inflammation recorded low or was there an analysis that demonstrated that those with missing data were not significantly different from the rest of the sample?)	<p>Yes (LOW RISK): Response rate >75% or <75% and analysis demonstrated no significant differences in non-responders.</p> <p>No (HIGH RISK): Response rate <75% and analysis demonstrated significant differences in non-responders or not done</p> <p>Not reported</p>	
Internal Validity		
5. Was an appropriate case definition used in the study? (Definitions: 1. Clinical evidence of SIRS: >2 SIRS criteria 2. Molecular evidence of inflammation: concentration of inflammatory	<p>Yes (LOW RISK): An appropriate case definition was used.</p> <p>No (HIGH RISK): An alternative definition was used or no definition was provided.</p> <p>Not applicable:(investigators did not impose a definition as aim of study was different from aims of review)</p> <p>Not recorded: A definition was clearly used but investigators did not record what it was.</p>	

biomarker > upper limit of normal)		
6. Was the study instrument that measured the parameter of interest shown to have reliability and validity? (Where the measure was SIRS criteria – assume reliable and valid; where a biomarker was measured, note kit name / manufacturer and check product data sheets)	Yes (LOW RISK): The study instrument has been shown to have reliability and validity. No (HIGH RISK): The instrument had NOT been shown to have reliability and validity. Not reported.	Measure of inflammation: Manufacturer: Validity: Reliability:
7. Was the same mode of data collection used for all patients?	Yes (LOW RISK): The same mode of data collection was used for all subjects No (HIGH RISK): The same mode of data collection was NOT used for all subjects Not reported	

Table 2-6 Review-specific risk of bias instrument

2.4.8 Reporting

The review was reported according to the relevant sections of the MOOSE guidelines for Meta-Analyses and Systematic Reviews of Observational Studies (Stroup et al., 2000).

2.5 Results

The main results are summarised in the data table that can be found at the end of the chapter (Table 2-12).

2.5.1 Study Selection

After electronic database searching and de-duplication, 7433 unique references were retrieved. In total, 3327 abstracts were screened and from these 208 articles fulfilling or potentially fulfilling eligibility criteria were retrieved for full text review. 57 papers (Akbas, Karakurt, Unluguzel, Celikel, & Akalin, 2010; Al-Subaie et al., 2010; Altrichter et al., 2011; Azevedo et al., 2011; Balci, Sivaci, Akbulut, & Karabekir, 2009; Barth et al., 2002; Bateman, McArdle, & Walsh, 2009; Book et al., 2007; Chen, He, & Li, 2009; Cho et al., 2005; Clark, Hentzen, Plank, & Hill, 1996; Damas et al., 2006; de Pablo et al., 2011; Dorizzi et al., 2006; Farah & Makhoul, 2011; Friedland et al., 1996; Grander et al., 2010; Hansen, Thiel, Wouters, Christiansen, & Van den Berghe, 2003; Heizmann, Koeller, Muhr, Oertli, & Schinkel, 2008; Hekimoglu Sahin, Memis, & Sut, 2009; K. M. Ho, G. J. Dobb, K. Y. Lee, S. C. Towler, & S. A. Webb, 2006; Ho et al., 2008; Iapichino, Marzorati, et al., 2010; Iapichino, Umbrello, et al., 2010; Jensen et al., 2006; A. Kaben et al., 2008; Kauss et al., 2010; Litton et al., 2007; Luzzani et al., 2003; Makris, Dulhunty, Paratz, Bandeshe, & Gowardman, 2010; Martensson et al., 2010; Memis et al., 2007; Oda et al., 2005; Parnaby, Eaton, Shafi, & Bell, 1994; Povia et al., 2006; Povia, Teixeira-Pinto, & Carneiro, 2011; Rauchschalbe et al., 2004; Reny et al., 2002; Rixen & Siegel, 2000; Schneider et al., 2011; Sihler, Raghavendran, Westerman, Ye, & Napolitano, 2010; Silvestre, Coelho, & Povia, 2010; Stegenga et al., 2010; Tsangaris et al., 2009; Tsuruta et al., 2010; Umbrello et al., 2010; Watanabe, Hirasawa, Oda, Shiga, et al., 2005; Weimann et al., 1998; Whang et al., 1998; Woehrle et al., 2008; Yousef, Amr, & Suliman, 2010; Yucel, Memis, Karamanlioglu, Sut, & Yuksel, 2008; Zeckey et al., 2011; Zenahlikova et al., 2010; Zugel, Kox, Lichtwark-Aschoff, Gippner-Steppert, & Jochum, 2011) fulfilled eligibility criteria (or did not contain enough information to exclude) from the review. A flow diagram is presented detailing exclusions at various stages of the review (Figure 2-1).

2.5.2 Data completeness

Of the 57 papers considered to be eligible after full text review, none had prevalence estimates for systemic inflammation. Therefore the authors of all these studies were contacted to provide further data. The authors for 34 (65%) of the articles responded (Akbas et al., 2010; Al-Subaie et al., 2010; Azevedo et al., 2011; Balci et al., 2009; Bateman et al., 2009; Cho et al., 2005; Damas et al., 2006; de Pablo et al., 2011; Grander & Dunser, 2010; Hansen et al., 2003; K. M. Ho et al., 2006; Ho et al., 2008; Iapichino, Marzorati, et al., 2010; Iapichino, Umbrello, et al., 2010; Jensen et al., 2006; Kauss et al., 2010; Litton et al., 2007; Makris et al., 2010; Martensson et al., 2010; Memis et al., 2007; Oda et al., 2005; Povia et al., 2006; Povia et al., 2011; Reny et al., 2002; Tsangaris et al., 2009; Tsuruta et al., 2010; Umbrello et al., 2010; Watanabe, Hirasawa, Oda, Shiga, et al., 2005; Weimann et al., 1998; Yousef et al., 2010; Yucel et al., 2008; Zugel et al., 2011). Nine studies were excluded at this stage. Seven of these did not measure inflammation at an appropriate time point (Cho et al., 2005; Oda et al., 2005; Povia et al., 2011; Reny et al., 2002; Tsangaris et al., 2009; Zenahlikova et al., 2010; Zugel et al., 2011). Two studies (Umbrello et al., 2010; Yucel et al., 2008) used the same data as other included studies (Iapichino, Umbrello, et al., 2010; Memis et al., 2007) and were excluded. Raw data to allow calculation of prevalence estimates was provided for 13 studies (23%) (Akbas et al., 2010; Al-Subaie et al., 2010; Azevedo et al., 2011; Damas et al., 2006; Grander et al., 2010; Heizmann et al., 2008; K. M. Ho et al., 2006; Iapichino, Marzorati, et al., 2010; Iapichino, Umbrello, et al., 2010; Litton et al., 2007; Makris et al., 2010; Martensson et al., 2010; Watanabe, Hirasawa, Oda, Shiga, et al., 2005). These studies were included in the analysis. Where prevalence data was not provided, summary estimates of biomarker concentrations were available for 8 studies and these were also included in the analysis (Balci et al., 2009; Bateman et al., 2009; Hansen et al., 2003; Hekimoglu Sahin et al., 2009; Ho et al., 2008; Jensen et al., 2006; A. Kaben et al., 2008; Silvestre et al., 2010). None of the studies measured physical function after ICU discharge. One investigator measured health-related quality of life but was unable to provide data to data to allow calculation of association with inflammatory

markers (Bateman et al., 2009). Finally, 1 author volunteered data from another study (Van den Berghe et al., 2006) and the summary data from this study was included.

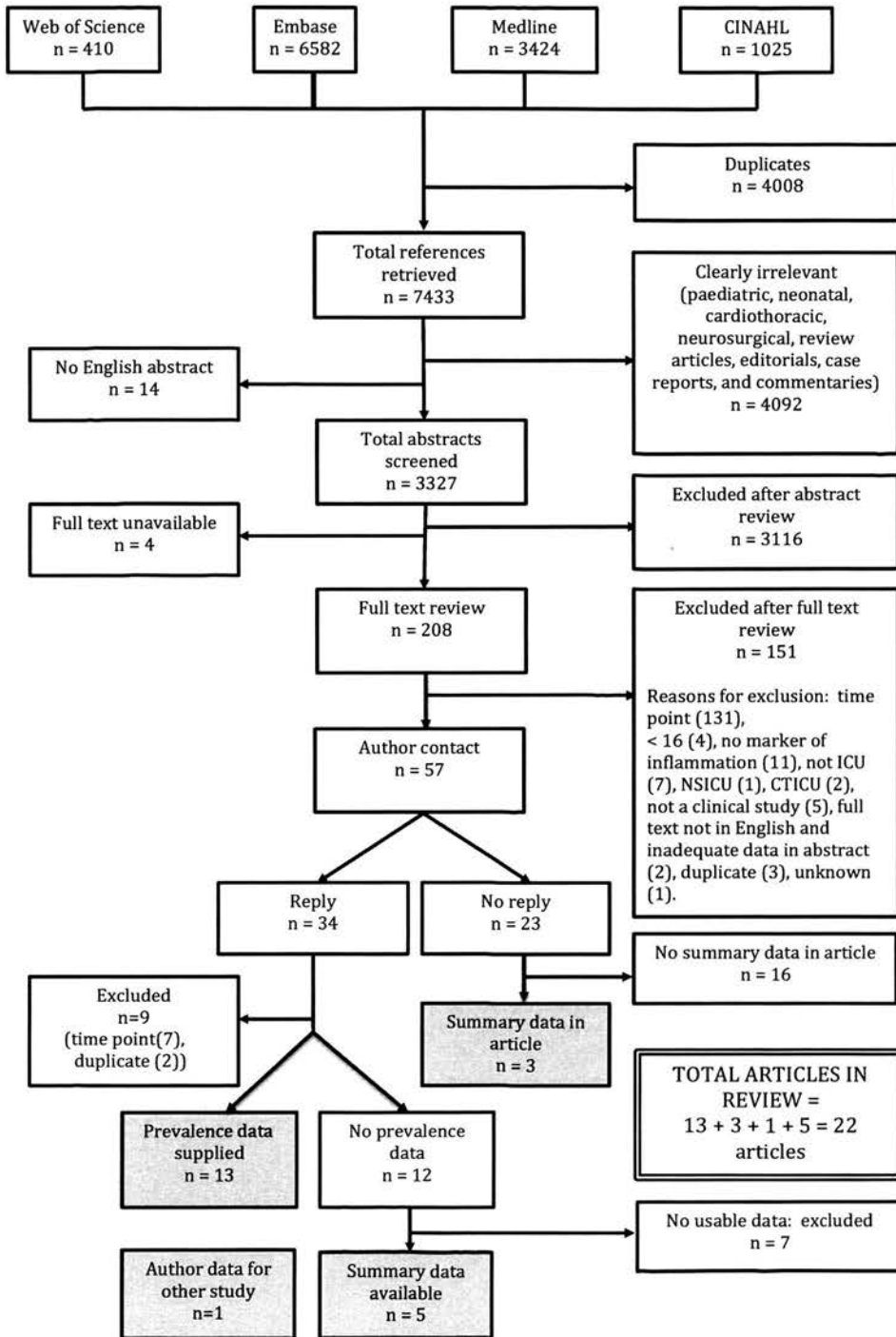


Figure 2-1 Systematic Review Flow Diagram

2.5.3 Design

Of the 22 analysed studies, 19 (86%) were observational and 3 (14%) were interventional. Of the observational studies, 3 (16%) were case-control studies, 1 (5%) was a cross-sectional study, and 15 (79%) were cohort studies.

2.5.4 Measures of inflammation and cut offs

The most common measure of inflammation was C-reactive protein (CRP), measured in 20 (91%) of studies. Pro-calcitonin (PCT) was measured in 3 (14%) studies. IL-6 was measured in 3 (14%) studies. TNF α was measured in 1 (5%) study. SIRS criteria were measured in 1 (5%) study. Myeloperoxidase (MPO) was measured in 1 study (5%). For a fuller discussion of inflammatory biomarkers see section 3.9).

For TNF α and IL-6, reference ranges from a population-based sample of community dwelling adults were used to determine a definition of 'evidence of inflammation' (Marques-Vidal et al., 2011). The authors report quartiles for those patients with detectable levels of these molecules in serum. In the absence of reported higher centiles, patients with TNF α and IL-6 levels above the 75th centile quoted in this study were deemed to have molecular evidence of systemic inflammation (4.5pg/mL for TNF α and 3.5pg/mL for IL-6).

The cut off for CRP was based on the distribution of CRP concentration observed in a healthy population. In healthy young adults, median CRP concentration is 0.8mg/L (90th centile 3mg/L, 99th centile 10mg/L) (Shine, de Beer, & Pepys, 1981). CRP rises slightly with age. Despite this, CRP concentration in healthy adults seldom rises above 3mg/L (Ballou et al., 1996; Delongui et al., 2013). A CRP cut off of 10mg/L was chosen to identify those patients with evidence of significant on going inflammation.

For PCT, the cut off was derived from a study of population of 451 healthy controls (Morgenthaler et al., 2002). In this population, the 97.5th percentile was 46.7ng/L (0.05ng/mL). The PCT cut off to detect systemic inflammation was therefore set at 0.05ng/mL.

The cut off for myeloperoxidase (MPO) concentration was set as the 75th centile of the control group for a nested case-control study of cardiovascular risk (Meuwese et al., 2007). This cohort consisted of 2,237 patients with a median concentration of 638 pg/mL (IQR 454-954pg/mL). The cut offs for each biomarker are summarised below (Table 2-7).

Biomarker	Cut off
IL-6	3.5 pg/mL
TNF α	4.5 pg/mL
CRP	10 mg/L
PCT	0.05 pg/mL
MPO	951 pg/mL (0.951ng/mL)

Table 2-7 Cut offs for systemic inflammation for the different biomarkers used in the review

2.5.5 Validity

Whilst adequate information was provided in the texts of most of the articles to make an assessment, inadequate reporting precluded full assessment in some cases. It should be noted that a lack of reporting was not interpreted as high risk of bias and is not a judgment on the quality of the work either. Prevalence of inflammation was not the primary research question of any study and therefore reporting of validity data relevant to this review may not have been relevant for the main research question of the article.

2.5.5.1 External validity

As anticipated at the planning stage, there was significant heterogeneity in the target populations chosen by the authors to meet the specific needs of their research question. As a result, the external validity of some of the included studies to all ICU patients was limited. Eight of the included studies (36%) aimed to study a target population that could be considered representative of general adult ICU survivors. The remaining 14 studies were more specialised in their target

populations. However, the subgroups have well defined in these studies and provide some information about inflammation in specific sub-groups. The target population for each study is shown in Table 2-8.

Target population	Study
Mixed medical / surgical ICU	Al Subai, Ho, Silvestre, Watanabe, , Povoia (Al-Subaie et al., 2010; Ho et al., 2008; lapichino, Umbrello, et al., 2010; Povoia et al., 2011; Silvestre et al., 2010; Watanabe, Hirasawa, Oda, Shiga, et al., 2005)
Chronic Obstructive Pulmonary Disease with respiratory failure	Akbas (Akbas et al., 2010)
Trauma	Balci (Balci et al., 2009)
Anaemia at ICU discharge	Bateman (Bateman et al., 2009)
Medical ICU	Grander, Van den Berghe (Grander et al., 2010; Van den Berghe et al., 2006)
Surgical ICU	Hansen, Kaben (Hansen et al., 2003; A. Kaben et al., 2008)
Severe Sepsis	Hekimoglu Sahin, Martensson, Memis (Hekimoglu Sahin et al., 2009; Martensson et al., 2010; Memis et al., 2007)
Unplanned ICU admissions	Ho, Makris (K. M. Ho et al., 2006; Makris et al., 2010)
Long term ICU patients	lapichino (lapichino, Marzorati, et al., 2010), Azevedo (Azevedo et al., 2011)
Unexpected in ward after ICU	Litton (Litton et al., 2007)
Ventilator associated pneumonia	Damas (Damas et al., 2006)
Matched control groups from case-	Ho, Makris, Litton (K. M. Ho et al., 2006;

control studies	Litton et al., 2007; Makris et al., 2010)
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Table 2-8 Target population of selected studies

Unlike large epidemiological studies, the available sampling frames for a study of inflammatory biomarker concentrations is limited. The unobtainable ideal would be to take a random sample of all ICU adult ICU survivors, everywhere in the world at exactly the same point in time after ICU discharge..

As a pragmatic compromise, it was considered that if the inclusion and exclusion criteria for a study did not exclude important subgroups of patients, the sampling frame would be considered adequate and therefore unlikely to be a source of bias. On this basis, fourteen studies (64%) were considered to be at low risk of bias and 6 studies (27%) were considered to be at high risk of bias.

Unlike epidemiological studies, in clinical studies, sampling does not occur at random. Instead, eligible patients are identified consecutively and then approached for consent. Therefore none of the studies fulfilled the criteria for random selection, therefore introducing a potential source of selection bias in all cases.

Follow up rates in the study were generally good. Therefore most of the patients enrolled in the studies had a measurement of inflammation carried out. On this basis, 15 studies (68%) were considered at low risk of non-response bias. Seven (32%) were considered at high risk of non-response bias..

Overall, external validity was poor. Excluding the 5 studies in which a full assessment was precluded by lack of data (Azevedo et al., 2011; Balci et al., 2009; A. Kaben et al., 2008; Memis et al., 2007; Van den Berghe et al., 2006) all the studies had 1 or more flaws in external validity, 11 (68%) had 2 or more flaws, and 4 (25%) had 3 flaws.

When target population was discounted (to allow for validity assessment in specific sub-groups), only 1 study lacked any flaws in external validity (Grander et al., 2010). 15 (93%) studies had 1 or more flaws, and 7 (44%) had 2 flaws.

2.5.5.2 Internal validity

Fifteen of the studies (68%) described their methods for ascertainment of the measure of inflammation. In one study, SIRS criteria were measured. Of the remaining studies, biomarkers were measured. In most cases, hospital biochemistry analysers capable of measuring a wide range of molecules were used. In a minority of cases, ELISAs and other laboratory-based techniques were used. All the described methods were considered reliable and valid methods for measuring the relevant biomarkers (see Table 2-9).

In fifteen of the studies (68%), the same instrument or method was used for all the measurements. The remainder did not report a method and made no comment about the consistency of the approach.

In summary, internal validity was considered to be good.

Biomarker	Measure / manufacturer	Type	Studies
CRP	Hitachi 917, Roche	Auto-analyser	Akbas, Ho 2006, Ho 2008, Litton 2007. Grander
	Hitachi 911, Roche	Auto-analyser	
	Hitachi Modular P	Auto-analyser	Hansen
	Roche Tina Quant (for use on Hitachi auto-analysers)	Auto-analyser	Silvestre
	Beckman Coulter LX-20	Auto-analyser	Al-Subai
	Beckman Coulter CX-9	Auto-analyser	Hekimoglu Sahin, Memis
	Boehringer-Mannheim	Nephelometric	Balci
PCT	Brahms AG Lumitest	Luminometric	Balci, Iapichino 2010
IL-6	Quantakine, R and D systems	ELISA	Bateman
	ELISA (Melenia)		Iapichino 2010

Table 2-9 Biomarker measurement methods

2.5.6 Evidence of inflammation at ICU discharge

2.5.6.1 CRP

Figure 2-2 illustrates the proportion of patients in each study with evidence of systemic inflammation at ICU discharge according to the definition derived in 2.5.4. Median CRP concentration at ICU discharge is then presented both according to study, then according to disease-specific subgroups to explore the ICU cohort on systemic inflammation at this time point.

Of the 22 included studies, 18 (82%) measured CRP at the point of ICU discharge. CRP concentration was elevated (>10mg/L) in the majority of patients ranging from 70% in a large study of mixed medical and surgical ICU patients (Grander et al., 2010) to 100% in patients with severe sepsis (Martensson et al., 2010) and a cohort of patients who subsequently were readmitted to ICU (K. M. Ho et al., 2006). The percentages observed in each sample are illustrated in bar chart format (Figure 2-2). For case-control studies, the cases and controls have been reported separately. In one of the cohort studies, data was only available for the subgroups and not for the cohort as a whole. In this case, data for the subgroups is shown.

The CRP concentration varied according to the population studied. The median (IQR) CRP concentration at ICU discharge for each study cohort is presented in Figures 2-3 to 2-8. The mean of the median concentrations of CRP at ICU discharge in the mixed medical / surgical cohorts was 60mg/L. Lower mean CRP concentration was observed in trauma ICU patients (23mg/L), patients with VAP (46mg/L), prolonged length of stay (45mg/L), and medical ICU patients (36mg/L). Higher mean CRP concentrations were noted in sepsis survivors (107mg/L) and surgical ICU patients (99mg/L). Unsurprisingly, the patients selected as cases for the observational studies of ICU readmission (K. M. Ho et al., 2006; A. Kaben et al., 2008) and unexpected death after ICU discharge (Litton et al., 2007) had high concentrations of CRP in their blood at ICU discharge (131 and 218mg/L respectively).

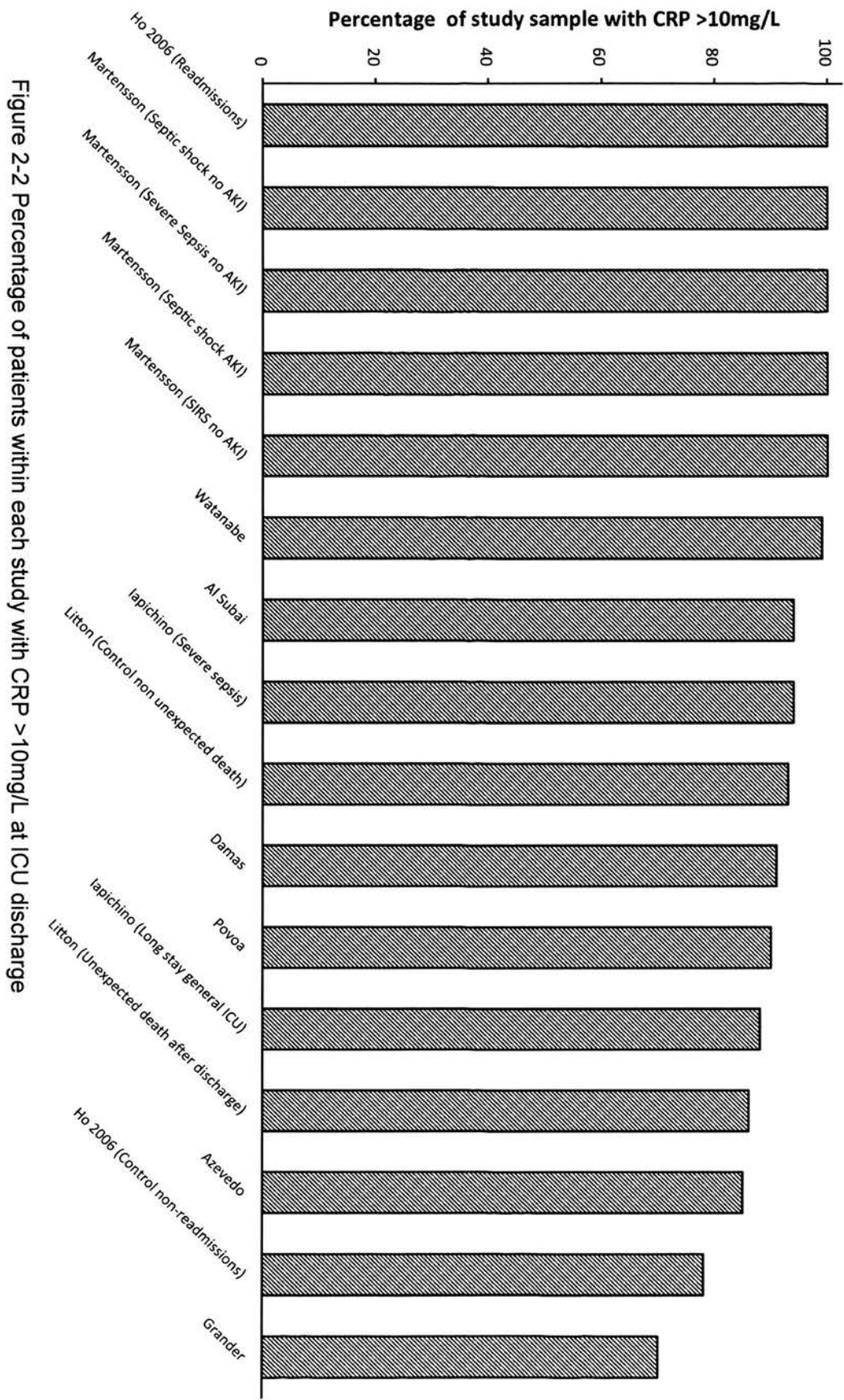


Figure 2-2 Percentage of patients within each study with CRP >10mg/L at ICU discharge

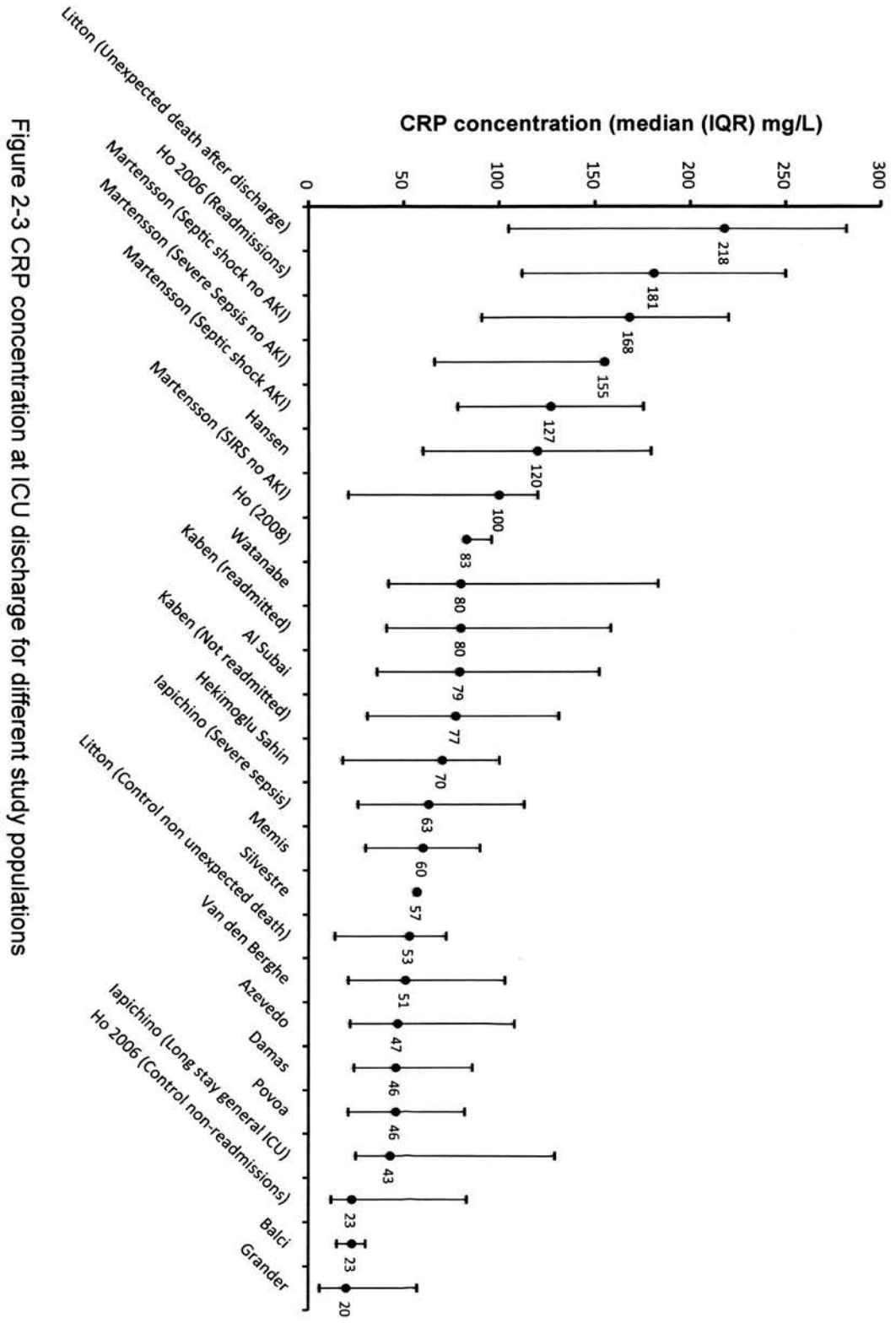


Figure 2-3 CRP concentration at ICU discharge for different study populations

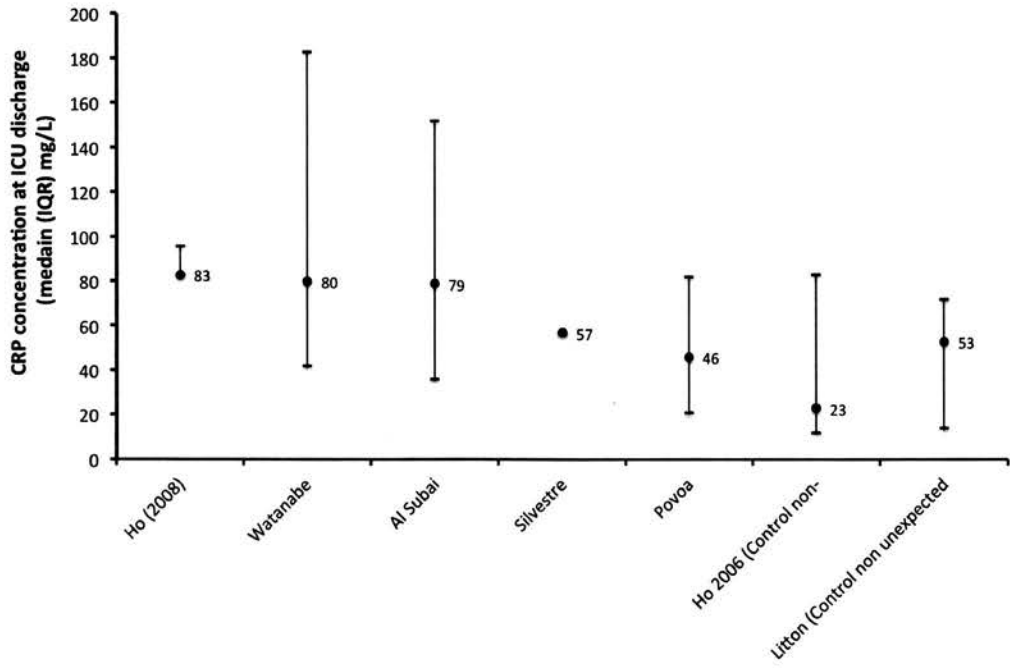


Figure 2-4 CRP concentration in General Adult ICU survivors

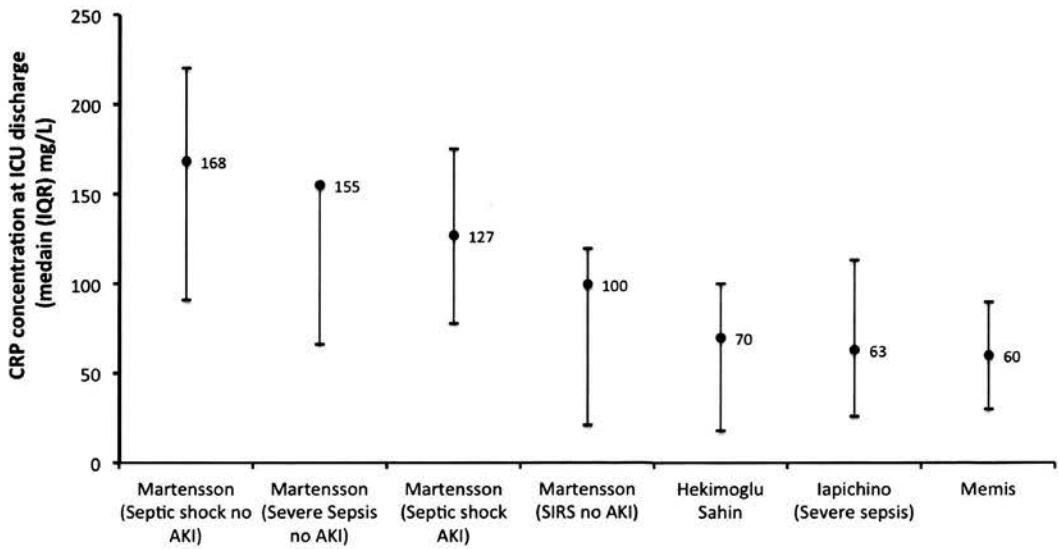


Figure 2-5 CRP concentration at ICU discharge in Sepsis Survivors

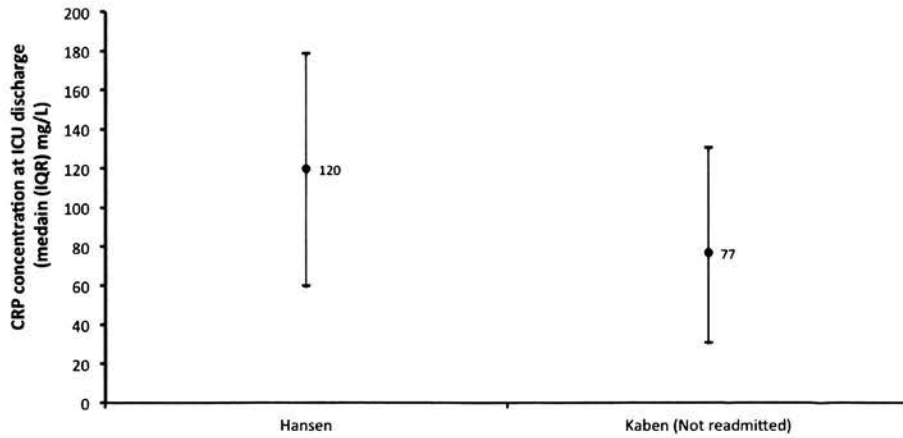


Figure 2-6 CRP concentration at ICU discharge in Surgical ICU survivors

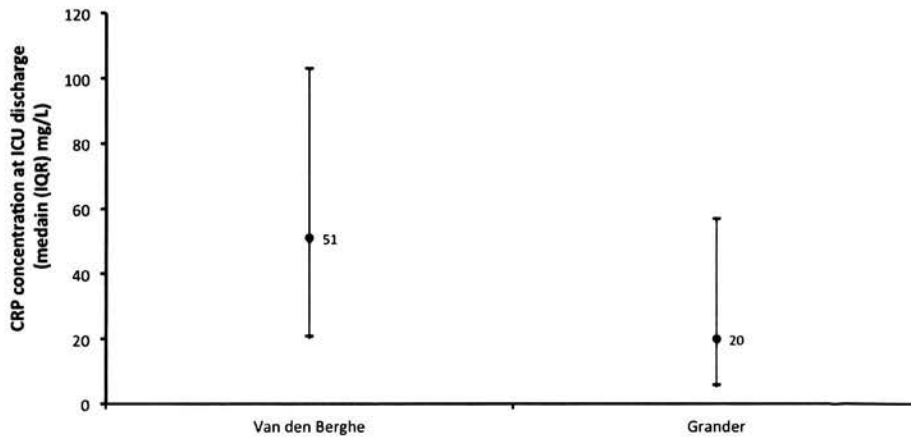


Figure 2-7 CRP concentration at ICU discharge in Medical ICU survivors

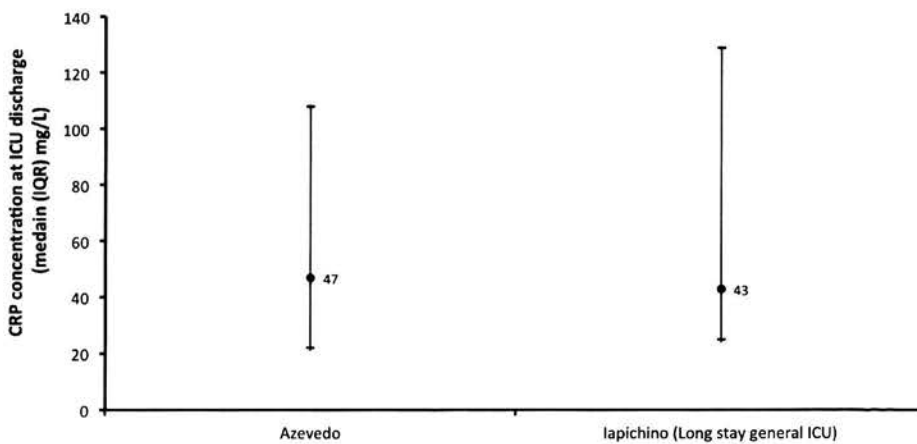


Figure 2-8 CRP concentration at ICU discharge in long stay ICU survivors

2.5.6.2 IL-6

Three studies (14%) measured IL-6 at ICU discharge (Iapichino, Marzorati, et al., 2010; Iapichino, Umbrello, et al., 2010; Watanabe, Hirasawa, Oda, Shiga, et al., 2005). These included one study of mixed medical and surgical ICU survivors (Watanabe, Hirasawa, Oda, Shiga, et al., 2005), one study of ICU patients with a length of stay longer than 6 days (Iapichino, Marzorati, et al., 2010), and one study of ICU patients with sepsis (Iapichino, Umbrello, et al., 2010). The percentage of patients in each of these samples with IL-6 concentration above 3.5 pg/mL was 99%, 63% and 100% respectively. Median (IQR) IL-6 concentration at ICU discharge in these samples were 80 (42-183) pg/mL, 76 (2-100) pg/mL, and 20 (15-39) pg/mL. There is therefore evidence of significant elevations in IL-6 concentration in the 3 studies at ICU discharge.

2.5.6.3 PCT

Study / cohort	Median (IQR) PCT concentration at ICU discharge (ng/mL)
Balci (Trauma ICU)	1.18 (0.99-1.44)
Iapichino (ICU stay >6 days)	0.17 (0.09-0.33)
Martensson (SIRS no AKI)	0.18 (0.15-0.54)
Martensson (Severe Sepsis no AKI)	0.53 (0.39-1.20)
Martensson (Septic shock no AKI)	0.58 (0.43-0.77)
Martensson (Septic shock AKI)	1.05 (0.48-2.89)

Table 2-10 PCT concentration at ICU discharge

Three studies (14%) measured PCT at ICU discharge (Balci et al., 2009; Iapichino, Marzorati, et al., 2010; Martensson et al., 2010). Only 2 of the authors of these studies provided data to allow a prevalence calculation (Iapichino, Marzorati, et al., 2010; Martensson et al., 2010). All the patients in the Iapichino study of ICU

patients with a stay of greater than 6 days had a PCT concentration greater than 0.05ng/mL (Iapichino, Umbrello, et al., 2010). All subgroups of sepsis survivors in Martensson’s study had elevated PCT concentrations according to this definition. In the subgroup of patients that had SIRS, 89% of patients had elevated PCT.

The median (IQR) concentrations of PCT in the various study groups is given in Table 2-10.

2.5.6.4 TNF- α

One study measured TNF- α at ICU discharge (Iapichino, Umbrello, et al., 2010). In this study of septic ICU patients the median (IQR) TNF α concentration was 20 (15-39) pg/mL. The percentage of patients with a TNF α concentration above 4.5 pg/mL was 100%.

2.5.6.5 MPO

MPO was measured at ICU discharge in 1 study of patients with SIRS and sepsis (Martensson et al., 2010). MPO was elevated at ICU discharge in 0%, 11%, 56% and 0% of ICU survivors with SIRS, severe sepsis without AKI, septic shock without AKI, and septic shock with AKI respectively. Median MPO concentrations for each sample are presented in Table 2-11. Notably, the concentrations measured are much lower than the expected reference ranges given in the control cohort from which the cut off was derived (951ng/mL).

Study / Cohort	Median (IQR) MPO concentration (pg/L).
Martensson (SIRS no AKI)	103 (77-112)
Martensson (Severe Sepsis no AKI)	72 (109-215)
Martensson (Septic shock no AKI)	70 (60-90)
Martensson (Septic shock AKI)	78 (106-145)

Table 2-11 MPO concentration at ICU discharge according to study population

2.5.6.6 SIRS Criteria

One study measured SIRS criteria at ICU discharge (Makris et al., 2010). Ninety-five percent of ICU survivors who were subsequently readmitted to ICU during the same hospital admission met criteria for SIRS. A comparable percentage (92%) of patients who were not subsequently re-admitted to ICU had SIRS.

2.5.7 Evidence of inflammation after ICU discharge

Three of the included studies measured inflammation after ICU discharge (Akbas et al., 2010; Bateman et al., 2009; Makris et al., 2010).

Makris and colleagues measured SIRS criteria 72 hours after ICU discharge in a case-control study comparing 244 patients who were subsequently re-admitted to ICU (cases), and 244 controls that were not readmitted (controls) (Makris et al., 2010). In the cases, 69% of the patients fulfilled criteria for SIRS whilst in the controls only 42% of the patients fulfilled criteria for SIRS.

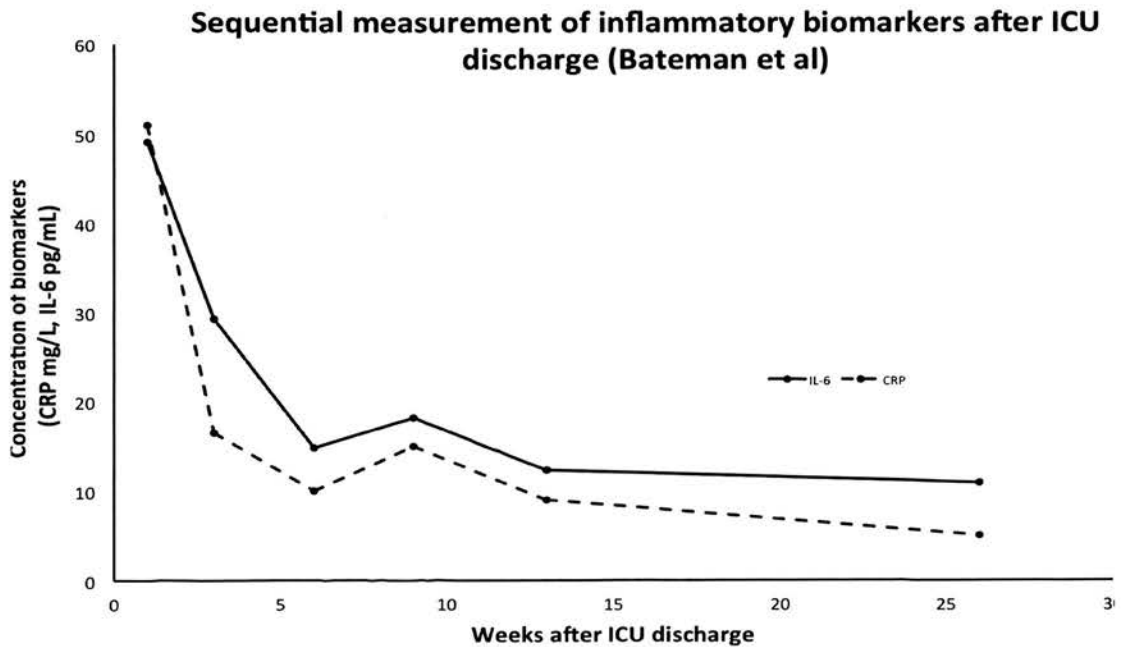


Figure 2-9 Sequential measurement of inflammatory biomarkers in ICU survivors with anaemia at ICU discharge

Akbas and colleagues measured CRP concentration in 10 ICU survivors at hospital discharge and found that 9 of them (90%) had a CRP concentration of greater than 10mg/L (Akbas et al., 2010). The median (IQR) of CRP concentration was 23 (16-93) in this sample.

Bateman and colleagues studied 24 ICU survivors that had evidence of anaemia at ICU discharge (Haemoglobin concentration <100g/dL) (Bateman et al., 2009). CRP and IL-6 were measured at weeks 1, 3, 6, 9, 13, and 26 after ICU discharge. Following a steep decline in both biomarkers, CRP concentration fell below 10mg/L at 13 weeks. IL-6 remained elevated even after 26 weeks (Figure 2-9).

2.5.8 Association of inflammation with physical recovery

None of the studies measured physical outcome measures as well as markers of inflammation after ICU discharge. There is therefore no known work that links inflammation after ICU discharge with physical recovery after ICU discharge. One study (Bateman et al., 2009) measured health-related quality of life after ICU discharge but were unable to provide data to test the association with systemic inflammation.

2.6 Discussion

This systematic review is a key part of the background work for the following chapters. It aimed to identify all previous studies that have measured systemic inflammation in survivors of critical illness, and all previous studies that have measured physical recovery in addition to systemic inflammation. From a basic scoping search carried out during the planning stage of the review, it was evident that very few investigators were likely to have posed either of these 2 research questions. It was considered of great importance to be inclusive as possible so that all potentially relevant studies would be identified even if they were subsequently excluded.

The review unearthed the data from 22 studies of ICU patients that had measured systemic inflammation at or after ICU discharge and found that in almost all ICU survivor populations, systemic inflammation is present at ICU discharge. Data pertaining to later time points is scarce and no previous studies had specifically explored the relationship between systemic inflammation and recovery of function after critical illness.

One major difficulty with the review was the setting of definitions. A large number of molecules are associated with inflammation and many of them are undetectable in the blood when the innate immune system is inactive. Conversely some investigators suggest that elevation of cytokines is a normal part of aging and that in some populations, cytokine induction might be the norm rather than the exception (Candore, Caruso, Jirillo, Magrone, & Vasto, 2010).

Using a threshold level of inflammatory mediators to define inflammation is therefore potentially misleading. Just like a diagnostic test, if the threshold is set too high, the test will become more specific but much less sensitive. This will result in a large number of patients with an activated immune system who are not identified by the biomarker measurement - the false negatives. Set too low, and the test becomes very sensitive but not specific. This results in a higher number of patients identified as being systemically inflamed but who are not - the false positives. Because there is no biochemical definition of systemic inflammation, and no 'gold standard' test to which investigators can refer, the author of this review had to rely on the best available healthy control population data.

Whilst the author accepts this limitation, especially for the biomarkers used less commonly in clinical practice, the steps taken to mitigate the risk posed by this limitation are emphasised. Firstly, in the case of CRP, a high cut off (>10mg/L) was chosen. This level is grossly abnormal even in very elderly patients and the author therefore has a degree of confidence that patients with CRP concentrations above this threshold have a significant degree of innate immune activation. Second, the quoting of a summary estimate (e.g. median (IQR)) next to the proportion in the data table gives the reader an indication central tendency of the measurement and

can compare this to the normal ranges quoted in the table of reference ranges (Table 2-7).

It is worth noting that the decision not to undertake a meta-analysis to combine and summarise the results was made in advance of carrying out the analysis. This decision was made on the basis that the study cohorts were likely to be heterogenous, and therefore would not improve the clarity of the results. Furthermore, the overall aim on the review was to identify what research others had carried out in this area in order to plan future work and this aim would not be addressed by a meta-analysis or meta-regression analysis.

One of the strengths of the review was the process of the literature search undertaken. The syntax for the search can be reviewed in the appendix. This was comprehensive, and was adapted for each relevant database search. It covered the breadth of the medical and clinical science literature and included searches databases of databases that listed sources of grey literature including conference proceedings and abstracts. The risk of missing studies was therefore extremely low.

Article selection was a long and detailed process. Over 7,000 articles were identified by the search, and many of these could be excluded immediately by reference screening on the basis that they did not describe clinical studies of general adult ICU patients. Of the remaining articles, 2 investigators reviewed abstracts of all abstracts. Following this, 2 investigators reviewed the full text articles of the included abstracts. Disputes between the 2 reviewers were settled by discussion. This 2-reviewer approach to article selection is a further strength of the review.

As emphasised in the results section, no single study set out to measure inflammatory markers in survivors of critical illness. The inclusion criteria allowed for any study that had measured a measure of systemic inflammation at any point starting from ICU discharge. A lack of detail in abstracts meant that even where inflammatory mediators had been measured sequentially in ICU patients, it was often not at all clear when measurements had stopped. To prevent the inadvertent loss of relevant studies during the abstract review stage, an inclusive approach was adopted so that exclusion could not occur at this stage due to lack of detail in the

abstract. This added to the workload of full text reviews. However, it reduced the risk of data loss.

None of the studies identified by the review process had in their reports enough data to carry out the proposed analysis. For this reason, as agreed at the protocol stage, the authors of the included studies were contacted by the email and postal addresses attached to the articles. In some cases, email addresses were invalid and postal addresses could not be found. For these studies, considerable effort was made to make contact. This included contact with co-authors and secretaries. As a result a relatively good response rate was achieved (65%) with authors very keen to assist with the project. Unfortunately, only about half of them had access to or were prepared to provide the raw data.

A further challenge of the review was the lack of a widely available evidence-based validity assessment instruments specific to the particular research question that could be easily and rapidly applied. A justification of the need for validity assessment has been given in addition to an extensive account of process of appraising available tools, and developing a review-specific tool. Criticism has been levelled at previous investigators who have modified other validity assessment checklists to suit the needs of a particular research question or disease. However the major challenge of the current review was the search for data pertaining to a research question that was not the aim of any of the included studies.

2.6.1 Systemic inflammation during recovery from critical illness

There is reasonable evidence that in survivors of critical illness, there is significant immune activation when they leave ICU. Despite the variable quality of the data, this is supported by numerous studies in different subsets of ICU survivors measuring primarily CRP but also SIRS criteria, IL-6, TNF-alpha, and PCT. The finding of persistent inflammation at this stage in a patient's illness is unsurprising.

ICU discharge is an arbitrary point in the patients hospital stay and will vary from country to country, hospital to hospital and from clinician to clinician. ICU

discharge often represents the point when the patient no longer requires organ support as part of their ongoing clinical management. It usually does not signify full resolution of the disease process. The finding of ongoing systemic inflammation at this time point therefore does not contribute significantly to medical knowledge.

One might expect that by the time a patient leaves hospital, organ failure and disease resolution might be nearly complete. A finding of significant elevations of inflammatory markers, or ongoing SIRS response at this stage might be more clinically important. Only one study of ICU survivors has measured inflammation at hospital discharge and this was focused on a very small (10 patients) COPD cohort that had been admitted to ICU for respiratory failure. The finding that 9 of these had CRPs above 10mg/L at ICU discharge is impossible to generalize to the wider ICU survivor population.

Beyond hospital discharge, evidence of inflammation may be even more significant. Whilst CRP and IL-6 remain elevated in ICU patients who were anaemic on ICU discharge in one small study, there is no evidence in the literature that in the wider ICU population, inflammation might be prevalent.

2.6.2 Systemic inflammation and physical recovery

After an extensive literature review process, there really are no studies that have tested the hypothesis that persistent inflammation after ICU discharge and recovery of muscle function might be interlinked. Despite the many studies that link inflammation to muscle function and weakness in other diseases, there is no previous work that has attempted to examine this link in a critical care cohort. In order to start to understand whether inflammation is important in recovery after critical illness, cohort studies testing such associations are required.

2.7 Conclusions

This systematic review marks the first stage in a programme of work exploring systemic inflammation during the convalescent phase of critical illness and its potential effects on the recovery of physical function. Whilst it has shown

without question that there is on-going activation of the innate immune system at the point a patient is discharged from the intensive care unit there is no available data that explains what happens beyond this point. Furthermore, there is no data to inform the hypothesis that inflammation during this phase of critical illness impacts on physical recovery.

Author	Design	Group	N (recruits)	Age (median (IQR) or mean (sd))	% Female	APACHE II (median (IQR) or mean (SD))	Inclusion criteria	Exclusion criteria	Measure of inflammation	Follow up	Fluid (Serum /Plasma)	Time-point (d/c = discharge)	% Inflamed	Summary (median (IQR) or mean (SD))
Akbas (Akbas et al., 2010)	Cohort	All	21/10	71 (66-76)	62	22 (16-25)	COPD patients, acute respiratory failure, admitted to ICU	Preexisting neurological, al, psych, metabolic, endocrine disease (excluding IC lesions, malignancy except BCC, liver disease, autoimmune disease, HIV positive patients, electrolyte disturbances, after CPR, heavy alcohol drinkers, pre-menopausal women,	CRP (mg/L)	10 (100)	S	Hosp d/c	90 (54-99)	23 (16-93)

		various drug treatments												
Al-Subai (Al-Subaie et al., 2010)	Cohort	All	1487 /1186	60 (16-97)	44	16 (12-20)	Medical/surgical ICU	Transferred to other ICU. Palliation	CRP (mg/L)	1186 (100)	P	ICU d/c	94 (92-95)	79 (36-152)
Azevedo (Azevedo et al., 2011)	Cohort	All	548/548	49 (20)	49	16 (10-21)	ICU survivors, LOS > 72 hours	-	CRP (mg/L)	548 (100)	S	ICU d/c	85 (82-88)	47 (22-108)
Balci et al., (2009)	Cohort	All	113/85	40 (5-85)	23	ND	ICU + trauma (>12 H survival)	CRF, thyroid or chronic hepatic disease.	CRP (mg/L)	85 (100)	ND	ICU d/c	ND	23 (15-30)
Bateman (Bateman et al., 2009)	Cohort	All	30/30	67 (55-77)	33	21 (16-24)	ICU survivors, >24 hours organ support, Hb <100g/L at ICU discharge,	Pre-existing haematological disorder, immunosuppressant/cytotoxic	PCT (ng/mL)	85 (100)	ND	ICU d/c	ND	1.18 (0.91-1.44)
									CRP (mg/L)	19 (79)	S	W1	ND	51 (21-108)
									CRP (mg/L)	19 (79)	S	W3	ND	17 (5-60)

Damas (Damas et al., 2006)	Trial	All	74/4 7	56 (19)	34	15(13- 19)	>16 years, mechanically ventilated >48 hours,	Non-VAP infection, antibiotic therapy in	CRP	43 (91)	S	ICU	91	46(24- 86)		
									(mg/L)	d/c	(77- 97)					
									>16 years	drugs, CRF, out with follow- up region, discharged for palliation, not expected to survive hospital discharge.	CRP (mg/L)	19 (79)	S	W6	ND	10 (5- 24)
									CRP (mg/L)	19 (79)	S	W9	ND	15 (5- 62)		
									CRP (mg/L)	19 (79)	S	W13	ND	9 (5-21)		
									CRP (mg/L)	19 (79)	S	W26	ND	5 (5-12)		
									IL-6 (pg/mL)	19 (79)	S	W1	ND	49 (31- 100)		
									IL-6 (pg/mL)	19 (79)	S	W3	ND	29 (13- 59)		
									IL-6 (pg/mL)	19 (79)	S	W6	ND	15 (9- 37)		
									IL-6 (pg/mL)	19 (79)	S	W9	ND	18 (9- 40)		
IL-6 (pg/mL)	19 (79)	S	W13	ND	12 (8- 27)											
IL-6 (pg/mL)	19 (79)	S	W26	ND	11 (9- 18)											

Study	Cohort	All	765/ 765	63 (15)	40	ND	All medical ICU patients	CRP (mg/L)	ND	P	ICU d/c	70 (66- 73)*	20 (6- 57)
Grander (Grande r et al., 2010)		All	765/ 765	63 (15)	40	ND	All medical ICU patients	CRP (mg/L)	ND	P	ICU d/c	70 (66- 73)*	20 (6- 57)
							Patients that died during ICU stay, ICU treatment <24 hours, <18 years, pregnancy, readmissio ns					*Calcu lated from 791 patient s	
Hansen (Hansen et al., 2003)	RCT	All	1569 /145 0	63 (14)	70	9 (7-13)	Adults receiving mechanical ventilation admitted to surgical intensive care unit	CRP (mg/L)	ND	S	ICU d/c	ND	120 (60- 179)
							Moribund patients Participatin g in other trials						
Hekimog lu-Sahin (Hekimo	Cohort	All	95/4 5	ND	56	-	ICU patients, bacteriologic al evidence	CRP (mg/L)	45 (100)	S	ICU d/c	ND	70 (18- 100)

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ppression,
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likely within
24 hours of
ICU
admission.

Ho (K. M. Ho et al., 2006)	Nested Case- Control	Cases 18/1 8	49 (19)	39	ND	Readmission s.	CRP (mg/L)	12 (67)	S	ICU d/c	100 (70- 100)	181 (112- 250)
	Control s	36/3 6	48 (21)	28	ND	Not readmitted	CRP (mg/L)	18 (50)	S	ICU d/c	78 (52- 93)	23 (12- 83)
Ho (Ho et al., 2008)	Cohort All	603/ 603	53 (19)	38	16 (7)	ICU patients	CRP (mg/L)	429 (71)	S	ICU d/c	ND	83 (83- 96)
lapichin o	Cohort All	26/1	61	54	ND	ICU LOS >6 days, "High	CRP	16 (89)	S	ICU	88 (60-	43 (25-

Study	Cohort	All	72/5 8	62 (14)	35	ND	Patients enrolled in RCT (glucose control), ICU, severe sepsis	<16 years, haematolo gical malignancy , BM transplant, DM, Likelihood of early death, LOOS <3 days	CRP (mg/L)	IL6 (pg/mL)	TNF alpha (pg/mL)	IL-6 (pg/mL)	CRP (mg/L)	IL6 (pg/mL)	PCT (ng/mL)	S	S	S	d/c	98)	129)
lapichin o, Marzorati, i, et al., 2010)																					
lapichin o, Umbrell o, et al., 2010)	Cohort	All	72/5 8	62 (14)	35	ND	Patients enrolled in RCT (glucose control), ICU, severe sepsis	<16 years, haematolo gical malignancy , BM transplant, DM, Likelihood of early death, LOOS <3 days	CRP (mg/L)	IL6 (pg/mL)	TNF alpha (pg/mL)	IL-6 (pg/mL)	CRP (mg/L)	IL6 (pg/mL)	PCT (ng/mL)	S	S	S	d/c	94 (83- 99)	63 (26- 113)
Kaben (A. Kaben et al.,	Cohort	All	2852 /285 2	62 (15)	36	ND	All patients admitted to surgical ICU	Discharged to other hospital, died during	CRP (mg/L)				CRP (mg/L)						ICU d/c	ND	ND

		ICU stay									
2008)	Not readmitted	ND	ND	ND	ND	CRP (mg/L)	ND	ND	ICU d/c	ND	77 (39-131)
	Readmitted	ND	ND	ND	ND	CRP (mg/L)	ND	ND	ICU d/c	ND	80 (41-158)
Litton et al., 2007)	Cases	29/29	63 (20)	52	ND	CRP (mg/L)	22 (38)	ND	ICU d/c	86 (64-96)	218 (105-282)
	Control	58/58	53 (17)	21	ND	CRP (mg/L)	14 (48)	ND	ICU d/c	93 (64-100)	53 (14-72)
Makris et al., 2010)	Cases	205/205	58 (19)	41	18 (7)	SIRS criteria	205 (100)	ND	ICU d/c-24H	95 (90-97)	NA
	Control	205/205	55 (20)	34	18 (7)	SIRS criteria	205 (100)	ND	ICU d/c+72H	69 (62-75)	NA
	Matched ICU survivors	205/205	55 (20)	34	18 (7)	SIRS criteria	205 (100)	ND	ICU d/c-24H	92 (87-95)	NA

Povoia (Povoia et al., 2011)	Cohort	All	63/5 1	57 (18)	36	19 (8)	ICU, >18 years, LOS >72 hours	-	CRP (mg/L)	51(100)	ND	ICU d/c	90 (78- 96)	46 (21- 82)
		Infected	35/2 3	62 (13)	65	21 (6)	ICU- acquired infection	-	CRP (mg/L)	28 (100)	ND	ICU d/c	90 (71- 97)	30 (15- 57)
		Non- infected	28/2 8	51 (22)	39	17 (9)	No antibiotics and survived to ICU discharge	-	CRP (mg/L)	23 (100)	ND	ICU d/c	91 (70- 98)	65 (28- 110)
Silvestre (Silvestre et al., 2010)	Cohort	All	156/ 156	55(18) (16)	35	15 (7)	ICU survivors, >17 years old, discharged from ICU, first admission only.	-	CRP (mg/L)	ND	ND	ICU d/c	ND	57 (42- 435)
Van Den Berghe (Van den Berghe et al., 2006)	RCT	All	1200 /904	64 (16)	39	23 (10)	Adult ICU expected to require >3 days ICU	Surgical ICU patients, medical patients receiving oral nutrition,	CRP (mg/L)	ND	S	ICU d/c	-	51(21- 103)

		DNAR orders												
Watana be (Watana be, Hirasaw a, Oda, Matsuda , et al., 2005)	Cross- section al	All	150/ 122	58 (18)	31	15 (4)	Critically ill patient admitted to ICU	Haematolo gical malignancy , autoimmun e disorders, immunosupr essive drug therapy	IL-6 (pg/mL)	96 (79%)	ND	ICU d/c	99 (94- 100)	80 (42- 183)
Yucel (Yucel et al., 2008)	Cohort	All	40 (20)	64 (17)	-	21 (5)	Sepsis, ICU	Burns, coronary care, trauma, steroids, bone marrow or organ transplant recipients, immunosu ppression, ICU acquired sepsis, condition considered irreversible , pre- existing	CRP (mg/L)	20 (100)	P	ICU d/c	-	86 (82)

<p>conditions associated with increased HbA1c and SHG plasma levels.</p>
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Table 2-12 Data table for systematic review

Chapter 3 - Persistent inflammation and the post-ICU syndrome: A Cohort Study

3.1 Introduction

The physical, social, and psychological consequences of critical illness place a real and significant burden on survivors of critical illness and their families. In the introduction to this thesis, risk factors for poor recovery were reviewed. Notably, these risk factors relate to patient characteristics, and severity of illness, factors that are largely un-modifiable. There is a lack of epidemiological study seeking to identify potentially modifiable risk factors occurring in the post-ICU period.

Low-grade 'chronic' inflammation is associated with poor physical function in the elderly and patients with chronic inflammatory disease. In addition, systemic inflammation has been implicated in psychiatric and psychological disease. In chapter 2, it has been shown that in a majority of patients show biochemical evidence of persistent inflammation at ICU discharge – but there is little knowledge about what happens beyond ICU discharge. There is also some evidence that circulating inflammatory markers may be detrimental to muscle function. It is therefore plausible that the presence of unresolved inflammation following an episode of critical illness (characterised by acute inflammation), may impact on physical, and possibly psychological outcomes.

The 'RECOVER' study is a randomised controlled trial evaluating a rehabilitation complex intervention for patients following intensive care discharge. The study protocol has recently been published (T. S. Walsh et al., 2012). Briefly, two hundred and forty survivors of critical illness were randomly allocated to receive standard NHS ward care (control group) or standard NHS ward care plus an enhanced recovery package (intervention group). Various measures of physical function, psychological comorbidity, and quality of life were measured at 3, 6, and 12 months after ICU discharge with comparisons made between intervention and control groups.

In addition to testing the effects of the RECOVER intervention, this trial provides a unique opportunity to study 'post-ICU' factors that may hamper rehabilitation. A blood sampling study was incorporated into the RECOVER trial design in order to investigate persistent inflammation in survivors of critical illness, and its relationship with rehabilitation outcomes.

This chapter aims to characterise the inflammatory profiles of ICU survivors and to investigate the effects of persistent inflammation on recovery from critical illness.

3.2 Hypotheses

- Systemic inflammation is prevalent in ICU survivors at ICU discharge, during the post ICU period of hospitalisation, and after hospital discharge.
- The presence of systemic inflammation in recovering ICU patients prevents physical and psychological recovery and quality of life.

3.3 Research Questions

- What is the prevalence of systemic inflammation in survivors of critical illness?
- How long does systemic inflammation persist in survivors of critical illness?
- What is the time course of individual biochemical markers of systemic inflammation after critical illness?
- Is persistent inflammation associated with the physical, psychological, and quality of life outcomes?

3.4 Patient selection

Patients were eligible for the biomarker study if they were enrolled in the RECOVER study. Inclusion and exclusion criteria for RECOVER are given in Table 3-1. Patients admitted to ICU for a short period of ventilation were excluded on the basis that they are not the group that experiences the greatest disability and could have weakened any observed impact of the trial intervention. Other excluded

groups included those that would not be able to get the intervention, those unlikely to survive, or those who could not be followed up.

Inclusion	<p>≥ 48 hours of invasive mechanical ventilation Consultant in charge of care deems patient fit for ICU discharge</p>
Exclusion	<p>Primary neurological admission diagnosis Palliative care only Currently receiving or planning to receive home ventilation Expected to be discharged to a non-study hospital where intervention could not be received Could not consent due to communication difficulties Currently enrolled in another trial with similar endpoints <18 years old at time of screening</p>

Table 3-1 Inclusion and exclusion criteria for the RECOVER trial.

3.5 Consent

During the consent process for RECOVER, patients were also provided with information about the biomarker sub-study. Participation in the study was not a pre-requisite of entry into the RECOVER study, and patients were permitted to withhold their consent for this part of the study.

3.6 Baseline data collected

Data was collected at baseline that served to describe the patients under study, track important functional changes, and to allow investigation of potential confounding and interacting factors. These were determined by the main trial and were not specific the biomarker component of the trial. This data can be subdivided into a number of categories and these have been outlined in Table 3-2.

Baseline data category	Data fields
Demographics	<p>Gender Age</p>
Evidence of ongoing disease process at study baseline	<p>Confusion assessment method (CAM) for the ICU Sequential Organ Failure Assessment Score</p>
Severity of illness	<p>Acute Physiology and Chronic Health</p>

	Evaluation (II) score (APACHE II) Use of vasopressors during ICU stay, Renal Replacement Therapy during ICU stay
Premorbid disease	Functional comorbidity index (FCI)
Function at baseline	Rivermead Mobility Index at baseline Hand Grip Strength at 1 week

Table 3-2 Baseline data collected in the RECOVER trial.

3.7 Blood sampling

Blood sampling was carried out at entry to the study (close to the point of ICU discharge). Following this, blood samples were drawn on a weekly basis until hospital discharge, and then a final sample was drawn 3 months after study entry.

3.8 Blood processing and storage

Blood samples were drawn by vene-puncture (or from arterial lines if they were still in situ to reduce patient discomfort) into pre-treated sample tubes (Serum Gel 4.7mL, and EDTA 4.9mL (Starstedt AG and Company, Nümbrecht, Germany). Samples were allowed to sit at room temperature for 30 minutes prior to processing as per the manufacturers guidance. Samples were then centrifuged at 2500G for 10 minutes at room temperature before being divided into aliquots in pre-labelled sample tubes. Samples were placed in boxes and stored at -70C until analysis. Freeze thaw cycles were kept to a minimum.

3.9 Selection of biochemical markers of inflammation

3.9.1 C-Reactive Protein

C-reactive protein (CRP) is an acute phase protein and is a short chain member of the pentraxin family. It is an extremely sensitive marker of systemic inflammation. CRP is produced primarily in hepatocytes. Its synthesis is rapidly up-regulated in response to tissue damage, infection, inflammation, and malignancy. Up-regulation is caused by inflammatory cytokines – particularly IL-1 and IL-6 originating at the site of injury. Serum concentration rises almost immediately and

peaks at 48 hours. Elimination half life of CRP is 19 hours (Pepys & Hirschfield, 2003). Baseline levels are very consistent in health. In healthy young adults, median CRP concentration is 0.8mg/L (90th centile 3mg/L, 99th centile 10mg/L) (Shine et al., 1981). CRP rises slightly with age. Despite this, CRP concentration in healthy adults seldom rises above 3mg/L (Ballou et al., 1996; Delongui et al., 2013). As such it is an excellent non-specific marker of systemic inflammation.

3.9.2 Human Neutrophil Elastase (HNE)

The granules of neutrophils are distinguished by their relative concentrations of cytotoxic peptides, and their ability to exocytose (discharge their cell contents out of the cell). Azurophil granules undergo limited exocytosis and are defined by the high concentrations of myeloperoxidase (MPO), bactericidal permeability-increasing protein, defensins, and serine proteases (cathepsin G, neutrophil elastase (HNE), and proteinase 3) that they contain.

The serine proteases have both intracellular and extracellular actions. They are involved in the degradation and elimination of microorganisms. In addition, they contribute to the coordination of the innate immune response, and may have a role in the development of non-infectious chronic inflammatory disease. These effects have recently been reviewed (Pham, 2006).

Neutrophils phagocytose microorganisms and imprison them within a compartment known as the phagolysosome. Following this, the granules of the neutrophil exocytose, releasing their cytotoxic contents including the serine proteases that kill the engulfed invading particles. Serine proteases are also a component of the extracellular fibrous protrusions of activated neutrophils known as neutrophil extracellular traps (NETs) that kill extracellular bacteria (Brinkmann et al., 2004).

Although the activity of serine proteases are controlled in the extracellular environment due to the presence of protease inhibitors, large amounts can inactivate those inhibitors leading to damage to the extracellular matrix (ECM) and proteolysis. In addition, serine proteases can alter the activity of chemokines through proteolytic degradation leading to an alteration in leukocyte recruitment. Serine

proteases can also alter the activation of cytokine precursors such as pro-TNF and pro-IL-1 β , modulating the availability of pro-inflammatory mediators (Pham, 2006).

HNE is an important mediator of inflammation-induced tissue injury. The epithelium is usually protected from damage by proteinase inhibitors such as elafin and secretory leukocyte protease inhibitor (SLPI) produced by epithelial cells and cells of the innate response. However other tissues are less well protected and an imbalance between HNE and inhibitors of serine proteases is implicated in the pathogenesis of ARDS and the organ failure associated with critical illness.

HNE is therefore an important component of the innate immune response, and also contributes to the collateral damage of critical illness. Plasma concentration of HNE may therefore be a useful marker of ongoing neutrophil driven immune system activation and may also contribute to impaired physical recovery. In healthy controls HNE concentration is around 77ng/mL (Z. Wang et al., 2009) and rises to 186 ng/mL in ICU patients with pneumonia (Tagami et al., 2011) and 640ng/mL in patients with ARDS (Z. Wang et al., 2009).

3.9.3 Myeloperoxidase (MPO)

Myeloperoxidase (MPO) is another leukocyte enzyme found in the largest quantities in the azurophil granules of neutrophils but is also found in monocytes and macrophages. It is released from neutrophils during inflammation and allows hydrogen peroxide conversion in to hypochlorous acid (HOCL). MPO is important in the pathophysiology of atherosclerotic plaque formation and instability. Elevated levels have been associated with an increased risk of atherosclerotic disease and cardiovascular events (Stankovifá & Majkifá-Singh, 2011). MPO can be measured by ELISA in the laboratory and has been chosen for use in this study as a second marker of neutrophil-driven inflammation.

3.9.4 Secretory leukocyte protease inhibitor (SLPI)

Secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor originally isolated from the bronchial secretions of patients with COPD. It is

secreted from epithelial surfaces of the respiratory and alimentary tracts and acts to protect them from damaging effects of proteases secreted by inflammatory leukocytes. SLPI is also produced systemically by macrophages and neutrophils, in response to bacterial products and increases the production of anti-inflammatory cytokines. It therefore plays 2 important roles in human inflammation: the localised protection of host tissue from serine proteases, and the induction of inflammatory resolution (Grobmyer et al., 2000a).

3.9.5 Pro-inflammatory interleukins

The pro-inflammatory interleukins – interleukin 1 β (IL-1 β) and interleukin 6 (IL-6) were chosen as biomarkers of innate immune response activation.

IL-1 β is a cytokine of the interleukin-1 family. It is produced by monocytes, macrophages, and dendritic cells in response to various stimuli in the form of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). Membrane associated toll-like receptors (TLRs) or nucleotide binding and oligomerisation domain (NOD)-like receptors (NLRs) recognise these PAMPs and DAMPs and lead to up-regulation and release of IL-1 β . The mechanisms involved in IL-1 β release have been recently reviewed (Eder, 2009).

IL-1 β is extremely important in the pathogenesis of acute inflammation. It initiates the pathways resulting in fever, reduced pain tolerance, erythema, and hypotension – the clinical hallmarks of inflammation. Through its effects on the vasculature, chemokine and cytokine production, and the bone marrow, IL-1 β promotes the infiltration of inflammatory cells into sites of tissue damage or infections (Dinarello, 2009).

Interleukin-6 (IL-6) is another cytokine important in the innate response to pathogens. It is released from numerous cells (macrophages, dendritic cells, mast cells, B cells, T cells, fibroblasts, astrocytes, and epithelial cells). It is produced in response to IL-1 and tumour necrosis factor α (TNF α). It causes activation of B and T cells, induction of fever, and it mediates the acute phase response. It also has anti-inflammatory effects by inhibiting IL-1 and TNF α release, and increases the

circulating concentrations of anti-inflammatory molecules (Schulte, Bernhagen, & Bucala, 2013).

3.9.6 Interleukin-8 (IL-8)

Chemokines are a group of around 50 small, structurally similar proteins that attract cells and control cell migration during inflammation. They are sub-classified according to structural characteristics. The CXC chemokine IL-8 is produced in tissue macrophages and is released in response to mediators of inflammation such as TNF and IL-1. IL-8 concentration is another marker of innate immune response activation.

3.9.7 Complement anaphylatoxin proteins

Complement proteins are involved in the initiation, propagation, regulation, and resolution of inflammation. Complement is the collective name given to a group of enzymes and proteins involved in both the innate and adaptive immune response and act together in the initiation and regulation of inflammation, the lysis of bacteria and viruses, and the clearance of immune complex from the circulation.

Complement responses can be initiated by the 'alternative' or 'manin-binding lectin' pathways (innate immune response) or the 'classical' pathway (adaptive immune response).

Complement is important in the clearance of apoptotic neutrophils during the resolution of inflammation. C1q deficiency has been shown to be a strong risk factor for SLE, a disorder characterized by reduced clearance of apoptotic neutrophils (Rossi book) and there also appears to be a role for 'downstream' complement proteins in this process. It is therefore reasonable to explore the association of serum levels of complement proteins in predicting inflammatory outcome (Doan, Melvold, Viselli, & Wlatenbaugh, 2012).

In this study, C3a, C4a, and C5a were chosen as markers of complement 'activation' to determine whether a dampened complement response during the

recovery phase of critical illness might be associated with a persistent inflammatory response.

3.9.8 Transforming growth factor beta (TGF- β)

Transforming growth factor beta (TGF- β) is an anti-inflammatory cytokine. It is released from macrophages during neutrophil phagocytosis during the resolution of inflammation and plays a key role in this process. TGF- β inhibits the secretion of pro-inflammatory cytokines and chemokines. It has been chosen as a marker of the inflammatory resolution process (M. O. Li, Wan, Sanjabi, Robertson, & Flavell, 2006).

3.10 Considerations for outcome measures used in the study

The outcome measures used in the RECOVER study were a mixture of questionnaires and clinical measurements. To consider the relative strength of these measures, it is useful to consider them in terms of reliability, validity, and responsiveness. These concepts are described in the following paragraphs.

3.10.1 Reliability

Reliability is the degree to which different independent assessors obtain the same result using the same measurement instrument (Streiner & Norman, 2008). As such it is a way of expressing measurement error. Interpretation of the clinical relevance of a measurement error is only possible in the context of the variability within the study population. Therefore reliability is typically expressed using the intra-class correlation coefficient (ICC):

$$Reliability = \frac{Subject\ Variability}{Subject\ Variability + Measurement\ Error}$$

The proportion produced by this calculation represents the proportion of variance in measurements that can be attributed to true differences between the

patients under study. It is important to note that reliability is only applicable to the context in which it was measured. Reliability of a measure in one population does not mean that it is equally reliable in another population.

3.10.2 Validity

Validity is the degree to which a measurement actually represents the quality of interest. A prerequisite to validity is reliability. If the reliability of a measure is only 0.5 (half of the variability between individuals can be attributed to true differences) then the validity cannot exceed that value. In a similar way to reliability, a measurement may only be valid in the population and context in which it was measured. Validity can be assessed by *criterion validation* (assessment of the extent to which a measure agrees with a Gold standard measure), *content validation* (an expert judgment about whether the content of a measure or scale is relevant or representative of the quantity being assessed), and *construct validation* (assessing the performance of a measure or scale with characteristics or behaviours that reflect unseen underlying factors known as constructs) (Streiner & Norman, 2008).

3.10.3 Responsiveness

Responsiveness is 'the ability of an instrument to measure a meaningful or clinically important change in a clinical state'. It has been distinguished from 'sensitivity', which is defined as 'the ability of an instrument to measure change in a state regardless of whether it is relevant or meaningful to the decision maker' (Liang, 2000). This can be considered part of validity – how much a change in a measure reflect a change in the characteristic of interest (Streiner & Norman, 2008).

3.11 Measures of physical outcome used in the study

3.11.1 Rivermead mobility index

The Rivermead Mobility Index (RMI) is the primary outcome of the RECOVER study. It was designed in 1991 by Collen and colleagues in the UK as an adaptation of the Rivermead Motor Assessment Gross Function Scale (Collen, Wade,

Robb, & Bradshaw, 1991). Its purpose was to give rehabilitation professionals a simple clinical test with which they could assess the effects of rehabilitation treatments.

The RMI consists of a 15-point scale consisting of 14 functional questions and 1 direct observation (Appendix D). A low score indicates poor mobility. Studies in stroke patients (Hsieh, Hsueh, & Mao, 2000; Hsueh, Wang, Sheu, & Hsieh, 2003) and mixed neurological patients (J. M. Walsh et al., 2010) have shown good reliability, validity and responsiveness. Reliability is consistently good (ICC 0.93, 0.92, and 0.96). Validity has been assessed against various 'gold standards' of mobility including the 10 minute walk test ($r = 0.86$) (J. M. Walsh et al., 2010), the Barthel Index ($r = 0.88, 0.6$) (Hsieh et al., 2000; Hsueh et al., 2003). It has also been assessed in patients with lower limb amputation in whom it is less useful (Franchignoni, Brunelli, Orlandini, Ferriero, & Trallesi, 2003; Ryall, Eyres, Neumann, Bhakta, & Tennant, 2003).

It is worth noting that the performance of the RMI has not been assessed in survivors of critical illness but all other measures of mobility are similarly lacking in validation studies in ICU survivors. RMI was therefore chosen as a pragmatic measure that has been shown to have very little inter-rater variability. Its simplicity and ease of use has allowed a very high follow-up rate in the RECOVER study and therefore extremely complete outcome data.

3.11.2 Hand grip strength

Maximal handgrip strength (HGS) was measured in the non-dominant hand positioned at the side of the body using a T.K.K. dynamometer 5401 D (Takei Scientific Instruments, CO., LTD. Tokyo, Japan). Measurements were taken 3 times and the maximum score was recorded.

Maximum handgrip strength has good concurrent validity with the 6 minute walk test that is a test of physical function (Enright, 2003; Reuter, Massy-Westropp, & Evans, 2011). HGS declines with age and is lower in females than in males (Massy-Westropp, Gill, Taylor, Bohannon, & Hill, 2011). HGS measurements in the

current study were therefore converted in to percentage predicted scores based on their age and gender for comparisons. Age and gender stratified measurements from a recent Australian study were used (Massy-Westropp et al., 2011). Tables showing age and gender stratified measurements from this study are shown in Appendix E. In addition, to identify particularly weak patients, we created binary categories of 'weak' and 'not weak' using 2 different definitions of 'weak' identified a priori. These were patients > 1 or 2 standard deviations below predicted HGS.

3.11.3 Timed up and go test

The Timed Up and Go test (TUG) (Podsiadlo & Richardson, 1991) is a modification of the 'Get up and Go' test designed by Mathias et al in 1986 (Mathias, Nayak, & Isaacs, 1986). It was intended as a simple method of assessing falls risk in the elderly. Subjects were asked to stand from sitting, walk 3 metres, turn and walk back and sit down again. The original test was a 5-point subjective observational rating. However, many observers felt that this scale lacked precision and the timed version has largely replaced it.

The TUG was initially validated in 60 elderly patients attending a day hospital (mean age 79.5 years). In this population, it was found to be reliable and had a high correlation with the Berg Balance Scale, a validated balance scale, and the Barthel index, a validated measure of mobility and functioning in relation to activities of daily living.

The TUG has subsequently been validated as a predictor of falls and physical function in institutionalized adults (Schoene et al., 2013) and Huntingdon's disease and is responsive to changes in disease severity (Rao, Muratori, Louis, Moskowitz, & Marder, 2009) but has been found to be a poor predictor of falls in patients with MS (Cattaneo, Regola, & Meotti, 2006) and non-institutionalised adults (Schoene et al., 2013).

3.12 Measures of psychological function used in the study

3.12.1 Davidson's Trauma Scale

The Davidson's Trauma Scale (DTS) is a scale that was developed as a simple diagnostic test for post-traumatic stress disorder (J. R. Davidson et al., 1997). The 17 items on the scale correspond to the diagnostic criteria listed in the Diagnostic and Statistical Manual for Mental Disorders 4th Edition (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DMS-IV-TR)*, 2000). Subjects were asked to rate their symptoms in terms of frequency and severity. It is reliable (test-retest reliability = 0.86), and shows very high validity when compared to the Structured Clinical Interview for DMS-III-R (SCID) ($r=0.83$ at a score of >40) (J. R. Davidson et al., 1997). In this study, DTS is analysed as continuous variable, the DTS score, and also in binary diagnostic categories according to a cut-off of 37. The DTS is in Appendix G.

3.12.2 Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) was developed in the early 1980s as a tool to rapidly screen patients for anxiety and depression in the non-psychiatric medical outpatient setting (Zigmond & Snaith, 1983). It is a self-administered questionnaire that consists of 14 questions addressing the domains of depression and anxiety. Each question is marked on a scale of 0-3 giving a maximum possible score of 21 for each of depression and anxiety. The scale can be found in Appendix F.

Since its inception, HADS has been used extensively (Bjelland, Dahl, Haug, & Neckelmann, 2002). The scale has been widely validated in a large number of populations (Annunziata, Muzzatti, & Altoe, 2011; Boyes, D'Este, Carey, Lecathelinais, & Girgis, 2013; Bratas, Gronning, & Forbord, 2013; Castro et al., 2006; G. Cheung, Patrick, Sullivan, Cooray, & Chang, 2012; Dawkins, Cloherly, Gracey, & Evans, 2006; Fatt, Atiya, Heng, & Beng, 2007; Golden, Conroy, & O'Dwyer, 2007; Gough & Hudson, 2009; Helvik, Engedal, Skancke, & Selbaek, 2011; Higashi et al.,

1996; Hinz, Zweynert, Kittel, Igl, & Schwarz, 2009; Honarmand & Feinstein, 2009; Julian, 2011; Karimova & Martin, 2003; Kuijpers et al., 2003; Leentjens et al., 2011; Loosman, Siegert, Korzec, & Honig, 2010; Lowe et al., 2003; McCue, Martin, Buchanan, Rodgers, & Scholey, 2003; Mitchell, Meader, & Symonds, 2010; Mondolo et al., 2006; Norton, Cosco, Doyle, Done, & Sacker, 2013; Poole & Morgan, 2006; Roberts, Bonnici, Mackinnon, & Worcester, 2001; Rodriguez-Blazquez, Frades-Payo, Forjaz, de Pedro-Cuesta, & Martinez-Martin, 2009; Sagen et al., 2009; Samaras et al., 2013; Smarr & Keefer, 2011; Snaith & Zigmond, 1986; Sukantarat, Williamson, & Brett, 2007; Terluin, Brouwers, van Marwijk, Verhaak, & van der Horst, 2009; Untas et al., 2009; White, Leach, Sims, Atkinson, & Cottrell, 1999; Woolrich, Kennedy, & Tasiemski, 2006; Zakrzewska, 2012) and has been used to evaluate the psychological impact of critical illness (Bienvenu et al., 2012; Garrouste-Orgeas et al., 2012; Kayambu, Boots, & Paratz, 2011; Kowalczyk, Nestorowicz, Fijalkowska, & Kwiatosz-Muc, 2013; H. Myhren, Ekeberg, Toien, Karlsson, & Stokland, 2010; Peris et al., 2011; Ringdal, Plos, Lundberg, Johansson, & Bergbom, 2009; Rosendahl, Brunkhorst, Jaenichen, & Strauss, 2013). Despite this, there has only been 1 validation study in this population and this study compared the HADS to a newer questionnaire (Sukantarat et al., 2007) without establishing the validity of the HADS that was being used as the gold standard.

A systematic review by Bjelland et al (Bjelland et al., 2002) found that optimal cut offs for both the depression (HADS-D) and anxiety (HADS-A) components to be 8/21 (giving sensitivity and specificity for both HADS-D and HADS-A of 0.8).

3.13 Measures of quality of life used in the study

3.13.1 SF-12v2

The Medical Outcomes Study Short Form 12 version 2 (SF-12v2) is a short, generic health status and health outcomes questionnaire containing 12 questions that can be administered in less than 2 minutes. It is a simplification of the Medical Outcome Study Short Form 36 (SF-36) which contains 36 items (Ware, Kosinski, &

Keller, 1996). The SF-12v2 yields physical and mental component summary scores (PCS and MCS respectively) as well as scores for a range of integrated health status scales (Physical Functioning, Role Physical, Bodily Pain, General Health, Vitality, Social Functioning, Role Emotional, and Mental Health). Reliability of the summary scores is very good (0.89 and 0.86 for PCS and MCS respectively). Raw scores are calculated according to an algorithm derived from the Medical Outcomes Study and then converted into norm based scores (Ware et al., 1996). SF-12v2 has been validated across a vast range of population groups (Ware, Kosinski, Turner-Bowker, & Gandek, 2008).

3.14 Assays

A summary of the assays used for each biomarker is given in Table 3-3. All assays were conducted according to manufacturers instructions.

Molecule	Assay type	Kit	Company	Sample	Optimal Dilution	
					Baseline	3 mo.
CRP	ELISA	Duaset	R and D	Serum	1:100000	1:10,000
IL-1 β	ELISA	Duaset	R and D	Serum	1:1	1:1
IL-6	ELISA	Duaset	R and D	Serum	1:1	1:1
IL-8	ELISA	Duaset	R and D	Serum	1:1	1:1
C3A	CBA	Anaphylatoxin	BD bioscience	Serum	1:1	1:1
C4A	CBA	Anaphylatoxin	BD bioscience	Serum	1:1	1:1
C5A	CBA	Anaphylatoxin	BD bioscience	Serum	1:1	1:1
MPO	ELISA	Duaset	R and D	Serum	1:100	1:100
HNE	ELISA	ELISA	Cambridge	Plasma	1:50	1:50
TGF β	ELISA	Duaset	R and D	Serum	1:20	1:20
SLPI	ELISA	Quantakine	R and D	Serum	1:100	1:100

Table 3-3 Characteristics of the assays used in the study

3.14.1 Sample types

Assays of CRP, interleukins, TFG beta, SLPI, MPO, and complement were carried out on defrosted serum. HNE was assayed on defrosted plasma.

3.14.2 Dilutions

IL-1 β , IL-6, IL-8, and complement assays were carried out undiluted because concentrations of these molecules in serum were low. All the other assays (all ELISAs) required dilution. A test analysis was carried out with each assay to establish the most appropriate dilution (Table 3-3). In some cases, optimal dilution depended upon the time point being analysed. Following this, each sample was assayed at the optimal dilution. If the optical density fell above the highest or below the lowest point on the standard curve, that sample was re-assayed at a higher or lower dilution.

3.14.3 Quality

All ELISAs were carried out in duplicate. The co-efficients of variation (CV) for optical density in each duplicate assay were assessed and assays were repeated where necessary to ensure CV <15%.

3.15 Statistical analysis and reporting

A signed, dated a priori analysis plan for this section was included in the request for RECOVER trial data. To further explore some of the signals seen on primary analysis, a number of post-hoc analyses are appended once the primary analysis had been carried out.

3.15.1 Prevalence of systemic inflammation in ICU survivors

In health, CRP concentration rarely rises above 3mg/L even in elderly cohorts. In a young adult cohort, a CRP concentration of greater than 10mg/L represents the 99th centile, and 3mg/L the 90th centile. Persistent inflammation was therefore defined according to these 2 definitions. The following time points of interest are arbitrarily defined:

- ICU discharge (study entry)
- Closest sample to hospital discharge (variable time after ICU discharge)
- Community sample (3 month follow up visit)

The number and proportion of surviving patients with evidence of systemic inflammation for the 3mg/L and 10mg/L threshold at each of these time points (CRP >3mg/L or >10mg/L) was reported.

3.15.2 Characterisation of the inflammatory and inflammatory resolution profile during recovery from critical illness

The blood markers of systemic inflammation and inflammatory resolution (CRP, IL-1 β , IL6, IL8, MPO, HNE, TGF beta and SLPI), were reported at 2 time points – ICU discharge and 3 months after ICU discharge. Serum concentrations of all biomarkers are non-parametric in distribution and were presented as median (IQR). Serum CRP concentration, IL-6, IL-1 β , IL-8, and TGF β were also reported at an additional time point (hospital discharge).

In an attempt to characterise the inflammatory resolution trajectory, a subgroup of ICU survivors who remained in hospital for similar lengths of time after ICU discharge was selected. Patients that remained in hospital for 1, 2, and 3 weeks after ICU discharge were compared. In these patients, median concentration of CRP, IL-6, IL-8, and TGF β were plotted against time. Serum concentration of IL-1 β was negligible at all time points. Therefore a line chart was not presented for this cytokine.

Biomarkers with concentrations falling below the level of detection of the assay were assigned a value of zero.

3.15.3 Physical and psychological outcomes of ICU survivors

Physical and psychological outcome measures were presented for the whole cohort at both 3 months and 6 months.

Mobility: RMI was reported as median (IQR).

Hand strength: Using age and gender stratified population norms, handgrip strength (HGS) measurements were converted into percentage predicted HGS and reported as mean (SD). In addition, 3 outcome groups were identified to identify patients with particularly poor physical recovery. Patients with a HGS that

decreases more than 2 standard deviations below mean predicted HGS for age and gender were considered 'very weak'. Patients with HGS between 1 and 2 standard deviations below the predicted HGS were considered 'weak'. All other patients were considered 'normal'. The number and proportions of patients falling into these categories are reported.

Quality of Life: Total, physical component and mental component scores of the SF-12v2 were presented as median (IQR).

Anxiety and Depression: Total Hospital Anxiety and Depression Scale (HADS) were reported as median (IQR). Patients scoring more than 8 points on the anxiety component were considered to have a positive screening test for anxiety. The same threshold is used for the depression component. The number and percentage of patients with a positive screening test for anxiety or depression was reported.

Post-traumatic stress disorder: Davidson's trauma scale (DTS) was reported as median (IQR). PTSD is defined as $DTS > 37$. Number and percentage of patients with PTSD was reported.

Falls risk: Number of seconds take to complete the timed up and go (TUG) test was reported as median (IQR).

3.15.4 Association of systemic inflammation with indices of physical and psychological recovery

A cross-sectional analysis of CRP concentration and indices of physical recovery at 3 months was performed. Scatter plots were drawn representing the relationships between CRP concentration and the outcome measures detailed in section 5. Pearson's correlation coefficients were reported with p values. Kendall's tau-b correlation coefficients were calculated for non-parametric correlation.

In addition, to explore the association of systemic inflammation and diagnostic subgroups (depression, anxiety, and PTSD) and patients with particularly poor physical recovery (lower quartile of RMI, weak HGS category),

CRP concentration are reported in binary groups and presented as boxplots. Comparisons are made with Mann Whitney U tests.

3.15.5 Post-hoc analyses

To explore the possibility that pre-morbid inflammation may contribute to the observation of persistent inflammation during the recovery phase of critical illness, comparisons were made between subgroups of patients who had pre-morbid conditions that could be considered 'inflammatory' diseases and those that did not. Two such analyses were carried out. The first compared those with a Functional Comorbidity diagnosis of 'arthritis' to those without. The second included other possible 'inflammatory' diagnoses (asthma, COPD, obesity, diabetes, and arthritis) compared to those without. Both median CRP concentration, and proportion of patients with inflammation were compared across subgroups.

3.15.6 Issues of study power and multiple testing

It is important to emphasise that the biomarker study reported here was an add-on to an RCT and was not powered to detect differences in inflammatory biomarkers between groups of patients. Positive results are regarded as hypothesis generating. Similarly, negative results may indicate lack of statistic power rather than a lack of affect. It is also acknowledged that multiple statistical tests were carried out to compare levels of biomarkers between groups and to take this into account, results are reported and discussed in relation to Bonferroni adjusted p value thresholds.

3.16 Results

3.16.1 Recruitment and loss to follow up

Figure 3-1 illustrates the recruitment of patients into the study and describes the small number of patients that dropped out during the study. A number of patients followed up at 3 months were lost to follow up at 6 months. Reasons for loss of follow up at 6 months are given in Table 3-4.

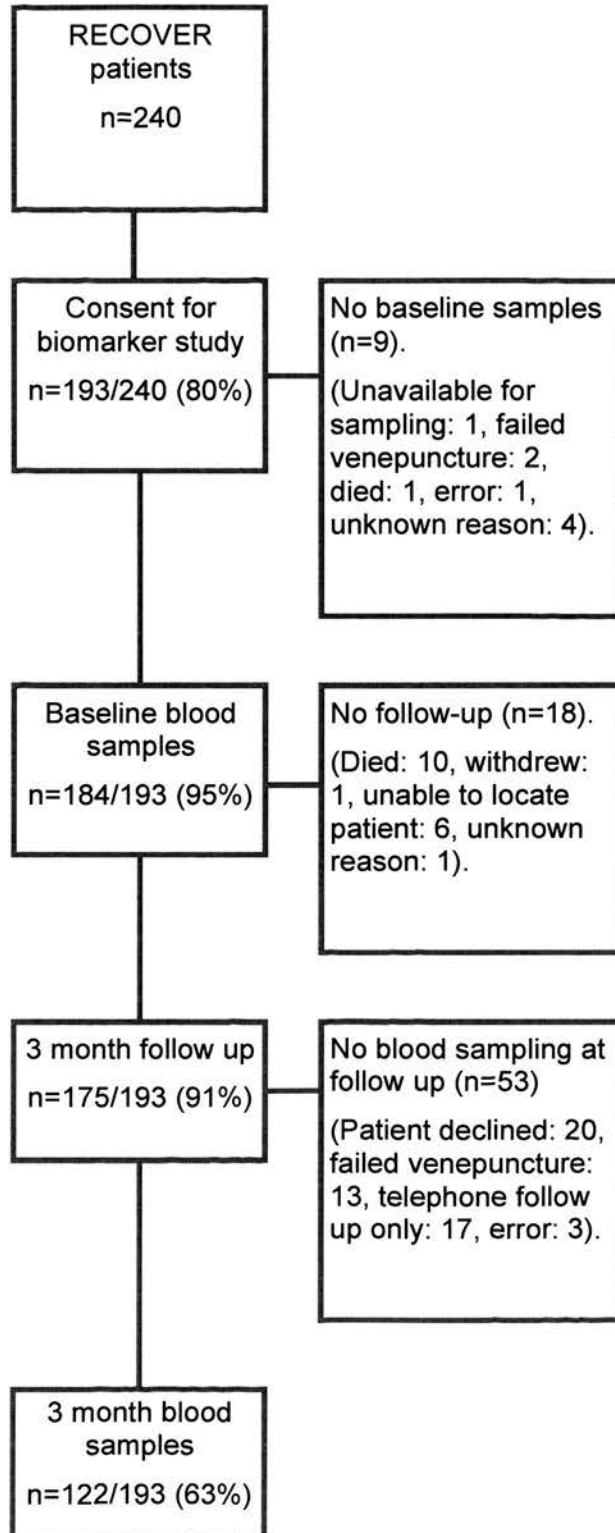


Figure 3-1 RECOVER biomarker study flow diagram up to 3 months

Unable to contact	7
Death	6
Declined	1
Not returned questionnaire / not able to contact	11
Unknown	11

Table 3-4 Reasons for loss of follow up at 6 months

Two hundred and forty patients were recruited to the RECOVER study. Of these, 193 participated in the inflammation sub-study. Of the 193 patients in the study, 175 patients were followed up to 3 months, and 157 patients were followed up to the 6 months. This represents 91% of those surviving to 3 months, and 82% of those surviving to 6 months. A comparison between those patients available for follow up at 3 months and those in whom follow up could not be arranged is shown in Table 3-5.

	Follow up blood taken (n=122)	No follow up blood taken (n=71)
Age	60 (13)	62 (15)
Gender (m/f (%m))	75/47 (62)	42/29 (59)
Functional comorbidity index	2.8 (2.0)	2.5 (1.9)
Functional comorbidity index subcategories (n (%))		
Arthritis	33 (27)	21 (30)
Osteoporosis	7 (6)	4 (6)
Asthma	23 (19)	10 (14)
COPD	24 (20)	8 (11)
Angina	12 (10)	5 (7)
Heart Disease	11 (9)	10 (14)
Heart Attack	14 (12)	4 (6)
Neurological	0 (0)	4 (6)
Stroke	15 (12)	7 (10)
PVD	1 (1)	5 (7)
Diabetes	22 (18)	8 (11)
Upper Gastro	39 (32)	23 (32)
Depression	36 (30)	19 (27)
Anxiety	30 (25)	16 (23)
Visual	18 (15)	7 (10)
Hearing	15 (12)	5 (7)
Disc Disease	12 (10)	5 (7)

Obesity	28 (23)	15 (21)
APACHE 2	21 (7)	21 (7)
SOFA score at baseline		
Total	3 (1,4)	3 (1,5)
Cardiovascular	0 (0,0)	0 (0,0)
Respiratory	2 (0,2)	2 (0,2)
Coagulation	0 (0,0)	0 (0,1)
Renal	0 (0,1)	0 (0,1)
Liver	0 (0,0)	0 (0,0)
Neurological	0 (0,1)	0 (0,1)
ICU stay (days)	11 (4,19)	11 (3,18)
Ventilation (days)	9 (3,16)	6 (3,13)
Vasopressors (n(%))	50 (70)	91 (75)
RRT (n(%))	31 (26)	21 (30)
Delirium (n(%))	19 (16)	10 (14)
RMI	3 (0,6)	2 (0,7)
HGS (kgs)	16 (6,22)	13 (6,20)

Table 3-5 Comparison of those patients followed up and those lost to follow up expressed as mean (standard deviation) or median (25th centile, 75th centile).

3.16.2 Baseline descriptive data

Table 3-6 illustrates the characteristics of the patients enrolled in the study. To explore the possibility of selection bias, comparisons were made between patients enrolled in the RECOVER study who agreed to take part in the blood study, and those that refused consent.

The mean age of the participants was 61 and a higher proportion were male than female. Women who were enrolled in RECOVER were less likely to consent to the blood sampling study than males. Mean APACHE 2 score was 21 and organ failure (defined by the sequential organ failure score (SOFA score)) was rare at the point of enrolment. Patients stayed a median of 11 days in the intensive care unit and were received mechanical ventilation for 8 days. A high proportion (73% received vasopressors) and just over a quarter (27%) received renal replacement therapy. Patients had poor mobility (median RMI of 3), and were weak (HGS of 13 kg).

Other than the gender differences already mentioned, the patients in the biomarker were a good representation of the RECOVER patients as whole.

	Biomarker study (n=193)	Refused consent (n=47)
Age	61 (14)	58 (15)
Gender (m/f (%m))	117/76 (61)	20/27 (43)
Functional comorbidity index	2.7 (2.0)	2.9 (2.0)
Functional comorbidity index subcategories (n (%))		
Arthritis	54 (28)	8 (17)
Osteoporosis	11 (6)	3 (6)
Asthma	33 (17)	10 (21)
COPD	32 (17)	5 (11)
Angina	17 (9)	4 (9)
Heart Disease	21 (11)	2 (4)
Heart Attack	18 (9)	1 (2)
Neurological	4 (2)	2 (4)
Stroke	22 (11)	3 (6)
PVD	6 (3)	1 (2)
Diabetes	30 (16)	7 (15)
Upper Gastro	62 (32)	14 (30)
Depression	55 (29)	22 (47)
Anxiety	46 (24)	16 (34)
Visual	25 (13)	9 (19)
Hearing	20 (10)	4 (9)
Disc Disease	17 (9)	13 (28)
Obesity	43 (22)	10 (21)
APACHE 2	21 (7)	19 (9)
SOFA score at baseline		
Total	3 (1,4)	2 (1,4)
Cardiovascular	0 (0,0)	0 (0,0)
Respiratory	2 (1,2)	2 (1,2)
Coagulation	0 (0,0)	0 (0,0)
Renal	0 (0,0)	0 (0,0)
Liver	0 (0,0)	0 (0,1)
Neurological	0 (0,1)	0(0,1)
ICU length of stay (days)	11 (4,18)	12(3,22)
Hospital length of stay (days)	29 (11,43)	35 (10,56)
Days of mechanical ventilation	8 (3,14)	9 (3,17)
Received vasopressors (n(%))	141 (73)	37 (78)
Received renal replacement (n(%))	52 (27)	12 (25)
Delirium at enrolment (n(%))	29 (15)	6 (13)

RMI at enrolment	3 (0,6)	3 (0,7)
HGS (kgs)	15(6,21)	12 (0,26)

Table 3-6 – Baseline characteristics of patients in biomarker study with comparison shown between those that refused consent for the study expressed as mean (standard deviation) or median (25th centile, 75th centile).

3.16.3 Prevalence of systemic inflammation

Prevalence of systemic inflammation was measured at 3 points during the period of recovery from critical illness: at the point of study recruitment when patients are ready for discharge from critical care, at the point of hospital discharge, and 3 months after ICU discharge.

3.16.3.1 Inflammation at ICU discharge

Median CRP concentration at the point of ICU discharge was 26.7 mg/L (IQR 11.0-60.7). 141/184 (77%) patients had a CRP greater than 10mg/L. 173/184 (94%) had a CRP greater than 3mg/L.

3.16.3.2 Inflammation at hospital discharge

Median CRP concentration at hospital discharge was 20.5mg/L (IQR 8.4-41.7). 133/188 (71%) of patients had a CRP greater than 10mg/L. 169/188 (90%) of patients had a CRP greater than 3mg/L.

3.16.3.3 Inflammation 3 months after ICU discharge

Median CRP concentration at the 3-month follow up was 4.4 mg/L (IQR 1.1-11.9). 34/123 (30%) of patients had a CRP greater than 10mg/L. 72/123 (59%) of patients had a CRP greater than 3mg/L.

3.16.4 Characterisation of the inflammatory resolution profile during recovery from critical illness

Median and interquartile ranges for the concentrations of CRP, IL-1 β , IL-6, IL-8, TGF β , MPO, and SLPI in serum and HNE in plasma at ICU discharge and 3 months after ICU discharge are shown in Table 3-7. Median CRP concentration at ICU discharge was 27 mg/L, decreasing to 20mg/L at hospital discharge, and 3 mg/L at 3 months. When compared to the normal values noted in section 2.5.6.1, the median values at ICU discharge and hospital discharge were above the 97th centile of normal healthy adults. The values at the 3-month follow up stage were at the 90th

centile of normal healthy adults. Therefore, whilst CRP concentration decreases drastically during the first 3 months, there remains evidence of significant on-going inflammation during the months of recovery after ICU discharge.

The pro-inflammatory cytokine IL-1 β was detectable in only a small number of samples at ICU discharge and hospital discharge. IL-6 was detectable. Median concentration was 27 pg/mL at ICU discharge, decreasing to 16 mg/L by hospital discharge, and 5 pg/mL at the 3 month follow up visit. These levels are higher than the upper quartile level of 3.5 pg/L measured in a healthy adult population in a previous study (see section 0).

The chemokine IL-8 was also detectable at all time points. At ICU discharge, the median concentration was 28 pg/mL decreasing to 11 pg/mL at hospital discharge, and 8 pg/mL at the 3 month follow up point. Again, this represents a higher concentration of IL-8 in the serum of ICU survivors than that seen previously in a healthy population sample.

Low concentrations of TGF β were found at all time points. Median concentration was 12ng/mL at ICU discharge decreasing to 8 ng/mL at hospital discharge, then rising to 10ng/mL at the 3 month follow up point. When compared to studies of healthy individuals (median concentration 35ng/mL) by previous investigators, these levels were extremely low.

HNE concentrations fell from 159ng/mL at ICU discharge to 106 ng/mL at the 3 month follow up visit. This is elevated in comparison to the 77ng/mL previously measured in healthy controls. Similarly, MPO concentration fell from a median of 191ng/ml at ICU discharge to 127ng/mL 3 months later. This is far in excess of the 951pg/mL (1ng/mL) measured in healthy controls in other studies indicating significant ongoing neutrophil driven inflammation.

Median SLPI was 59ng/mL at ICU discharge, decreasing to 47ng/mL 3 months later. In a previous study, the mean (SD) plasma concentration of SLPI in healthy elderly patients was 38 (10) ng/mL. In patients surgical patients with sepsis, SLPI rises above 100ng/mL, with serum concentration correlating with severity of illness. In healthy patients with COPD, mean concentration was 51 (6)

ng/mL (Grobmyer et al., 2000b; Hollander et al., 2007). The values observed at ICU discharge in the current study are unlikely to be elevated to a physiologically significant degree compared to the background level seen in the community. By 3 months, levels are almost normal.

Marker / Time point		Median	IQR	n
CRP (mg/L)	ICU discharge	27	11 – 60	184
	Hospital discharge	20	8 – 42	185
	3 months	4	1 – 11	123
IL-1 β (pg/mL)	ICU discharge	0	0 – 15	180
	3 months	0	0 – 15	120
IL-6 (pg/mL)	ICU discharge	27	7 – 80	180
	Hospital discharge	16	5 – 65	183
	3 months	5	0 – 31	120
IL-8 (pg/mL)	ICU discharge	28	13 – 64	180
	Hospital discharge	11	73 – 183	183
	3 months	8	1 – 28	119
TGF β 1 (ng/mL)	ICU discharge	12	8 – 16	181
	Hospital discharge	6	4 – 11	182
	3 months	10	8 – 13	120
HNE (ng/mL)	ICU discharge	159	116 – 228	180
	3 months	106	88 – 138	120
MPO (ng/mL)	ICU discharge	191	115 – 292	184
	3 months	127	79 – 213	120
SLPI (ng/mL)	ICU discharge	59	45 – 76	184
	3 months	47	37 – 60	123

Table 3-7 Biomarker concentrations at ICU discharge, hospital discharge, and 3 months after ICU discharge.

Figure 3-2 shows the in-hospital longitudinal trajectory of the acute phase protein CRP for patients staying for 1, 2 and 3 weeks after ICU discharge. The sampling points are displayed relative to hospital discharge rather than ICU discharge. Time point '0' represents hospital discharge; time point '-1' is 1 week prior to hospital discharge with '-2' and '-3' being 2 weeks and 3 weeks prior to hospital discharge respectively. In the subgroups presented, CRP resolves linearly

with time, but does not fall below 20mg/L prior to hospital discharge. This is supported by the median CRP concentration of 20.5mg/L quoted above for the whole sample. Interestingly, CRP concentrations are more similar when time points relative to hospital discharge rather than ICU discharge are studied. For example patients CRP concentration at ICU discharge in patients that stay in hospital for a further 2 weeks, is lower than CRP concentration at ICU discharge in patients staying for 3 weeks. One week after ICU discharge patients who stay for 3 weeks have CRP concentrations similar to patients that only stay 2 weeks.

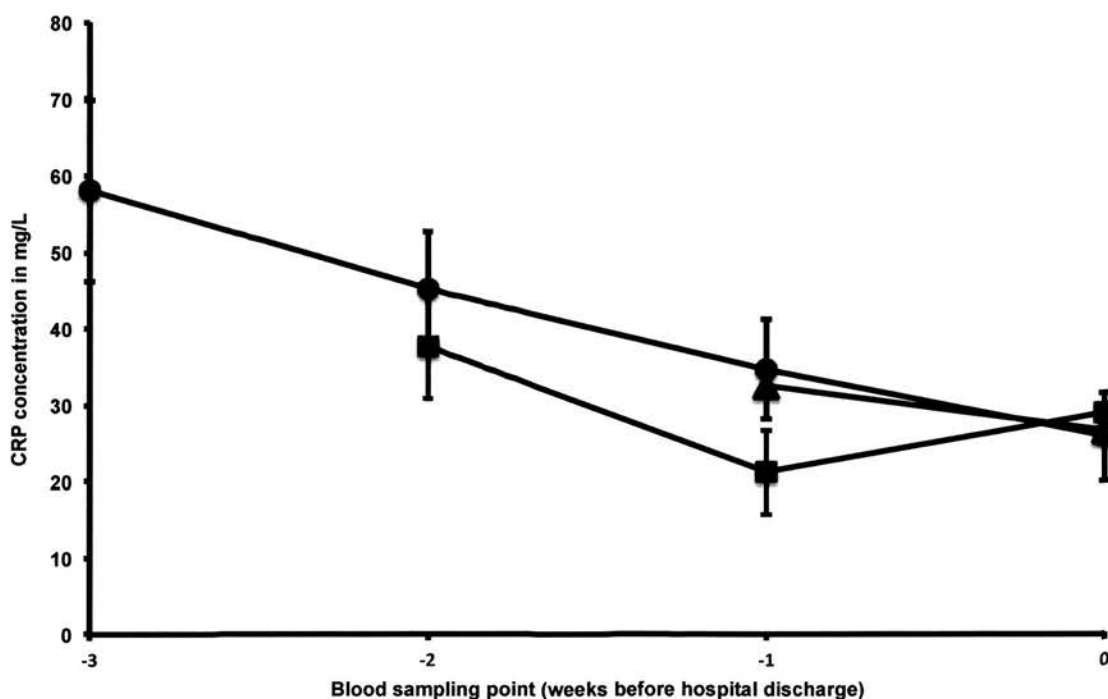


Figure 3-2 Median (IQR) CRP Concentration after ICU discharge in patients staying 3 weeks (n=17; closed circles), 2 weeks (n=27; squares) and 1 week (n=50; triangles) after ICU discharge.

Figure 3-3 shows a similar chart for IL-6 concentration over time. As with CRP concentration the chart is presented with the sampling time points on the x axis plotted relative to hospital discharge (weeks before hospital discharge), rather than ICU discharge (weeks after ICU discharge). In the subgroups presented, IL-6 concentration decreases exponentially as patients approach hospital discharge. Median values of IL-6 concentration are elevated in patients staying 3 weeks in hospital after ICU discharge when compared to those staying 1 or 2 weeks beyond

ICU discharge. By one week prior to hospital discharge, median IL-6 concentration decreases to around 20pg/mL where it remains until hospital discharge. When compared to other studies of healthy community dwelling adults, this value represents a significant elevation in IL-6 at the point of hospital discharge (see upper quartile of 3.5 pg/L noted in section 2.5.4).

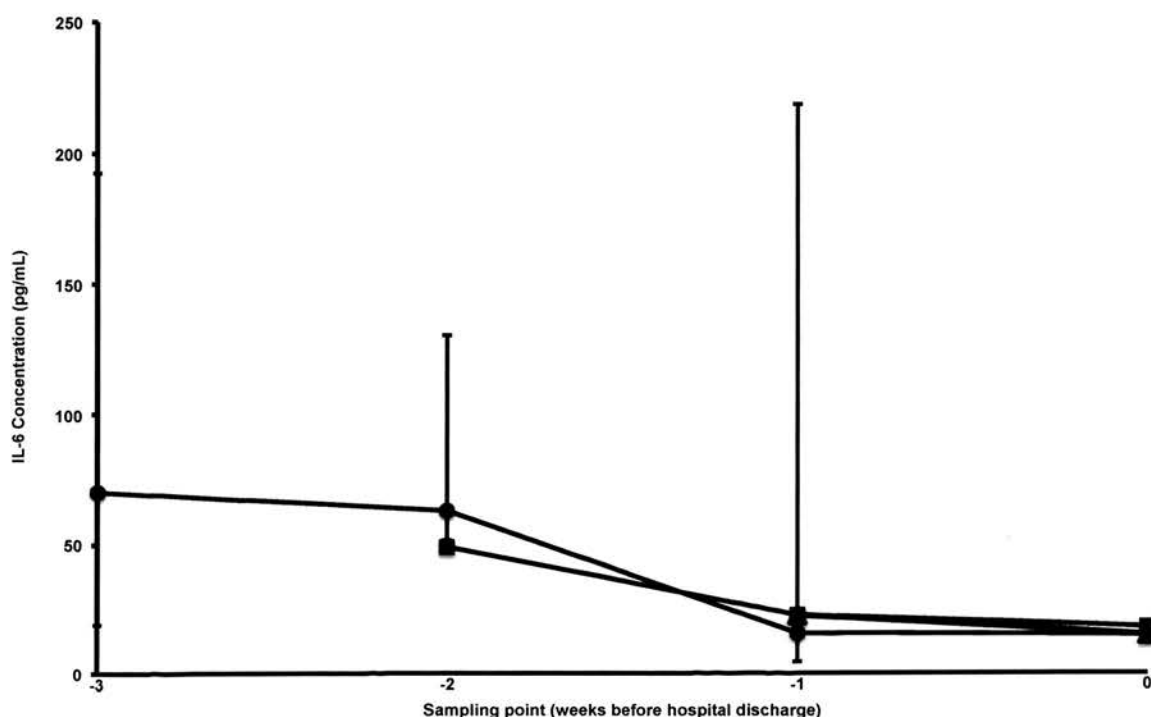


Figure 3-3 Median (IQR) IL-6 concentration in patients staying in hospital 3 weeks (closed circles; n=15), 2 weeks (squares; n=26), 1 week (triangles; n=40) after ICU discharge.

The time course chart for IL-8 concentration after ICU discharge (Figure 3-4) is again presented with sampling time point relative to hospital discharge. Concentration of IL-8 does not follow a clear pattern of resolution. IL-8 concentration fluctuates during the period of hospitalisation after ICU discharge around a level of 40pg/mL. The 18 patients staying for 3 weeks after ICU discharge (closed circles) have a median IL-8 concentration that peaks 1 week after ICU discharge and decreases again towards hospital discharge. At all time points, IL-8 is significantly elevated when compared to the levels found in healthy community

dwelling adults in whom the median concentration is about 3.5pg/mL (Boekholdt et al., 2004).

The TGF β time course chart is presented differently to the other 3 biomarkers studied (Figure 3-5). Sampling time points are presented relative to ICU discharge because TGF β concentration decreases exponentially after ICU discharge regardless of hospital length of stay. This is in direct contrast with IL-6 and CRP. TGF β concentration decreases to around 4ng/mL by about 1 week after ICU discharge where it remains until hospital discharge. These concentrations are far lower than the 35ng/mL median seen in other studies of healthy individuals living in the community (Lin et al., 2009).

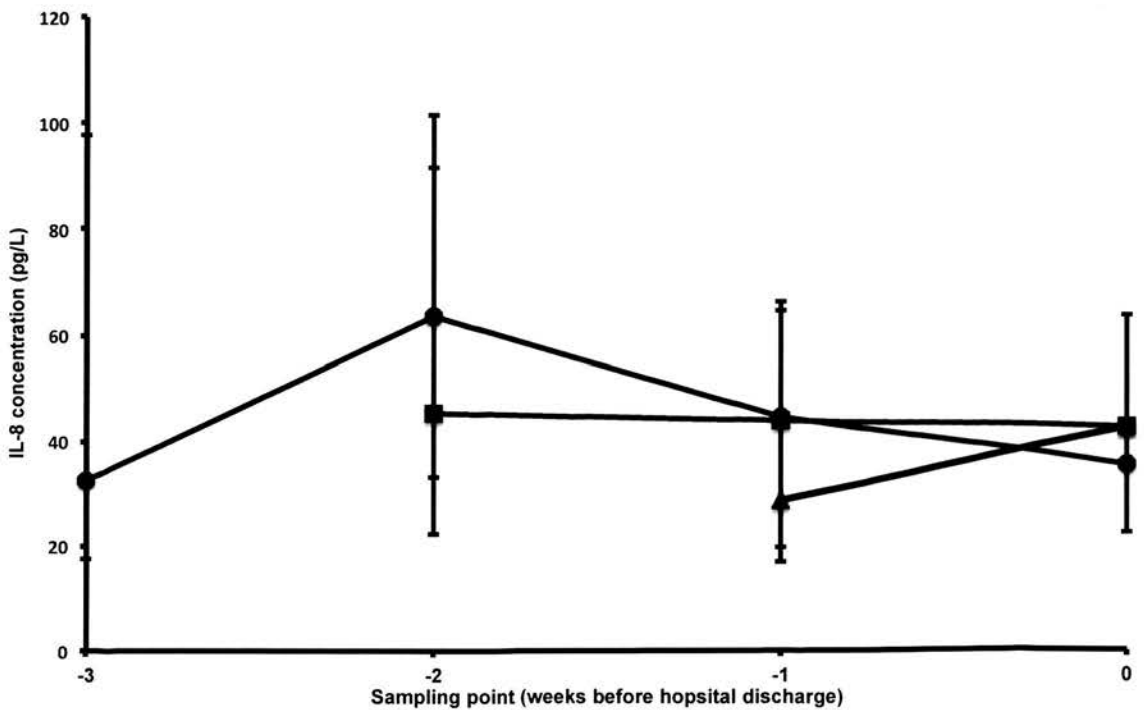


Figure 3-4 Median (IQR) IL-8 concentration in patients staying 3 weeks (closed circles; n=18), 2 weeks (squares; n=26), and 1 week (triangles; n=50) after ICU discharge.

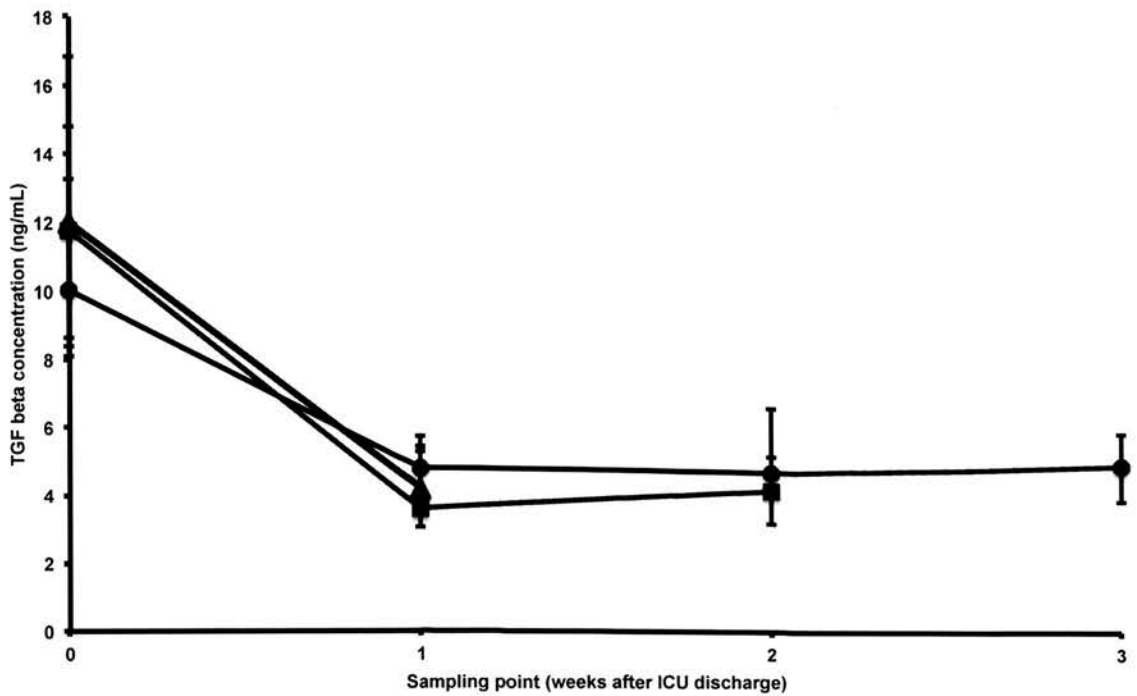


Figure 3-5 Median (IQR) TGF beta concentration in patients staying 3 weeks (n=17; closed circles), 2 weeks (n=27; squares), and 1 week (n=50; triangles) after ICU discharge.

3.16.5 Physical and psychological outcomes of ICU survivors

A summary of the main outcome measures is provided in Table 3-8.

3.16.5.1 Mobility

Patients are immobile at ICU discharge with a median RMI of 3 (IQR 0-6). Over the first 3 months, this rises to 13 (IQR 11-15) and by 6 months it is 14 (IQR 11-15). Due to the nature of the RMI, the frequency distribution is positively skewed at baseline and negatively skewed at the follow up points. The majority of patients at the follow point have very high scores on the RMI. This disguises the very significant impairments in physical function known to affect survivors of critical illness.

3.16.5.2 Hand Strength

Mean (SD) HGS at 3 months is 22 (8). This represents a mean percentage predicted HGS of 64%. The percentage of patients who would be considered weak when compared to a population of age and gender matched controls is 53%.

3.16.5.3 Quality of life

According to the SF-12 v2 questionnaire, both physical and mental components of quality of life are significantly lower than that seen in the US reference population used to calibrate the scale at both 3 months and 6 months. At 3 months, mean physical component score was 35, 1.5 standard deviations below the reference population mean. Mean mental component score was 45, 0.5 standard deviations lower than the reference population. At 6 months, SF-12 scores were very similar with a PCS of 37 and an MCS of 44.

3.16.5.4 Anxiety and depression

Mean (SD) HADS anxiety and depression scores at 3 months were 7 and 6 respectively. Thirty-three per cent had a HADS anxiety score of greater than 8 and met screening criteria for anxiety. Twenty-eight per cent had a HADS depression score of greater than 8 and met screening criteria for depression.

Mean (SD) HADS anxiety and depression scores at 6 months were also 7 and 6 respectively. At this later time point, 36% met screening criteria for anxiety and 31% met screening criteria for depression.

This represents a high proportion of patients suffering from anxiety and depression that does not resolve over the 6-month time horizon.

3.16.5.5 Post-traumatic stress disorder

At 3 months, the median Davidson's Trauma Scale value was 11 (IQR 0-25). Using the cut off of 37, 24 patients (18%) would be considered to have PTSD. At 6 months, median DTS was 27 (IQR 6-56). At this time point, 48 patients (39%) would be considered to have PTSD.

3.16.5.6 Falls risk

At 3 months, the median time to carry out the 10 metre timed up and go test was 10 seconds (IQR 8-13 seconds).

Outcome measure	Baseline (n=193)	3 months (n=175)	6 months (n=137)
RMI	3 (0,6)	13 (11,14)	14 (11,15)
HGS	-	22 (8)	-
% Predicted HGS	-	64 (21)	-
% 'Weak'	-	53 (34.6)	-
SF-12 PCS	-	35 (11)	37 (12)
SF-12 MCS	-	45 (13)	44 (13)
HADS A	-	7 (5)	7 (5)
HADS D	-	6 (4)	6 (4)
%HADSA>8	-	50 (33)	48 (36)
%HADSD>8	-	43 (28)	41 (31)
DTS	-	11 (0,25)	27(6,56)
%PTSD (DTS>37)	-	24(18)	48 (39)
TUG (seconds)	-	10 (8,13)	-

Table 3-8 Physical and Psychological outcomes of critical illness ('-' means that the outcome was not measured at a particular time point) Mean (SD) or median (1st, 3rd quartile).

3.16.6 Association of systemic inflammation and outcome measures at 3 months

A summary of correlation coefficients and inference testing can be found in Tables 3-9 and 3-10. To take into account the 8 outcome measured assessed, a Bonferoni-adjusted p value threshold of $p < 0.006$ was used to infer statistical significance.

3.16.6.1 CRP concentration and mobility

CRP concentration and RMI exhibit a statistically significant negative correlation when measured together at the 3-month time point (Kendall's Tau correlation coefficient = -0.2; $p < 0.001$). Both RMI and CRP concentration were severely skewed in their distribution – hence the use of Kendall's Tau. The relationship between the 2 variables is therefore illustrated using a log

transformation of the inverted RMI (to normalise the extreme negative skew in this variable), and presenting CRP on a logarithmic scale (Figure 3-6)

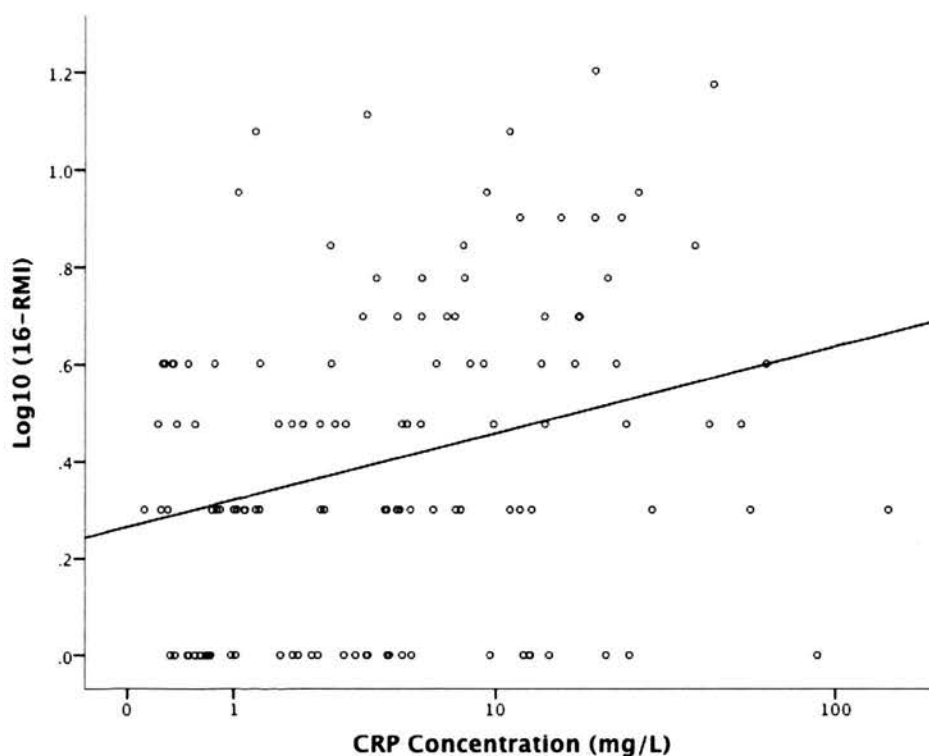


Figure 3-6 Scatter plot illustrating relationship of RMI and CRP concentration at 3 months

Higher levels of the inflammatory marker are associated with poorer mobility as measured by RMI. Median CRP concentration in patients with poor mobility outcomes (lower quartile of RMI) was 9.4 mg/L (IQR 4.8-20) compared to 3.0 (0.8-9.3) in those with good outcomes (upper 3 quartiles of RMI). This represented a significantly higher CRP concentration in those with poor outcomes ($P=0.002$). A box plot illustrating CRP concentration in these 2 groups is shown in Figure 3-7.

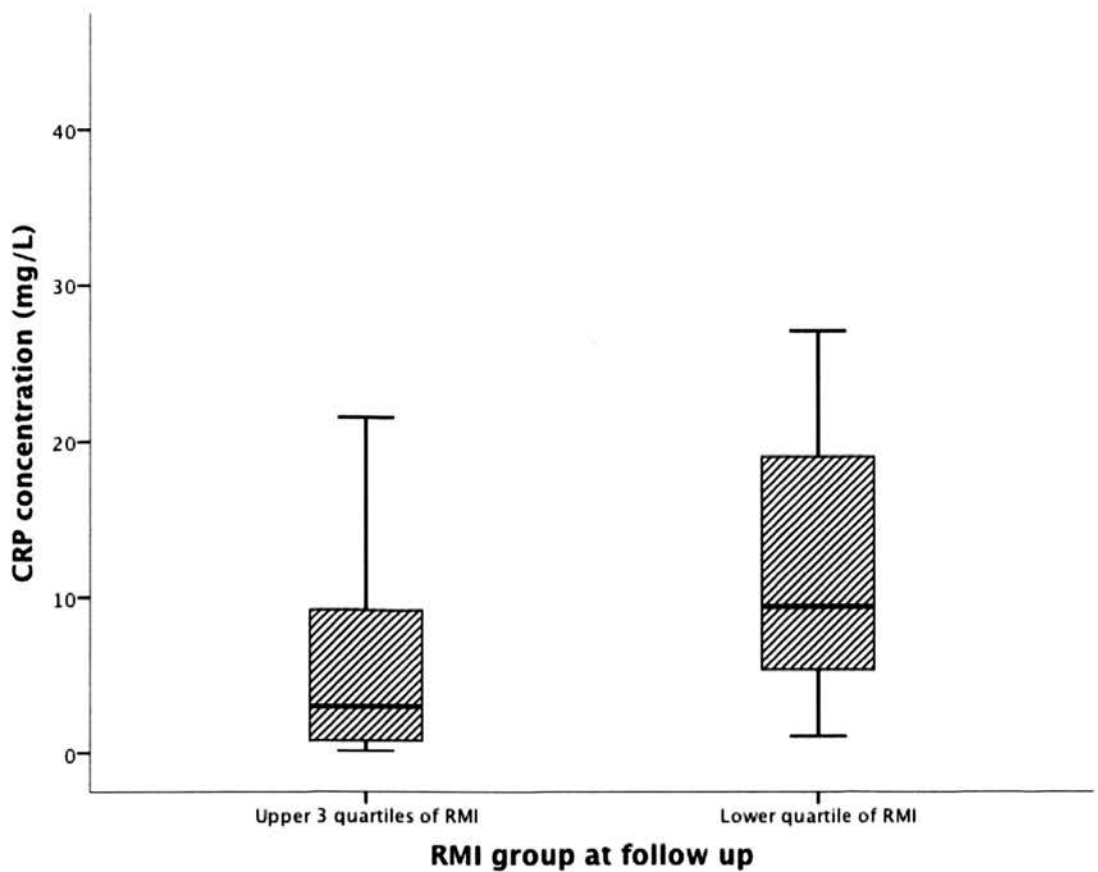


Figure 3-7 CRP concentration in patients in the upper 3 quartiles versus the lowest quartile of RMI at follow up. ($p=0.002$). Lower quartile $n=19$; Upper quartile $n=102$.

3.16.6.2 CRP concentration and hand strength

CRP concentration and percentage predicted HGS are also negatively correlated at the 3-month time point (Spearman's rho -0.19 ; $p=0.03$) but did not quite reach statistical significance according the Bonferoni adjusted threshold. Weak patients (HGS >2 SD lower than predicted) also had higher CRP concentration than those with higher HGS (6.3 versus 3.1mg/L) but this difference did not reach statistical significance ($p=0.10$).

3.16.6.3 CRP concentration and falls risk

The Kendall's Tau correlation coefficient for the relationship between CRP concentration and the number of seconds taken to perform the TUG was 0.11

(p=0.10). Patients with higher levels of CRP trended towards a longer time taken doing the TUG test but this did not reach statistical significance.

3.16.6.4 CRP concentration and psychiatric symptoms

Neither HADS anxiety nor HADS depression scores were correlated with CRP concentration at 3 months (Spearman’s rho correlations coefficients of 0.04 (p = 0.70) and 0.03 (p=0.73) respectively). Patients considered to be depressed (HADS-D >8) had a CRP concentration that was comparable with those who weren’t depressed (3.6 versus 4.4 mg/L p=0.68 respectively). Patients considered to be anxious (HADS-A >8) had a similar CRP concentration to those that weren’t anxious (4.1 versus 4.4mg/L p=1.0 respectively).

3.16.6.5 CRP concentration and post-traumatic stress disorder

DTS was not significantly correlated with CRP concentration at 3 months (Kendall’s Tau B correlation coefficient -0.07 (p=0.49)). Interestingly, patients with PTSD symptoms had lower CRP concentration than those without (1.3 versus 5.0 mg/L) a difference that met statistical significance for a single test but does not account for multiple testing (p=0.05).

3.16.6.6 CRP concentration and quality of life

Spearman’s rho correlation coefficients for the relationship between physical and mental components of the SF-12v2 with CRP concentration were small and insignificant.

Outcome measure		Correlation coefficient (CRP concentration at 3 months).	P value
Physical	RMI	-0.21**	0.00
	% Predicted HGS	-0.19*	0.03
	2m TUG	0.11**	0.10
Psychological	HADS A	0.04*	0.70
	HADS D	0.03*	0.73

	DTS	-0.07**	0.49
Quality of Life	SF-12 v2 PCS	0.02*	0.86
	SF-12 v2 MCS	-0.09*	0.31
* Spearman's rho **Kendall's Tau B			

Table 3-9 Relationship between CRP concentration at 3 months and physical, psychological, and quality of life outcome measures

Diagnostic subgroup		CRP concentration (mg/L) median (IQR)	P Value
Physical	HGS Weak Not weak	6.3 (1.5-10.3) 3.1 (1.0-12.3)	0.10
	RMI Poor Good	9.4 (4.8-20.0) 3.0 (0.8-9.3)	
Psychological	HADS-D Depression No depression	3.6 (0.8-9.2) 4.4 (1.1-12.7)	0.68
	HADS-A Anxiety No anxiety	4.1 (1.0-12.7) 4.4 (1.1-9.6)	
	DTS PTSD No PTSD	1.3 (0.6-4.8) 5.0 (1.3-12.0)	

Table 3-10 CRP concentration at 3 months according to categorical outcomes

3.16.7 Post-hoc analyses

3.16.7.1 Concentration of other biomarkers at 3 months according to RMI outcome groups

To further explore the relationship between the innate immune system and recovery of physical mobility, biomarker concentrations in patients with a good recovery (upper quartile of RMI) were compared with those with a poor recovery (lower 3 quartiles of RMI). The median values with 95% confidence intervals and Mann-Whitney U p values are presented in cross-reference.

Biomarker	RMI ≤ 12	RMI >12	p
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IL-1 β (pg/mL)	0 (0-0)	0 (0-0)	0.36
IL-6 (pg/mL)	7 (2-14)	3 (1-12)	0.56
IL-8 (pg/mL)	9 (4-14)	6 (4-12)	0.44
TGF β (ng/mL)	99 (84-112)	102 (94-114)	0.46
MPO (ng/mL)	137 (118-177)	107 (88-141)	0.06*
HNE (ng/mL)	114 (105-138)	105 (100-117)	0.02*
SLPI (ng/mL)	48 (40-57)	14 (41-49)	0.48

Table 3-11 Biomarker concentrations at 3 months according to RMI category

3.16.7.2 Biomarker trajectory in patients with good and poor outcome

For this analysis, the subgroup of patients who stayed in hospital for 3 weeks following ICU discharge was studied. The 3-week subgroup was chosen because it was considered that at least 3 readings after ICU discharge would be required to plot a meaningful trajectory and after 3 weeks, the number of patients still in hospital dropped off significantly. Unfortunately, after categorising patients according to RMI outcome, there were very few patients in each of these categories. Median concentration of CRP, IL-6, IL-8, and TGF β were plotted against time, however, due to the very small number of patients, the confidence intervals were widely overlapping. The graphs produced below are therefore of interest but do not give definitive characterisation of the inflammatory resolution profile. Formal statistical comparisons between biomarker profiles in patients with good and poor recovery were not carried out.

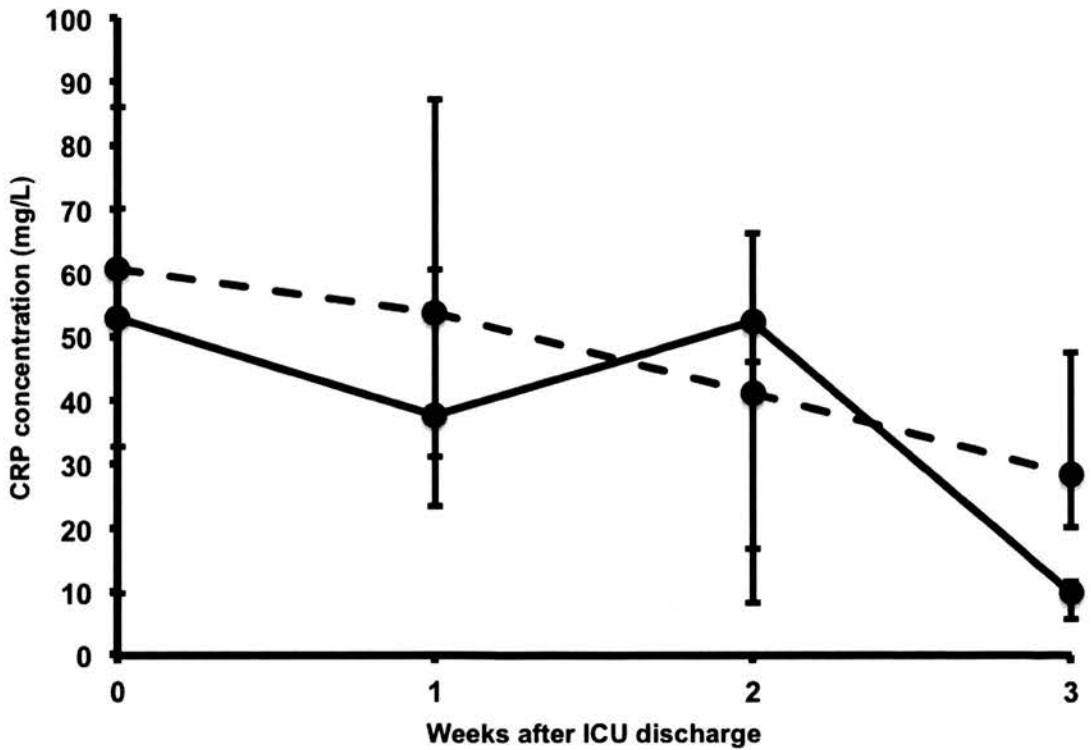


Figure 3-8 Median (IQR) CRP concentration in patients staying in hospital for 3 weeks after ICU discharge comparing patients with good recovery (RMI >12 (solid line; n=6)) and poor recovery (RMI ≤12 (dashed line; n=9)).

Figure 3-8 shows the changes in median CRP concentration over time in patients staying in hospital for 3 weeks after an ICU discharge. In this very small sample, there does not seem to be a dramatic difference in CRP trajectory between people with good recovery and poor recovery.

In this subgroup, IL-6 shows a delayed resolution profile in patients with a good outcome compared to those with a poor outcome (Figure 3-9). This observation conflicts with the finding in the whole cohort that patients with poor RMI scores have a higher IL-6 concentration at 3 months after ICU discharge. Similarly, IL-8 is higher and resolves more slowly during the hospital stay in patients good RMI scores (Figure 3-10). There seems to be very little difference in TGF beta concentrations during the post-ICU hospital stay (Figure 3-11).

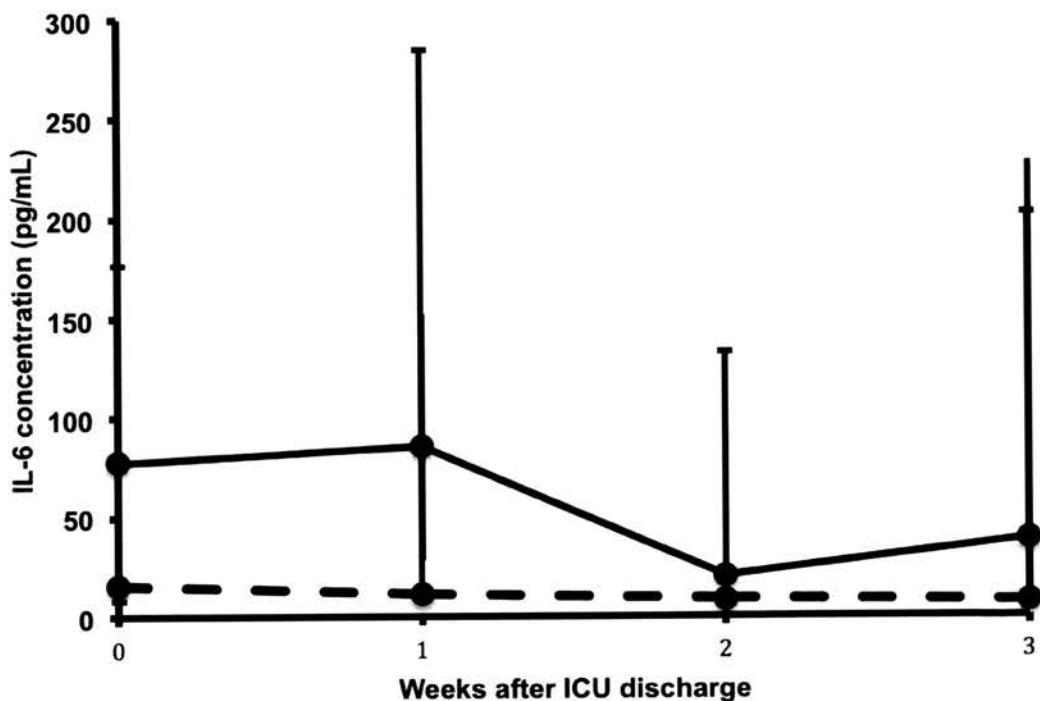


Figure 3-9 Median (IQR) IL-6 concentration in patients staying in hospital for 3 weeks with good recovery (RMI ≤ 12 (solid line; $n=6$)) versus poor recovery (RMI > 12 (dashed line; $n=6$)).

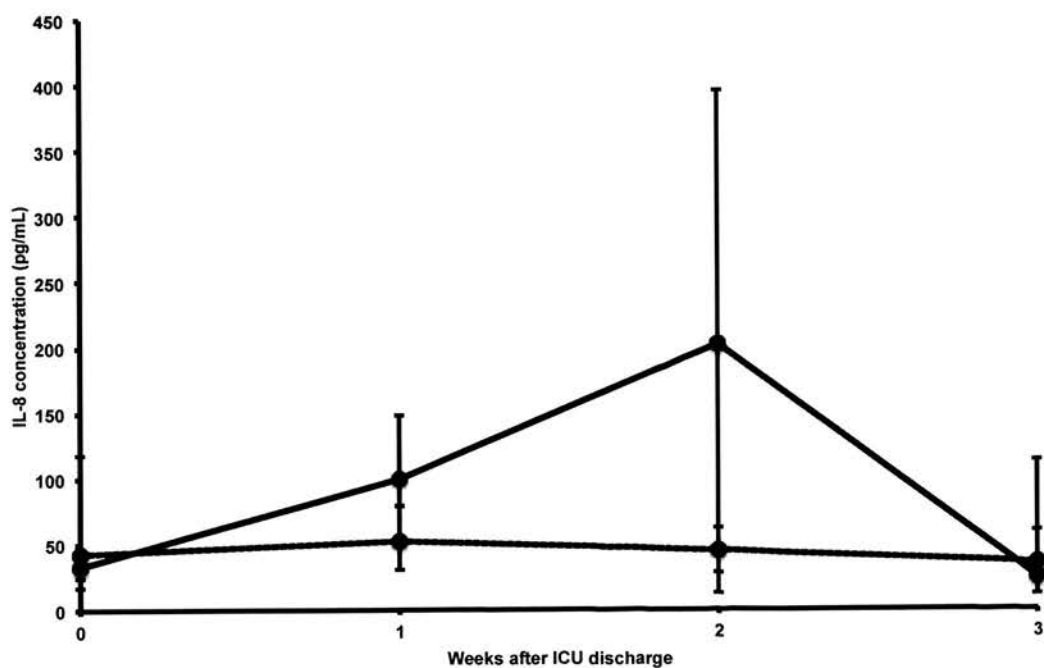
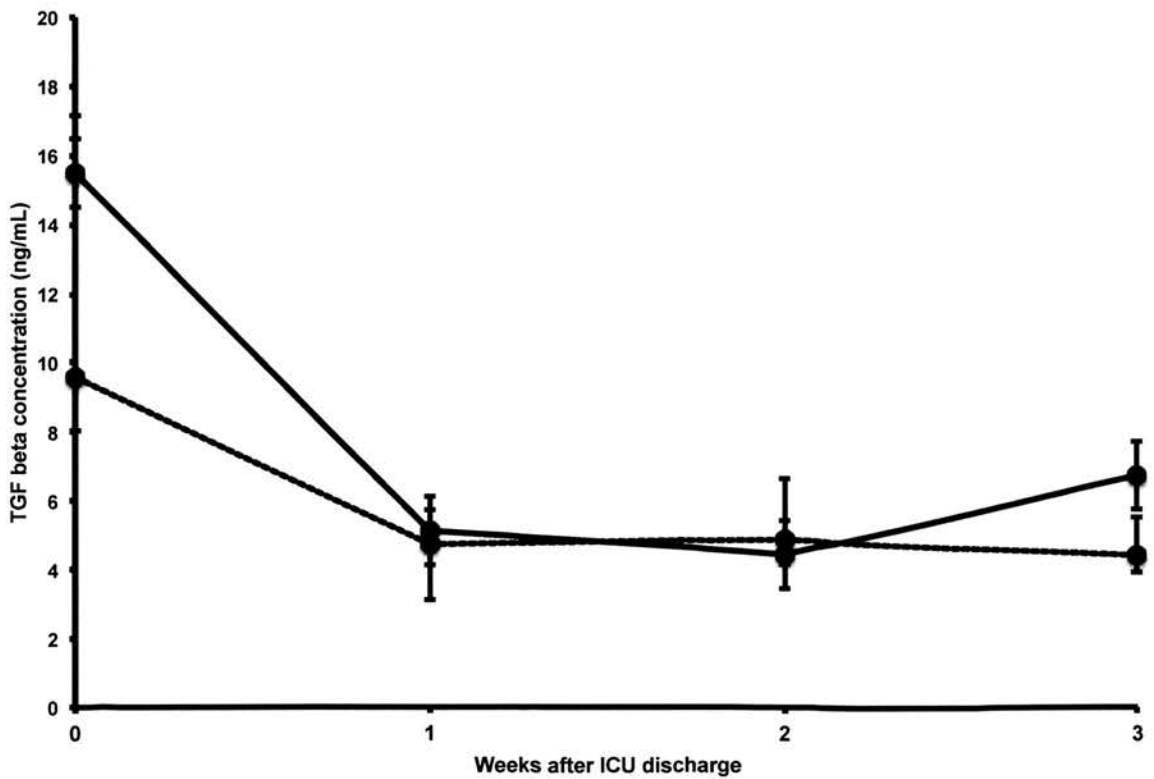


Figure 3-10 Median (IQR) IL-8 concentration after ICU discharge in patients staying in hospital for 3 weeks with good recovery (RMI > 12 ; solid line) ($n=6$) and poor recovery (RMI ≤ 12 ; dashed line) ($n=9$).



e 3-11 Median (IQR) TGF beta concentration after ICU discharge in patients staying in hospital for 3 weeks with good (RMI>12 (solid line; n=9), and poor (RMI<12 (dashed line; n=6) recovery.

3.16.7.3 Pre-existing inflammatory conditions

Patients with a pre-existing diagnosis of arthritis according to the FCI had similar serum concentrations of CRP at 3 months, compared with those that did not (3.6 (1.1-14.1) versus 4.4 (1.0-11.9) mg/L respectively (p=0.906)). Similarly, those with any of the FCI diagnoses considered to be 'inflammatory' (arthritis, diabetes, asthma, COPD, obesity) compared to those that had none of these conditions were similar (3.7(1.3-11.9) versus 4.9 (0.8-11.0) mg/L respectively (p=0.929)).

3.17 Discussion

This chapter describes a cohort study, built into a randomised trial and studied inflammation in ICU survivors and its relationship with patient relevant

outcomes. It found a high prevalence of systemic inflammation lasting up to 3 months after ICU discharge with a significant relationship with physical recovery.

The systematic review reported in Chapter 2 reported many studies that have explored inflammation during critical illness. In those that measured inflammation at the point of ICU discharge, elevation of inflammatory biomarkers was virtually ubiquitous at this treatment landmark. It was proposed that this was an unsurprising finding given that ICU discharge is an arbitrary point in an ICU patient's journey dictated mainly by the successful weaning from organ support, but not necessarily the point of full resolution of the disease process. It was also noted that there was a lack of data pertaining to the post-ICU period – either during hospitalisation or after discharge to the community. Furthermore, there was no data to support or refute the hypothesis that systemic inflammation during recovery from critical illness contributes to the post-ICU syndrome.

The analysis carried out in this chapter therefore represents the first specific exploration of the inflammatory fate of ICU survivors. It documents the prevalence of systemic inflammation according to pre-specified criteria and the concentrations of key inflammatory biomarkers at pre-specified time points after ICU discharge. In addition, it explores for the first time the interplay between persistent inflammation and the physical and psychological consequences of critical illness.

3.17.1 What is the prevalence and duration of systemic inflammation in survivors of critical illness

Almost all the patients in the sample had a CRP concentration that was greater than 3mg/L both at ICU discharge and hospital discharge. Even when a higher reference threshold was used representing the 97th centile in healthy adults (10mg/L), a significant majority of patients (more than 70%) were inflamed at these time points. By the 3-month follow up visit, median CRP concentration decreases almost within the normal range. Despite this nearly a third of patients had a CRP concentration that was greater than 10mg/L and two thirds have a CRP concentration that was greater than 3mg/L.

These data were obtained from a cohort of patients enrolled in a randomised controlled trial. A potential criticism of the work, and a possible barrier to the generalisability of the data was the use of a target population that was potentially very selective and one that might not represent ICU patients in general. These patients were all ventilated for greater than 48 hours and were therefore a sicker cohort than the 'average' ICU survivor. Despite this, the RECOVER trial excluded very few patients as a result of its protocol design. Furthermore, very few eligible patients in the study declined consent, most probably due to the perceived benefits of the 'enhanced' recovery package. From this cohort 80% of patients were enrolled in the biomarker study and analysis of the baseline data confirmed that those who were not enrolled were a very similar group. The population were likely to be a good representation of the RECOVER target population.

At the earlier time points (ICU discharge and hospital discharge) rates of subject attrition were very low. At 3 months, there was very complete data for the functional outcome measures. However, a significant number of patients did not have blood sampling for reasons of patient refusal or failed venepuncture introducing a potential source of attrition bias. A comparison of baseline features between those with complete and incomplete blood sampling was carried out and this did not reveal any significant differences between the groups at least in terms of the measured variables. The risk of attrition bias was considered to be minimal.

These data therefore provide convincing evidence of persistence of systemic inflammation in an inclusive cohort of ICU survivors with low overall risk of bias.

Evidence of inflammation in ICU survivors could represent distinct pathophysiological possibilities. Patients who are at risk of becoming critically ill may represent a subgroup that has higher basal levels of circulating inflammatory mediators. In this study, patients were enrolled in the study only after they had become unwell and after they had survived an episode of critical illness. It is therefore impossible to speculate whether they had raised levels of inflammatory markers or not before they became unwell. However, in the post-hoc analysis carried out in section 3.16.7.3, CRP concentrations at 3 months after ICU discharge

was found to be similar in patients with and without pre-existing inflammatory disease. This provides support for the hypothesis that inflammation persisting beyond ICU is related to the episode of critical illness.

It is possible that this pattern of inflammatory resolution represents the normal pattern for all patients following acute illness even if they do not become so unwell that they require ICU admission. To test this hypothesis, a comparison between ICU patients and a cohort of comparable hospitalised non-ICU patients would be necessary. It was not possible to make this comparison in the current study. Moreover, studies tracking inflammatory markers following hospital discharge are lacking in the literature except for a small study that looked at CRP concentration in patients discharged from hospital after an admission for unstable angina. Of the 53 patients studied, 26 (49%) had an elevated CRP concentration ($>3\text{mg/L}$) at hospital discharge with the same number having an elevated CRP concentration 3 months later. The 3-month data from this study are comparable to 59% of patient with CRP $>3\text{mg/L}$ seen in the RECOVER patients. However, the 2 samples are not comparable in terms of demographics and are starkly different in terms of underlying pathophysiological processes.

Another possibility is that the observed immune activation is an appropriate physiological response to a persistent pathogen or on-going tissue injury that is not evident clinically. No follow-up imaging, microbiological screening, or other diagnostic screening was carried out during the follow up of the study sample and so it is difficult to address this question.

Chronic inflammation following critical illness could represent recurrent episodes of infection – secondary and tertiary inflammatory ‘hits’ in an immunologically susceptible individual. It is known that down regulation of the innate immune response, termed ‘immunoparalysis’, is observed in both adults and children following critical illness and is associated with morbidity and mortality (Frazier & Hall, 2008). This immunosuppression could potentially lead to chronic low-grade activation of the immune system as the body is infected by pathogens that are incompletely combatted by a weakened innate immune response.

Immunoparalysis is formally defined as a reduction in the capacity of monocytes to present antigen (monocyte HLA-DR+ <30%), or less formally as a reduction in the cytokine production capacity of whole blood in response to a stimulus. Neither definition was tested in the current cohort of patients therefore it was not possible to test whether immunoparalysis might be causative in the inflammatory persistence observed.

Reactivation of latent viral infection is another potential mechanism for persistent innate immune activation and this topic is explored in chapter 4. A final potential explanation is that critical illness is associated with delayed or dysfunctional inflammatory resolution mechanisms that result in the persistence of acute inflammation in the absence of pathological stimulus. What is the time course of biochemical markers of systemic inflammation after critical illness?

Serum concentrations of molecules representing distinct aspects of the acute inflammatory response after ICU discharge were studied. This analysis served 2 purposes. Firstly, it documented for the first time in human survivors of critical illness, what happens to the blood concentrations of these substances as the body switches from combating critical illness to the new challenge of recovery and rehabilitation. Secondly, having identified a high prevalence of systemic inflammation, study of the individual components of the inflammatory response helped to explore which parts of the system might be contributing.

The granular neutrophil products HNE and MPO, the chemokine IL-8, the pro-inflammatory cytokine IL-6, and the acute phase protein CRP were found in high concentrations both at ICU discharge and 3 months after ICU discharge. Although there was no control group in the study, the comparison made with other populations of healthy subjects suggests that these are higher than what would normally be observed. This suggests that the innate immune system is activated at multiple levels, and that neutrophil degranulation, or neutrophil necrosis might be a key characteristic of the on-going inflammatory process.

These findings are interesting but must be interpreted with some caution. The lack of a control group prevents any formal statistical comparison between

patients who have experienced an ICU admission, and patients with similar illnesses who have not required organ support. These observations may represent the natural history of the diseases studied, and ICU admission 'event' may be of little consequence.

It was not possible in this study to follow the longitudinal time course of these inflammatory biomarkers in a rigorous manner. The main limitation to this was one of study design. Patients only had serial measurements of inflammatory biomarkers carried out during the post ICU, in-hospital stay. Following discharge, there were not further blood samples until the follow up point. In addition, a large number of patients only stayed a week or 2 after ICU discharge. For these reasons, the scope to plot biomarkers over time was limited.

A compromise was to compare patients who had stayed for a similar length of time, and this was carried out for patients staying 1, 2 and 3 weeks after ICU discharge. The interesting and perhaps surprising finding in this analysis was that biomarker concentration seemed to be dictated by time from hospital discharge and not ICU discharge. Despite following a very similar trajectory, patients staying for shorter lengths of time in hospital after ICU discharge, had drastically different biomarker concentrations at the point of ICU discharge. Assuming that discharge from hospital is dictated at least in part by clinical status, the fact that the time course of inflammatory resolution mirrors this is supportive of the theory that inflammatory resolution is a key 'event' in recovery.

3.17.2 Is persistent inflammation in survivors of critical illness associated with post-ICU recovery?

Another important finding of the study was that systemic inflammation and physical function after critical illness were significantly associated. CRP concentration was significantly correlated with RMI at 3 months with patients with higher concentrations of CRP having poorer function. The serum concentration of CRP measured in patients with a good mobility outcome (according to the pre-specified RMI threshold of greater than 12) was a third of the concentration of those

with a poor mobility outcome. Furthermore, a significant negative correlation was observed between hand grip strength and CRP concentration with the weakest patients (>2 standard deviations below the mean HGS) having a median CRP concentration that was twice that of the other patients in the sample.

These findings lend support to the hypothesis that a persistent inflammatory process impedes recovery. Despite this, it is important to stress that such a conclusion cannot be drawn from this data alone. Using an epidemiological framework proposed by Bhopal, the case for persistent inflammation as a candidate aetiological factor in the pathophysiology of delayed physical recovery can be evaluated (Bhopal, 2008).

Temporality. In epidemiology, temporality refers to the relationship in time between a proposed risk factor or variable and the outcome of interest. For a risk factor to be causative, its occurrence must predate the occurrence of the disease or outcome. In this study, inflammation was significantly associated with both hand strength and mobility at the same moment in time: 3 months after ICU discharge. The study design that led to this observation was dictated by the randomised controlled trial on which the sample was drawn. It was not possible to know with certainty, which occurred first, the immobility or the inflammation. Patients may have had significant decrements in their physical function prior to their ICU admission. Indeed some of these physical impairments may have been accompanied by chronic inflammation – rheumatoid arthritis being an obvious example. This possibility was explored in the study with CRP concentration in patients with potential pre-existing inflammatory conditions being compared to the rest of the sample. This analysis, though underpowered, did not give any indication that these patients were different in terms of their inflammatory response.

Alternatively, inflammation may have occurred as a *result* of muscle dysfunction, with recovering, regenerating muscle being the focus of a restorative inflammatory response. The finding of an association between inflammation and poor recovery may reflect the greater regenerative inflammatory process (higher

levels of inflammatory molecules) in patients with weaker and more damaged muscles at study baseline.

Strength of relationship. Provided the other conditions are met, a stronger relationship between potential risk factor and an outcome, the greater the likelihood that the relationship is causative. Although there was a significant correlation between inflammation and physical recovery, the correlation co-efficients and scatter plots show that the relationship is fairly weak, at least using the chosen outcome variables.

The RECOVER study was not originally designed to investigate inflammatory outcomes and therefore aspects of the study design are likely to have had an impact on the precision of the estimates. For example, the timing of blood samples was dependent on patient convenience and not standardised by time of day. In addition, the primary outcome measure of the RECOVER study (RMI) displayed some ceiling effect – i.e. many patients had scores of 15. Therefore, whilst RMI was used as a linear scale, lots of information will have been lost at the top end of the scale. It is possible that a study designed specifically to assess the relationship between inflammation and recovery addressing these potential flaws may have improved the strength of the observed relationship.

Dose response. Another indicator of causality is dose response – an increase in the exposure to or ‘dose’ of a risk factor (in this case inflammation) should lead to an increased expression of the disease (in this case poor recovery). This has certainly been demonstrated in this study. As CRP concentration increases, RMI and percentage hand grip strength decrease. This observation strengthens the case for a causative relationship.

Specificity. The case for a causative relationship is strengthened if the risk factor has an effect that is limited to a narrow number of diseases – so called specificity. In this situation, specificity is difficult to assess because neither ‘critical illness’ nor ‘physical disability after critical illness’ could be considered to be ‘diseases’. If a potential risk factor is associated with a wide range of conditions, then it is much less likely to be causative. Specificity is difficult to assess,

especially when studying a phenomena that has not been explored much in the past. The current study is the first to explore the role of inflammation in post-ICU comorbidity and was limited to the post-ICU syndrome. It is possible that future studies will demonstrate associations between post-ICU inflammation and other diseases – reducing specificity. At this stage, it is probably most accurate to state that specificity cannot be assessed at this time.

Other considerations are ‘consistency’ – the repetition of findings in other studies, ‘experimental confirmation’, and ‘biological plausibility’. So far, the findings of this study have not been replicated in other studies because no other studies asking this question have been carried out. No studies have confirmed the phenomenon in an experimental study design, and doing so, at least in clinical studies would not be feasible or ethical. Finally, the plausibility of the hypothesis has been extensively argued above.

In summary, an association between functional outcome, and systemic inflammation has been observed lending support to the hypothesis of a causative role of inflammation in the post-ICU syndrome. Further studies will be required to strengthen confidence or refute this hypothesis.

3.18 Conclusions

CRP concentration is elevated in almost all survivors of critical illness at the point of discharge from ICU, remains elevated until discharge from hospital, and is persistently elevated in up to 60% of patients 3 months after discharge from intensive care unit. Contrary to expectation, CRP concentration does not decrease exponentially as the patient recovers, but instead fluctuates considerably, in some cases having an overall downward trend, but in many has an upward or a persistently elevated course.

The exact mechanisms of CRP production and elimination and therefore the main determinants of serum concentration in this cohort of patients cannot be determined from this study. However, it is likely that ongoing release of pro-inflammatory mediators drives ongoing synthesis and release and therefore it is

reasonable to suggest that ongoing acute inflammation or chronic inflammation is at least partly responsible.

In this study, a significant relationship was observed between CRP concentration, and measures of physical recovery. As with all observational studies, it is impossible to determine whether these relationships signify a causative role of systemic inflammation on poor recovery after critical illness. However, biological plausibility, and the lack of confounding influences mean that this is at least a reasonable line of enquiry for future studies.

In addition, the fact that this study does not demonstrate a relationship between other measures (in particular measures of psychological illness), does not rule out the role for these factors in their aetiology and should not deter future enquiry, but that this study was not powered to detect these relationships.

Chapter 4 - CMV infection, systemic inflammation, and post-ICU recovery

4.1 Introduction

4.1.1 Human Cytomegalovirus

Human cytomegalovirus (CMV) is a virus of the Herpesviridae family. Owing to common biological characteristics such as restricted number of hosts, long reproductive cycle, slow culture times, sites of latency and its tendency to cause cytomegalia, CMV is classified in the betaherpesevirinae subfamily which also contains Muromegalovirus, Roseolovirus, and Proboscivirus (Pellett & Roizman, 2007).

Human transmission is via contact with the infected body fluids of an infected individual (saliva, breast milk, urine, vaginal secretions, semen). In an American population sample (Staras et al., 2006), overall sero-prevalence of CMV IgG was 59% increasing from 36% in 6-11 year olds to 91% in those older than 80 (Table). Female gender, low household income and certain racial / ethnic groups (e.g. Hispanics) carry increased risk of sero-positivity.

Age	CMV seroprevalence (%)
6-11	36
12-19	42
20-29	49
30-39	54
40-49	65
50-59	74
60-69	83
70-79	89
≥80	91

Table 4-1 Seroprevalence of CMV IgG in a non-institutionalised, civilian US population (Staras et al., 2006)

Following entry into the human body, the first line of defence encountered by a viral pathogen is the innate immune system. Interferons and natural killer cells (NK cells) are key components of the early defense against infection. Interferons exert their antiviral effects via a number of pathways that cause blockade of viral mRNA translation, apoptosis of virus-infected cells, degradation of viral RNA, and inhibition of viral transcription. In addition, interferons stimulate major histocompatibility complex (MHC) protein class 1 and 2 expression thus assisting in the orchestration of the adaptive immune response. Non-specific proliferation of NK cells is mediated by type 1 interferons and IL-15 in the immediate stages of infection. NK cells kill virus-infected cells directly via direct cytolysis. However they also produce interferon gamma that helps protect cells from infection and activates macrophages.

As infection continues, the adaptive immune responses take over involving both cell mediated and humoral mechanisms. The interaction of antibody and complement with viruses and virus-infected cells prevents the spread of virus into cells and mediates antibody dependent cellular cytotoxicity (ADCC) via NK cells, macrophages, and neutrophils. In addition, the T cell response is crucial for mounting a highly effective and specific cytotoxic effect against infected cells (Nash, 2006).

As a result of these immune responses, CMV infection is almost always a subclinical disease in the immunocompetent host. Rarely, patients develop symptoms of cytomegalovirus mononucleosis – manifesting as fever, lymph node swelling, sore throat, rash, and spleno- or hepatomegaly. Even more rarely, patients develop a severe multisystem disease (Pellett & Roizman, 2007).

In patients lacking a fully functioning immune system, unabated CMV replication can lead to severe life-threatening CMV infection. Acute CMV illness is classically recognised in 2 specific situations – vertical transmission from infected mother to unborn foetus causing foetal damage; and reactivation (and rarely primary infection) in patients with AIDS or following solid organ or bone marrow transplantation. In these scenarios CMV infection can cause pneumonitis, retinitis,

hepatitis, and colitis. Pneumonitis in particular is associated with high mortality (>80%).

The replication phase of viral infection is dependent on the host cell. After cell entry, a complex series of events leads to replication of the viral genome. Following this, the virus particle is packaged within a protein and released from the host cell. This process leads to death or 'lysis' of the host cell and this phase is therefore known as the 'lytic' phase of viral infection. Some viruses are able to enter cells and stop in a static or latent phase. All herpes viruses including CMV are able to do this (Traylen et al., 2011).

4.1.2 CMV latency and persistence

Once infected, CMV is a life-long infection persisting in the human host in 2 states. These states are best described as viral latency –'the maintenance of the viral genome in the absence of production of infectious virions' (Sinclair, 2008) and chronic infection, where viral replication continues at low levels but does not cause disease due to the surveillance and killing functions of host T cells.

The most studied site of HCMV latency is the myeloid cell lineage (Sinclair, 2008), a site that gained considerable interest following the discovery that transfusion of leukocyte-depleted blood reduces the risk of transfusion-related CMV disease (de Graan-Hentzen et al., 1989). There are likely to be other sites of CMV latency but so far, these have been not been consistently demonstrated.

The precise mechanisms by which herpes viruses evade detection and elimination by the host immune system remain largely a mystery. However, it has been recently shown that cells containing latent CMV virus secrete an increased quantity of the chemokine CCL8 that recruits CMV specific T cells and inhibit their cytotoxic function (Mason, Poole, Sissons, Wills, & Sinclair, 2012). In addition the CMV genome encodes IL-10 production within myeloid cells inhibiting the function of CD4 cells (A. K. Cheung et al., 2009).

Although CMV reactivation and its consequences have historically been considered a phenomenon isolated to those with immune-compromise particularly

organ transplant recipients and patients with the acquired immunodeficiency syndrome (AIDS), reactivation also occurs in immune-competent individuals in certain situations including critical illness (Chiche et al., 2009; Cook et al., 2003; Cook et al., 1998; Desachy et al., 2001; Heininger et al., 2011; Heininger et al., 2001; Jaber et al., 2005; Kutza et al., 1998; Limaye et al., 2008; von Muller et al., 2006).

It is known that the lytic phase of CMV infection is critically dependent on immediate early (IE) gene expression. IE genes are the first viral genes expressed following infection with CMV. In latent myeloid cells there is evidence of IE gene suppression. Virus-encoded genes including the CMV IE gene promoter control the switch from a latent to a lytic replication phase of CMV infection. NF-kappa B and C-Jun (both stimulated by cytokines and stress-related proteins during critical illness) up-regulate the production of these genes leading to CMV reactivation (Traylen et al., 2011).

4.1.3 CMV infection and reactivation in ICU patients

Various aspects of CMV infection have been studied in populations of ICU patients over the past 20 years. These studies have attempted to describe the rates of previous CMV exposure, the incidence of active CMV infection, the potential clinical consequences of active and latent infection, and the risk factors for CMV reactivation.

4.1.3.1 Evidence of previous CMV exposure

In healthy community dwelling adults, evidence of prior CMV exposure (presence of IgG antibody to CMV) is widespread and rates rises steadily with age (Staras et al., 2006) (Table 4-1). In ICU patients, prevalence rates are similarly high (up to 94%), and although direct age based comparisons have not been done, they are likely to reflect the background prevalence rates of the population (Table 4-2).

In an early study of 34 surgical ICU patients with sepsis, 33 were screened for CMV IgG on the day of sepsis onset and 31 (94%) were CMV IgG positive at this time (Kutza et al., 1998). Of 104 patients in an observational study of surgical ICU

patients with a length of stay of more than 5 days, 76 patients (73%) were CMV IgG positive (Cook et al., 2003). In another study of 242 immuno-competent medical ICU patients that were mechanically ventilated for at least 48 hours, 194 patients (80%) were CMV IgG positive at the point of ICU admission (Chiche et al., 2009).

Author	Year	Population	Sample size	CMV IgG positive n (%)
Kutza (Kutza et al., 1998)	1998	Surgical ICU Severe Sepsis	33	31 (94)
Cook (Cook et al., 2003)	2003	Surgical ICU Length of stay >5 days	104	76 (73%)
Chiche (Chiche et al., 2009)	2009	Medical ICU Ventilated > 48 hours	242	194 (80%)

Table 4-2 Baseline CMV IgG seropositivity of ICU patients

4.1.3.2 Evidence of CMV reactivation

Some investigators have examined the incidence of active CMV infection in ICU patients with evidence of prior CMV exposure (CMV IgG positive). These studies are summarised in Table 4-3. Whilst it is possible that these patients have experienced newly acquired CMV infections, due to the presence of dormant infections, these patients are usually described as patients experiencing CMV ‘reactivation’.

In a series of 23 immuno-competent critically ill mechanically ventilated patients, CMV DNA was not detected in the peripheral blood leukocytes or in the cells of broncho-alveolar lavage (BAL) samples from any of the patients (Stephan et al., 1996). This was an observational study and the sampling regimen was not standardised. Only half of the patients had BAL sampling, and whilst all the patients had blood sampling, it was not clear how often this was carried out. Certainly, it appears that only 8 of the 23 patients had sequential blood sampling.

There is therefore a high likelihood that active CMV infection if it had been present could have been under-diagnosed.

In another study, this time of severely ill surgical intensive care unit patients, 36% of CMV IgG positive patients were found to have active CMV infection defined by CMV detection by PCR or viral culture of plasma, leukocytes, or respiratory tract secretions (Heininger et al., 2001). Limaye and colleagues found a reactivation rate of 33% measured by CMV plasma DNA PCR in their study of 120 CMV IgG seropositive ICU patients (Limaye et al., 2008). Finally, in 86 immunocompetent CMV seropositive patients with sepsis, 35 patients (41%) had evidence of CMV reactivation (CMV DNA detected by PCR in leukocytes, plasma, or tracheal secretions)(Heininger et al., 2011).

Author	Year	Population	Sample size	Reactivation n (%)	Method	Sampling interval
Stéphan (Stephan et al., 1996)	1996	Mechanical ventilation	23	0 (0)	PCR (lysates of BAL and peripheral blood leukocytes) Viral culture (plasma and BAL)	Variable
Heininger (Heininger et al., 2001)	2001	Surgical ICU	56	20 (36)	PCR (plasma, leukocytes, respiratory secretions) Viral culture (plasma and respiratory secretions)	Weekly
Limaye (Limaye et al., 2008)	2008	Mixed ICU	120	39 (33)	PCR (plasma)	Thrice weekly
Heininger (Heininger et al., 2011)	2011	Severe Sepsis	86	38 (41)	PCR (plasma, leukocytes, tracheal secretions)	Weekly

Table 4-3 CMV reactivation in CMV IgG positive ICU patients

4.1.3.3 Evidence of active CMV infection

Other investigators have described active CMV infection regardless of IgG status. These studies are summarised in Table 4-4. In a study of patients with sepsis, found that 11 out of 34 patients (33%) had CMV DNA in their leukocytes and 6 (17%) had CMV antigen in their leukocytes (Kutza et al., 1998). In another study of patients with multiple organ dysfunction syndrome (MODS), CMV infection was detected in only 1 of the 96 ICU patients studied using a similar definition. The authors of this study conceded that the likely explanation for the disparate result was the single sampling point early during the ICU admission (mean 1.8 days after ICU admission) in contrast to the serial sampling regimen undertaken in other studies (Desachy et al., 2001). In a surgical ICU population, Cook and colleagues cultured CMV in the respiratory samples of 15% of patients and found CMV viraemia in 5.8% using a weekly sampling regimen (Cook et al., 2003). In a heterogenous ICU population of 242 patients ventilated for greater than 48 hours, 38 (16%) of patients had active CMV infection. Diagnosis was based on the presence of CMV pp65 antigenaemia, positive bronchoalveolar lavage (BAL) viral culture, a histological diagnosis of CMV, or signs and symptoms of lung disease combined with CMV detected in BAL fluid.

In a highly selective population, Jaber and colleagues studied 237 patients in whom CMV infection was suspected on clinical grounds and in whom CMV pp65 antigen was measured in peripheral blood leukocytes. Of these patients 40 patients (17%) had evidence of active CMV infection (Jaber et al., 2005).

Author	Year	Population	Sample size	Infection N (%)	Method	Sampling interval
Kutza (Kutza et al., 1998)	1998	Sepsis	34	11 (33) 6 (17)	PCR CMV antigen staining (both leukocytes)	Twice weekly
Desachy	2001	MODS	96	1 (0)	PCR	Single

(Desachy et al., 2001)					(serum)	sample
Cook (Cook et al., 2003)	2003	Surgical ICU Severe sepsis	104	16 (15) 6 (6)	Viral culture (respiratory secretions) CMV antigen (blood)	Weekly
Chiche (Chiche et al., 2009)	2009	Medical ICU Ventilated >48 hours	242	38 (16)	CMV antigen (leukocytes) Viral culture (BAL) Histology Clinical signs+CMV in BAL	Weekly
Jaber (Jaber et al., 2005)	2005	ICU patients in whom CMV infection suspected	237	40 (17)	CMV antigen (leukocytes)	Determined by clinical suspicion of CMV infection

Table 4-4 CMV infection in ICU patients

4.1.3.4 CMV infection and mortality

Few of the observational studies exploring the effects of CMV infection in critical illness on mortality have employed statistical techniques to take into account potential confounders. Heining reported higher mortality in patients with active CMV infection (55% versus 36% $p=0.17$) (Heining et al., 2001). This may reflect the greater proportion of CMV positive patients that had sepsis (50% versus 17%). In Cook's study, an excess mortality was seen in CMV positive patients (50% versus 27% $p=0.15$) (Cook et al., 2003). Jaber and colleagues also reported excess ICU mortality (50% versus 28% $p=0.02$). Chiche and colleagues reported a greater proportion of patients with CMV infection in those that died during ICU stay (Chiche et al., 2009).

Limaye and colleagues performed a multivariable analysis to examine the effect of CMV infection on the composite endpoint of death or hospitalisation at 30 days (Limaye et al., 2008). In univariable analysis, mechanical ventilation, serious infection, ventilator days and CMV viraemia but not age, type of intensive care unit, race, severity of illness, or blood transfusion were associated with the composite outcome. In their multivariable model including intensive care unit, mechanical ventilation, major infection, ventilator days, and various quantitative measures of CMV load, all variables were independently associated with the composite endpoint. On the basis of this analysis, it would seem plausible that CMV infection is associated with mortality or prolonged hospital length of stay. Unfortunately the presence of multiple potentially interacting variables introduces the risk of a type 1 error so the results of this regression must be interpreted with caution.

Heininger and colleagues used Cox proportional hazards modelling to explore the relationship of CMV reactivation with hospital mortality. They found severity of illness, presence of septic shock, and CMV reactivation had significant bivariate relationships with hospital mortality. After adjustment for measured potential confounders (SAPS II score, presence of septic shock, length of ICU stay before enrolment, and HSV detection in respiratory secretions), CMV reactivation did not have a significant association with hospital mortality (Heininger et al., 2011).

4.1.3.5 CMV infection and other outcomes

Similar to the data reported for mortality, most of the studies examining other outcomes in CMV-infected ICU patients did not adjust for potential confounding factors. Heininger and colleagues noted a longer ICU length of stay in patients with CMV infection. This observation is likely to be confounded by the preponderance of septic patients in the CMV group who may have had a longer length of stay (Heininger et al., 2001). Cook and colleagues also found that patients who developed CMV infections had a longer length of ICU stay, in addition to longer hospital lengths of stay, and longer duration of ventilation (Cook et al., 2003). Jaber and colleagues reported longer length of stay, longer hospital length of stay

after ICU, longer duration of ventilation, and a greater number of nosocomial infections. Chiche and colleagues reported longer ICU length of stay, greater number of ventilator-associated pneumonia (and other bacteraemia) in patients with active CMV infection (Chiche et al., 2009).

In the only study to perform multivariable analysis on outcomes other than mortality, Heininger and colleagues found CMV reactivation had a significant association with ICU length of stay. This association remained significant after adjustment for SAPS II, septic shock, ICU stay before enrolment, and HSV detection in respiratory secretions (Heininger et al., 2011).

4.1.3.6 CMV infection after ICU discharge

No studies were found that measured CMV infection in patients after ICU discharge. It is therefore unclear, how many of the large proportion of CMV infected patients have on-going CMV infection.

4.1.3.7 Risk factors for CMV infection

Several studies have explored potential risk factors for CMV infection during critical illness. Investigators have done this by comparing patient characteristics between patients who do and do not subsequently develop CMV infections. In some cases, multivariable analyses have been conducted to adjust for measured confounders.

In risk factor analysis such as this, temporality is important – i.e. the characteristic must exist prior to the onset of the disease. For baseline characteristics such as age, gender, and pre-existing comorbidity, this is straightforward. However for illness-related factors such as severity of illness and drug treatments given during an illness, temporality is difficult to demonstrate. Any risk factor analysis examining these factors should therefore be interpreted with caution.

Using a case-control study design where patients were matched for gender, age, severity of illness, pre-ICU hospital length of stay, and ICU diagnosis, Jaber

and colleagues found that patients with suspected CMV infection who had the diagnosis confirmed were more likely to have renal failure requiring renal replacement therapy, or receive corticosteroids during their ICU stay (Jaber et al., 2005). Whilst no analysis of confounding was carried out in this study, the matched design suggests that these differences were independent of the matched factors. It is not known whether these differences contributed to or were a consequence of CMV infection.

In Heininger's study of severely septic patients, there were no differences noted in terms of age, gender, diagnosis, or severity of illness at baseline (Heininger et al., 2011).

Kutza and colleagues study of septic patients (Kutza et al., 1998) noted higher rates of severe sepsis in those patients who subsequently developed CMV infections (73% versus 44%).

In Heininger's study of surgical patients, gender, age, severity of illness (SAPS II), presence of malignant disease, pre-ICU treatment duration, and a diagnosis of sepsis were compared between CMV infection groups. The group who subsequently developed CMV infections had higher proportions of patients with a diagnosis of sepsis and pre-existing malignant disease. More of these patients had pre-ICU treatment of longer than a week. When SAPS II score, age, female gender, malignant disease, and acute sepsis were entered into a multivariable logistic regression equation with CMV infection as the outcome, malignant disease and a diagnosis of sepsis had a statistically significant association with CMV infection status and could therefore be considered potential independent risk factors (Heininger et al., 2001).

In a heterogeneous group of ICU patients, those that subsequently experienced CMV reactivation were more likely to be male, more likely to have received a blood transfusion and be mechanically ventilated prior to study entry. Severity of illness (APACHE 2) race, age, type of ICU were similar in both infection groups. In a multivariable logistic regression analysis including type of ICU, and gender, male gender remained independently associated with subsequent CMV

reactivation. Patients in cardiac ICU were less likely to be become reactivated (Limaye et al., 2008).

In Chiche and colleagues' ventilated medical ICU cohort, age, gender, severity of illness, ICU diagnosis, and prior comorbidities were compared. Patients who subsequently developed CMV infection were slightly older (68 versus 62 years $p=0.018$). They were more likely to have a diagnosis of pneumonia and more likely to have pre-existing chronic renal failure, COPD, or have taken corticosteroids in the preceding 1 month. No multivariable analyses were carried out to adjust for potential confounders and therefore these observations are speculative (Chiche et al., 2009).

Cook and colleagues tested the associations of various baseline characteristics with CMV infection as the outcome in a multivariable logistic regression model (Cook et al., 2003). Of the variables included in the model (APACHE 2 score, age, female gender, previous CMV exposure, steroid exposure, multiple blood transfusions, ICU length of stay, and bacterial pneumonia), female gender, previous CMV exposure, steroid exposure, and bacterial pneumonia were significantly associated with CMV infection (Cook et al., 2003).

4.1.4 Effect of CMV infection on the immune system

CMV infection is associated with elevated levels of inflammatory cytokines and induces systemic inflammation that persists beyond the acute infection and into viral latency (van de Berg et al., 2010). It is therefore plausible that patients who have evidence of active CMV infection during the recovery phase of critical illness will have inflammatory consequences. In addition, the mere presence of latent CMV infection might increase a patient's risk of persistent inflammation, and by extension, worsened physical outcome as a consequence.

4.1.5 Other viruses in critically ill patients

The reactivation of other viruses (Herpes Simplex Virus (HSV), Epstein Barr Virus (EBV), and torque teno virus (TTV) is also observed during critical illness.

Studies of these viruses have shown possible association with patient relevant outcomes such as secondary bacterial infections, and prolonged ICU length of stay (Bruynseels et al., 2003).(Walton et al., 2014). CMV was chosen as the virus that has most frequently been studied in a critical care population, has shown the strongest associations with clinical outcome including mortality, and also has been shown to have an impact on the inflammatory profile of again patients. Thus as a potential aetiological factor in the pathogenesis of inflammatory persistence in ICU survivors, it carries the most promise.

4.1.6 Summary and outline of aims

In summary, the majority of ICU patients are likely to have been previously exposed to cytomegalovirus and a significant proportion of these will experience viral reactivation during critical illness. The consequences of CMV infection in critical illness requires further investigation as most observational studies do not control for confounding variables. CMV infection is associated with longer ICU length of stay and possibly also increased mortality.

No studies have explored CMV infection in ICU survivors. Specifically, it is not known how many ICU patients (who have high rates of reactivation) still have evidence of active infection at ICU discharge and at later time points.

This study therefore aimed to document the prevalence of active CMV infection at ICU discharge and at 3 months after ICU discharge in a heterogeneous population of 193 survivors of critical illness. Furthermore, the author aimed to study the characteristics of patients with evidence of active infection, and compare them to non-infected patients. Finally, the authors set out to explore the associations between CMV status and markers of inflammatory resolution and physical recovery.

4.2 Hypotheses

- A high proportion of ICU survivors have evidence of prior CMV exposure.

- In some patients, it may be possible to detect evidence of CMV reactivation at ICU discharge.
- Prior CMV exposure is associated with persistent inflammation and dysfunctional inflammatory resolution after ICU discharge.
- CMV reactivation is associated with persistent inflammation and dysfunctional inflammatory resolution after ICU discharge.
- CMV status at ICU discharge is associated with post-ICU complications such as depression, anxiety, PTSD, and poor physical function.

4.3 Research Questions

- What is the proportion of ICU survivors that have evidence of prior exposure to CMV at ICU discharge?
- What proportion of patients in the sample has evidence of active CMV infection at ICU discharge?
- What are the characteristics of these patients?
- How long does CMV infection persist after ICU discharge?
- Is CMV infection associated with resolution of inflammation?
- Is CMV infection associated with the post-ICU syndrome?

4.4 Methods

This chapter analyses CMV reactivation on the same cohort of patients described in chapter 3. The reader is therefore directed to the methods section of this chapter for information on patient selection, consent, and blood sampling. In addition, the methods employed for processing and storage of the blood samples is identical.

4.4.1 Diagnosis of active CMV infection

To establish exposure to CMV prior to ICU discharge, stored serum samples were analysed using a quantitative indirect anti-globulin enzyme immunoassay (VIDAS CMV G, Biomerieux, Lyon, France), CMV antibody was quantified in

arbitrary units/mL (aU/mL). As per the manufacturers instructions, patients with <4 aU/mL were considered to have negative CMV IgG serology. Patients with CMV titres of greater than or equal to 4aU/mL or less than 6aU/mL were considered equivocal. Those with titres of greater than or equal to 6aU/mL were considered to have positive serology.

Those patients with positive or equivocal CMV IgG titres had quantitative PCR carried out on stored plasma samples.

4.4.2 Systemic inflammation and inflammation resolution

The inflammatory biomarkers measured in RECOVER have been justified previously (section 3.9).

Serum concentrations of CRP, complement enzymes, IL-1 β , IL-6, IL-8, TGF beta, SLPI, MPO, and HNE were determined using the methodology described in chapter 3.

4.5 Statistical Methods

A signed, dated a priori analysis plan for this section was included in the request for RECOVER trial data (Appendix H).

4.5.1 Evidence of previous CMV exposure and active CMV infection

The number and percentage of patients with previous CMV exposure was reported. Previous CMV exposure was defined as a positive test for CMV IgG antibody at ICU discharge.

The number and percentage of patients with evidence of CMV reactivation was reported. CMV reactivation was defined as the number of patients with previous CMV exposure that had detectable CMV DNA in plasma at ICU discharge.

4.5.2 Investigation of factors associated with evidence of prior CMV exposure

Baseline data was presented for patients with evidence of prior CMV exposure at ICU discharge. Comparison was made with patients that did not have evidence of prior CMV exposure using the Student's t-test for parametric data and Mann-Whitney U Test for non-parametric data.

4.5.3 Investigation of factors associated with evidence of CMV reactivation at ICU discharge

Baseline data was presented for patients with evidence of CMV reactivation at ICU discharge. Comparison was made with patients that did not have evidence of CMV infection at this time using the Student's t-test for parametric data and Mann-Whitney U test for non-parametric data.

4.5.4 Duration of CMV infection

In those patients who did have evidence of CMV infection at ICU discharge, the number and percentage of patients of those patients that still had CMV DNA in plasma at 3 months was reported.

4.5.5 Association of prior CMV exposure / active infection and inflammatory resolution

Comparisons CRP, IL-1 β , IL-8, IL-6, TGF β , MPO, HNE, and SLPI, concentration at 3 months grouped according to previous CMV exposure and active CMV infection are presented. Groups were compared with Mann Whitney U tests. These comparisons represented cross-sectional analyses of 2 groups of patients. As such an un-paired statistical test was chosen.

4.5.6 Association of CMV status with indices of physical recovery, psychological sequelae, and quality of life outcomes

Outcomes of physical (RMI, % predicted HGS, 2 minute timed up and go), psychological (HADS A, and D; DTS), and quality of life (SD12 v2 PCS and MCS) were compared according to previous CMV exposure group. In addition these outcome measures were compared according to CMV reactivation group. The Mann-Whitney U test was used for non-parametric data and Student's t-test was used to compare parametric data.

4.5.7 Association of CMV status and other outcomes

The association of CMV status with post ICU hospital length of stay was explored using Student's t-test.

4.5.8 Issues of study power and multiple testing

As with chapter 3, this study was an add-on to an RCT and is also likely to be underpowered to detect differences between study groups. To take into account issues of multiple testing, results are reported and discussed in relation to Bonferroni adjusted p value thresholds.

4.6 Results

4.6.1 Patients

For details of patients enrolled in the blood sampling study see Figure 3-1. At study baseline (ICU discharge) CMV serology was carried out on 183 of the 184 patients (99%) in RECOVER that had blood sampling. One patient had an insufficient quantity of remaining sample due to previous testing to carry out the CMV assays.

At study follow up (3 months after ICU discharge) CMV serology was carried out on all 122 patients of the RECOVER patients that had blood samples at this time point.

4.6.2 CMV serology

At ICU discharge, 115/183 (63%) had positive CMV serology indicating prior CMV infection and 1 patient had equivocal CMV serology.

Three months later, 77/122 (63%) had positive serology and 1 patient had equivocal CMV serology. Of the 77 CMV IgG positive patients at 3 months, 74 patients had CMV IgG measured at ICU discharge. Seventy-two patients were IgG positive at ICU discharge, 1 patient had equivocal serology. Only 1 patient had negative CMV IgG at baseline and represented a single case of CMV sero-conversion during the 3 month follow up period.

4.6.3 CMV reactivation

CMV PCR was carried out on 114 (99%) of the patients with positive CMV serology at ICU discharge. In one case, there was inadequate sample to perform PCR analysis. Of these, 13 patients (11.4% of IgG positive patients or 7.2% of all ICU survivors) had detectable CMV on PCR consistent with reactivation of latent CMV infection.

4.6.4 Characteristics of patients with evidence of prior CMV exposure

The baseline characteristics of ICU survivors who had positive CMV IgG were compared with those who were IgG negative (Table 4-5). Patients in these groups were similar in terms of age, gender distribution, previous comorbidity, and severity of illness. IgG positive patients spent a similar period of time in ICU and received mechanical ventilation and vasopressors as often as IgG negative patients. IgG positive patients were more likely to receive renal replacement therapy than IgG negative patients (30% versus 22% $p=0.25$). This difference did not reach statistical significance, however given the wide confidence intervals around the percentage, it is possible that this represents a type 2 error resulting from the secondary analysis of trial data that was not powered to demonstrate such a difference.

Similarly, IgG positive patients were twice as likely to have on-going delirium at ICU discharge (19% versus 9% $p=0.06$) and had worse scores on the RMI (2 versus 4 ($p=0.07$)). Whilst these results also fail to reach statistical significance, they provide a potentially interesting signal that may warrant future exploration.

	CMV IgG + (n=115)*	CMV IgG – (n=68)*	P***
Age	61 (14)	61 (13)	0.97
Gender (m/f (%m))	69/46 (60)	43/25 (63)	0.66
Functional comorbidity index	2.8 (2.1)	2.6 (1.9)	0.60
Functional comorbidity index subcategories (n (%))			
Arthritis			
Osteoporosis	37 (32)	16 (24)	0.21
Asthma	7 (6)	3 (4)	0.75
COPD	20 (17)	13 (19)	0.77
Angina	20 (17)	12 (18)	0.97
Heart Disease	12 (10)	5 (7)	0.49
Heart Attack	13 (11)	8 (12)	0.93
Neurological	13 (11)	3 (4)	0.11
Stroke	4 (3)	0 (0)	0.30
PVD	14 (12)	7 (10)	0.70
Diabetes	3 (3)	3 (5)	0.67
Upper Gastro	17 (15)	10 (15)	1.00
Depression	37 (32)	20 (30)	0.69
Anxiety	32 (28)	21 (31)	0.66
Visual	25 (22)	20 (30)	0.24
Hearing	16 (14)	9 (13)	0.90
Disc Disease	12 (10)	7 (10)	0.98
Obesity	9 (8)	6 (9)	0.81
	27 (24)	14 (21)	0.65
APACHE 2	21 (7)	21 (8)	0.98
SOFA score at baseline			
Total	3 (2,5)	2 (2,4)	0.18
Cardiovascular	0 (0,0)	0 (0,0)	0.69
Respiratory	2 (1,2)	2 (1,2)	0.39
Coagulation	0 (0,1)	0 (0,0)	0.26
Renal	0 (0,1)	0 (0,0)	0.36
Liver	0 (0,1)	0 (0,0)	0.12
Neurological	0 (0,1)	0 (0,1)	0.56
ICU length of stay (days)	11 (6,18)	11 (6,20)	0.81

Days of mechanical ventilation	8 (5,13)	7 (4,16)	0.66
Received vasopressors (n(%))	83 (72)	49 (72)	0.91
Received renal replacement (n(%))	34 (30)	15 (22)	0.25
Delirium at enrollment (n(%))	22 (19)	6 (9)	0.06
RMI at enrollment	2 (1,6)	4 (1,5)	0.07
HGS (kgs)	16 (8)	16 (8)	0.99

Table 4-5 Baseline characteristics of CMV IgG negative/equivocal versus IgG positive CMV ICU survivors. *Expressed as mean (sd), median (25th, 75th centile) or n (%) unless otherwise stated. **For continuous data, mean difference and 95% confidence intervals are presented. For percentage data, percentage differences and 95% confidence interval for the differences are presented. *** p values: T test for parametric data, Mann Whitney U for non-parametric data, Chi-squared test for proportion unless any cell value <5 in which case Fishers exact test was employed.

4.6.5 Characteristics of patients with active CMV infection

The baseline characteristics of those IgG seropositive patients who had evidence of active CMV infection (CMV PCR positive) at ICU discharge are compared with IgG seropositive patients without evidence of CMV infection in Table 4-6.

	CMV PCR + (n=13)*	CMV PCR - (n=101)*	p***
Age	61 (13)	61 (13)	0.93
Gender (m/f (%m))	9/4 (69)	60/41 (59)	0.71
FCI	2.8 (2.0)	2.4 (2.6)	0.47
FCI subcategories (n (%))			
Arthritis	4 (31)	32 (32)	1.00
Osteoporosis	1 (8)	6 (6)	0.58
Asthma	2 (15)	18 (18)	1.00
COPD	2 (15)	18 (18)	1.00
Angina	2 (15)	10 (10)	0.63
Heart Disease	3 (23)	10 (10)	0.17
Heart Attack	1 (8)	12 (12)	1.00
Neurological	0 (0)	12 (12)	1.00
Stroke	0 (0)	4 (4)	0.36
PVD	0 (0)	3 (3)	1.00
Diabetes	1 (8)	16 (16)	0.69

Upper Gastro	5 (39)	32 (32)	0.62
Depression	2 (15)	30 (30)	0.35
Anxiety	2 (15)	23 (23)	0.73
Visual	0 (0)	16 (16)	0.20
Hearing	1 (8)	11 (11)	1.00
Disc Disease	1 (8)	8 (8)	1.00
Obesity	4 (31)	23 (23)	0.50
APACHE 2	20 (11)	21 (6)	0.65
SOFA score at baseline			
Total			
Cardiovascular	3 (2,6)	3 (2,5)	0.50
Respiratory	0 (0,0)	0 (0,0)	0.65
Coagulation	2 (1,2)	2 (1,2)	0.48
Renal	0 (0,2)	0 (0,1)	0.60
Liver	0 (0,1)	0 (0,1)	0.75
Neurological	0 (0,2)	0 (0,1)	0.47
	0 (0,1)	0(0,1)	0.63
ICU length of stay (days)	16 (10,28)	11 (6,18)	0.06
Days of mechanical ventilation	11 (4,26)	8 (5,13)	0.51
Received vasopressors (n(%))	10 (77)	72 (72)	1.00
Received RRT (n(%))	3 (23)	31 (31)	0.75
Delirium at enrolment (n(%))	4 (31)	18 (18)	0.27
RMI at enrolment	1 (0,2)	2 (1,6)	0.06
HGS (kgs)	12 (6)	17 (8)	0.03

Table 4-6 Baseline characteristics of CMV PCR positive versus CMV PCR negative/equivocal CMV seropositive ICU survivors. *Expressed as mean (sd), median (25th, 75th centile) or n (%) unless otherwise stated. **For continuous data, mean difference and 95% confidence intervals are presented. For percentage data, percentage differences and 95% confidence interval for the differences are presented. *** p values: T test for parametric data, Mann Whitney U for non-parametric data, Chi-squared test for proportion unless any cell value <5 in which case Fishers exact test was employed.

Patients with evidence of CMV infection were of a similar age but were more likely to be male. Small differences were noticed between the 2 groups in FCI subcategories reflecting small differences in pre-existing comorbidity. Due to the small sample size, it is impossible to determine whether these would translate to significant differences in a larger samples size. Overall FCI score was the same in the PCR positive and PCR negative groups indicating a similar level of pre-existing comorbidity.

Severity of illness as assessed by APACHE 2 score was the same in both groups. SOFA scores at ICU discharge were also the same in both groups. PCR positive patients trended towards a longer stay in ICU (16 days versus 11 days $p=0.059$), and were ventilated for longer (11 days versus 8 days $p=0.511$).

Of note, physical function at baseline was reduced. Despite the highly skewed distribution of RMI data at this time point, with most patients having vary poor function, RMI was a point higher in CMV PCR negative patients compared with CMV PCR positive patients (RMI 1 versus 2 $p=0.06$) (Figure). In addition, handgrip strength at baseline was significantly lower (HGS 12 versus 16 kg $p=0.032$) (Figure 4-2).

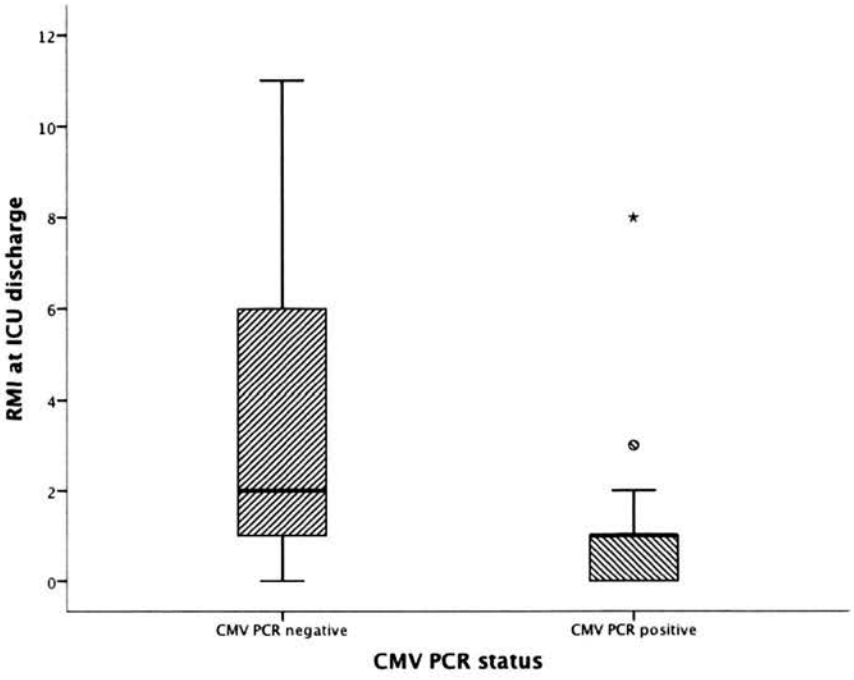


Figure 4-1 Comparison of RMI at ICU discharge across CMV reactivation groups ($p=0.06$). PCR positive $n=13$; PCR negative $n=105$.

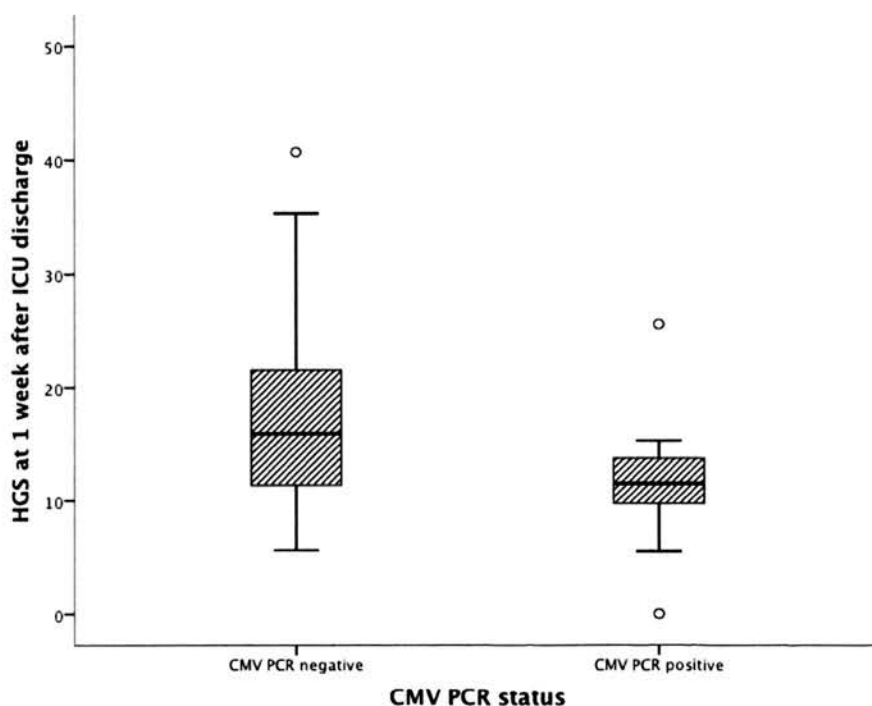


Figure 4-2 HGS across CMV reactivation groups (p=0.03). PCR positive n=11; PCR negative n=69.

As discussed in the introductory section (4.1.3.7), potential risk factors for CMV reactivation and infection are sepsis, severity of illness, blood transfusion, corticosteroid use, organ transplantation, and other causes of immunosuppression. A case note review was therefore carried out to further assess these potential risk factors. Case notes were available for 11/13 (85%) of the patients in whom CMV reactivation was diagnosed. A summary of this review is presented in Table 4-7 and Table 4-8.

Risk factor	n	%
Blood transfusion	2/11	18
Organ transplant	2/11	18
Blood borne virus	1/11	8
Sepsis	4/11	36
Corticosteroids	2/11	18
Other immunosuppression	2/11	18

Table 4-7 Summary of risk factors from ICU survivors with evidence of CMV reactivation

Six out of 11 (55%) of patients had 1 or more of the risk factors described in Table 4-7. Two of the 11 patients (18%) received a blood transfusion during their ICU admission. Two patients (18%) of the patients had received an orthotopic liver transplant and were receiving immunosuppression. One patient had a diagnosis of hepatitis C - potentially putting them at risk of other blood borne viruses and particularly HIV. Four patients (36%) had an admission diagnosis of sepsis, and 2 patients (18%) had received systemic corticosteroids whilst in ICU. One patient had had a splenectomy. Five patients (45%) had no known risk factors for CMV reactivation other than critical illness.

Case	Diagnoses	Age	Sex	APACHE 2	Vasopressors	RRT	Steroids	BBV	Organ t/p	Transfusion	Other immunosuppress
1	Acute pancreatitis Ventilator Associated pneumonia Multi organ failure	20	M	13	Y	N	N	N	N	N	N
2	Smoke inhalation CO Poisoning ARDS	56	M	22	Y	N	N	N	N	N	N
4	Out of hospital cardiac arrest	78	M	26	Y	N	N	N	N	N	N
5	Post op repair mycotic aneurysm	76	M	21	Y	N	N	N	N	Y	N
6	Alcoholic liver disease Aspiration Pneumonia Bleeding	44	F	ND	Y	N	N	N	N	N	N

Case	Diagnoses	Age	Sex	APACHE 2	Vasopressors	RRT	Steroids	BBV	Organ t/p	Transfusion	Other immunosuppress
	oesophageal varices										
7	Hepatic abscess	83	F	24	Y	Y	Y	N	N	Y	N
8	Post op Whipples Pancreatic cancer LRTI	79	M	20	N	N	N	N	N	Y	N
9	OLT Septic shock (GI tract) CMV pneumonitis	50	M	ND	Y	Y	Y	Y	Y	Y	N
10	Bleeding oesophageal varices Splenic aneurysm bleed Laparotomy Splenectomy	60	F	22	N	N	N	N	N	Y	Y
11	TAA repair Ventilator associated pneumonia Acute kidney injury	74	M	22	Y	N	N	N	N	Y	N
12	Sepsis – subdiaphragmatic collection AKI ARDS	70	F	22	N	N	N	N	N	N	N

Table 4-8 Summary of case note review of ICU survivors with CMV reactivation (ND=no data)

4.6.6 Time course of CMV infection following ICU discharge

Of the 13 patients that were CMV PCR positive at ICU discharge, only 6 had repeat sampling at 3 months. The research team was unable to contact 2 patients, one of who was known to have moved abroad. Four patients received a home visit but the research nurses were unable to obtain a blood sample due to difficulties with venepuncture.

Of the 7 patients in whom a sample was obtained, none had on-going evidence of persistent CMV viraemia.

There were no cases where patients tested PCR negative at ICU discharge and PCR positive at 3 months after ICU discharge.

4.6.7 CMV status and systemic inflammation

4.6.7.1 Systemic inflammation and CMV IgG

Comparison of biomarker concentrations between ICU survivors who were CMV IgG positive and those that were negative are shown in Table 4-9 (see page 164). CRP concentration at baseline was similar in both groups (28 versus 25mg/L $p=0.90$). At the 3-month follow up point, IgG positive patients had a CRP concentration that was nearly twice that of the IgG negative patients (5mg/L versus 3mg/L $p=0.07$) with the difference not reaching statistical significance (Figure 4-3). Given that this was an exploratory analysis that was not powered to detect such differences, there is a reasonable chance that this represents a type 2 error and that with a larger sample size a difference may be evident.

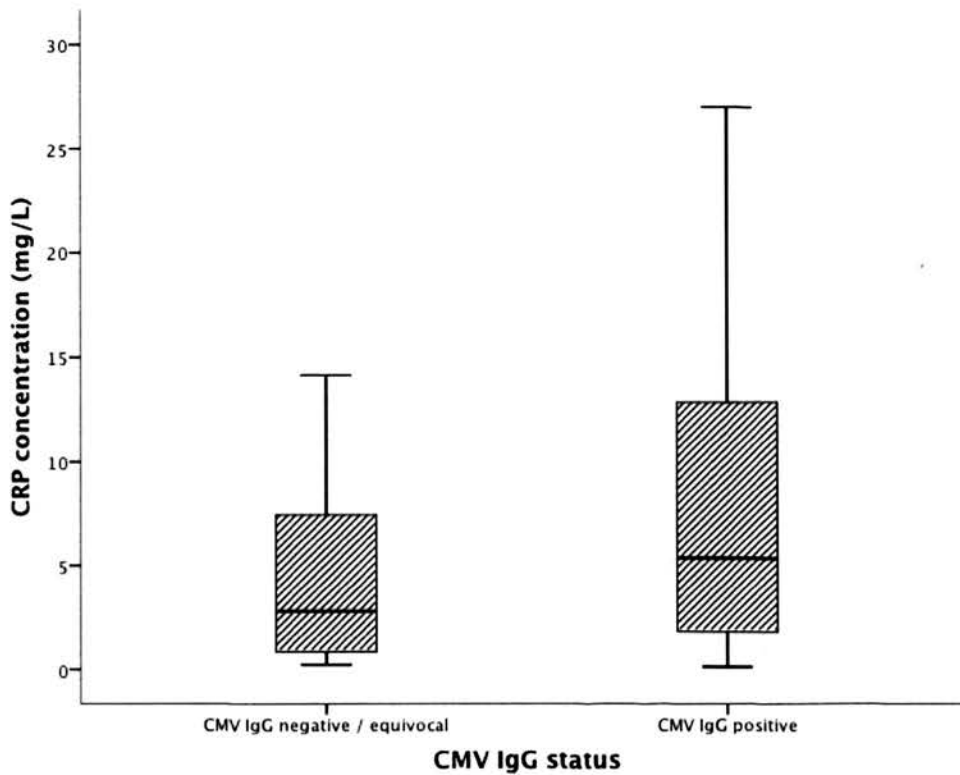


Figure 4-3 CRP concentration at 3 months according to CMV IgG status. IgG positive n=76; IgG negative/equivocal n=41.

At ICU discharge, HNE (Figure 4-4), IL-6 (Figure 4-5), and IL-8 (Figure 4-6) were elevated in IgG positive patients, reaching statistical significance in the case of HNE, even accounting for a Bonferoni adjusted p value of $p < 0.004$. IL-6 almost achieved statistical significance according to this threshold. TGF β on the other hand was found in lower concentrations in the serum of IgG positive patients and had a p value that was almost significant to the Bonferoni adjusted threshold ($p = 0.01$) (Figure 4-7). IL-1 β was virtually undetectable in the serum of ICU survivors. No differences in concentration were observed in the concentrations of MPO or SLPI. However, the median MPO concentration of 192ng/mL across the entire sample is significantly greater than the upper limit of normal of 951pg/mL (0.9ng/mL) seen in studies of healthy patients.

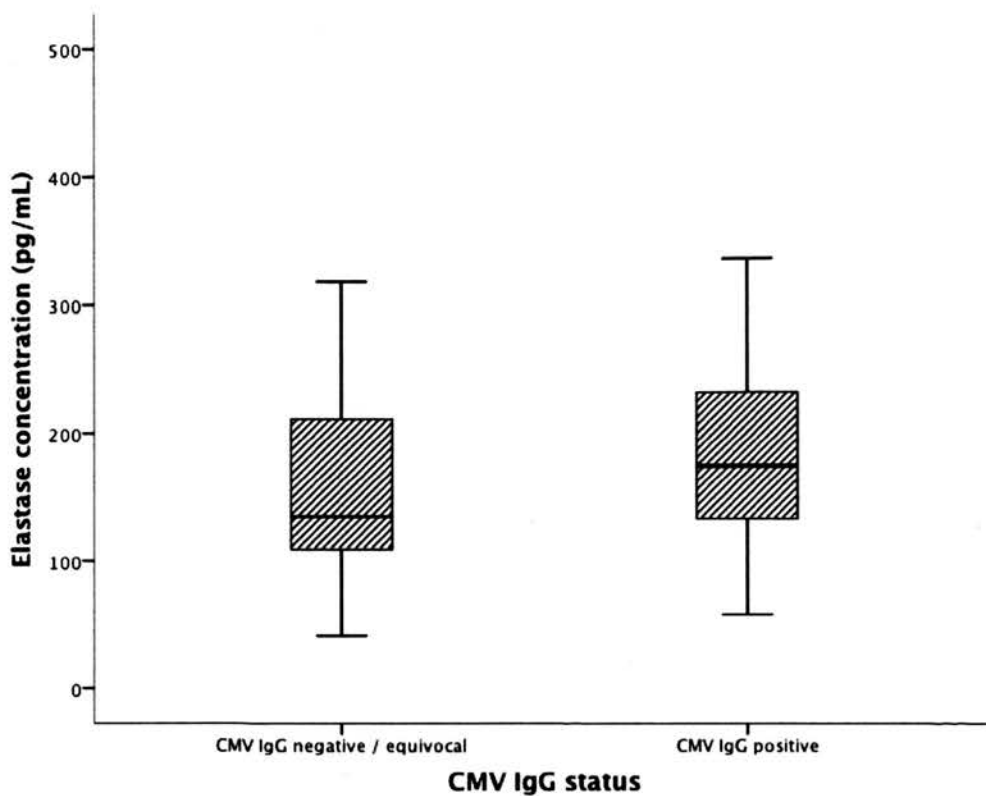


Figure 4-4 HNE concentration at ICU discharge according to CMV status $p=0.00$. IgG positive $n=113$; IgG negative/equivocal $n=66$.

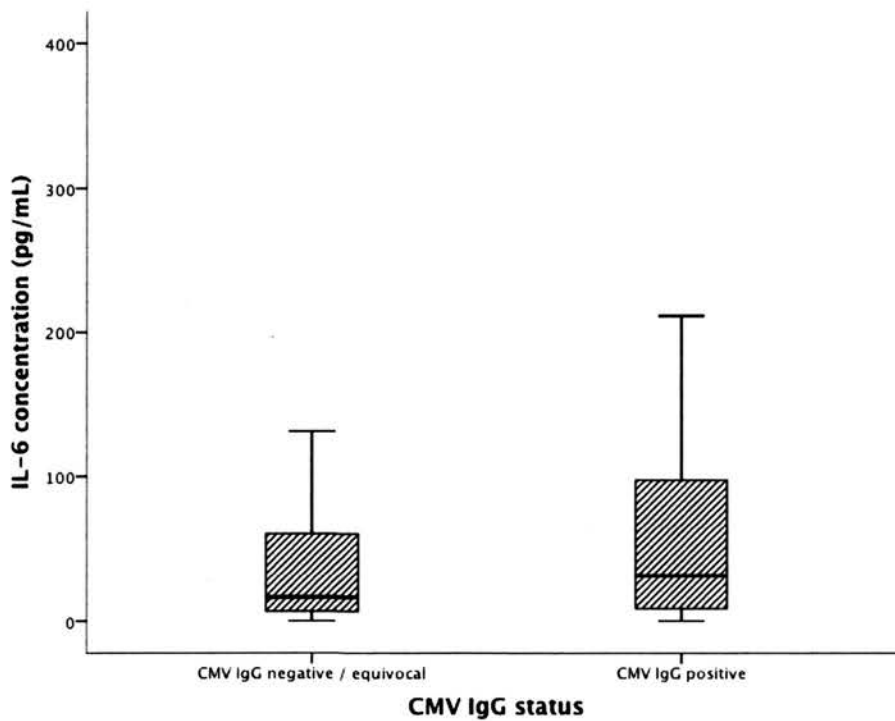


Figure 4-5 IL-6 concentration at ICU discharge according to CMV IgG status $p=0.05$. IgG positive $n=112$; IgG negative/equivocal $n=67$.

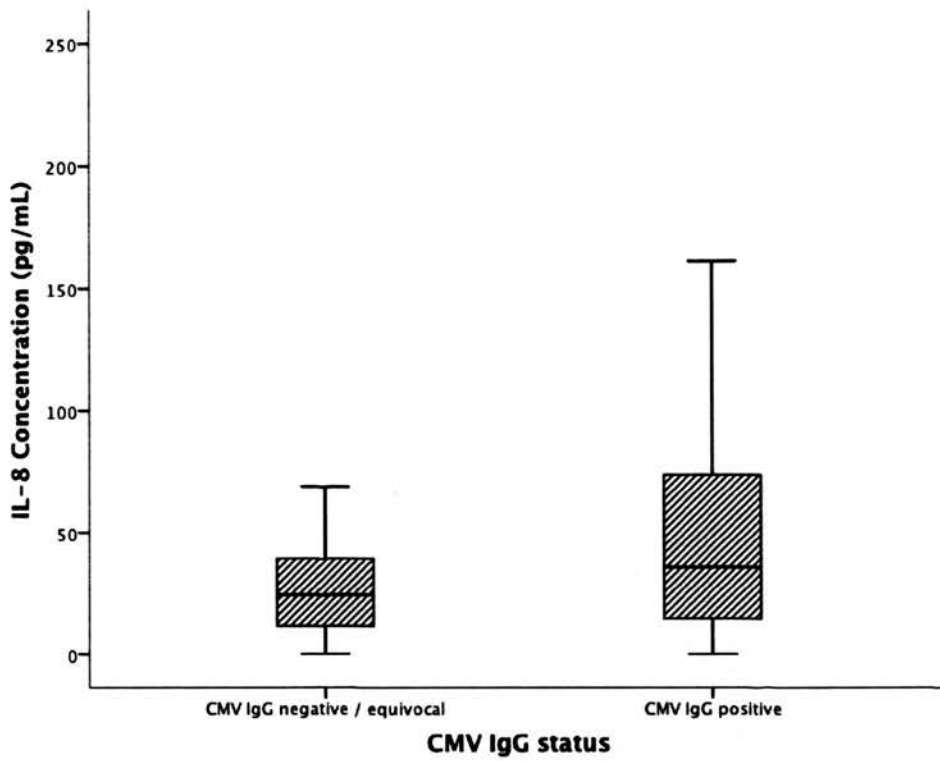


Figure 4-6 IL-8 concentration at ICU discharge according to CMV IgG status $p=0.06$. IgG positive $n=112$; IgG negative/equivocal $n=67$.

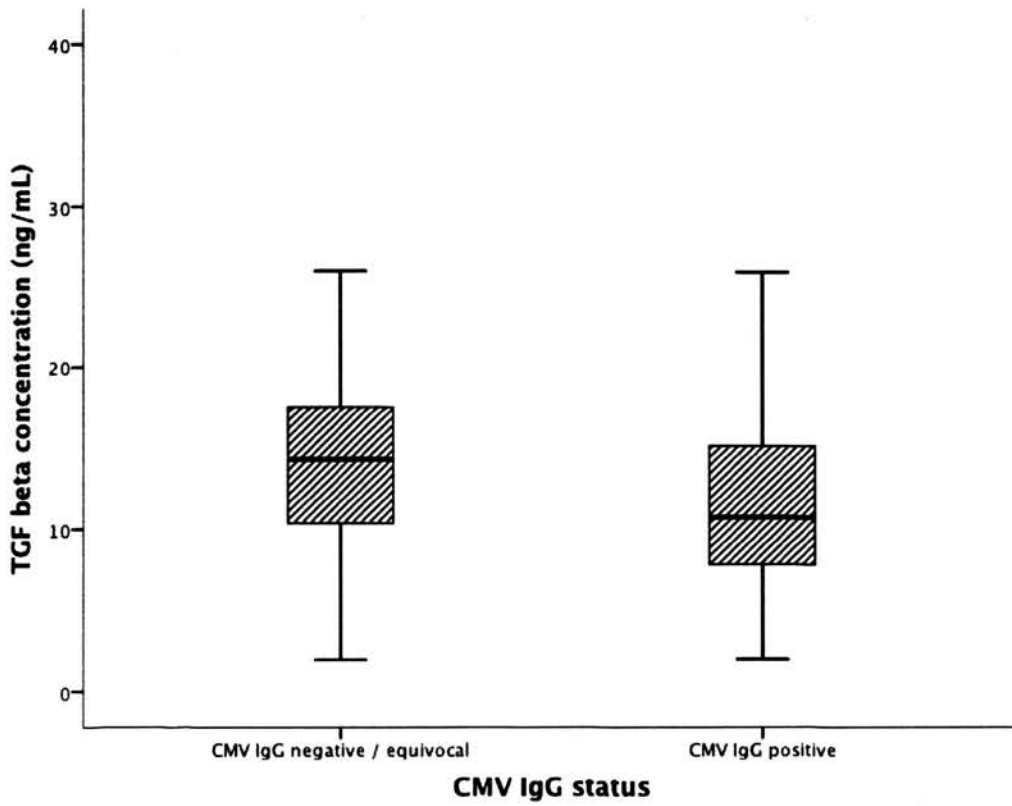


Figure 4-7 TGF beta concentration at ICU discharge according to CMV IgG status p=0.01. IgG positive n=113; IgG negative/equivocal n=67.

At the 3-month sampling point, the biomarker patterns observed at ICU discharge were preserved. Patients who were CMV IgG positive had higher concentrations of HNE, IL-6, and IL-8 and lower concentrations of TGF beta in their serum. There were not differences in the concentrations of SLPI or MPO but as at ICU discharge, the levels of MPO were significantly elevated compared to the observations made by others in healthy adults.

In summary, ICU survivors with evidence of previous CMV exposure had elevations in their inflammatory markers when compared to patients without prior exposure. Given that these 2 groups were similar in terms of their severity of illness, demographics, and prior comorbidity, it is possible that latent CMV infection does have some impact on inflammatory resolution.

	CMV IgG positive (n=115) Median (95% CI)	CMV IgG negative (n=68) Median (95% CI)	p value
CRP (mg/L)			
Baseline	25 (20-40)	28 (21-44)	0.90
3 months	5 (4-8)	3 (1-5)	0.07
SLPI (ng/mL)			
Baseline	56 (52-63)	61 (55-66)	0.43
3 months	47 (41-53)	48 (43-51)	0.91
MPO (ng/mL)			
Baseline	191 (149-228)	193 (149-228)	0.47
3 months	132 (117-160)	106 (90-141)	0.32
HNE (ng/mL)			
Baseline	175 (161-197)	135 (125-154)	0.00
3 months	118 (108-134)	91 (82-101)	0.00
IL-1 β (pg/mL)			
Baseline	0 (0-3)	0 (0-0)	0.11
3 months	0 (0-0)	0 (0-0)	0.35
IL-6 (pg/mL)			
Baseline	31 (22-56)	16 (10-37)	0.05
3 months	7 (2-16)	1 (0-10)	0.06

IL-8 (pg/mL)			
Baseline	36 (24-36)	24 (17-29)	0.07
3 months	9 (5-16)	5 (1-10)	0.06
TGFβ (ng/mL)			
Baseline	11 (10-12)	14 (12-15)	0.01
3 months	9 (9-10)	11 (10-12)	0.01

Table 4-9 Comparison of inflammatory biomarkers according to serum IgG at ICU discharge.

4.6.7.2 Effect of latent CMV infection on biomarker recovery trajectory

To further explore this hypothesis, a post hoc analysis was carried out on a subgroup of patients that remained in hospital for at least 3 weeks after ICU discharge. It was considered necessary to have at least 3 samples after ICU discharge to plot a 'trajectory' and three weeks was the time point with the maximum number of patients fulfilling this criterion. These patients had weekly blood sampling during their post-ICU hospital stay and line charts of median concentration were constructed to depict the recovery trajectory of several of the inflammatory biomarkers – CRP (Figure 4-8), IL-6 (Figure 4-9), IL-8 (Figure 4-10), and TGF-beta (Figure 4-11).

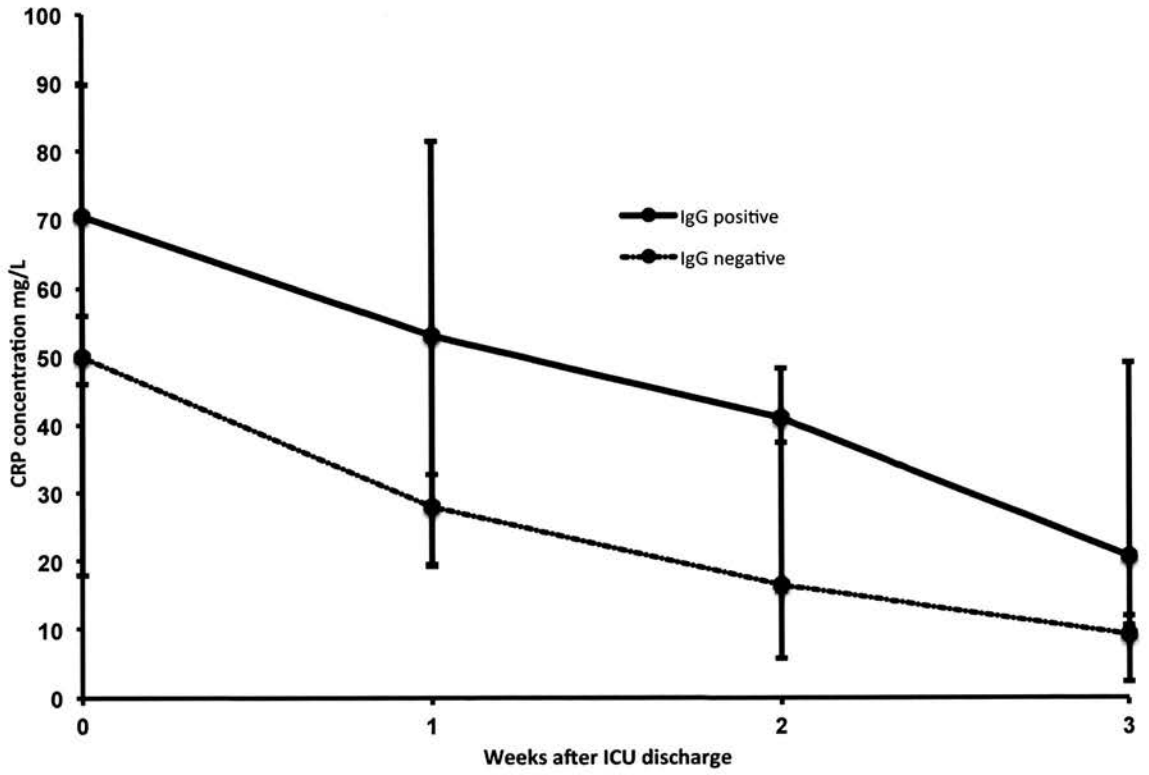


Figure 4-8 Median (IQR) CRP concentration after ICU discharge according to CMV IgG status. IgG positive n=10; IgG negative n=5.

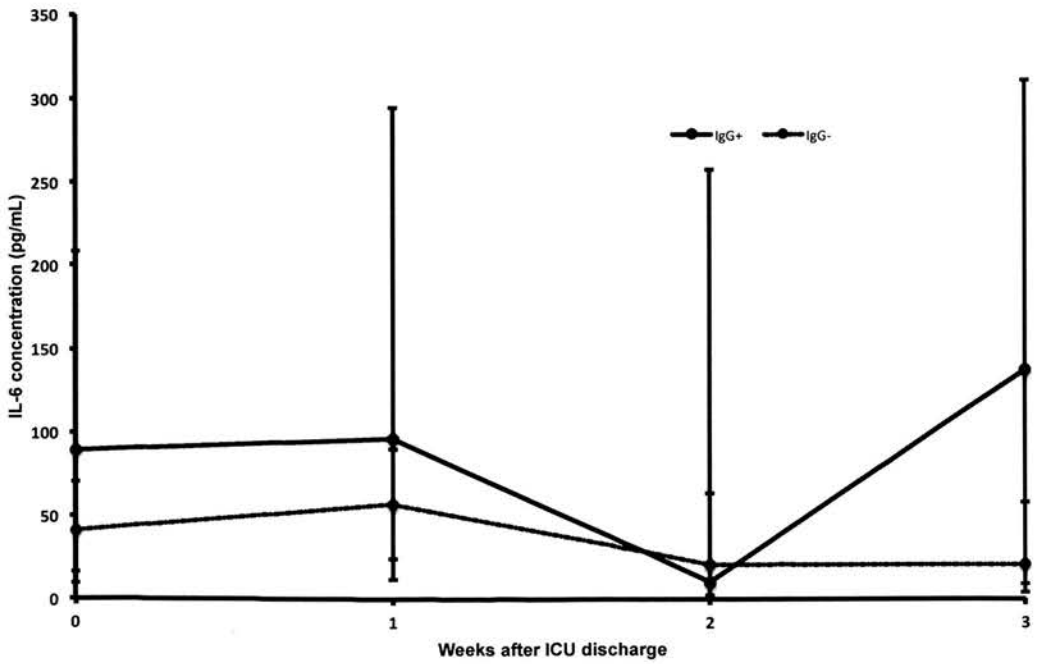


Figure 4-9 Median (IQR) IL-6 concentration after ICU discharge according to CMV IgG status. IgG positive n=8; IgG negative n=5.

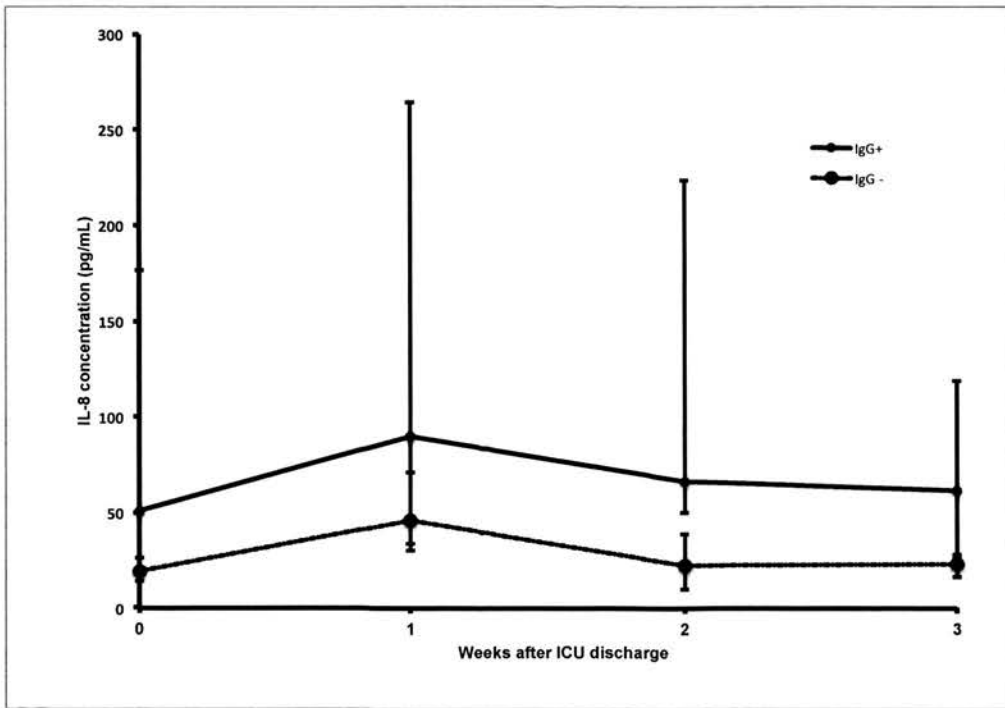


Figure 4-10 Median (IQR) IL-8 concentration after ICU discharge according to CMV IgG status. IgG positive n=10; IgG negative n=4.

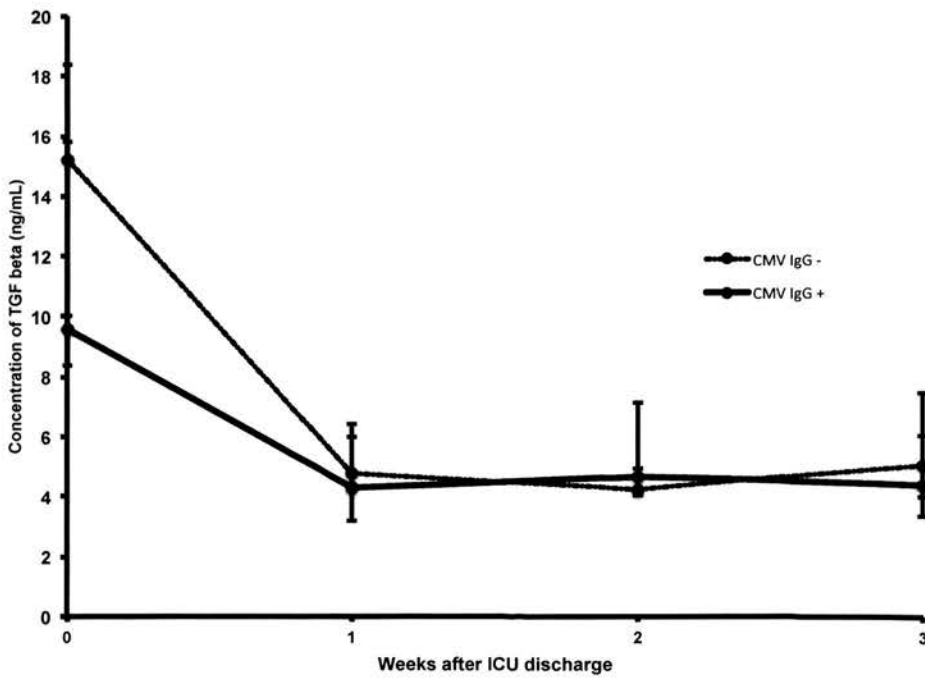


Figure 4-11 TGF β concentration after ICU discharge according to CMV IgG status. IgG positive n=9; IgG negative n=5.

In this subset of patients with prolonged post-ICU length of stay (3 weeks), CRP concentration remained elevated in IgG positive patients compared to IgG negative patients at all time points and decayed at a similar rate. Due to the small sample size (10 patients and 5 patients respectively), statistical inference testing was not carried out and the values observed have widely overlapping confidence intervals (median (95% CI) CRP concentration at baseline in this subgroup was 50 (5-17) in the IgG positive group versus 70 (6-91). It is therefore possible that this observation was made purely as a result of chance. However, given the elevations seen in other biomarkers at baseline and follow up time points, it is also possible that this signals a difference in inflammatory resolution in the IgG subgroups. Similar differences were noted in the IgG subgroups when Il-6 and IL-8 were examined.

As in the whole sample population, TGF beta concentration was higher in the IgG negative patients compared to the IgG positive patients at the point of ICU discharge. During the first week after ICU discharge, serum concentration of TGF beta dropped in both IgG positive and IgG negative patients, reaching a plateau of 4-6 ng/mL for the remaining weeks of the hospital admission. As a substance that is released during resolution from inflammation, its suppression in CMV IgG positive patients who have elevations in pro-inflammatory cytokines some 3 months after ICU discharge may represent an inflammatory resolution defect.

4.6.7.3 Systemic inflammation and CMV PCR

In this section, only patients who were CMV IgG positive were included in the analysis. Patients with evidence of CMV reactivation (CMV IgG positive patients who were also PCR positive) were compared with those without evidence of CMV reactivation (CMV IgG positive patients that were PCR negative). This analysis is summarised in Table 4-10 (see page 168). There was no difference in CRP concentration between these 2 groups at baseline (24 versus 27mg/L p=1.00) or at 3 months (6 versus 5mg/L p=1.00).

Due to the small number of events, the differences in the concentrations of the other biomarkers were difficult to interpret. There were small differences in the median values of some of the biomarkers that had widely overlapping confidence intervals. It is therefore impossible to draw many conclusions from this data. There is no evidence in this sample that patients with latent CMV infection that have evidence of CMV reactivation at ICU discharge are different in terms of their inflammatory biomarkers at ICU discharge or at 3 months after ICU discharge.

	CMV PCR positive (n=13) Median (95% CI)	CMV PCR negative (n=101) Median (95% CI)	p value
CRP (mg/L)			
Baseline	24 (14-79)	27 (18-40)	1.000
3 months	6 (1-24)	5 (3-8)	1.000
SLPI (ng/mL)			
Baseline	56 (42-86)	58 (52-63)	1.000
3 months	62 (32-175)	46 (41-52)	0.407
MPO (ng/mL)			
Baseline	228 (151-293)	181 (161-223)	0.556
3 months	117 (23-198)	138 (118-172)	0.121
HNE (pg/mL)			
Baseline	227 (137-496)	170 (155-193)	0.555
3 months	119 (108-134)	118 (106-134)	0.970
IL-1 β (pg/mL)			
Baseline	2.0 (2.0-14.1)	2.0 (2.0-16.6)	0.587
3 months	0 (0-130)	0 (0-0)	0.645
IL-6 (pg/mL)			
Baseline	24 (9-76)	35 (11-61)	0.785
3 months	11 (0-290)	7 (2-16)	0.709
IL-8 (pg/mL)			
Baseline	36 (15-175)	35 (20-47)	0.944
3 months	5 (0-91)	10 (5-16)	0.938
TGF β (ng/mL)			
Baseline	9 (6-17)	11 (10-13)	0.238
3 months	10 (6-14)	9 (9-10)	0.671

Table 4-10 Comparison of inflammatory biomarkers between CMV IgG positive patients with evidence of CMV reactivation (CMV PCR positive) and those without.

4.6.7.4 Effect of CMV reactivation on biomarker recovery trajectory

Of the 10 patients identified who were CMV IgG positive who stayed in hospital for at least 3 weeks after ICU discharge, only 2 had evidence of CMV reactivation. It was not therefore possible to make a meaningful comparison of biomarker recovery trajectories in this subgroup.

4.6.7.5 Complement proteins

As was the case in the main RECOVER analysis, complement proteins were analysed using a cytometric bead array technique. Due to technical difficulties with this assay, and the resulting loss of sample, no interpretable results are available for complement and as such are not included in the analysis.

4.6.8 Association of CMV status and the post-ICU syndrome

4.6.8.1 Recovery after critical illness and CMV IgG

Measures of physical function, psychological comorbidity and quality of life 3 months after ICU discharge were compared between patients with and without evidence of prior CMV infection (CMV IgG positive versus CMV IgG negative patients). TUG was 10 seconds in IgG negative patients versus 11 seconds in IgG positive patients ($p=0.03$).

There was a clinically small but statistically significant difference in the time taken to perform the Timed Up and Go test. RMI was 1 point lower in IgG positive patients compared to IgG negative patients ($p=0.09$) and % predicted HGS was also slightly lower (63% versus 65% $p=0.42$). Neither of these met the pre-specified threshold for statistical significance. Otherwise there were no differences between the 2 groups. There was therefore no definitive evidence that CMV IgG positivity on its own has a negative impact on physical recovery after critical illness, although there were perhaps some signals that physical function might be poorer.

Psychological outcome as measured by the HADS score showed no difference between the 2 groups. IgG positive patients scored almost twice the

number of points on the DTS signalling a potential increased risk of PTSD symptomology at the 3 month follow up stage.

Finally physical and mental quality of life as measured by SF-12 version 2 were no different between the 2 groups.

	CMV IgG + (n=115) mean* or median** (95% CI)	CMV IgG – (n=68) mean* or median** (95% CI)	p
Physical			
RMI	13 (11-14)**	14 (12-15)**	0.09
HGS (kg)	21 (19-23)*	23 (20-25)*	0.28
2 m TUG (seconds)	11 (10-12)**	10 (8-10)**	0.03
Psychological			
HADS A	6 (5-8)**	6 (4-8)**	0.66
HADS D	7 (5-7)**	6 (4-8)**	0.99
DTS	12 (5-1)**	6 (3-14)**	0.40
Quality of Life			
SF12 v2 PCS	35 (33-37)*	35 (32-38)*	0.85
SF12 v2 MCS	45 (43-48)*	45 (41-48)*	0.82

Table 4-11 Comparison of physical, psychological, and quality of life outcomes in ICU survivors according to CMV IgG status

4.6.8.2 Recovery after critical illness and CMV reactivation

There were no significant differences between the patients who had evidence of CMV reactivation when compared to those that didn't (Table 4-12). The number of IgG patients who had evidence of active CMV infection was small and the follow up incomplete therefore rendering this analysis grossly underpowered. It is therefore difficult to draw any meaningful conclusions about the effects of CMV reactivation in the post-ICU period and recovery after critical illness.

	CMV PCR positive (n=13) mean* or median** (95% CI)	CMV PCR negative (n=101) mean* or median** (95% CI)	p
Physical			
RMI	11 (7-14)**	13 (12-14)**	0.76
HGS (kg)	66.0 (50.8-78.1)*	52.6 (42.2-63.0)*	0.09

2 m TUG (seconds)	13 (9-23)**	11 (10-12)**	0.31
Psychological			
HADS A	5 (2-10)**	6 (5-8)**	0.63
HADS D	7 (5-7)**	6 (1-9)**	0.71
DTS	16 (0-19)**	11 (4-15)**	0.73
Quality of Life			
SF12 v2 PCS	37 (32-42)*	35 (33-37)*	0.50
SF12 v2 MCS	43 (34-53)*	46 (43-48)*	0.63

Table 4-12 Comparison of physical, psychological and quality of life outcomes in CMV IgG positive patients according to CMV PCR status

4.6.8.3 CMV status and hospital length of stay

Hospital length of stay was increased in CMV IgG positive patients who had evidence of CMV reactivation (median of 57 days versus 28 days $p=0.05$). To further explore this, the number of post-ICU days in hospital was compared to test whether CMV PCR positive patients had a more protracted course following ICU discharge that could explain the prolonged hospital length of stay. Patients with evidence of CMV reactivation at ICU discharge stayed in hospital twice as long (median 26 days versus 13 days $p=0.02$).

4.7 Discussion

In chapter 3, significant correlations between serum markers of inflammation and measures of physical recovery were reported. Thus it was speculated that inflammatory outcome and recovery of physical function might be inter-related. Understanding why inflammation is evident in the blood of ICU survivors months after ICU discharge may therefore be of crucial importance if reductions in post-ICU morbidity and enhanced post-ICU recovery are to be realised.

One mechanism that could explain the presence of chronic inflammation in this cohort is the reactivation of latent viral infection either through direct activation of the innate immune system, or by increasing susceptibility to inter-current infections and illnesses.

Numerous studies have reported reactivation of latent CMV infection in ICU patients assumed to have normal immune systems and these have been extensively discussed in the introduction (page 137). These patients spend longer in ICU, longer in hospital, and seem to require a longer duration of mechanical ventilation. They may also be more likely to die. The mechanisms linking CMV reactivation to these adverse clinical outcomes are a matter of speculation at the present time. However as a relatively common occurrence with known clinical consequences, studying CMV reactivation as a potential contributor to inflammatory outcome and/or rehabilitation outcomes is a reasonable line of enquiry.

It could be argued that the ideal study design to explore this hypothesis would be to identify all seropositive patients within an ICU cohort that go on to develop reactivated CMV infection and then follow them up after ICU discharge. As this study was limited to the post-ICU period, it was not possible to do this. On the other hand, by limiting the analysis to ICU survivors only, it is possible to limit the survivor bias attributable to ICU mortality. The fact that very few of the patients in this cohort died subsequent to ICU discharge reduces the risk of this kind of bias – and is a notable strength of the study.

4.7.1 What is the proportion of ICU survivors that have evidence of prior exposure to CMV at ICU discharge?

This study has shown that sixty-three percent of critically ill adults who are ventilated for more than 48 hours have evidence of prior CMV exposure (CMV latency) at the point of ICU discharge. Referring back to the American population described in Table where prevalence rates raise from 65% in 40-49 year olds to nearly 90% in 70-79 year olds, this finding most likely represents that background sero-prevalence rate.

This is lower than the rates seen in a severe sepsis cohort (Kutza et al., 1998) that had a prevalence rate of 94%, a surgical ICU cohort (Cook et al., 1998) that had a prevalence rate of 73%, or a medical ICU cohort (Chiche et al., 2009) that had a prevalence rate of 80%. This is likely to reflect pathological and demographic

differences in these cohorts. From the current study data, there is no reason to suspect that ICU patients have higher rates of prior CMV exposure although it is acknowledged that this statement is speculative only.

4.7.2 What proportion of ICU patients has evidence of CMV reactivation after ICU discharge?

Of the 65% of ICU survivors that had positive CMV IgG serology, 11.4% had evidence of CMV infection at ICU discharge and were considered to have CMV reactivation. This represented 7.2% of the whole sample.

When compared to the studies that examined samples from patients still in ICU, the prevalence of CMV reactivation is low. In 120 IgG positive ICU patients' patients in a mixed unit, 39 (33%) of patients had evidence of CMV reactivation determined by CMV DNA in plasma (Limaye et al., 2008).

None of the previous investigators have sought to determine the duration of CMV infection in their studies. The observation of 11.4% reactivation prevalence at ICU discharge is therefore interesting. Although from the available data in the current study, it is impossible to determine the total number of patients who experience reactivation during their ICU stay, from the work of others, it may be as high as 30%. One explanation for the low rates of CMV infection found at ICU discharge is that most patients with CMV reactivation during critical illness, resolve that infection whilst they are in ICU. For some, the battle to curtail viral replication lasts longer. Another explanation is that patients with CMV reactivation are more likely to die in ICU and the low rates observed reflect this.

From the case note review carried out on CMV PCR positive cases, it is known that only in the case of 1 patient, CMV infection had been suspected and a diagnosis made. In this case, antiviral treatment (Gancyclovir) was given. In all the other cases, the clinical team did not know about the CMV infection. Despite this, all the patients followed up managed to resolve their CMV infection over the following 3 months without receiving specific treatment.

4.7.2.1 What were the characteristics of patients with evidence of prior exposure to CMV?

ICU survivors with evidence of prior CMV exposure did not differ significantly from the other patients in the sample. Patients were of a similar age, and gender distribution, had similar levels of pre-existing comorbidity, and had experienced critical illness of a similar severity.

The finding that patients were in receipt of RRT more commonly if they were IgG positive is worth noting, particularly because patients with renal failure have been observed to have higher circulating levels of inflammatory mediators (Raj 2003). Renal replacement therapy is therefore a potential confounder of any relationship between IgG status and inflammation. Given the very small difference observed, and the lack of statistical significance, it is unlikely to have had a major impact in the current analysis.

Another difference observed was that CMV IgG positive patients had rates of delirium that were more than twice those observed in CMV IgG negative patients. Whilst it is difficult to ascertain the significance of this observation, delirium has been associated in previous studies with prolonged hospital length of stay and increased mortality. The causative relationship between delirium and these outcomes has not been established and some have argued that delirium is really a marker of severity of illness. It is therefore possible that the increased rates of delirium observed in the IgG positive patients in the current study are a subtle indicator of a more severe illness in this group – therefore raising another potential (unquantifiable) confounder of the relationship between inflammatory outcome, and CMV latency.

In a study where most patients had a very low RMI at study baseline, it was interesting to observe that the RMI at ICU discharge was 2 in the CMV IgG positive patients when compared with an RMI of 4 in the CMV IgG negative patients. Although the difference did not quite meet statistical significance, the difference was striking. If the observed difference does reflect a true difference within the population, there are several possible explanations.

Firstly, patients who have previously been exposed to CMV might have poorer pre-existing physical function. Secondly, prior CMV exposure might predispose patients to a more severe illness that results in a greater insult to physical function. Thirdly, the IgG positive patients, a proportion of whom may have experienced CMV reactivation during critical illness, are more likely to have experienced damaging pulmonary insults (caused by CMV pneumonitis) and therefore have a cardiopulmonary reason for poor baseline mobility. It is impossible to determine which of these is the explanation and it warrants further exploration in future studies. The first 2 are probably more likely than the third due to the relative rarity of CMV pneumonitis.

4.7.2.2 What were the characteristics of ICU survivors with evidence of CMV reactivation at ICU discharge?

Before considering the differences in the patients with and without evidence of CMV reactivation at the time of blood sampling, it is worth considering the possible circumstances that could have led to these 2 states.

The most likely sequence of events given the findings of the previous literature is that a proportion of patients previously exposed to CMV at an earlier stage in their life, reactivated latent CMV infection during critical illness. These reactivations are likely to have been triggered by pro-inflammatory stimuli in susceptible individuals. Following reactivation, the immune system responds firstly by activation of the innate immune response, and secondly by adaptive responses. In the majority of patients, these responses bring the reactivation under control so that by ICU discharge – the point at which organ failure has resolved, only a minority of patients still have evidence of on-going viral replication – the CMV PCR positives in this study. Those patients who are CMV negative are likely to represent 2 groups of patients. One group may consist of patients that have reactivated their CMV and in whom the immune response has curtailed viral replication. The other group may consist of patients that never reactivated. Those

patients in the CMV negative group may therefore represent patients at the end of distinct pathological processes.

The other less likely explanation for the observation is that the arbitrary single sampling time point at ICU discharge picks up patients at different stages of their CMV reactivation. Assuming that all ICU patients resolve an episode of CMV reactivation over a similar time frame (and this is unlikely to be the case), this theory would mean that positives and negatives represent patients that have reactivated earlier and later in their ICU stay. This is unlikely due to the sampling point at ICU discharge where patients could be considered to be at the same stage of their recovery from acute illness.

Patients who had evidence of CMV reactivation (CMV IgG positive patients that were CMV PCR positive) did not differ significantly from the other patients in the sample. Patients were of a similar age, and gender distribution, had similar levels of pre-existing comorbidity, and had experienced critical illness of a similar severity according to APACHE 2 score after 24 hours, vasopressor use, and use of renal replacement therapy.

Patients with CMV reactivation had a longer length of ICU stay and had poorer physical function at ICU discharge as indicated by a lower score on the RMI and a lower HGS. These findings suggest that patients with evidence of CMV reactivation had a more protracted illness, and it is possible that this had a negative impact on physical function. There is no evidence of any difference in prior functional comorbidity in the reactivation group making it possible, but unlikely that pre-existing functional deficits explain this observation. Unfortunately the current study cannot answer this question definitively.

In the present study, active CMV infection was observed in 11.4% of IgG seropositive ICU survivors at the point of ICU discharge. Other than the criteria imposed by the RECOVER study, the sample included all ICU patient groups. Patients with risk factors for CMV reactivation were not identified in advance and excluded. Despite this, in only 55% of cases of CMV reactivation were specific risk factors found on case note review – the most common of these being sepsis, present

in 36% of the cases studied. Other risk factors identified were blood transfusion (2 cases), organ transplant (2 cases), corticosteroids (2 cases) and splenectomy (1 case).

4.7.3 Is CMV infection associated with resolution of inflammation?

Perhaps the most interesting observations made in this study were the differences in inflammatory biomarkers in ICU survivors depending on whether they were CMV IgG positive or negative. At ICU discharge, HNE, IL-6, and IL-8 were all elevated in IgG positive patients and whilst IL-6 and IL-8 could only be considered to have achieved borderline statistical significance, the highly significant elevation in HNE lends support to a more severe on-going inflammatory process in the IgG positive patients. Furthermore, the highly significant difference in pro-resolution biomarker TGF beta concentration at the same time point with lower levels in the IgG positive patients supports the theory that there is delayed switching from a pro-inflammatory to a pro-resolution state.

Three months after ICU discharge, the persistent activation of the immune system of the ICU survivor is more pronounced in IgG positive patients. Again IL-6, IL-8 and HNE are found in greater concentrations and TGF beta is found in lower concentrations in these patients. In addition, a small difference in CRP concentration is evident with IgG positive patients having a CRP that is almost twice that of IgG negative patients. This strongly suggests that CMV IgG positive patients resolve inflammation at a different rate to IgG negative patients.

The exploratory longitudinal analysis of inflammatory biomarkers whilst statistically underpowered also supports this hypothesis.

The limitations of this analysis should be emphasised. Firstly, it is acknowledged that issues pertaining to multiple testing may have affected the analysis. Despite this, the difference in HNE concentration observed both at ICU discharge and 3 months later would have achieved significance at a Bonferonni adjusted p-value threshold of 0.004. Moreover, this analysis was pre-specified and strongly hypothesis driven both of which add considerable strength to the analysis and its findings.

Another weakness is one that is shared by all studies of an observational design – that of confounding. This feature contributes a significant limitation to the interpretation of the results. From the measured baseline characteristics, there are very few indications that IgG positive patients are a different group to those who are IgG negative. There are more patients that received RRT during their ICU stay in the IgG positive group and it is conceivable that this could contribute to a prolonged ICU state. However, the subgroup analysis carried out in chapter 3 does not support this.

Finally, it should be recognised that this is a study of ICU survivors and some patients that have CMV disease may have died in ICU before study recruitment. This limits the external validity of our findings to ICU survivors and should not be applied to all ICU patients.

4.7.4 The effect of CMV reactivation on inflammatory profile

In a subgroup of IgG positive patients (114 patients), inflammatory markers were not significantly different at any of the time points studied between patients with or without evidence of CMV reactivation. In these comparisons, there were subtle differences in the median values of biomarkers but the wide confidence intervals made their interpretation impossible.

The most likely explanation for this observation is that the sample size simply wasn't large enough to give an accurate impression of the biomarker behaviour. The number of patients with reactivation was only 13. In addition, the large number of patients without evidence of CMV reactivation could represent 2 separate groups of patients (see also p175) – those who have not reactivated, and those who have reactivated, but the immune system has managed to curtail viral replication. To properly assess the impact of CMV reactivation during critical illness as a risk factor for persistent inflammation, serial sampling designed to capture all episodes of CMV reactivation with a prolonged post-ICU longitudinal study of biomarkers would be more suitable.

4.7.5 Is latent CMV infection associated with recovery from critical illness?

There were some small signals that CMV latency might be a risk factor for physical but not psychological comorbidity 3 months after an episode of critical illness. CMV IgG positive patients scored 1 point less on the RMI ($p=0.09$) and were 1 second slower in performing the 2-minute timed up and go test ($p=0.03$). Despite the apparently small difference in 2m TUG, a 1 second difference is likely to represent a significant difference in frailty. In the case of RMI, the majority of patients in RECOVER had very high scores on RMI at 3 months despite experiencing significant disability. The observation of even this small difference may therefore be of some clinical significance.

The other observation of relevance was that IgG positive patients had a significantly longer post-ICU length of stay. This observation may be the key to understanding the observed inflammatory resolution defect. These patients may be more at risk of recurrent infections that may prolong hospital stay, and lead to prolonged elevations in inflammatory biomarkers.

4.7.6 Is CMV reactivation associated with recovery from critical illness?

The question of whether CMV reactivation is associated with recovery from critical illness has not been adequately addressed by this study. As was the case with the exploration of inflammatory resolution in this subgroup, the small number of reactivation events detected limited the analysis. A further limitation was the possibility that the negatives might represent 2 different pathophysiological groups as outlined above.

There were some small differences noted. Median RMI was lower in the patients with evidence of reactivation and the 2m timed up and go took 2 seconds longer. Mean HGS was slightly higher in the CMV reactivation patients. None of these differences were supported by significant p values, and confidence intervals were widely overlapping. It was therefore difficult to draw any conclusions from this analysis.

The most significant finding in this analysis was that post-ICU hospital length of stay was far longer in patients with CMV reactivation.

4.8 Conclusions

A large proportion of ICU survivors have evidence of prior CMV exposure when tested at the point of ICU discharge. Despite being similar in terms of severity of illness, demographics and prior comorbidity, prior CMV exposure is associated with differences in serum concentrations of both pro-inflammatory and pro-resolution biomarkers in a pattern that would support the theory of a defect in inflammatory resolution caused by latent CMV. Furthermore, there are signals in the data that suggest that CMV latency may also be a risk factor for delayed functional recovery after ICU discharge – a finding that warrants further exploration.

A small but significant proportion of these patients also have evidence of active CMV infection (CMV reactivation) at ICU discharge. Many of these patients do not have obvious risk factors for reactivation, and may represent up to a third of patients who have reactivation triggered by an episode of critical illness. The significance of this subgroup in terms of post-ICU outcome remains unclear.

These patients experienced longer ICU stays and more prolonged hospital stays than seropositive patients that did not have evidence of CMV reactivation. Levels of physical function were poorer at baseline but this did not translate into statistically significant differences in function at follow up.

Chapter 5 - Derivation of a predictive tool to identify patients at risk of delayed physical recovery after critical illness

5.1 Introduction

An increasing awareness of the physical, psychological and social problems faced by ICU survivors has led to a surge in research activity in this area – the RECOVER study being a good example. The hope is that some of these studies will identify therapeutic interventions that could improve the overall quality of life for ICU survivors.

Intensive care research is limited by the heterogeneity of its patients, and the illnesses that result in a critical care admission. Some patients may benefit from a certain intervention, and some may not. In some cases, these competing effects may cancel each other out – therefore failing to demonstrate overall benefit for the population studied. Even in studies that do demonstrate benefits to patients, it is likely that certain groups of patients may benefit more than others. Subgroup analysis is an important component of trial design that can enable greater understanding of the effects of an intervention, particularly when a trial fails to show a significant overall result.

In the case of functional recovery after critical illness, some patients may have a good functional outcome regardless of the treatment that they receive. Investment in a complex rehabilitation package for these patients may therefore be an unjustifiable expense as well as an unnecessary burden.

There is an urgent need to be able to identify at an early stage in a patient's illness their likely recovery trajectory and individual need for enhanced rehabilitation. Despite the widespread recognition of the post-ICU syndrome, surprisingly little is known about risk factors for persisting disability. The following paragraphs summarise previous studies in this area.

5.1.1 Risk factors for persisting disability after ICU discharge

In a Canadian follow up study of acute lung injury (ALI) patients, a number of patient characteristics were associated with decrements in physical function (greater number of organ failures, longer ICU stay, surgical admission diagnosis, lower blood glucose concentration, lower doses of sedative, depression at follow up). However, only depression remained significantly associated when multivariable adjustment was carried out to control for the effects of known confounding factors (Bienvenu et al., 2012).

Another observational study of 194 American cardiothoracic ICU patients measured predictors of persistent dependency as defined by a Karnofsky score of less than 80 points (Gersbach et al., 2006; Grieco & Long, 1984). This study identified the occurrence of a peri-operative neurological event as the major risk factor for long-term disability – relevant in an a post cardiac surgery setting, but not useful in a general adult ICU population.

In a study of 516 survivors of severe sepsis, an American group demonstrated significant increases in cognitive impairment and functional limitations as a result of an episode of severe sepsis. This study did not demonstrate that severe sepsis provided an increased risk of these problems in addition to the effects of critical illness (Iwashyna, Ely, Smith, & Langa, 2010).

Patients who have pre-existing deficits in physical function cannot be expected to recover to the pre-illness functional levels of fitter patients. Therefore pre-illness characteristics are of vital relevance to the prediction of function in the months following ICU discharge. The functional comorbidity index (FCI) was developed in a cohort of nearly 40,000 individuals drawn from 2 databases in the USA and Canada (Groll, To, Bombardier, & Wright, 2005). The index consists of 18 comorbidity categories each of which has been shown to be strongly associated with physical function (as measured by the physical component of the Medical Outcome Study SF-36 (P-SF36)). This score has subsequently been validated in 73 survivors of ARDS (Groll, Heyland, Caeser, & Wright, 2006). It has never been evaluated in an unselected ICU cohort. This study also had a relatively small sample size, and

the linear multivariable model used to evaluate the FCI also included 10 other variables (age, gender, APACHE2 score, P/F ratio, baseline SOFA score, ICU and total hospital length of stay, cause of lung injury, and smoking status). Whilst none of these other factors were associated with physical outcome as measured by the P-SF-36 it is likely that the analysis was underpowered.

5.1.2 Clinical prediction tools

Clinical prediction tools can be derived using multivariable data to allow identification of patients who are at risk of a certain outcome. These rules assist and in some cases improve the decision-making ability of clinicians (Adams & Leveson, 2012). As described by Adams et al (Adams & Leveson, 2012), rule development requires a number of mandatory steps, all of which are necessary for a rule to become useful in the clinical context. These steps are development, validation, impact analysis, and implementation and are summarised in Table 5-1.

Step	Description
Development	Identification of risk factors from an observational study
Validation	Testing of the tool in another clinical setting to ensure reliability
Impact	Measure clinical usefulness in terms of cost benefit, patient satisfaction, and time resource allocation
Implementation	Widespread acceptance and adoption of the tool

Table 5-1 Stages of clinical prediction rule design as described by Adams et al

5.1.3 Available clinical prediction tools for post-ICU outcome

A literature search carried out in the MEDLINE database returned 1 study that had attempted to develop a clinical prediction tool for the purpose of identifying patients at risk of post-ICU complications (Schandl, Bottai, Hellgren, Sundin, & Sackey, 2013). The investigators reported the development of a prediction tool for psychological comorbidity after critical illness and represented a component of a research degree thesis that also described the development of a clinical prediction tool for physical disability (Schandl, 2013).

The author reported a process of selecting candidate variables for both prediction models using a combination of literature review and multidisciplinary input. Eighteen risk factors were selected for consideration in both the psychological and physical prediction tools. Additional specific risk factors were added as a result of expert opinion. These risk factors were assessed individually in univariable logistic regression models for binary outcomes of psychological complications (anxiety, depression, or PTSD) and physical complications (physical dependency according to the ADL staircase).

Major pre-existing disease, having young children, previous psychological problems, being agitated in ICU, pre-morbid unemployment, and depressive symptoms in ICU were predictors of PTSD, anxiety or depression and were included in the final psychological prognostic tool. Education level, reduced core stability at ICU discharge, fractures, and ICU length of stay >48 hours were identified as independent risk factors for physical dependency and were included in the final physical prognostic tool. Both tools performed well from a statistical point of view. The predictive ability of the psychological and physical prediction tools were measured by area under the receiving operator characteristic curves of the derived indices and were 0.72 and 0.82 respectively.

Despite being impressive statistically, these scores have yet to be validated in other patient groups. In addition there were a number of methodological problems, some of which were acknowledged by the author that limit their applicability. Firstly, there were a large number of patients who were excluded from the derivation cohort for reasons that may have introduced selection bias. Of 389 eligible ICU survivors studied over a 6 month period, 137 patients (35%) were excluded. 41 (11%) were excluded on the basis of language, and 10 (3%) were excluded on the basis of pre-existing cognitive impairment.

In addition, there was a high drop out rate leading to a high risk of attrition bias. One hundred and two patients (41%) were lost to follow up. Nineteen of these were deaths. However, 83 patients were non-responders. Although there were no statistically significant differences in this group of non-responders compared to the

study population, bias introduced by this level of loss of follow up cannot be excluded and will make generalisation difficult.

There were also some issues with the model derivation. Following the work by Peduzzi et al that demonstrated problems with inference testing and estimation of coefficients at low numbers of events per variable (EBV) in logistic regression analysis, it has been recommended that an EBV of 10 is the minimum required to make the model function appropriately (Peduzzi, Concato, Kemper, Holford, & Feinstein, 1996). In the derivation of the psychological prediction tool, there were 46 'events' and 7 variables were included in the multivariable analysis. This gives an EBV of 6 that makes the model at high risk of this sort of problem. In the thesis, the author does not report the physical event rate so one cannot comment on EBV in this case.

A final limitation of the work is the lack of a clear definition for physical dependence. The author states the use of the ADL ladder to diagnose dependence. However there are varying degrees of dependence described by this score and the level of dependence is not stated in the thesis text.

Overall this single prognostic rule derivation study has derived strong diagnostic tests for physical and psychological disability within the limitations described. Importantly, it has identified some potential candidate risk factors for future work. Further exploration of risk factors and derivation of a new prognostic scale whilst taking into account the limitations of previous studies can now be justified.

5.1.4 The RECOVER study sample – an opportunity to derive a clinical prediction tool for post-ICU complications

The RECOVER study investigators collected a rich database of baseline variables relating to pre-existing patient factors (age, gender, Functional Comorbidity Index), critical illness (diagnosis, severity scoring, duration, organ support), and clinical data (delirium scoring, muscle strength, and baseline mobility).

As already discussed, some of these factors have been tested as risk factors for physical disability after critical illness. Some have not been previously tested. The availability of such a complete and rich dataset in a heterogeneous ICU population presented an opportunity for the development of a predictive model that might be useful for the detection of patients with good and poor function after critical illness.

In previous chapters, it has been shown that inflammatory outcome and latent CMV infection is associated with physical function after critical illness. It is therefore reasonable to suspect that measurement of inflammatory biomarkers and CMV IgG status at the point of ICU discharge could add additional strength to a prognostic tool.

The aim of this chapter was to identify potential risk factors for poor physical recovery after critical illness and attempt to incorporate them into a clinical prediction tool. The performance of this tool was evaluated with and without the addition of blood biomarkers data.

5.2 Research Questions

- Which risk factors for post ICU physical immobility can be identified from the RECOVER study baseline data?
- Can physical recovery after critical illness be predicted using readily available data at ICU discharge?
- Can prediction of physical outcomes be improved by the addition of markers of inflammation and inflammatory resolution measured at ICU discharge?

5.3 Methods

5.3.1 Patients

As with chapters 3 and 4, patients participating in the RECOVER study were approached for consent to participate in the biomarker study. Consenting patients

who were not lost to follow up and did not have missing data in the target variables were included in the analysis.

5.3.2 Analysis plan

In order to minimize the risk of researcher bias creeping in during the analysis stage, a signed and dated analysis plan (Appendix H) was produced prior to the trial outcome data being made available to the author. For various reasons this analysis plan required modification. This analysis plan and modifications are described below

5.3.3 Number of candidate variables

To minimise problems with bias errors in coefficient calculation, the rule of 1 variable per 10 cases was used. In the original analysis plan, this allowed for the assessment of a maximum of 15 candidate variables in a linear regression model on a projected sample size of 150 patients.

In the RECOVER biomarker study, the final sample size was 175 patients. This allowed for the testing of a maximum of 17 candidate risk factor variables.

The study aimed to produce 2 tools, one using the available clinical data, and the other adding inflammatory biomarker concentrations to the available clinical data, to assess whether this improved predictive ability. The author wished to assess 7 biomarkers and CMV IgG status in the second tool. For this reason, a maximum of 9 clinical data variables were permitted in the first tool, and 17 in the second.

5.3.4 Identification of candidate independent variables

The design of the original analysis plan pre-dated the publication of the prognostic model work by Schandl and colleagues (Schandl et al., 2013). This selection of candidate clinical variables therefore followed a pragmatic approach. It was hypothesised that physical outcome at 3 months might be predicted by pre-existing characteristics, characteristics of critical illness, duration of critical illness,

evidence of the physical consequences of critical illness at ICU discharge, and inflammatory biomarker concentrations at ICU discharge. Clinical data available at ICU discharge were evaluated, looking for variables that represented each of these potential contributing factors.

5.3.4.1 Pre-illness characteristics

FCI and age were included in the original analysis plan. In the modified model, social deprivation category was also included to take into account the association found between education level and functional outcome in Schandl's study (Schandl, 2013). Although social deprivation category and education level are not the same thing, social deprivation is one of the strongest predictors of educational attainment and is therefore a valuable surrogate (Perry & Francis, 2010).

In chapter 4, it was found that latent CMV infection was associated with higher concentrations of pro-inflammatory mediators, poorer mobility, and reduced muscle strength (Schandl, 2013). CMV IgG was therefore included as an independent variable. Another variable, worst sequential organ failure score (SOFA score) was initially considered. Although this variable was included in the original analysis plan, due to a protocol change, it was not actually recorded as part of the RECOVER dataset and was excluded.

Diagnosis was not considered in the original model due to the limitations placed by the proposed sample size. However due to the increase in the final sample size, and the loss of the worst SOFA variable, diagnosis of sepsis was tested in the revised model to reflect the importance placed on sepsis as a cause of reduced function described by Iwashyna et al (Iwashyna et al., 2010). A surrogate indicator of sepsis was derived from the available data using the following method. ICU admission diagnoses were obtained from the Scottish Intensive Care Society Audit Group codes obtained at study baseline. Codes associated with infection (e.g. septic shock – respiratory) were grouped into the binary categorical variable 'infection'.

5.3.4.2 Evidence of the detrimental effects of critical illness at ICU discharge

Physical function at ICU discharge was assessed by means of the Rivermead mobility index (described in detail in section 3.10.1) and was included in the proposed analysis. Another physical assessment carried out at this timepoint was the physical examination component of the Subjective Global Assessment tool (SGA). This SGA was originally described in the late 1980s and is a validated tool for assessing nutritional status (Lawson et al., 2013). The physical component of SGA was included in the RECOVER study as a screening tool for the physical affects of protein malnutrition. Although not included in the initial proposal, due to the limitations imposed by the projected sample size, this is one of the additional variables tested in the modified model due to the larger actual sample size.

5.3.4.3 Biomarkers of inflammation and inflammatory resolution

A prognostic model was built and assessed initially using the 8 variables described in the first 3 sections. Following this, a further model was built by adding 7 biomarkers of inflammation, and CMV IgG status.

These variables were CRP (section 3.9.1), IL-6 (section 3.8.4), IL-8 (section 3.9.6), TGF beta (section 3.8.8), MPO (section 3.8.3), neutrophil HNE (section 3.8.2) and SLPI (section 3.8.7). Their use as markers of inflammation and inflammatory resolution has been justified in the indicated sections.

Category	Planned analysis	Revised analysis
Pre-illness characteristics	Age FCI	Age FCI SIMD quintile CMV IgG status
Characteristics of critical illness	APACHE2 ICU length of stay Worst SOFA score in ICU	APACHE2 Ventilator days Diagnosis of sepsis
Evidence of detrimental effects of critical illness at	Baseline RMI	Baseline RMI Physical component of

ICU discharge		SGA
Biomarkers of inflammation and inflammatory resolution	CRP	CRP
	IL-6	IL-6
	IL-8	IL-8
	TGF-beta	TGF-beta
	MPO	MPO
	HNE	HNE
	SLPI	SLPI

Table 5-2 – details of the proposed and revised independent variables.

5.3.5 Outcome variable

The primary outcome measure was Rivermead Mobility Index (RMI) discussed in detail in section 3.10.1. RMI is a 15 point scale consisting of 14 functional questions and 1 direct observation (see also appendix C).

5.3.6 Choice of regression technique

The choice of technique for deriving a prognostic model is determined by the characteristics of the data to be modelled, the sample size, and the desired ease of use of the final prognostic tool. Binary outcomes such as mortality can be modelled using linear regression techniques. However, because the outcome can only take 2 levels (e.g. alive or dead), the resulting models are difficult to interpret. Logistic regression models circumvent this issue by converting the outcome into the ‘logit’ – the natural logarithm of the odds of an outcome. The linear relationship between a particular variable and the logit is then modelled. An additional benefit is the derivation of odds ratios to describe the influence of particular variables. Odds ratios are conceptually easier to interpret clinically. As alluded to in the introduction, because logistic regression relies on probabilities, if the event rate is very low, or the number of modeled variables is high resulting in a low EBV, logistic regression models become biased. Therefore for small samples, linear models are preferable if the data will support it.

Linear outcome variables are best modelled using linear regression techniques. Linear regression is not subject to the same issues relating to sample size and event rates that logistic regression suffers from. Therefore, a greater number of independent variables can be tested.

The Rivermead Mobility Index (RMI) was the chosen outcome measure for this study. The RMI is a categorical variable with 15 levels and therefore does not lend itself to either a logistic regression or a linear regression technique. The potential options were to dichotomize RMI into good and poor categories as was carried out in 3.14.4 and apply logistic regression. However, given the loss of power that would result from this categorisation and the reduction in the permitted number of independent variables, a linear regression approach was adopted. This approach is considered acceptable practice provided that the data meet the model assumptions imposed by a linear regression technique.

5.3.7 Choice of independent variable selection method

Various automatic variable selection methods have been proposed to reduce a list of candidate variables into a smaller number of variables in multivariable model. Generally speaking, their use is discouraged, particularly when investigating the effects of known or suspected confounding factors in risk factor analysis. However, the development of a prognostic model is one situation where variable selection methods can be useful. Reducing the number of variables in a model can be useful to reduce the complexity of a prognostic score and by doing so increasing the likelihood that the model will be used in clinical practice.

All automatic variable selection techniques include or exclude independent variables depending on various pre-set statistical conditions. Two examples of automatic variable selection techniques are forward selection, and backward selection. In forward selection, the model starts with no variables in it. Bivariate associations are tested and the variable that is most significantly associated with the outcome variable is included first provided it meets a pre-specified level of significance. Following this the variable that has the greatest association with the

outcome variable taking into account the effects of the first variable is entered into the model. Again, entry of the second variable depends on it reaching a pre-specified level of significance. The process continues until there are no more variables that have a significant association with the outcome variable.

Backward selection starts from the position of having all variables in the model. Variables that have significance levels above a pre-specified value are assessed. The variable that has the weakest association with the outcome variable is then removed from the model. This process continues until none of the included variables have a significance level above the pre-specified value.

Variable selection methods do not always end up drawing the same conclusions and consistency of results between methods has been used as an indicator of model strength. The forward method tends to produce more restrictive models with fewer variables. The backward selection technique is considered to be more inclusive. For this reason a backward selection technique was chosen for use in this study. This differs from the stepwise technique originally proposed.

5.3.8 Conduct of the multivariable regression

Having defined a list of candidate variables for inclusion in the multivariable linear regression model, bivariate relationships with RMI were assessed. Variables with a p value of less than 0.25 were taken forward for assessment in the multivariable models. The first multivariable model tested only the non-serological variables using data readily available at ICU discharge. Following this, any biomarkers considered significant on bivariate testing were added to those tested in the first model to determine whether the addition of biomarkers to the model could increase its prognostic significance.

As discussed in section 6.3.6, variable selection was carried out using backward selection with a p value for removal of 0.1.

5.3.9 Checking model assumptions

The reliability of a linear regression model depends on the extent to which the data meets the assumptions of the chosen technique. Accepted linear regression model assumptions are:

1. The relationship between the independent variables combined in the linear regression equation and the outcome variable is linear in nature.
2. The regression equation defines the mean value of the outcome variable for a set of independent variables.
3. Predicted values of the outcome variable are normally distributed around the regression line.
4. The variance around the regression line is the same for each independent variable.
5. The independent variables included in the model are not related to each other.

5.3.9.1 Linearity

To assess linearity within the fitted model, residual analysis was undertaken. A residual is the distance between the mean value of the regression equation and the observed value of the outcome variable. The standardised residual is the residual divided by the standard error of the residual. To test the assumption of linearity, standardised residuals were plotted against each of the independent variables and the predicted value of the outcome variable. Linearity was assumed if the spread of residual values was similar at all levels of each independent variable.

5.3.9.2 Normal distribution of predicted values of the outcome variable around the regression line

Q-Q plots and histograms were used to assess normal distribution of the data points of the residuals around the regression line. Q-Q plots present quantiles of a theoretical normal distribution for a variable against the observed data points.

Normal distribution can be assumed if the coordinates of the Q-Q plot closely fit (or 'hug') the reference line.

5.3.9.3 Equal variance

Equal variance was assessed from the plots of the standardised residuals against predicted outcome. Equal variance was assumed if the spread of the residuals was even above and below zero.

5.3.9.4 Collinearity

Collinearity was assessed using a scatter plot matrix, and calculating the correlation coefficients. If the correlation coefficient between 2 variables exceeded 0.7, collinearity would be suspected. The effect on the model standard error of including the 2 variables to the other variables in the model will be observed. If there is no increase in standard error, the impact of any collinearity was assumed to be negligible and both variables were included in the model. If significant effects on the model were detected, only one of the variables was included.

5.3.10 Analysis of outlying and potentially influential data points

Plots of standardised residuals against case number were used to identify potential outliers. Any residuals exceeding 2 standard deviations were examined to check for errors in data entry. Leverage values were calculated and any values greater than $3(p+1/n)$ (where p = number of variables in the model including the constant and n = number of subjects in the sample) were examined as potential influential data points. To test the actual effects of potentially influential data points, Cook's distances were calculated. Any points with a Cook's distance greater than 1 were considered influential.

5.3.11 Assessing goodness of fit

As a method for comparing regression models, goodness of fit was compared by use of the R^2 statistic. The R^2 statistic is a measure of how much of the

observed behaviour of an outcome variable is explained by the regression equation. An R^2 statistic is a numeric value ranging from 0 to 1. A value of 0 indicates that none of the observed behaviour can be explained by the regression equation indicating that it is of no value. A value of 1 indicates a perfect fit – where all the behaviour is explained by the regression equation. A value of 0.5 indicates that half of the behaviour is explained by the regression equation. To provide some context from intensive care research, the APACHE 2 score had an R^2 value of 0.319 (Knaus, Draper, Wagner, & Zimmerman, 1985).

5.3.12 Assessing the clinical utility of the linear regression model

One potential application of a prognostic index is the prediction of ICU survivors that will have very good functional outcomes regardless of intervention. Complex rehabilitation interventions for these patients may be expensive, unnecessary, and an unwanted burden. To assess the potential clinical impact of the prediction tools, a group of patients deemed to have ‘good’ outcomes was defined. The current practice for triaging patients to rehabilitation within NHS Lothian was identified. Following this, the performance of each index was compared to current practice.

5.3.12.1 Identifying patients with good outcomes

The RMI describes the full range of physical disability describing patients with minimal functional limitation right through to patients who are entirely bed bound. Patients scoring less than 4 are likely to be bed bound. Patients scoring less than 6 are unlikely to be able to stand and are limited to bed and chair. Patients scoring less than 8 are unlikely to be able to walk with or without an aid. Patients scoring less than 10 are likely to be limited in function to such an extent that they are unlikely to be able to function independently. Patients with an RMI above 10 whilst possibly exhibiting deficits in physical function are likely to be able to function independently in their home environment provided climbing stairs is not a

pre-requisite. For the current analysis, patients who had returned to independence, defined by an RMI of greater than 10 were considered to have a 'good' outcome.

5.3.12.2 Identifying current practice for triaging patients to rehabilitation

The physiotherapy department at NHS Lothian was contacted, and information regarding rehabilitation triage was discussed. Current practice within NHS Lothian is to determine through history taking a patient's prior level of function. When a patient's function differs significantly from normal, they are considered to require rehabilitation. Because there is no formalised mechanism for making this assessment the reality is that all ICU patients are seen in ICU before they leave and are subsequently followed up on the ward. In the RECOVER trial, this process was formalised so that in the intervention arm, treatments were triggered based on an assessment of prior function, and a patient's deviation from this normal.

In essence, an assessment of function at ICU discharge is used as to predict function 3 months later. For this reason, RMI at ICU discharge was used as a surrogate for current practice and compared with the derived prognostic indices.

5.3.12.3 Comparing triage methods

Observed RMI at 3 months was dichotomised according to the threshold score of 10 to identify 2 groups of patients – those with good outcomes, and those with poor outcomes. Receiver operating characteristic (ROC) curves were constructed using RMI at ICU discharge (current practice), and each of the derived prognostic indices. Prognostic accuracy was determined by calculating the area under the curve (AUC) for each of the ROC curves. AUC is equal to the probability that in individual with a particular condition of interest (in this case good functional outcome) has a higher (or lower) value of a measurement than an individual without (Altman & Bland, 1994)

For any diagnostic test, a trade off must be made between sensitivity and specificity. Sensitivity is defined as proportion of true positives (those identified by

the test who actually have the condition of interest) that are identified by the test. Specificity is the proportion of true negatives (those identified by the test as not having the condition who actually do not have the condition of interest).

The author was interested in identifying patients with particularly good outcome – those patients in whom rehabilitation may be less important. To accurately identify these patients, a test with good sensitivity was required. To prevent exclusion of patients in whom rehabilitation might be beneficial, a high level of specificity was required. To explore the potential utility of each model, different cut-off values for each mode were selected according to pre-defined levels of specificity (60%, 70%, 80%). Cut-off values were determined using the coordinate points on the ROC curves. The sensitivity achievable at these cut-offs was assessed.

Using cut-off values corresponding to a specificity of 80%, pre-test and post-test probabilities for a negative and positive test result were compared for different triage methods. Pre-test probability was defined as the prevalence of good physical recovery (proportion of patients with RMI<10). Post-test probability was calculated using likelihood ratios (Deeks & Altman, 2004).

5.4 Results

5.4.1 Bivariate relationships of candidate predictive variables with RMI

A summary of bivariate relationships between RMI and each of the candidate independent variables is presented in Table 5-3. The clinical variables significantly associated with RMI at a p value of less than 0.25 were age, FCI, ventilator days and baseline RMI. These were taken forward for assessment in the first multivariable model. The serological markers associated with RMI at a p value of less than 0.25 were CMV IgG status, CRP, and SLPI. These were added to the variables considered in the first model to assess whether a model with greater predictive accuracy could be derived using serological markers in addition to clinical variables.

Candidate variable	Beta coefficient	p
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Age	-0.03	0.06
FCI	-0.20	0.08
SIMD Quintile	0.08	0.63
CMV IgG status	-0.79	0.12
APACHE 2	-0.02	0.56
Ventilator days	-0.05	0.02
Diagnosis of Sepsis	-0.40	0.39
Baseline RMI	-0.33	0.00
Physical component of SGA	-0.32	0.37
CRP	-0.01	0.04
IL-6	0.00	0.54
IL-8	0.00	0.46
TGF β	-0.04	0.39
MPO	0.00	0.84
HNE	0.00	0.56
SLPI	-0.02	0.04

Table 5-3 Bivariate relationship of candidate baseline variables with RMI. Variables taken forward for consideration in the multivariable models are shaded.

5.4.2 Multivariable analysis testing baseline clinical variables only

The results of the multivariable analysis using a backwards variable selection technique (as proposed in the modified analysis plan) including age, FCI, baseline RMI, and ventilator days are presented in Table . Ventilator days, baseline RMI, and FCI were significantly associated with RMI at 3 months and were included in the final model (Model 1)

Variable	Beta coefficient	Significance
Constant	12.444	-
Ventilator Days	-0.047	0.02
Baseline RMI	0.301	0.00
FCI	-0.231	0.03

Table 5-4 Results of the multivariable model using clinical data only (Model 1)

5.4.3 Multivariable model testing clinical variables and serological markers

Interestingly, the serological markers (CRP, SLPI, and CMV IgG status) that had p values below the inclusion threshold on bivariate testing were not associated with RMI at 3 months when the confounding effects of the clinical data were taken into account. The model yielded a multivariable model was identical to that produced in section 5.4.3.

5.4.4 Testing linear regression model assumptions for Model 1

On an initial assessment of the data, it was noted that the outcome variable RMI was very severely skewed. This is illustrated in Figure 5-1. As a result, there was considerable concern that the model produced in section 5.4.2 might not be valid due to violations of the assumptions of linear regression.

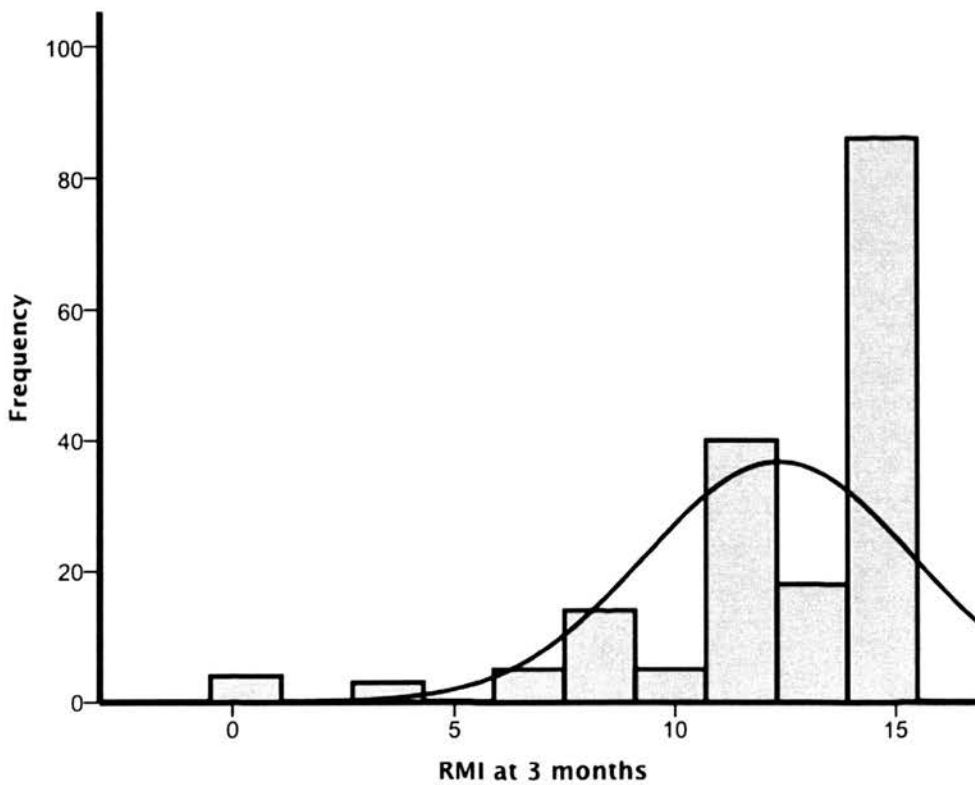


Figure 5-1 Distribution of RMI at 3 months

5.4.4.1 Linearity

Plots of standardised residuals of the regression equation against the included independent variables and the predicted value of the regression equation are shown in the figures below. The spread of residuals is consistent across all levels of ventilator days and FCI. However, the spread of residuals is inconsistent across levels of baseline RMI, suggesting non-linearity in the relationship between RMI at baseline and RMI at 3 months. Furthermore, the graph depicting residuals plotted against the predicted value of the regression equation is extremely abnormal suggesting that the relationship between the independent variables combined in the model, and RMI at 3 months is non-linear.

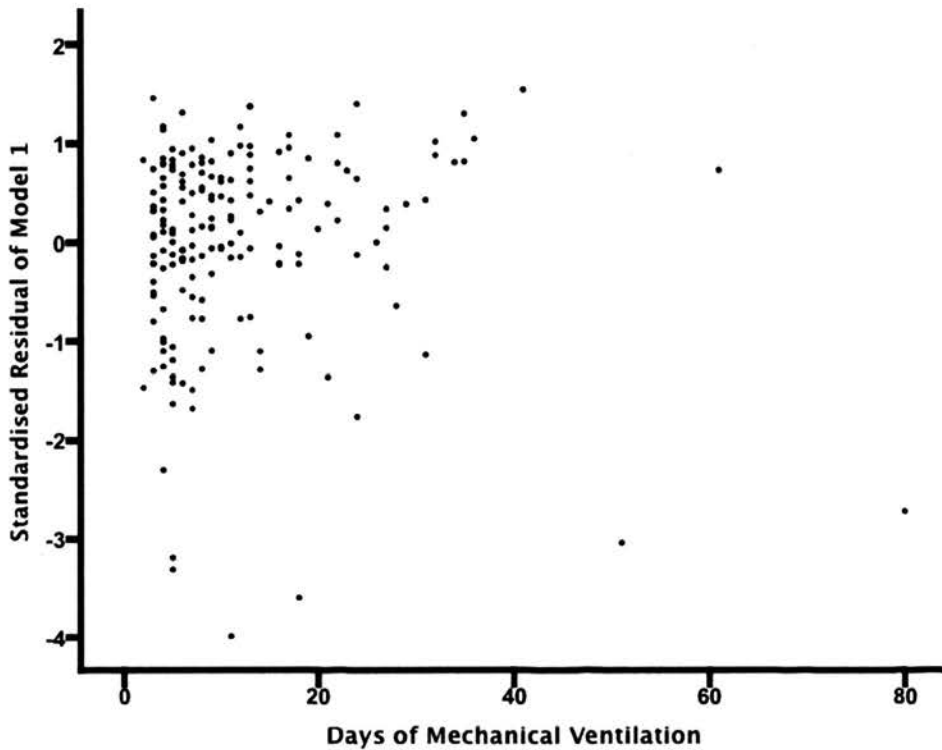


Figure 5-2 Standardised residuals of Model 1 plotted against days of mechanical ventilation

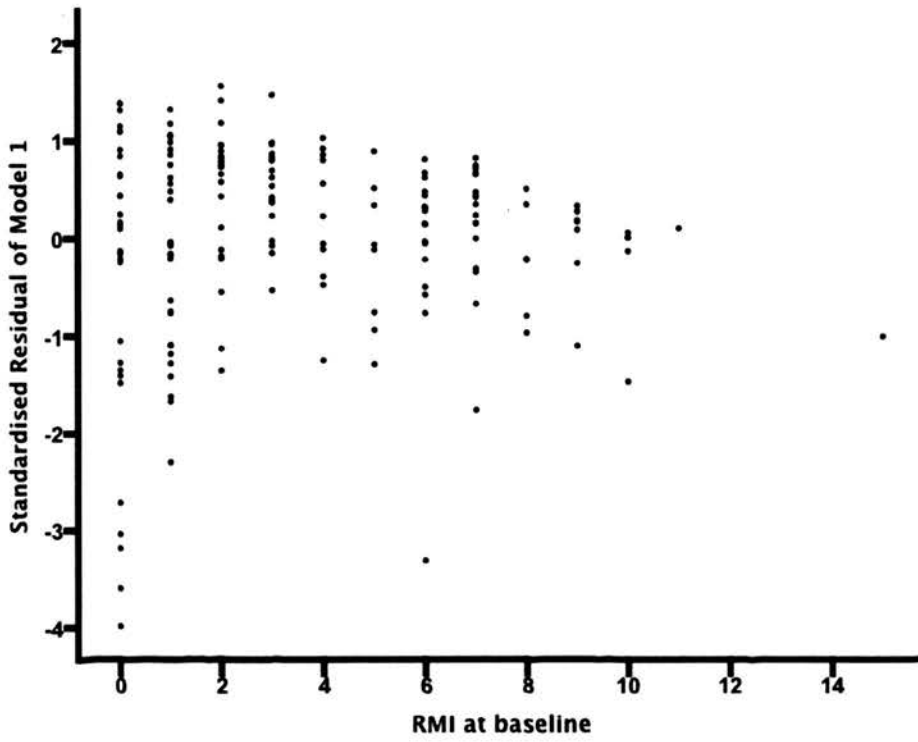


Figure 5-3 Standardised residuals of Model 1 plotted against baseline RMI

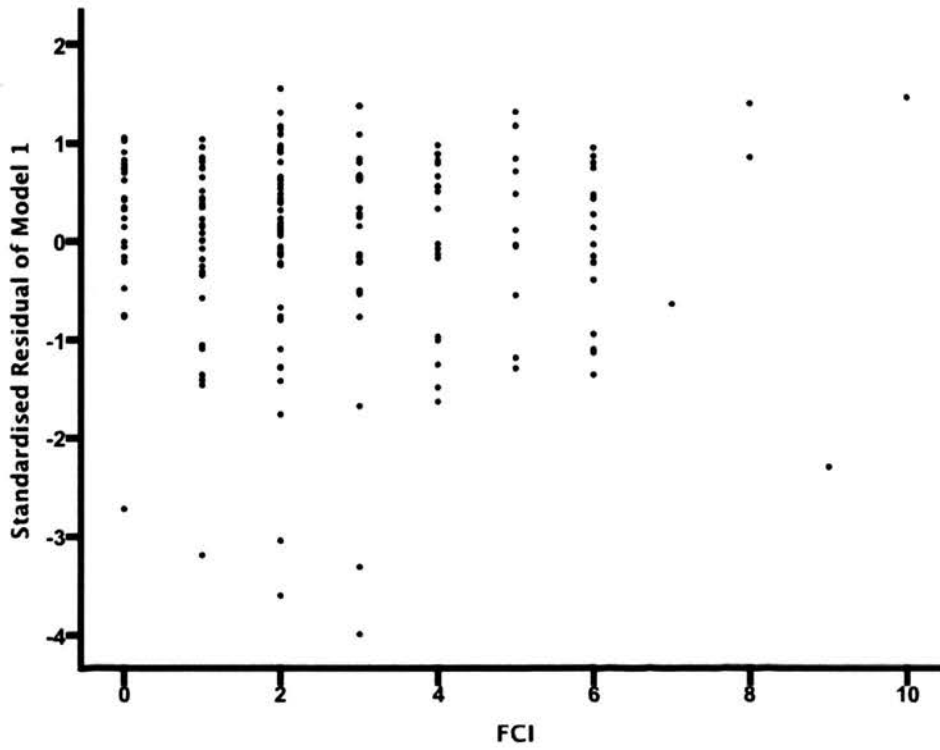


Figure 5-4 Standardised residuals of Model 1 plotted against FCI

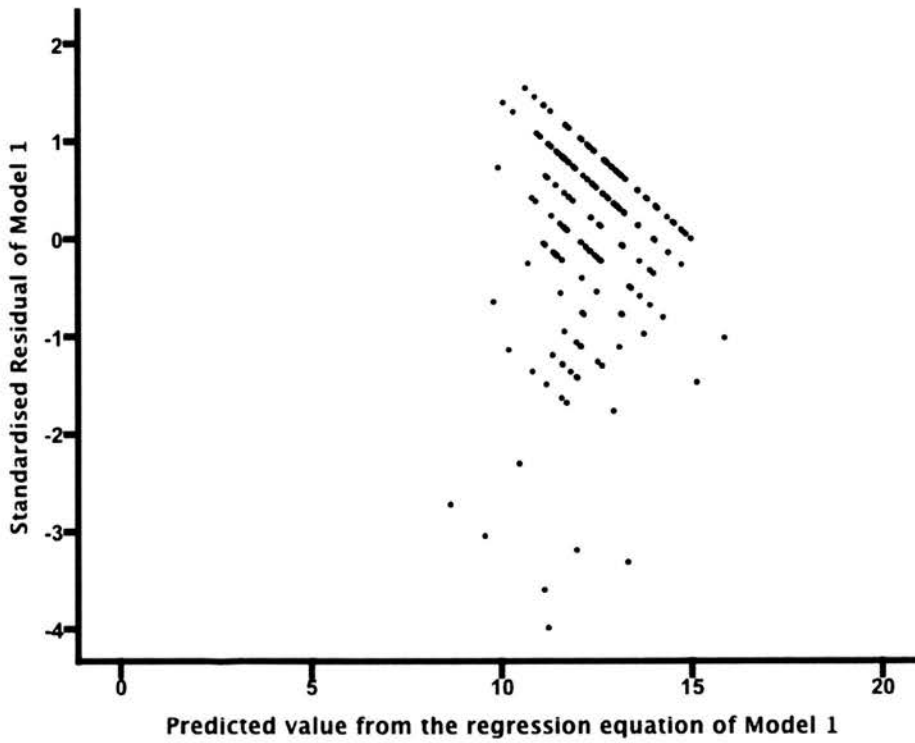


Figure 5-5 Standardised residuals of Model 1 plotted against the value predicted by the regression equation

5.4.4.2 *Normal distribution around the regression line*

The Q-Q plot and histogram of the residuals of model 1 are presented below. This plots demonstrate that the residuals deviate significantly from a normal distribution – hence violating the assumption of normal distribution of predicted values around the regression line.

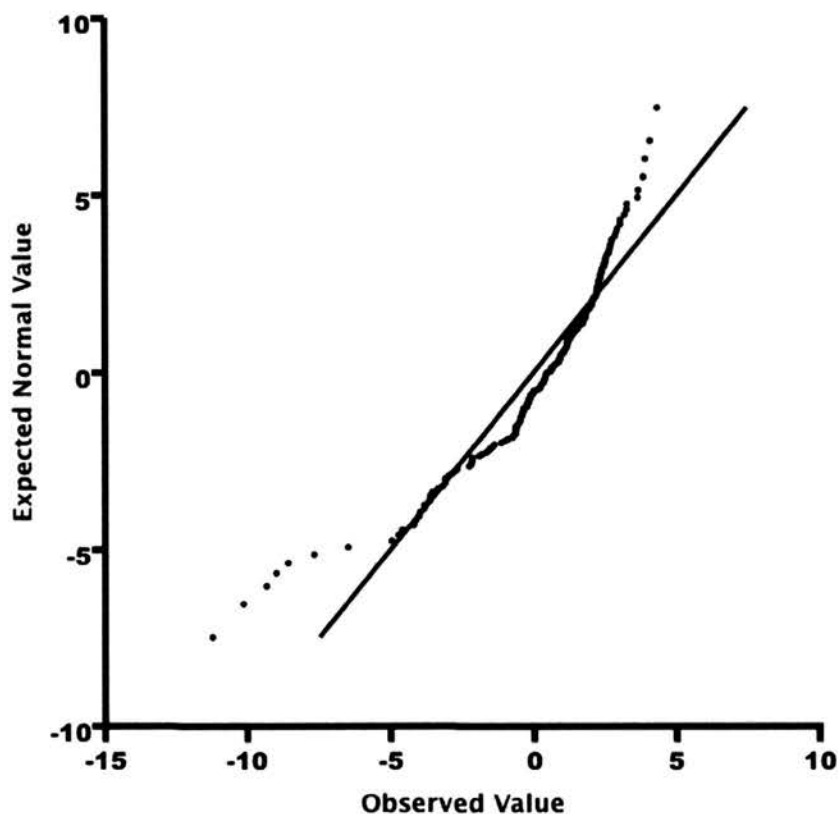


Figure 5-6 Q-Q plot illustrating relationship of Model 1 residuals to a theoretical normal distribution

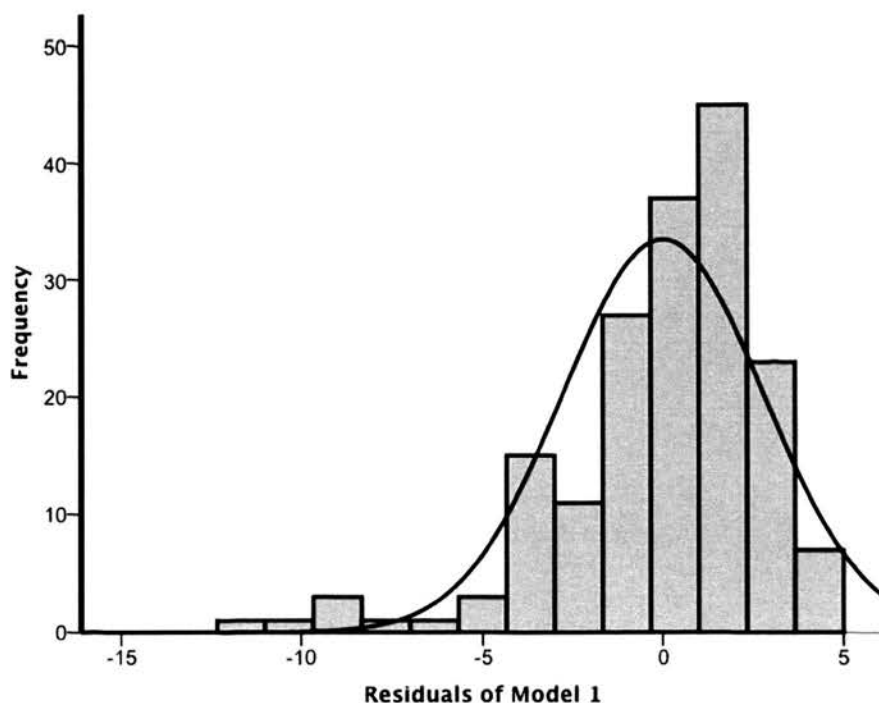


Figure 5-7 Histogram of the residuals of Model 1 demonstrating a skewed frequency distribution

5.4.4.3 Equal variance

The residual plots in section 5.3.9.1 show that the spread of residuals above and below zero is not equal for any of the independent variables suggesting a violation of the assumption of equal variance in the model.

5.4.4.4 Collinearity

The scatter plot matrix and correlation coefficients for the independent variables included in Model 1 are presented below. There was no collinearity observed between the include variables.

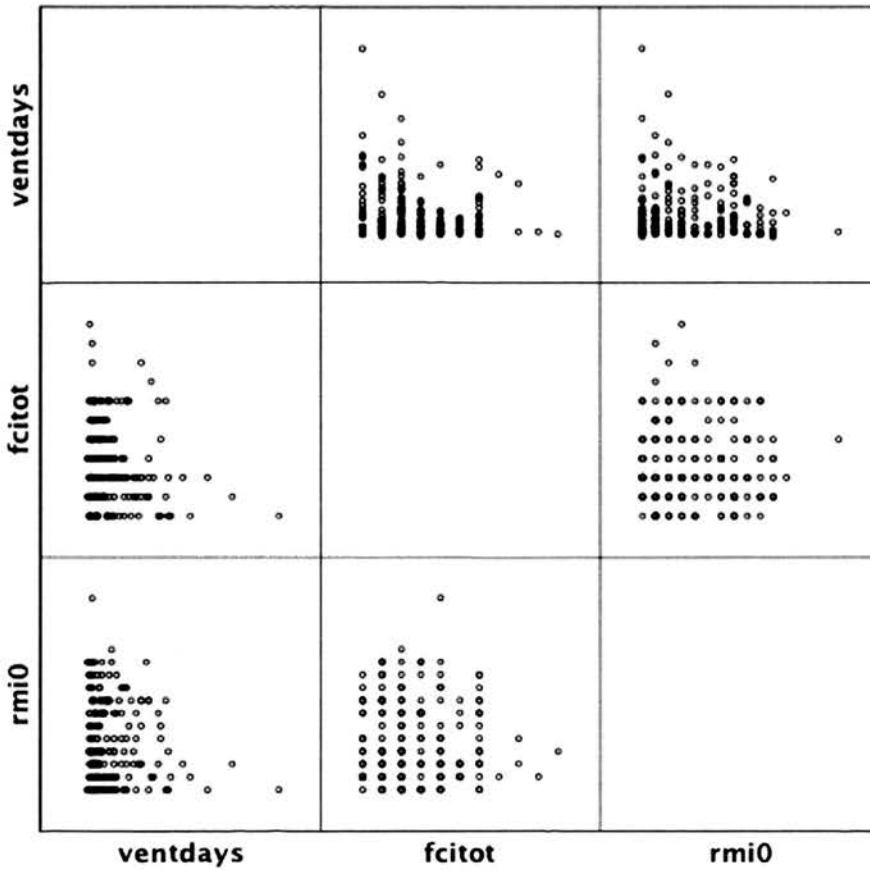


Figure 5-8 Scatter plot matrix for Model 1 independent variables (rmi0 = Baseline RMI; fcitot = FCI; ventdays = Days of mechanical ventilation).

	Ventilator Days	FCI	Baseline RMI
Ventilator Days	-	-0.2	-0.1
FCI	-0.2	-	-0.0
Baseline RMI	-0.1	-0.0	-

Table 5-5 Correlation coefficients to assess colinearity of included variables in Model 1.

5.4.4.5 Conclusions from linear model assumption testing of Model 1

The assumption testing carried out in this section demonstrated violations of the assumptions of linearity, equal variance, and normal distribution of predicted values around the regression line. For these reasons, it was considered that there was a moderate to high risk that the estimation of coefficients was biased, and therefore a possibility that important variables were not included or unimportant variables had been included in Model 1.

Model 1 was therefore not considered further, and the analysis was repeated after transformation of the severely negatively skewed outcome variable (RMI at 3 months), and the severely positively skewed independent variable (RMI at baseline).

5.4.5 Outlier assessment of model 1

Outlier analysis was not carried out for model 1 because the model violated the assumptions of linear regression.

5.4.6 Transformations

The negatively skewed outcome variable RMI at 3 months was transformed using a base 10 logarithm transformation of the inverted RMI score which is a recognised method for normalising negatively skewed data. The formula for this transformation is shown below. This transformed outcome variable was named tRMI, and the improved normal distribution of this variable is illustrated in Figure 5-9.

$$\text{Transformed RMI} = \log_{10}(16 - \text{RMI})$$

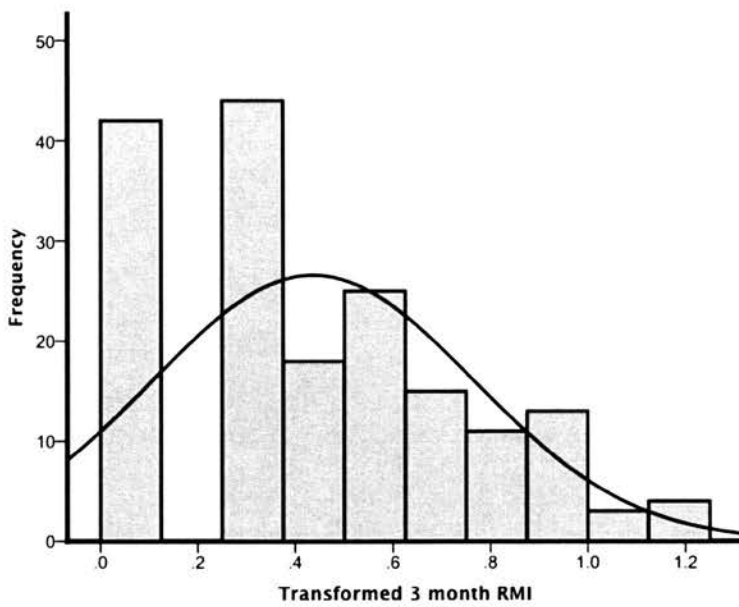


Figure 5-9 Histogram showing frequency distribution of tRMI with an improvement in normality after transformation.

This frequency distribution can be compared with the distribution of RMI illustrated in Figure 5-1.

The positively skewed independent variable RMI at baseline was transformed by adding 1 and then applying a base 10 logarithm transformation which improved normality (Figure 5-10).

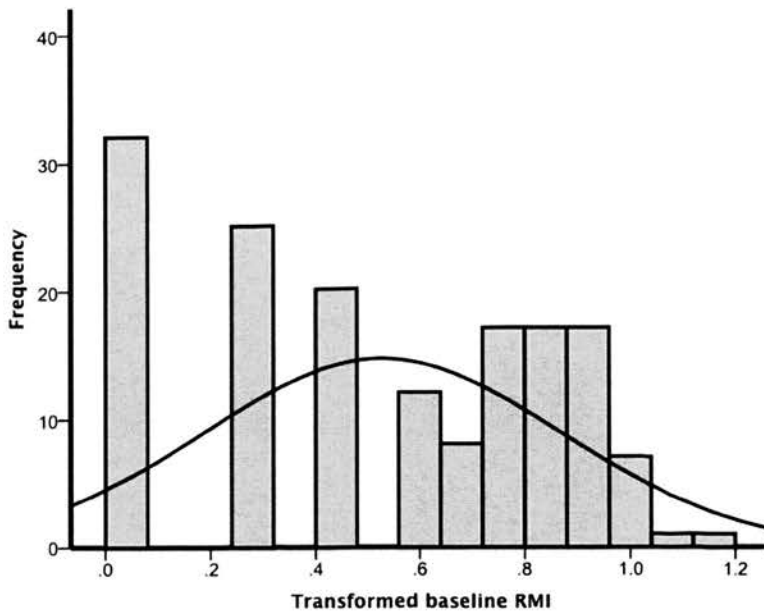


Figure 5-10 Histogram of the transformed baseline RMI showing an improvement in normal distribution

5.4.7 Bivariate relationships of candidate predictive variables with the transformed RMI

A summary of the bivariate relationship of candidate predictive variables and the transformed outcome variable tRMI are presented in Table 5-6. The clinical variables age, FCI, SIMD quintile, $\text{Log}_{10}(1+\text{Baseline RMI})$, and physical component of SGA and the serological variables CMV IgG status, CRP, and SLPI all had significant associations with tRMI and were considered further as candidate variables in the multivariable analysis.

Candidate variable	Beta Coefficient	p
Age	0.00	0.07
FCI	0.03	0.02
SIMD Quintile	-0.03	0.06
CMV IgG status	0.09	0.09
APACHE 2	0.00	0.58
Ventilator days	0.00	0.17
Diagnosis of Sepsis	0.01	0.87
$\text{Log}_{10}(1+\text{RMI})$	-0.37	0.00

Physical component of SGA	0.04	0.24
CRP	-0.01	0.10
IL-6	0.00	0.36
IL-8	0.00	0.28
TGFβ	0.00	0.84
MPO	0.00	0.34
HNE	0.00	0.65
SLPI	0.00	0.01

Table 5-6 Bivariate association between candidate predictive variables and the transformed outcome variable tRMI. Shaded variables were tested in the multivariable models.

5.4.8 Multivariable analysis using clinical variables only and the transformed outcome variable tRMI – Model 2

In this analysis, age, FCI, SIMD quintile, ventilator days, $\log_{10}(1+\text{RMI})$, and physical component of the SGA were tested in the multivariable model. The results are presented in Table 5-7. SIMD quintile, $\log_{10}(1+\text{Baseline RMI})$, and FCI were the variables that met criteria for inclusion in the model using a backwards selection technique and were included in the final model.

Variable	Beta coefficient	Significance
Constant	0.660	-
FCI	0.024	0.03
$\log_{10}(1+\text{RMI})$	-0.367	0.00
SIMD Quintile	-0.030	0.08

Table 5-7 Results of the multivariable model using clinical data only and the transformed variables – Model 2.

5.4.9 Multivariable analysis using clinical and serological variables and the transformed outcome variable tRMI – Model 3.

In this analysis, CMV IgG status, CRP, and SLPI were added to the clinical variable above and tested in another multivariable model. The results of this model are presented in Table 5-8. The resulting model included $\log_{10}(1+\text{RMI})$, FCI, and SLPI concentration.

Variable	Beta coefficient	Significance
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Constant	0.447	-
FCI	0.027	0.02
Log10(1+RMI)	-0.343	0.00
SLPI	0.002	0.05

Table 5-8 Results of the multivariable model using clinical and serological variables and the transformed outcome variable tRMI - Model 3

5.4.10 Testing linear regression model assumptions for Model 2

5.4.10.1 Linearity

Plots of standardised residuals of the regression equation against the included independent variables and the predicted value of the regression equation are shown in the figures below. The spread of residuals is reasonably consistent across levels of the both the independent variables and the predicted outcome variable. This indicates reasonable adherence to the assumption of linearity.

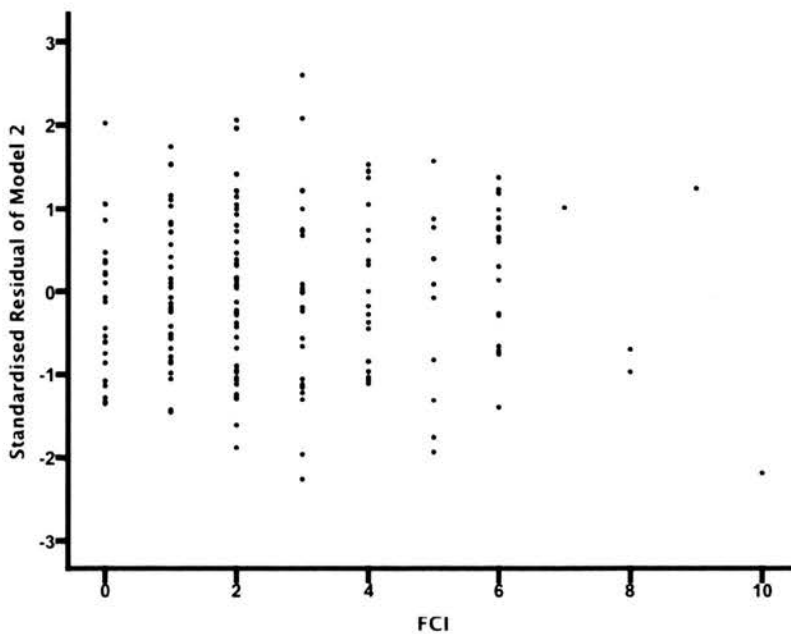


Figure 5-11 Standardised residuals of Model 2 plotted against FCI

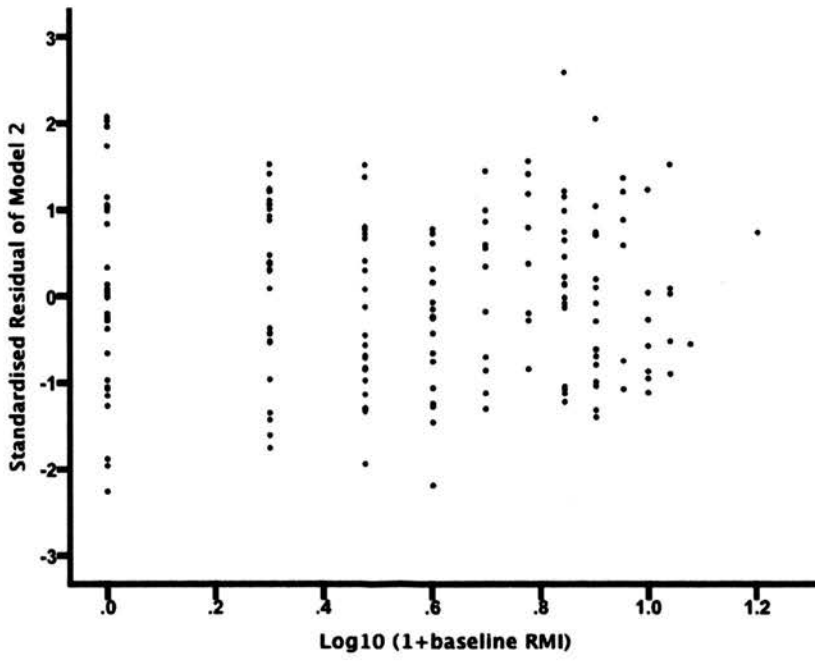


Figure 5-12 Standardised residuals of Model 2 plotted against tRMI at baseline

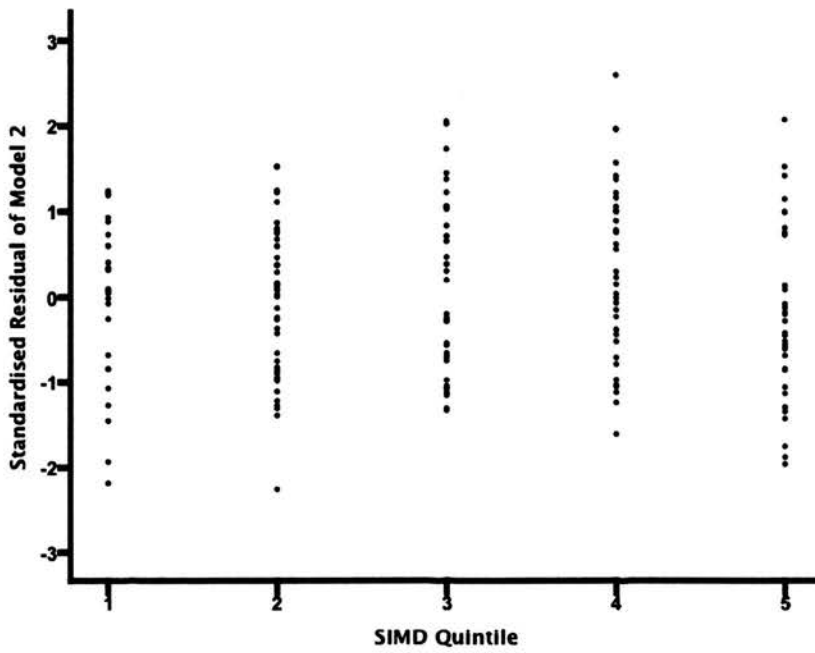


Figure 5-13 Standardised residuals of Model 2 plotted against SIMD quintile

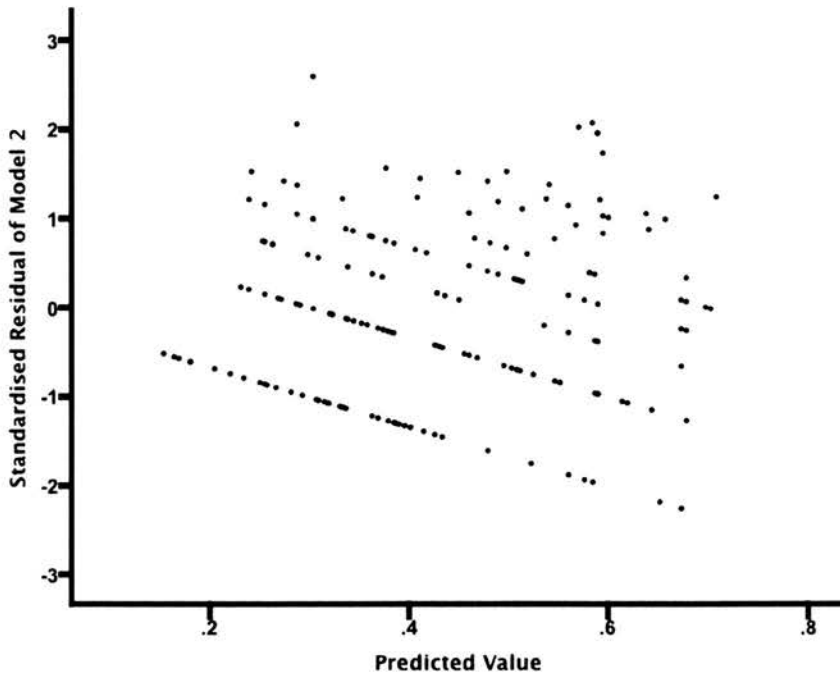


Figure 5-14 Standardised residuals of Model 2 plotted against the value predicted by the regression equation

5.4.10.2 Normal distribution around the regression line

The Q-Q plot of the residuals of Model 2 is presented in Figure 5-15. The distribution much more closely represents a normal distribution than the Q-Q plot of residuals for Model 1 (Figure 5-6). The histogram is shown in Figure 5-16. Both of these indicate better adherence to the assumption of normal distribution of predicted values around the regression line.

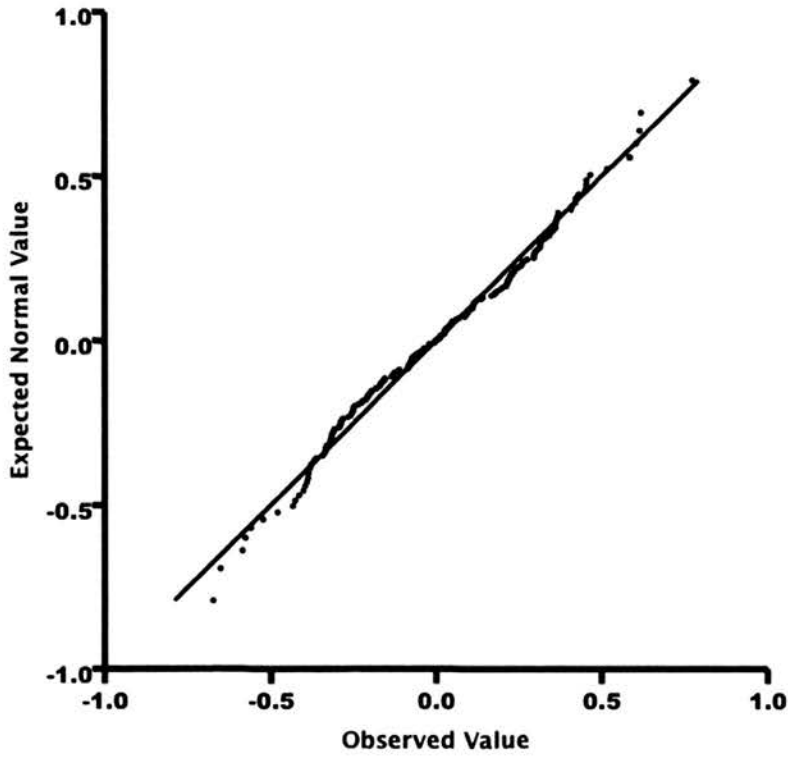


Figure 5-15 Q-Q plot illustrating the relationship of Model 2 residuals to a theoretical normal distribution

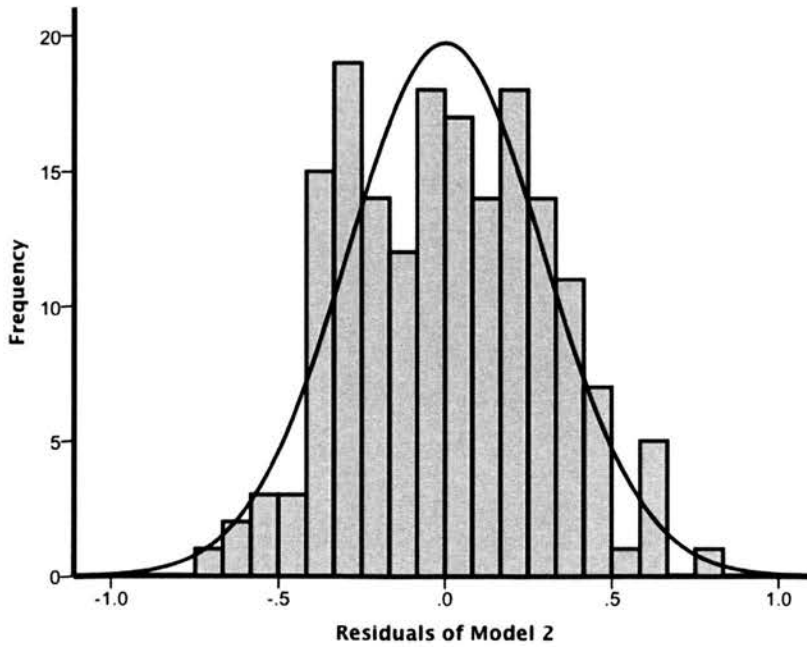


Figure 5-16 Histogram of the residuals of Model 2 demonstrating normal distribution

5.4.10.3 Equal variance

The residual plots in section 5.4.10.1 show that the spread of residuals above and below zero is relatively equal suggesting adherence to the assumption of equal variance in the model.

5.4.10.4 Collinearity

The scatter plot matrix and correlation coefficients for the independent variables included in Model 2 are presented below. There was no evidence of collinearity observed between the included variables.

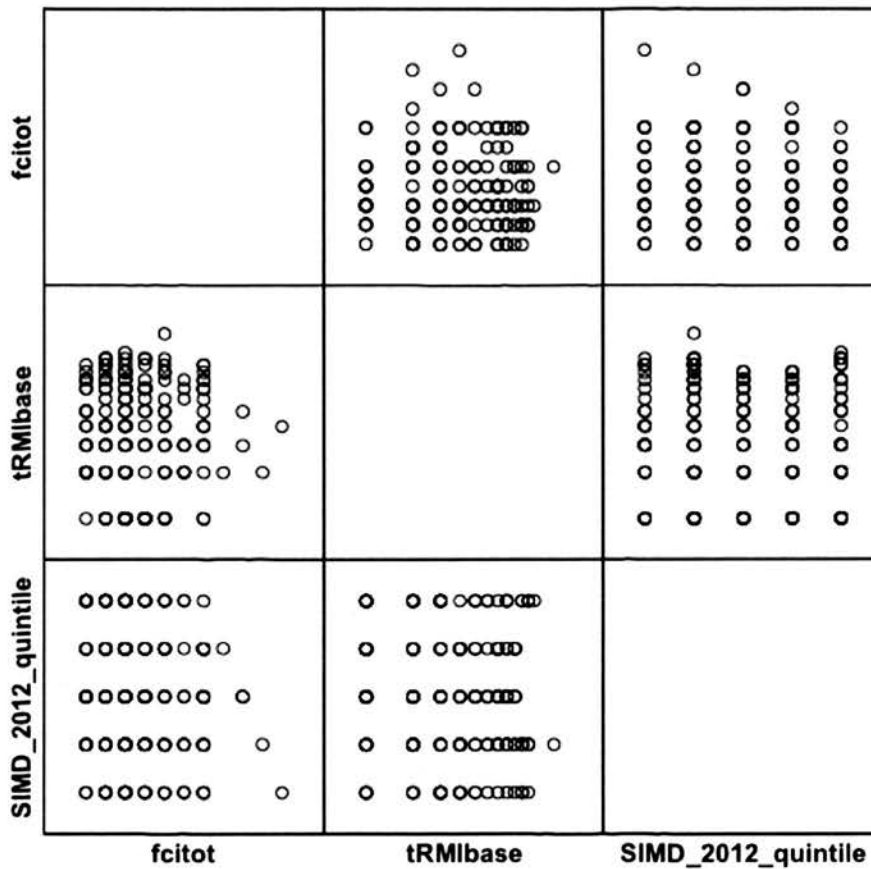


Figure 5-17 Scatter plot matrix for Model 2 independent variables (fcitot = FCI; tRMlbase = Log10(1+RMI at baseline); SIMD_2012_quintile = SIMD quintile)

	FCI	Log10 (1+RMI)	SIMD Quintile
FCI	-	-0.0	-0.2
Log10 (1+RMI)	-0.0	-	-0.1
SIMD quintile	-0.2	-0.1	-

Table 5-9 Correlation coefficients to assess colinearity in Model 2

5.4.10.5 Conclusions from linear model assumption testing of Model 2

Assumption testing of Model 2 suggested good adherence of Model 2 to the assumptions of linear regression modelling. This model showed a significant improvement on Model 1, supporting the use of the transformed outcome variable tRMI, and the transformed baseline RMI as an independent variable. The author had reasonable confidence in the accuracy of the coefficient estimates and variables included in this model.

5.4.11 Outlier analysis of Model 2

Figure 5-18 is a plot of the standardised residuals for Model 2. Six of the residuals fell outwith 2 standard deviations and were considered potential outliers. The data for each variable was examined to help identify any data errors. No errors were identified in the data entry for any of the potential outliers.

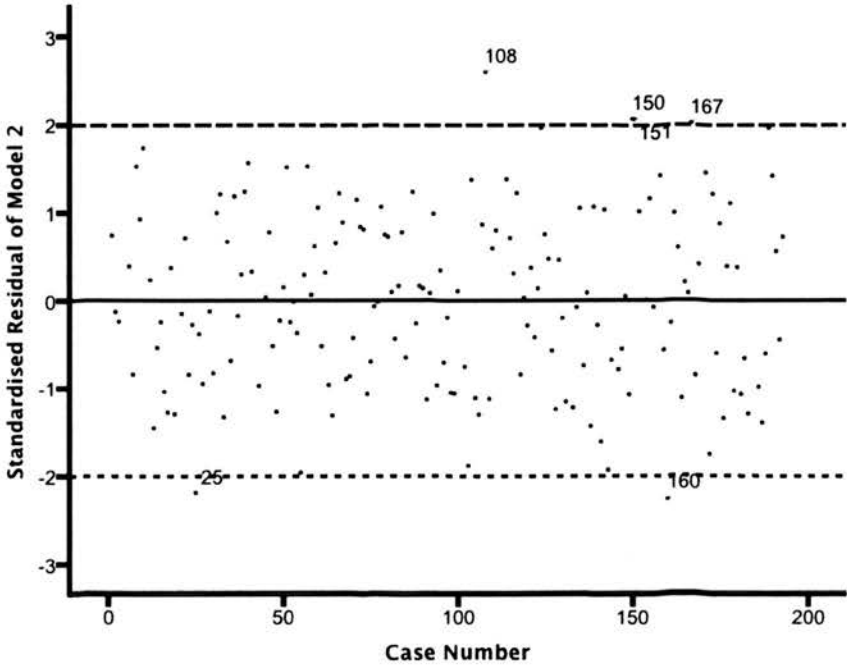


Figure 5-18 Standardised residuals of Model 2 plotted against case number

Leverage values were calculated to assess the potential impact on outlying data points on the regression equation. Leverage values for Model 2 are plotted

against case number in Figure 5-19. Only 1 outlier – case 25 was considered to be a leverage point because it had a leverage value of greater than $3((p+1)/n)$. This case represented an ICU survivor that had particularly high levels of previous comorbidity according to an FCI of 10.

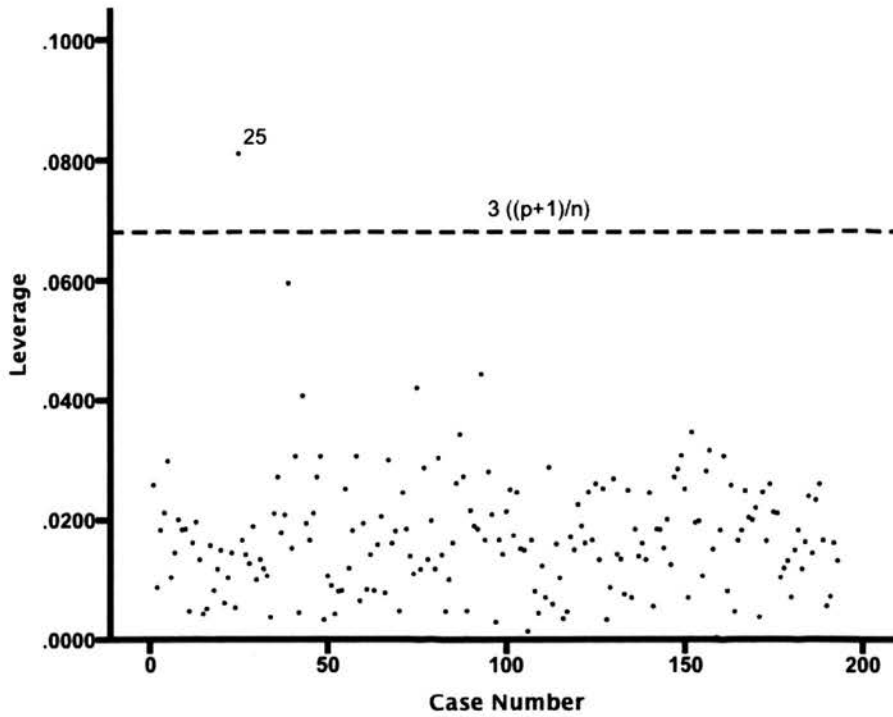


Figure 5-19 Leverage values of Model 2 plotted against case number

Leverage points do not always have a significant impact on the fitting of the regression line. To determine this, the effect of deleting each variable on model fit can be assessed using the Cook's distance. Cook's distances greater than 1 were considered to have a significant influence on the regression analysis. Cook's distances for Model 2 are illustrated in Figure 5-20..

The Cook's distances were low. The outlier identified by the leverage values was also the most influential data point in the analysis. However, the Cook's distance of less than 1 indicated that this data point did not have a significant impact on the regression model fit. This case was therefore left in the analysis.

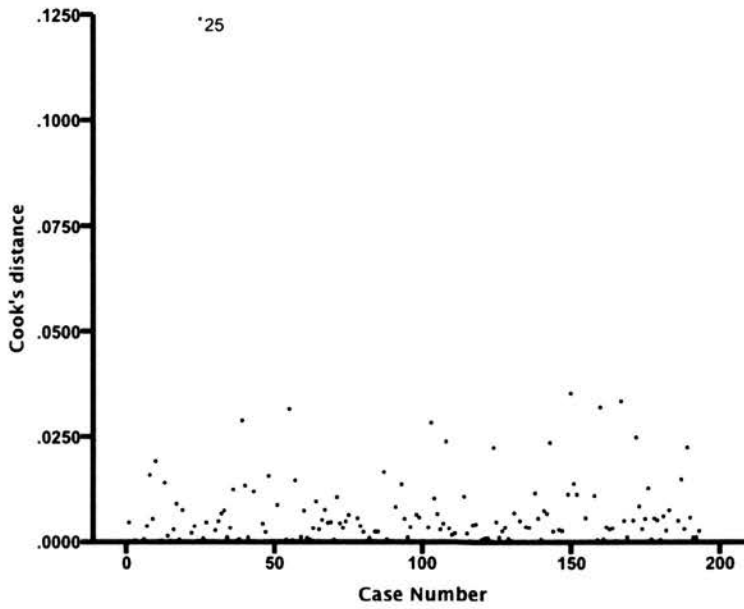


Figure 5-20 Cook's distances for Model 2

5.4.12 Testing linear regression model assumptions for Model 3

5.4.12.1 Linearity

Plots of standardised residuals of the regression equation against the included independent variables and the predicted value of the regression equation are shown in the figures below. The spread of residuals is reasonably consistent across levels of the both the independent variables and the predicted outcome variable. This indicates reasonable adherence to the assumption of linearity.

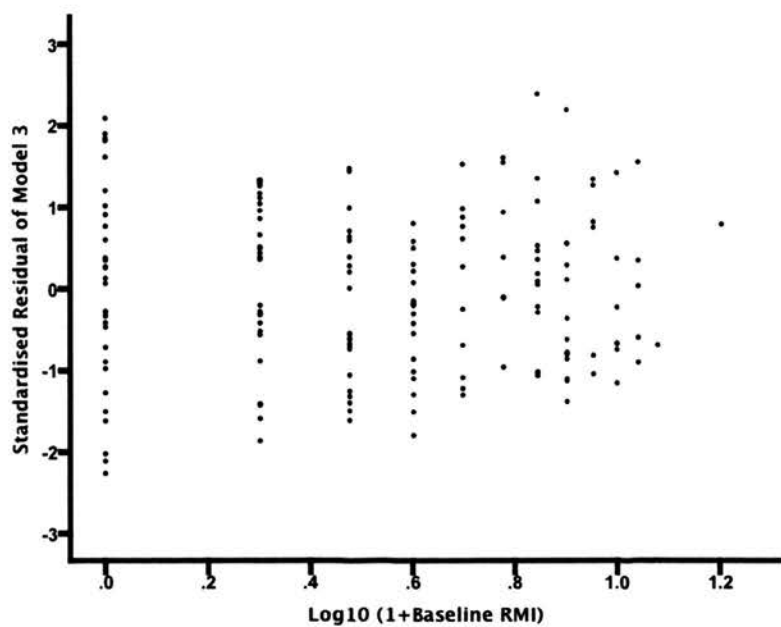


Figure 5-21 Standardised residuals of Model 3 plotted against FCI

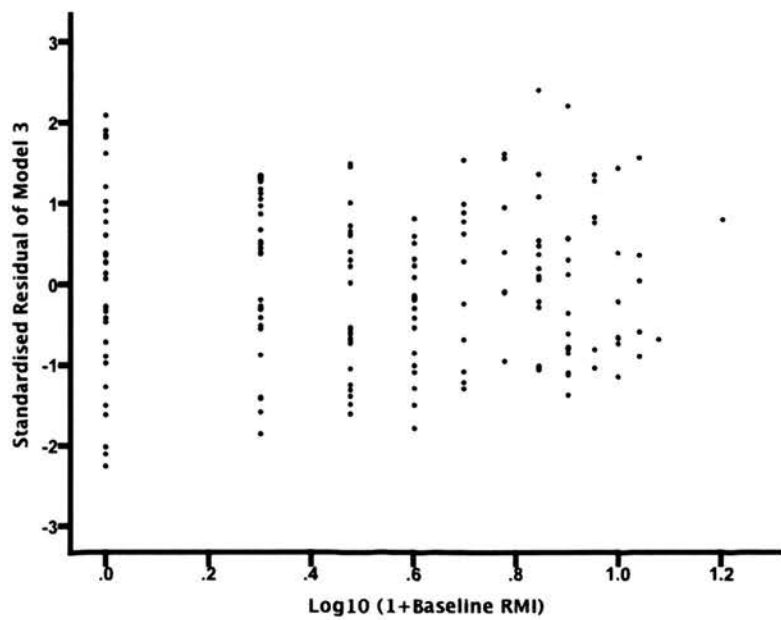


Figure 5-22 Standardised residuals of Model 3 plotted against $\text{Log}_{10}(1+\text{RMI})$ at baseline

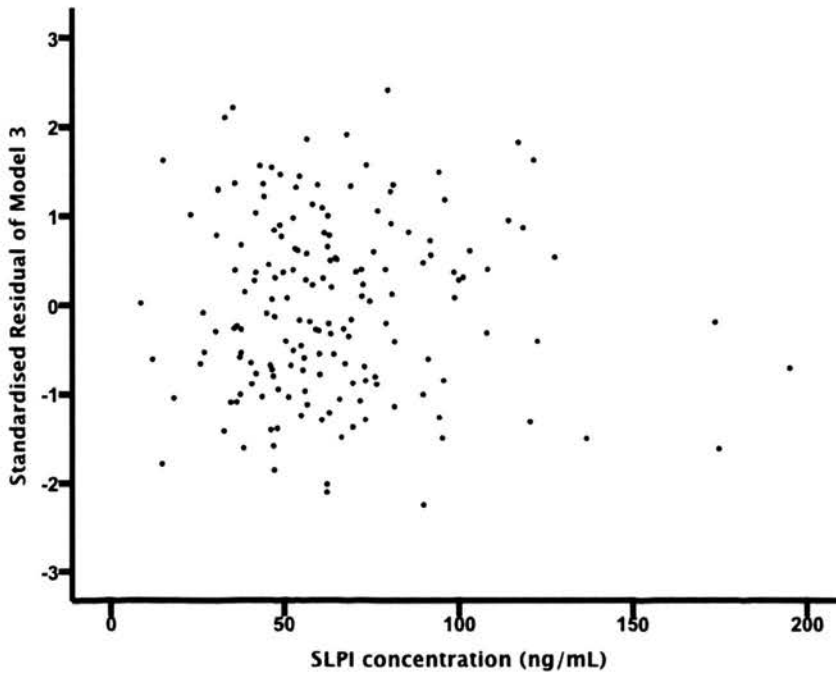


Figure 5-23 Standardised residuals of Model 3 plotted against SLPI concentration

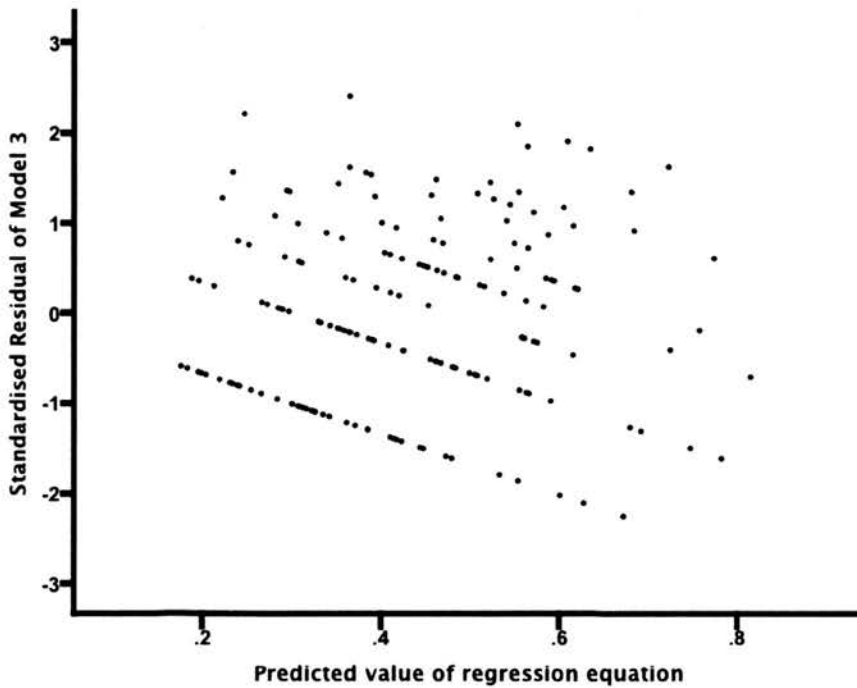
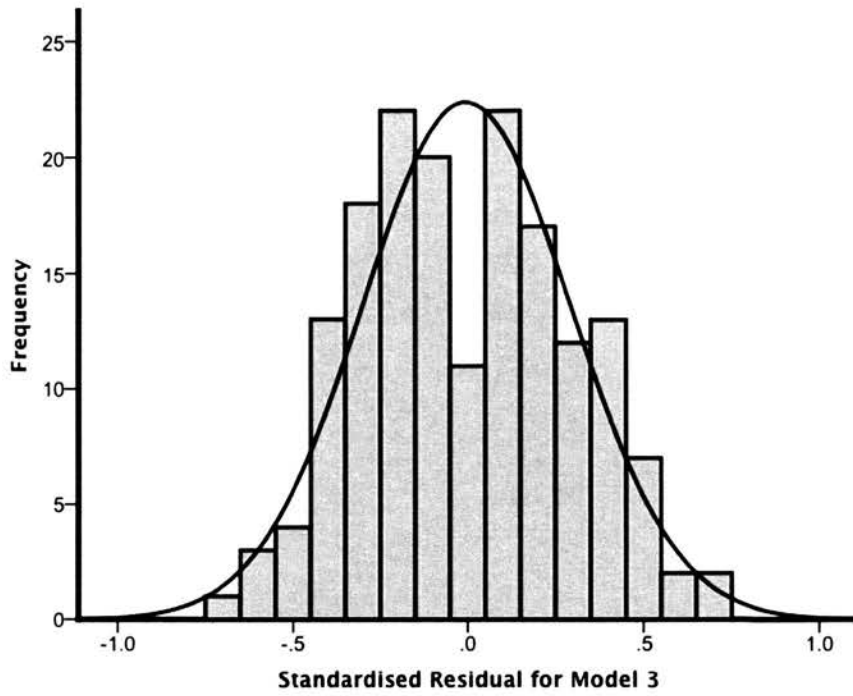


Figure 5-24 Standardised residuals of Model 3 plotted against the value predicted by the regression equation

5.4.12.2 Normal distribution around the regression line

The Q-Q plot and histogram of the residuals of Model 2 are presented in Figure 5-25 and Figure 5-26. As with model 2, the distribution closely represents a

normal distribution. This indicates good adherence to the assumption of normal distribution of predicted values around the regression line.



5-25 Histogram of the residuals from Model 3 showing a normal distribution

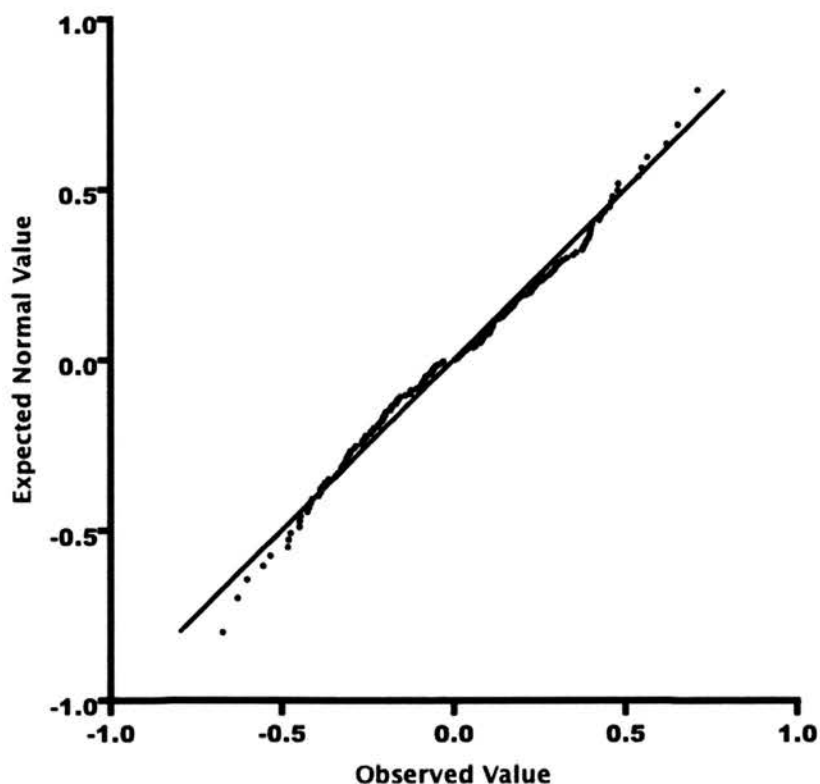


Figure 5-26 Q-Q plot illustrating relationship of Model 3 residuals to a theoretical normal distribution

5.4.12.3 Equal variance

The residual plots in section 5.4.12.1 show that the spread of residuals above and below zero is relatively equal suggesting adherence to the assumption of equal variance in Model 3.

5.4.12.4 Collinearity

The scatter plot matrix and correlation coefficients for the independent variables included in Model 3 are presented below. There was no evidence of collinearity observed between the included variables.

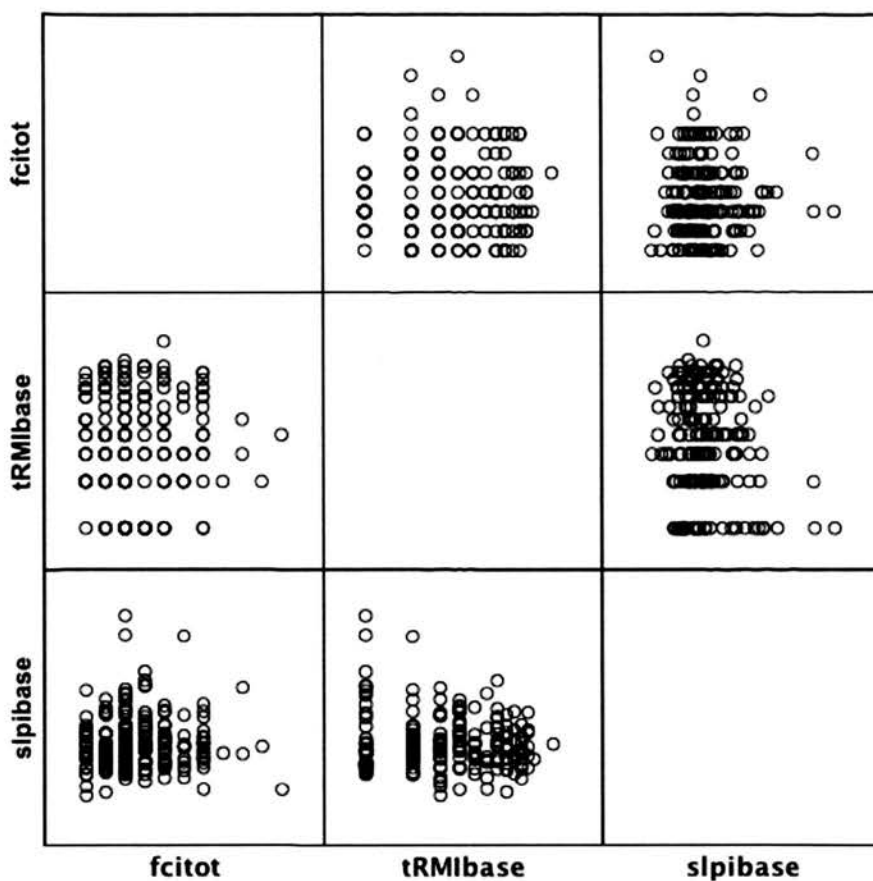


Figure 5-27 Scatter plot matrix for independent variables in Model 3

	FCI	Log10 (1+RMI)	SLPI
FCI	-	-0.0	-0.0
Log10 (1+RMI)	-0.0	-	-0.1
SLPI	-0.0	-0.1	-

Table 5-10 Correlation coefficients for the assessment of collinearity in Model 3

5.4.12.5 Conclusions from linear model assumption testing for Model 3

Assumption testing of Model 3 suggested good adherence of Model 3 to the assumptions of linear regression modelling.

5.4.13 Outlier analysis of Model 3

The scatter plot of standardised residuals against case number is shown in Figure 5-28. Six of the residuals fell outwith 2 standard deviations and these are

labelled in Figure 5-29. These data points were checked for errors in data entry and no issues were found.

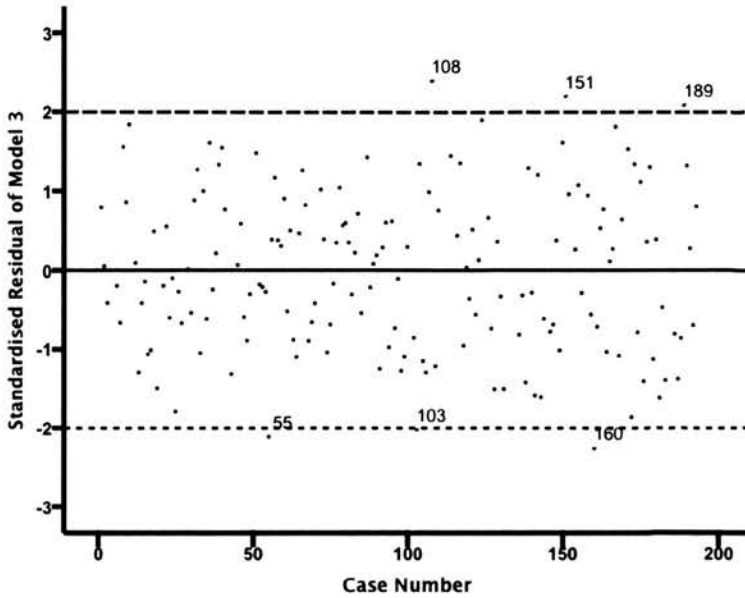


Figure 5-28 Standardised residuals of Model 3 plotted against case number

Leverage values were calculated for Model 3 and these are plotted against case number in Figure . Six of the points were considered to be leverage points because they have a value that was greater than $3((p+1)/n)$. The cases representing these points were checked for errors and none were found.

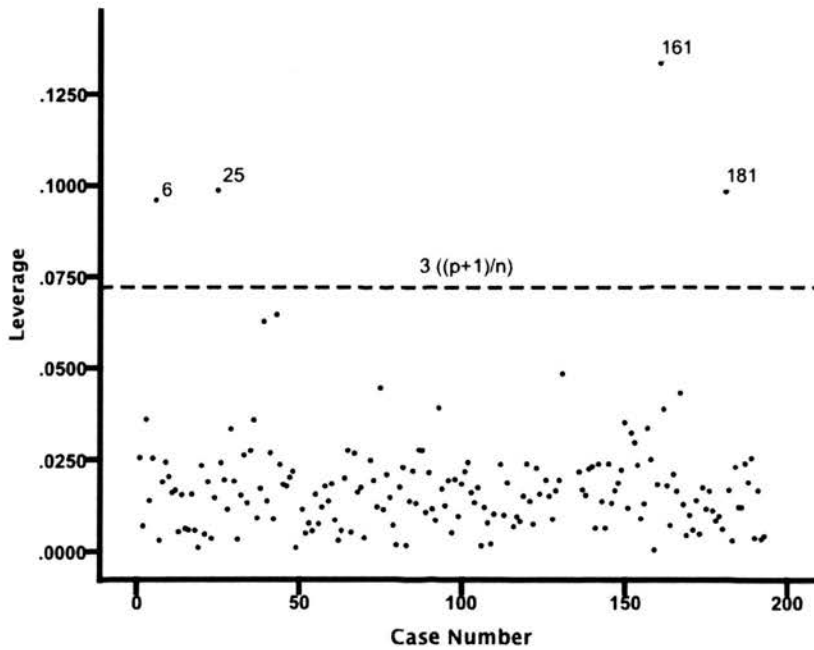


Figure 5-29 Leverage values for Model 3

Cook's distances were calculated and these are plotted in Figure 5-30. There were 2 outlying points. Neither of these were greater than 1. Therefore, none of the outlying points were considered to have a significant impact on model fit.

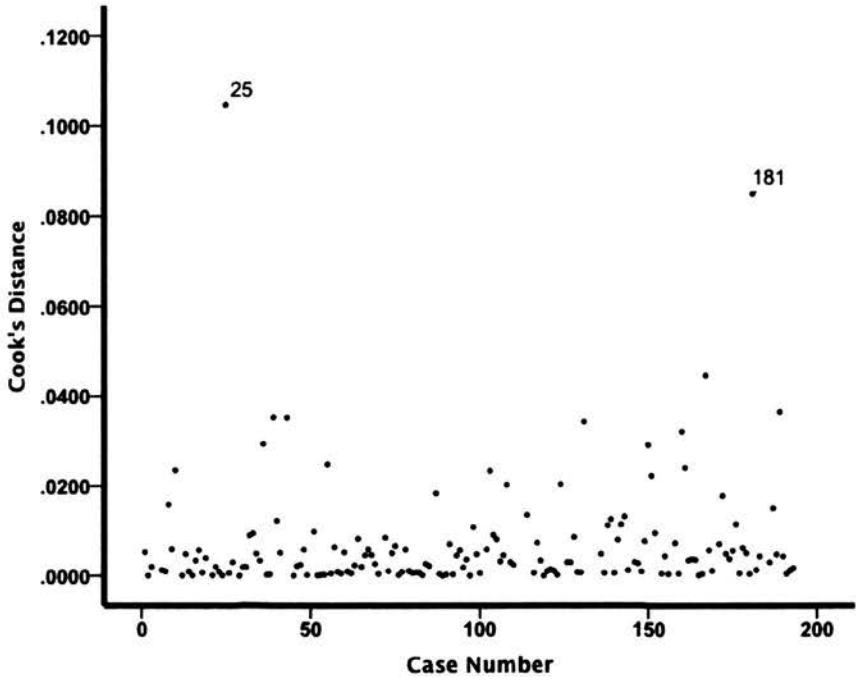


Figure 5-30 Cook's Distances for Model 3

5.4.14 Assessment of model fit

R² values for Models 2 and 3 alongside p values (calculated from the F statistic) are presented in Table 5-11. The independent variables included in Model 2 account for 17.5% of the variation in the outcome variable tRMI. Model 3 improves on this slightly with an R² value of 0.193 (accounting for 19.3% of the variation). Both regression models were significantly better than chance for predicting mobility 3 months after discharge from the ICU.

Model	R ²	P
2	0.175	0.000
3	0.193	0.000

Table 5-11 Goodness of fit assessment

5.4.15 Assessing the diagnostic accuracy of the linear regression models

As described in the methods, ROC curves were constructed using the predicted values from the two regression equations and the binary outcome variable 'Good Outcome'. Good outcome was defined as an observed RMI of greater than 10 after an interval of 3 months after ICU discharge. The accuracy of the 2 regression models created was compared using the area under curve (AUC) statistics. Similarly, RMI measured at baseline (a surrogate of current practice) was plotted against the binary outcome measure 'Good Outcome' and an AUC statistic calculated to determine any improvement in prognostic accuracy over clinical observation.

To recap, Model 1 was a linear regression model that did not meet the assumptions of linear regression modelling and was therefore not considered further. Model 2 was a linear regression model constructed using clinical variables only. Model 3 was a linear regression model constructed testing clinical and serological variables. In contrast to Model 1, Models 2 and 3 used transformed variables to improve the compliance of the regression model with linear regression modelling assumptions. Model 2 included the independent variables FCI,

$\text{Log}_{10}(1+\text{RMI})$, and SIMD quintile. Model 3 included the independent variables FCI, $\text{Log}_{10}(1+\text{RMI})$, and SLPI.

The ROC curves for Model 2 and 3, and for RMI at baseline are shown in the figures below. The regression models have moderate accuracy in predicting the rehabilitation outcome after 3 months and both are better than chance at doing so (AUC of 0.76 and 0.73 respectively). The AUC for model 2 that incorporates clinical variables (RMI, SIMD quintile, and FCI) is greater than that for model 3 that substitutes SIMD quintile for SLPI, suggesting that the addition of a blood test did not assist in mobility prediction. Furthermore, the use of RMI alone has an AUC that is only slightly lower than that produced by Model 2 suggesting that a structured clinical assessment may be an adequate triage tool in itself.

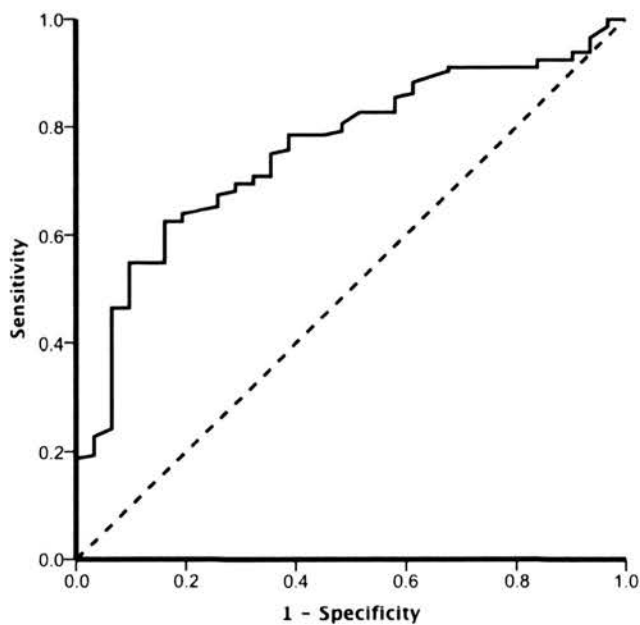


Figure 5-31 ROC curve illustrating the accuracy with which regression model 2 predicts RMI >10 at 3 months. (AUC=0.759).

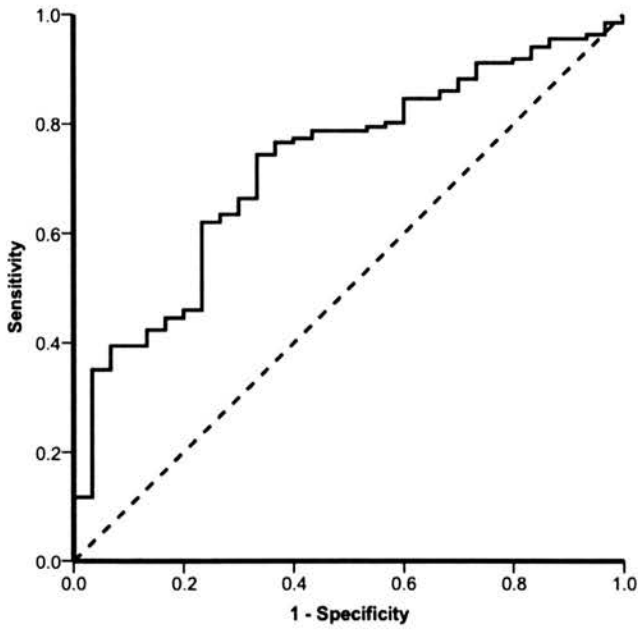


Figure 5-32 ROC curve illustrating the accuracy with which regression model 3 predicts RMI >10 at 3 months. (AUC = 0.725).

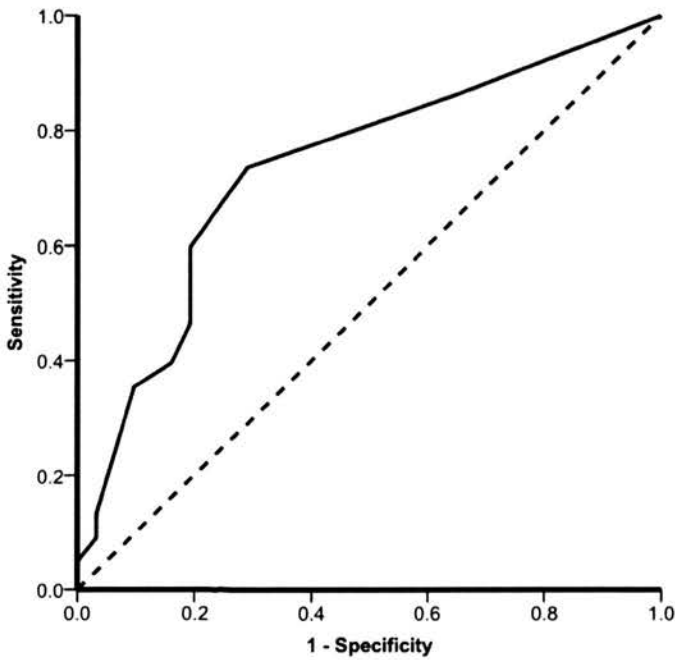


Figure 5-33 ROC curve illustrating the accuracy with which RMI at baseline predicts RMI >10 at 3 months (AUC = 0.734).

The sensitivity achieved for each triage tool at different cut-offs is show in Table 5-12. Model 2 (clinical data only) outperforms Model 3 at all the cut-offs. At a specificity of 80%, a sensitivity of 63.9% was achieved with this tool.

Triage tool	Cut-off	Specificity	Sensitivity
Model 2	0.54	60.4	78.5
	0.49	71.0	69.4
	0.46	80.6	63.9
Model 3	0.53	60.0	77.4
	0.47	70.0	66.4
	0.39	80.0	46.0
RMI	*		
	1.5	71.0	73.6
	2.5	80.6	59.7

Table 5-12 Sensitivity at different test cut-off values. *Given the small number of data points for RMI; there was no available cut-off value on the RMI ROC plot that corresponded to a specificity of 60%.

5.4.16 Assessing the clinical utility of the triage tools

Based on the above analysis, cut-off values corresponding to specificities of 80% were chosen, and the change in pre-test to post-test probability of having a RMI at 3 months of greater than 10 was calculated to demonstrate the diagnostic utility of each tool. In the absence of any previous work in this area, pre-test probability was set as prevalence of RMI greater than 10 3 months after ICU discharge in the reference population (0.82). Positive and negative post-test probabilities were calculated using likelihood ratios according to a published methodology (Deeks & Altman, 2004).

Triage tool	Cut off	Pre-test probability	Positive Likelihood Ratio	Negative Likelihood Ratio	Positive post-test probability	Negative Post-test probability
Model 2	0.46	0.82	3.29	0.45	0.94	0.67
Model 3	0.39	0.82	2.22	0.69	0.91	0.76
RMI	2.5	0.82	3.08	0.50	0.93	0.70

Table 5-13 Results of Likelihood analysis for each triage method

A positive test result based on regression model 2 altered the probability of having an RMI of greater 10 3 months after ICU discharge from 82% to 94%. This was no better than could be achieved by measuring RMI at baseline alone (82% to

93%). A negative test result based on regression model 3 had a poorer performance still. Probability of a good functional outcome was increased from 82% to 91%. Thus clinical prediction of good functional outcome was improved using a structured assessment of mobility at ICU discharge. This probably reflects current practice. The use of other baseline data including biomarker concentration does not improve on this.

5.5 Discussion

The aim of this chapter was to identify risk factors for physical disability after ICU discharge and incorporate them into an a predictive model In doing this, it was hoped that a simple clinical tool could be created that could identify patients that would see the greatest potential benefit from novel rehabilitation programmes. The work was carried out using data from the RECOVER study database, supplemented with the results of blood tests taken at the point of ICU discharge.

A review of the literature was carried out to identify risk factors for physical disability after intensive care unit discharge and also to identify and appraise previous attempts at predicting physical outcome. The aim of the review was to identify potential candidate variables for use in a predictive score. The results of this review have been summarised in the introduction to this chapter.

Very few studies have managed to identify these risk factors. Characteristics related to severity of illness such as illness severity scores, organ failure, and ICU length of stay are associated with physical outcome. However in previous studies, these associations have not been evident after adjustment for confounding has been incorporated into the analysis. There is a strong association between symptoms of depression and physical recovery however a causal pathway has not been confirmed. It is therefore not clear whether depressive symptoms prevent physical recovery, or whether poor physical function leads to depression. In the previously derived predictive score, education level, reduced core stability, at discharge, fractures, and ICU length of stay were important predictors of ICU recovery.

The selection of the candidate variables for the multivariable model was informed by the literature review, the available data fields in the RECOVER trial database, and the perceived practicalities of obtaining such data in a real life setting. With a plethora of potential data fields available for inclusion in the model, it was recognised that indiscriminately testing all available variables might result in the identification of spurious associations that would weaken the final prognostic model. An analysis plan was therefore designed in advance of receiving any data to prevent this potential problem.

5.5.1 Which risk factors for post ICU physical immobility can be identified from the RECOVER study baseline data?

This study adds to the understanding of risk factors for physical disability after ICU discharge. Perhaps unsurprisingly, the level disability at the point of ICU discharge is the most important risk factor. Patients with the lowest RMI scores at ICU discharge are more likely to be the patients who have poor RMI scores 3 months later. In some respects, this finding is reassuring since in the current healthcare model, at least in the UK, allocation of rehabilitation is likely to be directed by the perceived needs of patients as determined by physical assessment. Less reassuring is the finding that poor physical function at ICU discharge is not the only predictor of functional outcome. This is important because currently patients who are not considered to be the most severely disabled may not be identified at ICU discharge and may therefore miss out on potential beneficial rehabilitation interventions.

Patient characteristics that precede ICU admission are of particular importance. This study has shown that functional comorbidity (as measured by the FCI), and social deprivation category (as measured by SIMD quintile) are significant risk factors for post-ICU physical disability.

High scores on the FCI are likely to indicate patients who have struggled with physical disability prior to ICU admission, and potentially may have less rehabilitation potential in terms of improvement of physical function. Despite this,

FCI seems to be associated with poor outcome irrespective of the observed functional deficits seen at ICU discharge. It is therefore feasible that the accumulation of comorbidities described by the FCI could be important in preventing full recovery after critical illness. For patients with pre-existing disability, small changes in function could lead to a big change in quality of life. For example a change in an RMI score of just 2 points could mean the difference between being unable to stand to being able to walk with an aid around the house.

Social deprivation category was included in the analysis as a surrogate for education level, a variable that had been found in a previous study to be associated with poor physical recovery. Patients living in more deprived areas of Edinburgh had more significant levels of physical disability after 3 months. This suggests that regardless of comorbidities, duration of illness, and level of function at ICU discharge, deprivation has a significant role to play in the recovery of ICU survivors. The reasons for this association are likely to be multifactorial and the analysis is likely to have omitted important potential confounders such as smoking status and diet.

In keeping with previous studies of risk factors, traditional measures of severity of illness were not significantly associated with disability. Duration of illness as measured by days of mechanical ventilation did show an association on bivariate testing. However after adjustment for FCI, baseline RMI and SIMD quintiles, these factors were not significantly associated with RMI at 3 months.

With regard to disease-specific risk factors, sepsis was singled out as a potential risk factor for post ICU disability, based on the literature. In the current study, a diagnosis of sepsis as defined by consensus diagnostic criteria was not recorded. The presence or absence of the admission diagnosis of 'infection' did not make any difference to physical outcome. Whilst there were problems with the definition in this study that may limit its generalisability to other populations, the lack of any association between a diagnosis of an admission diagnosis of infection and post-ICU disability makes it unlikely that septic patients are at increased risk of long-term disability compared to other ICU survivors.

A number of other factors were considered for inclusion in the multivariable models but were not found to be significant independent risk factors for disability. Age was one such factor. An association was suggested on bivariate testing, with increasing age being associated with poorer function 3 months after ICU discharge. This association was not evident when adjusted for prior comorbidity, ICU length of stay, and baseline RMI. This suggests that the relationship between age and disability is confounded by at least one of these other factors. This too is an important finding. Firstly, triaging patients into rehabilitation pathways based on age is unlikely to identify those most at need. Secondly, it strengthens the argument that treatment limitation decisions, and access to critical care should not be informed by the chronological age of the patient.

Another factor that was considered was the physical component of the subjective global assessment score – a surrogate marker of nutritional status. This test is simple and quick to carry out but has not previously been validated in an ICU survivor cohort. Interestingly there was a trend towards poorer functional outcome with greater scores on the SGA physical examination. One potential explanation for this is the challenge of carrying out this assessment on patients that have significant oedema – a feature of many patients recovering from critical illness.

5.5.2 Risk factors for physical disability after 3 months – the role of inflammatory biomarkers

In chapter 3, a significant association was found between RMI and CRP concentration in a cross sectional analysis at a time point 3 months after ICU discharge. This supported the hypothesis that inflammation and physical function are related. One possible explanation for this finding is that inflammatory persistence after ICU discharge hampers physical recovery in some way. It is further hypothesised that the active process of inflammatory resolution might be dysfunctional in some patients, making them more likely to have persistence of the inflammatory response, and therefore make them more at risk of physical disability. It therefore seemed feasible that patterns of inflammatory molecules measured at

ICU discharge might be able to extra information to a clinical parameter based prediction tool and improve its performance.

A number of markers of the inflammation, inflammatory resolution, and chronic inflammation were measured at ICU discharge and their relationship with physical disability 3 months after ICU discharge was tested. Higher CRP concentration at ICU discharge was associated with physical disability at 3 months although the associated p value was 0.1. This association was no longer evident when adjusted for ICU length of stay, FCI, baseline RMI, and SIMD quintile. Higher serum concentration of SLPI was significantly associated with increased levels of physical disability 3 months after ICU discharge and remained significant after adjustment for confounders.

5.5.3 Can physical disability be predicted using clinical data available at ICU discharge?

A rigorous prognostic model development procedure was carried out in order to make best use of the available data to predict physical outcome after ICU discharge. A linear regression technique was used for reasons of statistical power. Transformation of the outcome variable and one of the independent variables was necessary to satisfy the assumptions of the regression technique. Using clinical variables alone, FCI, baseline RMI, and SIMD quintile were significantly associated with RMI 3 months after ICU discharge. These variables were combined in a regression equation that performed well statistically as a predictor of post-ICU recovery. Furthermore, the regression model included 2 variables that represented characteristics of the patient prior to ICU admission, and a physical assessment at the time of ICU discharge that fitted well with factors hypothesised to be important during the study design stages.

To test the clinical utility of the tool, a potential application was explored. It was considered important to be able to distinguish patients that had particularly good outcomes (who may have good outcomes regardless of intervention) from patients who had poor outcomes and would require rehabilitation. A RMI cut-off

was defined, and the outcome variable was dichotomised according to this cut-off. ROC analysis was then carried out to determine the prognostic accuracy of the regression equation in differentiating 'good' from 'bad' outcome. Again, statistically, the model performed well and moderately good accuracy (assessed by AUC which was 0.76). On the basis that current rehabilitation triage practices are essentially an assessment of immobility at ICU discharge, analogous analyses were carried out using RMI at ICU discharge as a triage tool. The ROC curve and likelihood ratio analysis suggested that RMI alone provided as much prognostic information as the triage tool based on the regression model.

In summary, there is evidence from this study that both comorbidity preceding critical illness, social class, and physical function at ICU discharge are important determinants of subsequent physical recovery. Despite this, assessment of physical disability at the point of ICU discharge emerges as the most important factor. The data suggest that very few (if any) patients would be miss-identified if social class or FCI were not known.

5.5.4 Can prediction of physical outcomes be improved by the addition of markers of inflammation and inflammatory resolution measured at ICU discharge?

Previous chapters of this thesis have suggested that both inflammation and CMV IgG status might be important variables in post-ICU physical recovery. It was therefore considered perceivable that both these factors might be useful in predicting physical recovery after ICU discharge.

If either of these factors significantly improved prognostic ability, a blood test at the point of ICU discharge might be justified. However, if the additional prognostic information is limited, an additional blood test might become a burden. For this reason, prediction tools were developed sequentially. Firstly, the tool described above including only clinical variables was developed. Following this, biomarker data and CMV IgG status were incorporated.

Only SLPI, CMV IgG and CRP met statistical criteria for consideration in the regression model. Only SLPI was retained in the model after inclusion of clinical variables and this was at the expense of SIMD quintile. This regression model explained slightly more of the variation in the outcome variable than the model containing only clinical variables as determined by a slightly higher R^2 statistic. However, the ROC curve and likelihood ratio analyses suggested that clinically, the tool was inferior to both the clinical predictive model, and RMI measured at ICU discharge.

There was therefore little to support the addition of biomarker measurements, or CMV IgG titre to clinical data when assessing patients for rehabilitation especially given the extra cost and burden to patients that additional blood testing might impose.

5.5.5 Further Reflection

. The current study has suggested a number of risk factors identified at ICU discharge that were associated with poor physical mobility 3 months later. These were current level of function, previous comorbidity, and social class. The identification of these risk factors supports the assertions made by other studies that pre-morbid function and level of education influences physical outcome, regardless of the level of function at the time of the assessment.

Whilst these findings are interesting, several points of caution relating to study design and conduct should be emphasised. Firstly, the analysis was an opportunistic use of data from the RECOVER trial database. The baseline data fields were originally chosen by the trial steering group to allow comparison of baseline characteristics between the 2 arms of the RCT. For this reason the variables available for consideration in the multivariable models were limited by the design of the RECOVER study. Despite this, careful literature review, and consideration of biologically plausible risk factors for on-going disability after ICU suggest that most of the important known confounders were included. In some cases (diagnosis of sepsis, education level) compromises had to be made and these may have

influenced the analysis. On balance, it would appear that attempts to control for known confounders are likely to have been adequate. However, as in all observational studies, this does not rule out the impact of unknown confounders.

Secondly, it should be emphasised that the associations observed within this cohort can only be generalised to patients who are similar to the patients in the current sample. These were general ICU patients who were ventilated for longer than 48 hours who by definition stayed longer in ICU, and had more significant degrees of organ failure than general adult ICU patients as a whole.

Thirdly, it should be remembered that this analysis was based on an interventional study aiming to improve physical outcome through a coordinated in-hospital rehabilitation package. Whilst the allocation to control or intervention was randomised, and outcome assessment was blinded, it is possible that within this setting both control and intervention patients received care that was better than the ward standard. Indeed a significant majority of patients had 'good' and many patients, regardless of randomisation, had maximum RMI scores at the 3 month follow up visit.

This point raises 2 issues. Firstly, that physical outcome may have been influenced by participation in the trial, leading to bias. Secondly, the sensitivity of the outcome measure RMI was likely to be affected by the 'ceiling effect' of the outcome measure. This issue was certainly apparent statistically and required transformation of the data to make it suitable for linear regression modelling.

With regard to the development of the clinical prediction tools, significant effort was made to ensure that the regression models were statistically robust. There was therefore confidence that the regression equations were better than chance at predicting outcome but no better than an assessment of mobility at ICU discharge. The explanation for this is probably a simple one. Disability observed at ICU discharge is likely to be the major determinant of recovery and whilst statistically significant, the clinical importance of the other components of the score is small.

It was disappointing that biomarker concentrations were of little clinical utility in predicting post-ICU recovery. None of the pro-inflammatory molecules

measured at ICU discharge had significant bivariate associations with RMI at 3 months. CRP did have show a weak association that lacked statistical significance on bivariate testing but after adjustment in the model this relationship was weakened further.

Of note, elevated levels of SLPI were significantly associated with RMI at 3 months and this relationship remained important even after adjustment for baseline function. This would tend to support the hypothesis generated in chapter 3 that suggested that inflammatory resolution, and particularly serum concentrations of substances that dampen down and neutralise systemic inflammation might be important in the pathogenesis of delayed recovery from critical illness.

It is possible that a shift of focus away from serum concentrations of pro-inflammatory markers to substances critical to inflammatory resolution might give clues to the inflammatory fate of patients, and by extension, their ability to recover physical function.

An important point to note, and one that has not been addressed by this study is the concept of rehabilitation potential. Current practice in the NHS, at least in Lothian is to triage patients by making an assessment of the degree to which function at ICU discharge differs from the normal function for an individual patient. Patients assessed, as having significantly poorer function than normal would be considered to have a rehabilitation requirement. This is a sensible approach. It would be completely unrealistic to expect a patient with severe pre-existing disability to return to near normal function after an episode of critical illness. Conversely, patients with minor degrees of disability may have life changing decrements in their quality of life.

The regression models in the current study worked on the assumption that a low score on the RMI scale represented a poor outcome for an individual. In fact, low RMI scores at 3 months may actually represent a return to normal function that would be considered a favourable outcome. This is a significant limitation of the work. It should be remembered therefore that the regression models produced are

robust in predicting absolute levels of function after ICU discharge, but not rehabilitation potential or favourable outcome for individual patients.

Chapter 6 - Overall conclusions and future avenues

6.1 Prevalence of systemic inflammation after critical illness

The systematic review reported in chapter 2 presents all available data relating to inflammatory markers in the post-ICU period. Whilst many studies reported high proportions of patients with elevated markers at ICU discharge, there were no studies of patients at or beyond hospital discharge.

The observational work presented in chapter 3 of this thesis confirms that the majority of patients surviving critical illness have biochemical evidence of systemic inflammation at ICU and hospital discharge and that many have on-going inflammation 3 months after ICU discharge.

The causes and consequences of this long lasting inflammatory process are yet to be fully appreciated. As a potentially relevant risk factor for post-ICU morbidity, it provides a long duration of exposure, and is also a potential avenue for pharmacological intervention in the future. Despite the previous failures in immunomodulation in the acute phase of critical illness, it is possible that addressing the redundant immune activation in the recovery phase might have greater impact.

The limitations imposed by the observational nature of the study design were extensively discussed in chapter 3, and to some extent in chapter 2. These issues make it difficult to attribute critical illness as the cause of the observed elevations in inflammatory markers, particularly at the later time point. It is possible that many of the patients with raised inflammatory markers had similar elevations prior to the acute illness. The post hoc analyses carried out in this study does not suggest this, but in patients it almost certainly plays a part.

Future studies of this issue are likely to be challenging and would require and epidemiological population based approach. Identification of inflammation in ICU patients before they become unwell is awkward and data from animal studies is difficult to extrapolate to human critical illness. A compromise would be an observational study that rigorously identified and excluded patients with chronic

diseases or conditions known to cause elevations in inflammatory markers. Unfortunately such a study could be similarly criticised for not addressing the 'unknown' causes of systemic inflammation in healthy adults that may make them more susceptible to critical illness.

6.2 Association between inflammation recovery from critical illness

Biochemical markers of inflammation measured 3 months after ICU discharge have a significant association with physical comorbidity but not psychological comorbidity after critical illness.

This conclusion was drawn from the cross sectional analysis carried out 3 months after ICU discharge and reported in chapter 3. As noted in the systemic review in chapter 2, no previous studies have examined the association between physical recovery and post-ICU inflammation and this work therefore represents the first report of this finding.

Whilst the current work has demonstrated that inflammation and recovery are inter-related in this population, it is not yet clear whether inflammation hampers physical recovery, or whether recovering damaged muscles are in themselves an inflammatory focus. Before attention turns towards therapy, this issue must be resolved.

Two potential future avenues of research might clarify this issue. Firstly, an experimental approach employing animal models of critical illness might allow the study of subjects without a prior inflammatory lesion. A previous study of sterile and bacterial lung injury in mice has shown accelerated inflammatory resolution and improvement in organ failure using drugs that target inflammatory cell apoptosis (Lucas et al., 2013). Experimental manipulation of the inflammatory response during recovery from critical illness in mice might allow the longitudinal study of muscle function in mass under different inflammatory conditions.

A second potential avenue is to make attempts to localise inflammatory foci in patients recovering from critical illness. This would help clarify the role of the

muscle as source, or target of systemic inflammation in these patients. Recent advances in magnetic resonance imaging (MRI) may permit this line of enquiry. A clinically available MRI contrast agent contains ultra-small super-paramagnetic particles of iron oxide (USPIO) that when administered systemically are engulfed by macrophages and are then transported to sites of inflammation. Subsequent MRI imaging can then identify these sites. This technology has been employed in the prediction of aortic aneurysm growth by the uptake of USPIO, and studies of the myocardium following acute myocardial infarction but it may also be possible to identify sites of inflammation in recovering skeletal muscle (Alam et al., 2012; Richards et al., 2011).

6.3 CMV infection and its role in post-ICU inflammation and recovery from critical illness

The majority of ICU patients show serological evidence of prior CMV infection at ICU discharge, reflecting the known background prevalence of CMV latency. It is known from previous studies that during critical illness, CMV reactivation occurs in up to 38% of CMV IgG positive patients. In the current study, 11% of IgG positive patients were found to have active infection (reactivated CMV) at ICU discharge, and none had evidence of on-going viral replication at 3 months after ICU discharge.

The low rates of CMV reactivation detected in this study might reflect a lower incidence of critical illness induced reactivation in the study population. A more logical explanation is that by ICU discharge, a much later sampling point than previous studies, human immune responses have brought any CMV infection under control in the majority of patients. This is a reassuring finding because it would suggest that despite potentially high rates of viral reactivation, in those that survive to ICU discharge CMV infection does not persist. This may explain why previous studies have failed to demonstrate any effect of CMV reactivation on mortality in ICU patients. Certainly it would suggest that intervening with antiviral therapy (e.g. Gancyclovir) in the post-ICU period would be unlikely to confer any advantage.

In the current study, patients previously exposed to CMV were very similar to unexposed patients. Despite this, there were some clinically relevant differences in how they responded to critical illness. These patients were more immobile, and had increased rates of delirium at ICU discharge. Most notably, they stayed in hospital nearly twice as long after ICU discharge as patients with no evidence of previous CMV exposure. Whilst the potential for a unrecognised confounder of the relationship between CMV IgG status and post-ICU length of stay cannot be ruled out, based on the baseline data studied, it is likely that this relationship is real.

The impact of CMV latency on systemic inflammation confirms the findings of previous studies. Patients who have previously been exposed to CMV infection have a different inflammatory marker profile compared to unexposed patients during recovery from critical illness. They had higher concentrations of circulating pro-inflammatory cytokines and granular neutrophil products. Furthermore, they have lower concentrations of TGF beta 1, a substance important for inflammatory resolution.

The clinical significance of these biochemical differences requires further elucidation. Statistically significant differences in physical outcome measures were observed between IgG positive and IgG negative ICU survivors supporting the hypothesis that CMV latency has important clinical effects.

The relevance of CMV reactivation was more difficult to establish in this study. The small number of patients identified at ICU discharge with CMV reactivation meant that comparisons of inflammatory markers and clinical outcomes were statistically underpowered. Furthermore, it is reasonably likely that a proportion of patients who had experienced CMV reactivation were not detected due to the late sampling time point. This group of 'misclassified' patients are likely to have further undermined the analysis.

Patients identified as having CMV reactivation had a more severe illness. They stayed longer in ICU and were weaker and more immobile at ICU discharge. They also stayed much longer in hospital after ICU discharge. Very little difference was noted between concentrations of inflammatory markers and there was no

difference in measures of physical or psychological comorbidity, or quality of life in patients with CMV reactivation.

In conclusion, CMV latency is likely to be important factor in inflammatory resolution in ICU survivors. It may also impact on physical recovery. This study has not fully addressed whether CMV reactivation is also relevant.

This study was an opportunistic exploratory study and as such was not designed to fully characterise CMV disease in ICU patients and its impact on post-ICU recovery. Further observational study is required to establish the natural history of CMV reactivation in critical illness, its effects on inflammatory resolution, and its impact on post-ICU recovery.

6.4 Prediction of functional outcome after ICU discharge

This thesis describes the most rigorous exploration of the ability of clinical and biochemical variables to predict functional outcome in the months following ICU discharge carried out to date. Despite a number of methodological limitations, the presence of pre-existing comorbidity, and social class appear to be important risk factors for post-ICU disability. Both of these factors are difficult to modify. Interestingly age was not associated with poor outcome.

The most important predictor of post-ICU disability was the level of function observed in patients at the point of ICU discharge. Rivermead mobility index measured at ICU discharge was so important that the other significant covariates added little to RMI used on its own as a means of identifying patients likely to regain independence in the months following critical illness.

The regression models produced showed reasonable accuracy. However, the R^2 values for the regression equations were modest at best suggesting that there were many unmeasured factors at play.

The current study was an opportunistic use of trial data in combination with biomarker data collected during an ancillary study. To fully explore risk factors for poor functional outcome after intensive care further observational studies are required. Any future study will need to consider a much more targeted panel of

exploratory variables. Furthermore, the insensitivity of the RMI and its distribution were major limitation of the work. More sensitive outcome measures may allow a better characterisation of the key events.

Appendix A: Search strategies for systematic review

Database (s)	Search stage	Search string
CINAHL Plus	1	(((((((((MH "Intensive Care Units")))) OR (((MH "Critical Care")) OR (((MH "Critical Illness")) OR (((MH "Respiratory Care Unit")) OR (("critical care")) OR (("intensive care")) OR (ICU) OR (ITU) OR (("critical illness")) OR (("critically ill")))) AND (((CRP) OR ("C-reactive protein")) OR (SIRS) OR ("Systemic Inflammatory Response Syndrome")) OR (Interleukin*) OR (tnf*) OR ("tumour necrosis factor")) OR (((MH"Interleukin 1") OR (MH "Interleukins")) OR (((MH "Tumor Necrosis Factor")) OR (((MH "Inflammation")) OR (((MH "Systemic Inflammatory Response Syndrome"))))))))
CPCI-S CPCI-SSH	1	Topic=(CRP* OR "C-reactive protein" OR Interleukin* OR IL1* OR IL6 OR IL8 OR TNF* OR SIRS OR "Systemic Inflammatory Response Syndrome") OR Title==(CRP* OR "C-reactive protein" OR Interleukin* OR IL1* OR IL6 OR IL8 OR TNF* OR SIRS OR "Systemic Inflammatory Response Syndrome")
	2	Topic=(Intensive care OR critical care OR ICU or ITU or Critical illness OR critically ill) OR Title=(Intensive care OR critical care OR ICU or ITU or Critical illness OR critically ill)
	3	1 AND 2

Appendix B: Example data request for Systematic Review



David Griffith. RoomW2.03
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Dr N Al-Subai
General Intensive Care Unit
St George's Hospital, London
SW17 0QT
UK
nalsubaie@gmail.com

2 November 2012

Dear Dr Al-Subai,

Request for further information in relation to your study:

Al-Subai et al C-reactive protein as a predictor of outcome after discharge from the intensive care: a prospective observational study. BJA. 2010 September 105(3):318-25

Background information

Our research group is pursuing several research avenues exploring the role of persistent inflammation in survivors of critical illness, and relating this to physical recovery. We are currently undertaking a large randomized controlled trial testing a complex rehabilitation intervention on physical outcomes after critical illness (RECOVER study) and as part of this, we are exploring the role of inflammation in preventing physical recovery. In addition we are conducting smaller clinical study (DIGRESS) examining immune cell dysfunction in critical illness survivors (see links below).

We are also undertaking a systematic review of the literature to answer the following questions:

1. What is the prevalence of systemic inflammation in survivors of critical illness?
2. What is the relationship between systemic inflammation in survivors of critical illness and markers of physical recovery?

We wish to collate the available data from the literature in order to provide evidence of an ongoing inflammatory process in ICU survivors, and where possible, to relate this to markers of physical recovery.

We are particularly interested in the values of inflammatory parameters at the following time points:

1. The point of ICU discharge
2. The period between ICU discharge and hospital discharge.
3. After hospital discharge.

Our request

Our request

In your study, you recorded CRP concentration in survivors of critical illness at the point of ICU discharge. You present these data as median (IQR) for your 2 groups of interest. We are interested in absolute values of CRP for those patients surviving to ICU discharge so that we can evaluate variability of the results, and calculate the proportion of patients who have ongoing systemic inflammation at the point of ICU discharge.

1. What were the absolute values of CRP concentration for your ICU survivors?
2. If you cannot provide absolute values, are you able to provide us with the following summary data?
 - a) The mean (with standard deviation) and/or median (with interquartile range) for CRP in those patients (combining both of your groups of interest) at the point of ICU discharge.
 - b) How many patients had a CRP >5mg/L at the point of ICU discharge?
 - c) How many patients had a CRP >10mg/L at the point of ICU discharge?

We would be very grateful if you could fill in the form attached and where relevant, send us the requested data.

If you are unable to help

Even if you are unable to provide data, we are interested to know the reason for this. We would be extremely grateful if you could respond by filling in and returning in the attached form indicating whether or not you are able to help and giving reasons if this is not possible.

Thanks for helping us with our study.

Yours Sincerely

Dr David Griffith

Prof Adriano Rossi

Prof Timothy Walsh

Further information:

Our research group:

<http://www.cir.ed.ac.uk/investigator/Professor-Tim-Walsh>

<http://www.cir.ed.ac.uk/investigator/Professor-Adriano-G-Rossi>

<http://www.ccm.mvm.ed.ac.uk/>

RECOVER study:

<http://bmjopen.bmj.com/content/2/4/e001475.full>

Appendix C: Data extraction table for Systematic Review

Appendix D: The Rivermead Mobility Index

Ask the patient each question. Observe for question 5. Score 1 for 'yes', 0 for 'no'.

Total possible score = 15

Topic	Question	Score
Turning over in bed	Can you turn over from your back to your side without help?	
Lying to sitting	From lying in bed, do you get up to sit on the edge of the bed on your own?	
Sitting balance	Do you sit on the edge of your bed without holding on for 10 seconds?	
Sitting to standing	Do you stand up from any chair in less than 15 seconds and stand there for 15 seconds, using hands and/or an aid if necessary?	
Standing unsupported <i>Ask to stand</i>	Observe standing for 10 seconds without any aid	
Transfer	Do you manage to move from bed to chair and back without any help?	
Walking in side (with and aid if necessary)	Do you walk 10 meters, with an aid if necessary, but with no standby help?	
Stairs	Do you manage a flight of stairs without help?	
Walking outside (even ground)	Do you walk around outside on pavements, without help?	
Walking inside, with no aid	Do you walk 10 meters inside, with no caliper, splint, or other aid (including furniture or walls) without help?	
Picking up off floor	Do you manage to walk five meters, pick something up from the floor, and then walk back without help?	
Walking outside (uneven ground)	Do you walk over uneven ground (grass, gravel, snow, ice etc) without help?	
Bathing	Do you get into/out of a bath or shower and wash yourself unsupervised and without help?	
Up and down four steps	Do you manage to go up and down four steps with no rail, but using an aid if necessary?	
Running	Do you run 10 metres without limping in fours seconds (fast walk, not limping, is acceptable)?	
	TOTAL	

Appendix E: Hand grip strength population norms

Age	MALE		FEMALE	
	Right	Left	Right	Left
20-29	47(9.5)	45(8.8)	30(7)	28(6.1)
30-39	47(9.7)	47(9.8)	31(6.4)	29(6)
40-49	47(9.5)	45(9.3)	29(5.7)	28(5.7)
50-59	45(8.4)	43(8.3)	28(6.3)	26(5.7)
60-69	40(8.3)	38(8.0)	24(5.3)	23(5)
70 +	33(7.8)	27.2 (3.9)	20(5.8)	19(5.5)

Age and gender stratified hand grip strength in kilograms (Massy-Westropp et al., 2011)

Appendix F: Hospital Anxiety and Depression Scale

Question / score	0	1	2	3
1. I feel tense or 'wound up'	Not at all	Time to time, occasionally	A lot of the time	Most of the time
2. I still enjoy the things I used to enjoy	Definitely as much	Not quite so much	Only a little	Not at all
3. I get a sort of frightened feeling like something awful is about to happen	Very definitely and quite badly	Yes but not too badly	A little but it doesn't worry me	Not at all
4. I can laugh and see the funny side of things	As much as I always could	Not quite so much now	Definitely not so much now	Not at all
5. Worrying thoughts go through my mind	A great deal of the time	A lot of the time	From time to time but not too often	Only occasionally
6. I feel cheerful	Most of the time	Sometimes	Not often	Not at all
7. I can sit at ease and feel relaxed	Definitely	Usually	Not often	Not at all
8. I feel as if I am slowed down	Not at all	Sometimes	Very often	Nearly all of the time
9. I get a sort of frightened feeling like "butterflies in the stomach"	Not at all	Occasionally	Quite often	Very often
10. I have lost interest in my appearance	I take just as much care as ever	I may not take quite as much care	I don't take as much care as I should	Definitely
11. I feel restless as if I have to be on the move	Not at all	Not very much	Quite a lot	Very much indeed
12. I look forward with enjoyment to things	As much as I ever did	Rather less than I used to	Definitely less than I used to	Hardly at all
13. I get sudden feelings of panic	Not at all	Not very often	Quite often	Very often indeed
14. I can enjoy a good book or radio or TV programme	Very seldom	Not often	Sometimes	Often

HADS-A = questions 1, 3, 4, 7, 9, 11, 13. HADS-D = questions 2, 5, 6, 8, 10, 12, 14. Total score for each domain = 21.

Appendix H: SF-12v2

- This survey asks for your views about your health. This information will keep track of how you feel and how well you are able to do your usual activities. For each of the answers, please mark an X in the one box that best describes your answer.

1. In general, would you say your health is:				
Excellent <input type="checkbox"/>	Very Good <input type="checkbox"/>	Good <input type="checkbox"/>	Fair <input type="checkbox"/>	Poor <input type="checkbox"/>
2. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?				
a. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf:				
Yes, limited a lot <input type="checkbox"/>	Yes, limited a little <input type="checkbox"/>	No, not limited at all <input type="checkbox"/>		
b. Climbing several flights of stairs:				
Yes, limited a lot <input type="checkbox"/>	Yes, limited a little <input type="checkbox"/>	No, not limited at all <input type="checkbox"/>		
3. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?				
a. Accomplished less than you would like:				
All of the time <input type="checkbox"/>	Most of the time <input type="checkbox"/>	Some of the time <input type="checkbox"/>	A little of the time <input type="checkbox"/>	None of the time <input type="checkbox"/>
b. Were limited in the kind of work or other activities:				
All of the time <input type="checkbox"/>	Most of the time <input type="checkbox"/>	Some of the time <input type="checkbox"/>	A little of the time <input type="checkbox"/>	None of the time <input type="checkbox"/>
4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling anxious or depressed)?				
a. Accomplished less than you would like:				
All of the time <input type="checkbox"/>	Most of the time <input type="checkbox"/>	Some of the time <input type="checkbox"/>	A little of the time <input type="checkbox"/>	None of the time <input type="checkbox"/>
b. Did work or other activities less carefully than usual:				
All of the time <input type="checkbox"/>	Most of the time <input type="checkbox"/>	Some of the time <input type="checkbox"/>	A little of the time <input type="checkbox"/>	None of the time <input type="checkbox"/>

5. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all A little bit Moderately Quite a bit Extremely

Appendix I: Data request / RECOVER analysis plan



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Edinburgh EH16 4SB
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01312426395

Dr Steff Lewis
Coordinating Statistician
Edinburgh Critical Care Trials Group
University of Edinburgh

6th August 2013

Cc. Tim Walsh, Julia Boyd, Ashma Krishan

Enc. Analysis plan including data request.

Dear Steff,

I am writing to request data from the RECOVER trial database to undertake analysis of the RECOVER biomarker study titled '*Persisting Inflammation following critical illness: prevalence, relationship to physical recovery, and exploration of defects in inflammatory resolution pathways*'.

I enclose a detailed request and analysis plan for the study. This serves as a justification for the database fields requested.

I understand that at this stage, 12-month follow up is not complete and the study has not been reported. I therefore do not request trial group allocations at this time. I am keen to perform additional analysis using this data after RECOVER has concluded and will send a further request for data at this time.

Please contact me if you have any queries regarding this data request

Yours sincerely,

Dr David Griffith
Research Fellow in Critical Care

ANALYSIS PLAN – RECOVER BIOMARKER STUDY

Author: David Griffith.

Date: 6th August 2013

Brief Description of Study

The RECOVER study is a randomised controlled trial testing the hypothesis that a complex rehabilitation intervention can improve physical outcome in survivors of critical illness. To investigate the role of persistent systemic inflammation in recovery from critical illness, a blood sampling sub study was incorporated into the trial design. Blood samples were taken at ICU discharge, weekly whilst patients were in hospital, and then at the 3-month follow up point. Blood was analysed for a panel of inflammatory biomarkers and markers of inflammatory resolution. As a further analysis, patients were screened for reactivation of cytomegalovirus (CMV) to determine whether active CMV infection inhibits inflammatory resolution and therefore physical recovery.

The a priori analysis plan is described in the following sections. Further explorative analysis will be carried out depending on the findings of this analysis. Requested data fields can be found in section 14.

1. Description of the study sample
2. Prevalence of systemic inflammation in ICU survivors
3. Characterisation of the inflammatory and inflammatory resolution profile during recovery from critical illness
4. Physical and psychological outcomes of ICU survivors
5. Association of systemic inflammation with indices of physical and psychological recovery
6. Evidence of previous CMV exposure and active CMV infection
7. Investigation of factors associated with evidence of CMV reactivation at ICU discharge
8. Duration of CMV infection
9. Association of prior CMV exposure / active infection and inflammatory resolution
10. Association of prior CMV exposure / active infection and indices of physical and psychological recovery
11. Prediction of physical recovery using clinical information available at ICU discharge
12. Addition of inflammatory biomarkers to clinical information to predict physical recovery after critical illness
13. Further notes on regression models
14. Requested data

1. Description of study sample

Details of study recruitment and follow-up will be provided in a flow diagram (figure 1). Baseline characteristics of recruited patients will be presented. Baseline variable that will be included will be:

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- Age (mean (SD))
- Gender (M/F (%F))
- Functional comorbidity index (mean (SD))
- Functional comorbidity index subcategories (n (%))
- ICU admission diagnosis (n (%))
- APACHE II score (mean (SD))
- ICU LOS (mean (SD))
- Hospital LOS in days (mean (SD))
- Ventilator days (mean (SD))
- Inotropes at any time (n(%))
- Days of inotropes (median (IQR))
- RRT at any time (n(%))
- Days on RRT (mean (SD))
- Worst SOFA score (median (IQR))
- SOFA subcategories (median (IQR))
- CAM ICU positive (n(%))
- RMI at randomisation (median (IQR))

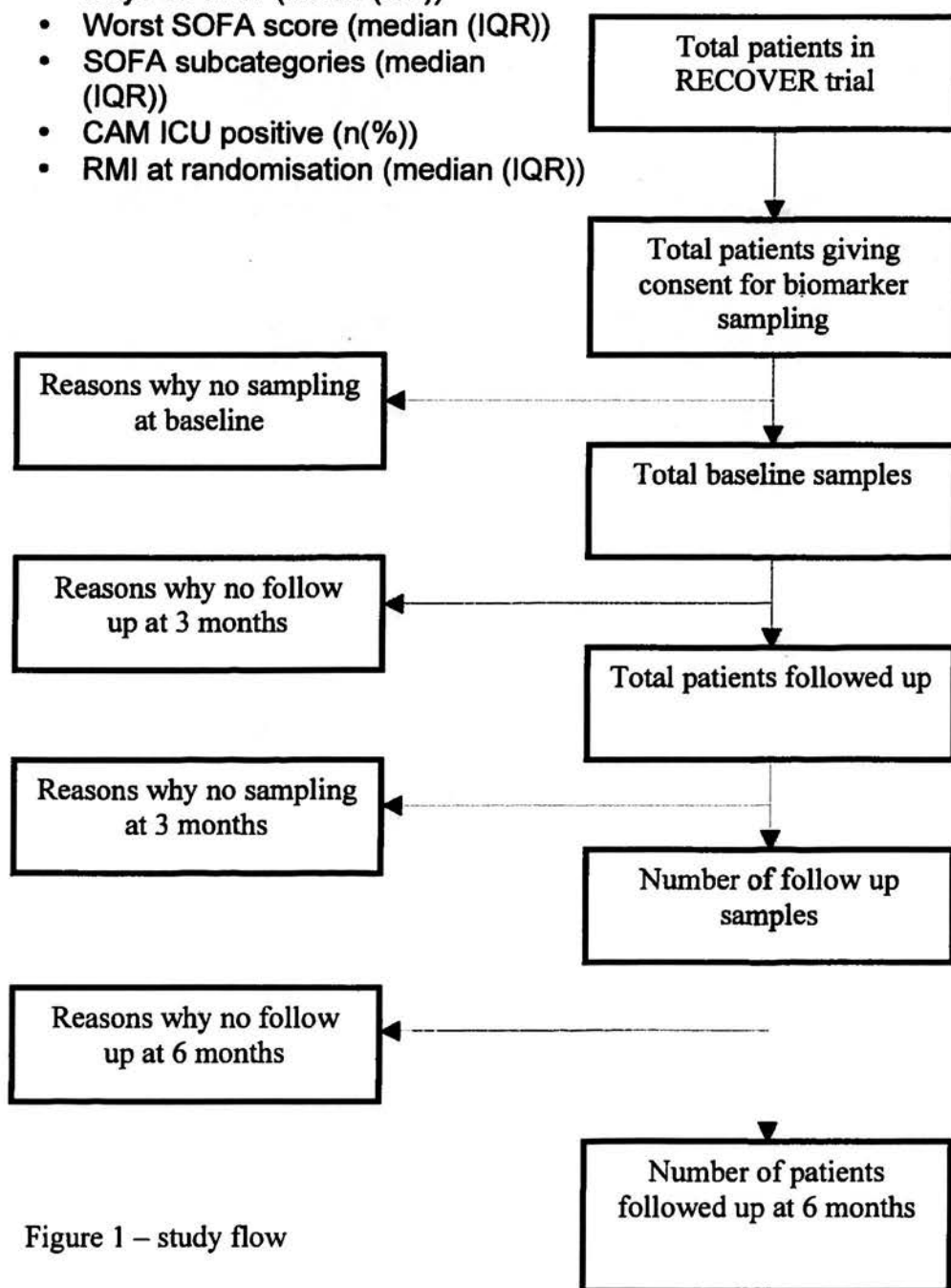


Figure 1 – study flow

To allow assessment of selection and attrition bias, comparison will be made with patients that declined consent, and those that were lost to follow up.

2. Prevalence of systemic inflammation in ICU survivors

In health CRP concentration rarely rises above 3mg/L even in elderly cohorts. In a young adult cohort, a CRP concentration of greater than 10mg/L represents the 99th centile, and 3mg/L the 90th centile. Persistent inflammation as therefore defined according to these 2 definitions. The following time points of interest are arbitrarily defined:

- ICU discharge (study entry)
- Closest sample to hospital discharge (variable time after ICU discharge)
- Community sample (3 month follow up visit)

The number and proportion of surviving patients with evidence of systemic inflammation for the 3mg/L and 10mg/L threshold at each of these time points (CRP >3mg/L or >10mg/L) will be reported.

3. Characterisation of the inflammatory and inflammatory resolution profile during recovery from critical illness

Blood markers of systemic inflammation and inflammatory resolution (CRP, IL1 beta, IL6, IL8, C3A, C4A, C5A, MPO, elastase, TGF beta and SLPI), will be reported at 2 time points – ICU admission and at the 3 month follow up period. Serum concentrations of all biomarkers are anticipated to be non-parametric in distribution and will be presented as median (IQR). Serum CRP concentration will also be reported at an additional time point (hospital discharge).

For patients surviving to the 3 month follow up visit, CRP concentration will also be measured at each in-hospital sampling point. Median values of CRP with interquartile ranges will be presented as a line chart to depict the inflammatory resolution trajectory.

4. Physical and psychological outcomes of ICU survivors

Physical and psychological outcome measures will be presented for the whole cohort at both 3 months and 6 months.

Mobility: Rivermead mobility index will be reported as median (IQR).

Hand strength: Using age and gender stratified population norms, handgrip strength (HGS) measurements will be converted into percentage predicted HGS and reported as mean (SD). In addition, 3 outcome groups will be identified to identify patients with particularly poor physical recovery. Patients with a HGS that falls more than 2 standard deviations below mean predicted

HGS for age and gender will be considered 'very weak'. Patients with HGS between 1 and 2 standard deviations below the predicted HGS will be considered 'weak'. All other patients will be considered 'normal'. The number and proportions of patients falling into these categories will be reported.

Quality of Life: Total, physical component and mental component scores of the Medical Outcomes Study Short Form 12 (SF-12) will be presented as median (IQR).

Anxiety and Depression: Total Hospital Anxiety and Depression Scale (HADS) will be reported as median (IQR). Patients scoring more than 8 points on the anxiety component will be considered to have a positive screening test for anxiety. The same threshold will be used for the depression component. The number and percentage of patients with a positive screening test for anxiety or depression will be reported.

Post-traumatic stress disorder: Davidson's trauma scale (DTS) will be reported as median (IQR). PTSD will be defined as DTS > 40. Number and percentage of patients with PTSD will be reported.

Falls risk: Number of seconds take to complete the timed up and go (TUG) test will be reported as median (IQR).

5. Association of systemic inflammation with indices of physical and psychological recovery

A cross-sectional analysis of CRP concentration at indices of physical recovery at 3 months will be performed. Scatter plots will be drawn representing the relationships between CRP concentration and the outcome measures detailed in section 5. Pearson's correlation coefficients will be reported with p values. Kendall's tau-b correlation coefficients will be calculated for non-parametric correlation.

In addition, to explore the association of systemic inflammation and diagnostic subgroups (depression, anxiety, and PTSD) and patients with particularly poor physical recovery (lower quartile of RMI, weak HGS category), CRP concentration will be reported in binary groups and presented as boxplots. Comparisons will be made with Wilcoxon rank-sum tests.

6. Evidence of previous CMV exposure and CMV reactivation

The number and percentage of patients with previous CMV exposure will be reported. Previous CMV exposure will be defined as a positive test for CMV IgG antibody at ICU discharge.

The number and percentage of patients with evidence of CMV reactivation will be reported. CMV reactivation will be defined as the number of patients with previous CMV exposure that have detectable CMV DNA in plasma at ICU discharge.

7. Investigation of factors associated with evidence of CMV reactivation at ICU discharge

Baseline data will be presented for each patient with evidence of CMV reactivation at ICU discharge. Comparison will be made with patients that do not have evidence of CMV infection at this time.

8. Duration of CMV infection

In those patients who have evidence of CMV infection at ICU discharge, the number and percentage of patients of those patients that still have CMV DNA in plasma at 3 months will be reported.

9. Association of prior CMV exposure / CMV reactivation and inflammatory resolution

Box plots of CRP concentration at 3 months grouped according to previous CMV exposure and active CMV infection will be presented. In addition the median (IQR) for these comparisons will be reported. The results of Wilcoxon rank-sum tests will be reported for each of the 2 comparisons.

10. Association of prior CMV exposure / CMV reactivation and indices of physical and psychological recovery

The outcome measures described in section 4 will be compared according to previous CMV exposure group. In addition the outcome measures will be compared according to CMV reactivation group.

Box plots for each comparison will be presented and comparisons will be performed using Wilcoxon rank-sum tests.

11. Prediction of physical recovery using clinical information available at ICU discharge

Linear regression analysis (bivariate) will be carried out using RMI as a continuous outcome variable. Factors considered potentially important in predicting physical outcome are age, pre-existing frailty, severe and prolonged illness, multiple organ failures, and physical function at ICU discharge. The following 6 variables will therefore be tested:

- Age
- Functional comorbidity index
- APACHE 2 score
- Worst SOFA score in ICU
- Length of stay in ICU
- Rivermead mobility index at baseline

Variables with predictive potential ($p < 0.25$ on bivariate testing) will be entered into a multivariable linear regression model using a stepwise approach. Non-significant variable ($p > 0.1$) will be discarded from the final model.

12. Addition of inflammatory biomarkers to clinical information to predict physical recovery after critical illness

A further 8 variables will be subjected to bivariate testing. These variables represent markers of persistent inflammation and inflammatory resolution. Variables with predictive potential ($p < 0.25$ on bivariate testing) will be entered into a multivariable linear regression model using a stepwise approach. Non-significant variables ($p > 0.1$) will be discarded from the final model.

Variables included will be:

- CRP
- IL6
- IL8
- TGF beta
- MPO
- Neutrophil elastase
- SLPI

13. Further notes on regression models

Power It is anticipated that 150 patients will have complete follow up for this analysis. A maximum of 15 variables (10 variables per case) will be permitted.

Model assumptions All linear model assumptions will be tested. Collinearity of variables will be tested by correlation prior to model fitting. Where collinearity is detected (correlation coefficient > 0.7), only one of the collinear variables will be tested in the regression model.

14. Requested data fields

Details of blood sampling

Date of randomisation

Date of follow up visit

Date of ICU discharge

Consent to blood sampling (yes/no)

Baseline sample taken (yes/no)

Reason if baseline sample not taken

Survival to 3 month follow up visit (yes/no)

Date of death

Follow up completed at 3 months (yes/no)

Reason if no follow up at 3 months

Follow up blood sample taken (yes/no)

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Reason if no follow up blood sample
Survival to 6 month follow up (yes/no)
Follow up completed at 6 months (yes/no)
Reason if no follow up at 3 months

Baseline data

Age
Gender
FCI
FCI subcategory
ICU diagnosis
APACHE II score
ICU length of stay
Hospital length of stay
Ventilator days
Inotropes at any time (yes/no)
Days of inotropes
Renal replacement therapy at any time (yes/no)
Days on renal replacement therapy
Worst SOFA score (and worst score in each category)
CAM ICU score at randomisation
Rivermead mobility index at randomisation
Handgrip strength at 1 week after randomisation (first HGS)

In hospital

Weekly hand grip strength and rivermead mobility index scores

3 months

RMI
HGS
SF-12 – total score, PCS, and MCS
HADS score – total score, depression score, anxiety score
DTS – total score
Timed up and Go

6 months

RMI
SF-12 – total score, PCS, and MCS
HADS score – total score, depression score, anxiety score
DTS – total score

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