SYSTEMIC INFLAMMATORY RESPONSE SYNDROME AND SEPSIS IN MAJOR VASCULAR SURGERY

Tiffany Alicia Hassen, MBBS.

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The University of Adelaide, The Queen Elizabeth Hospital, Adelaide, Australia

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Abstract

Background

Prediction of post-operative systemic inflammatory response syndrome (SIRS) and sepsis would assist risk stratification prior to major vascular surgery, and may positively affect morbidity and mortality rates. Reciprocal regulation exists between immuno-inflammatory and neuroendocrine responses. Like inflammatory events, the neuroendocrine stress response is an important component of surgical pathophysiology. Psychological variables may influence neuroendocrine function and therefore modulate the neuroendocrine response to major vascular surgery. Comparison of both immuno-inflammatory and neuroendocrine responses to open abdominal aortic aneurysm (AAA) repair and endovascular aneurysm repair (EVAR) would enhance understanding of biological mechanisms underlying the differing clinical outcomes from these surgical approaches.

Aims

The aims of the current study were: (1) to identify relationships between post-operative SIRS and sepsis and markers of immunological, neuroendocrine, nutritional and psychological status; (2) to examine relationships between psychological variables and neuroendocrine responses to surgery; and (3) to identify differences in immunological and neuroendocrine responses to open AAA repair compared with EVAR.

Methods

A prospective cohort study was performed involving patients undergoing elective open AAA repair (n = 36), EVAR (n = 17) and lower limb revascularisation (n = 17). Pre-operative neutrophil expression of CD11b and CD16b was determined, and CD32a and CD16b genotyping performed. Plasma cytokines were assayed pre-operatively, during maximal intra-operative ischaemia (T_0), and 4, 24 and 72 hours following T_0. Twenty-four hour urinary free cortisol (UFC) and catecholamine excretion was assayed pre-operatively [T(pre-op)], from anaesthetic induction [T(0-24)] and 72 hours later [T(72-96)]. Pre-operative nutritional status was assessed by dual energy x-ray absorptiometry (DEXA). Pre-operative depression and trait anxiety were evaluated using self-report inventories. SIRS severity and duration within five post-operative days and sepsis within 30 days were documented.

Results

A positive correlation was identified between CD11b expression and SIRS severity score amongst EVAR patients. Neither CD32a nor CD16b genotypes were associated with sepsis. Fluctuations in IL-6, IL-8, and IL-10 were frequently observed in association with AAA repair whereas elevations in TNF-α, Il-1β and IL-12p70 were infrequent. IL-10 production predicted sepsis in this cohort but could not be used to confidently predict sepsis for any individual. Negative correlations were identified between fat free mass (FFM) and both SIRS score following open AAA repair and SIRS duration following EVAR. A negative correlation existed between skeletal muscle mass (SMM) and both SIRS score and duration following open AAA repair. SIRS duration was significantly longer amongst non-depressed compared to depressed EVAR subjects categorised by the Beck Depression Inventory - II (BDI-

II), however no difference was apparent when the Center for Epidemiological Studies-Depression Scale (CES-D) was employed.

UFC excretion at T(0-24) was significantly greater amongst non-depressed compared to depressed subjects, but only when BDI-II classifications were used. Correlations between trait anxiety and neuroendocrine responses to surgery were weak and non-significant.

Open AAA repair stimulated greater production of IL-6, IL-8 and IL-10 than EVAR. Accordingly, SIRS was more frequent, severe and prolonged following open AAA repair. At T(72-96) both UFC and adrenaline excretion were greater amongst the open AAA than the EVAR cohort. These neuroendocrine responses were positively correlated with measures of SIRS and were significantly greater amongst those who developed sepsis.

Conclusions

Higher pre-operative CD11b may be associated with more severe SIRS following major vascular surgery. Lower pre-operative FFM and SMM are associated with more severe SIRS after AAA repair. Neuroendocrine responses reflect rather than predict post-operative SIRS and sepsis. No relationships between psychological parameters and neuroendocrine responses to surgery were identified. Robust evidence of a greater inflammatory response to open AAA repair than EVAR has clarified contradictory reports in existing literature.

Statement of Originality

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Tiffany Alicia Hassen

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Publications and Presentations

The following publication and presentations were derived from the research presented in this thesis:

Publication

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Endovascular repair of abdominal aortic aneurysms reduces the intra-operative stress response and post-operative morbidity.

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Department of Vascular Surgery, The Queen Elizabeth Hospital, Adelaide, South Australia.

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Endovascular abdominal aortic aneurysm repair reduces the neuroendocrine stress response and post operative complication rate compared with open repair.

TA Hassen, S Pearson, A Khanna, PA Cowled and RA Fitridge.

Department of Vascular Surgery, The Queen Elizabeth Hospital, Adelaide, South Australia.

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Systemic inflammatory response syndrome and sepsis following major vascular surgery – sequelae independent of pre-operative neutrophil integrin and immunoglobulin receptor expression.

TA Hassen, PA Cowled, S Pearson and RA Fitridge.

Department of Vascular Surgery, The Queen Elizabeth Hospital, Adelaide, South Australia.

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Predicting systemic inflammatory response syndrome and sepsis post major vascular surgery – the value of pre-operative neutrophil integrin and immunoglobulin receptor expression.

TA Hassen, PA Cowled, S Pearson and RA Fitridge.

Department of Vascular Surgery, The Queen Elizabeth Hospital, Adelaide, South Australia.

The Queen Elizabeth Hospital Research Day, Adelaide, South Australia, 21st October, 2005.

Cytokine profiles as intra-operative and post-operative predictors of the development of sepsis following surgery for abdominal aortic aneurysm.

J Ahern[1], T Hassen[1], P Cowled[1], R Fitridge[1], G Hodge[2] and H Zola[2].

[1]Department of Vascular Surgery, The Queen Elizabeth Hospital, Adelaide, South Australia and [2]The Women's and Children's Hospital, Adelaide, South Australia.

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CHAPTER 1

INTRODUCTION,

LITERATURE REVIEW

AND

AIMS OF CURRENT STUDY

1.1 Introduction

Abdominal aortic aneurysm (AAA) is a relatively common vascular pathology affecting 5-9% of males above 65 years of age.[1, 2] Epidemiological evidence from discrete periods between 1950 and 1995 suggests an increasing incidence of AAA's in Australia,[3] Sweden,[4] the United States,[5] Asia,[6] and the United Kingdom.[7, 8] Recently reported incidence rates range from 3.0 to 117.2 per 100 000 in the at-risk Caucasian population.[2]

The considerable burden of management of this pathology not only arises from its significant prevalence, however, but also from its natural history and the morbidity and mortality associated with AAA repair. In the absence of surgical intervention, the natural history of AAAs is one of progressive expansion and ultimately rupture[9], with those patients with aneurysms 7.0cm or more in diameter subject to a one-year incidence of probable rupture of 32.5%[10] and subsequently a mortality rate estimated to be approximately 48%[11] associated with emergency repair necessitated to salvage the otherwise uniformly fatal event of rupture. Furthermore, whilst elective surgical repair prevents the progression to an emergency procedure with its associated high risk of death and can prolong life, and despite improvements in post-operative outcome achieved over recent decades,[12-14] elective open repair continues to generate the significant overall and major morbidity rates of 17-29% and 5-14% respectively,[14, 15] with 30 day mortality rates reported by single-centre series ranging from 1.6% to 6.5%,[12, 13] increasing to between 6 and 8% for operative and hospital mortality rates reported by regional and population studies.[13, 16] Whilst the advent of the apparently less invasive endovascular approach to aneurysm repair results in more favourable short and mid-term morbidity profiles,[17-20] suggested benefits to

short-term mortality[17] have not been consistently demonstrated.[21-24] Given the substantial operative risks associated with AAA repair, combined with the fact that patients undergoing this procedure are predominantly elderly, the need for careful pre-operative risk stratification and case selection is clear. Furthermore, development of novel interventions to modify risk would certainly be welcomed by clinicians and their patients facing AAA surgery.

A number of studies have examined potential prognostic indicators of outcome, particularly 30-day mortality, following elective AAA repair. Poor lung function, indicated by factors including low forced expiratory volume in one second (FEV_1), a history of chronic obstructive pulmonary disease, dyspnoea or pulmonary surgery; and impaired renal function have been repeatedly identified as having prognostic value in predicting death following AAA repair.[25-31] Similarly, cardiac co-morbidity, indicated by factors including a history of myocardial infarction, congestive heart failure and electrocardiographic evidence of myocardial ischaemia, has been found to be a risk factor in many published studies.[26, 28, 30, 31] Associations between older age and high post-operative risk have been identified in univariate analyses, however the effect appears to be attenuated when confounding co-morbidities are considered.[28-30, 32] Cerebrovascular disease[33] and female gender may also predict a slightly higher risk of mortality[27, 30] as may reduced hospital volume[34] and surgeon's experience.[35] It is undeniable that an awareness of these risks is useful in obtaining a perception of an individual's operative risk. Furthermore, efforts have been made to utilize this awareness of risk factors to generate objective methods of pre-operative risk scoring. The validity and practical utility of these methods is variable.

The modified Goldman cardiac risk index is one of the earlier, and most widely known methods of cardiac risk stratification developed for patients undergoing noncardiac surgery, including those undergoing major vascular surgery.[36] Whilst its focus on cardiac risk did address one of the leading causes of post-operative mortality[26, 29, 37-39] and a frequent cause of morbidity[15, 40-42] following AAA repair, it does not attempt to address any of the other frequent causes of post-operative complications, and most importantly, its predictive value amongst vascular patients now appears doubtful,[43] prompting its use to be discouraged.[44] Lette *et al.*[43] identified seven clinical scoring systems for determining cardiac risk, however an analysis of the predictive capacity of these and an additional eighteen clinical parameters amongst vascular surgery patient suggested that only diabetes and one of the scoring systems considered correlated with cardiac morbidity and death amongst this patient cohort. More recent efforts have been made to utilize available knowledge about risk factors to develop more inclusive risk scoring tools derived in, and dedicated to, patients undergoing AAA repair. The most notable of these are the Glasgow aneurysm score[33] , the Eagle Score[45] the Leiden score[30] , the Modified Leiden score[46] and the Vanzetto score[47] , each determining overall operative risk based on assessments of various combinations of criteria which account for age, gender, cardiac, pulmonary, renal, and cerebrovascular disease, diabetes and centre-specific mortality. Nesi *et al.* recently examined the validity of these five risk scoring methods and encouragingly found that all methods predicted the risk of in-hospital mortality with reasonable accuracy, however these systems did not perform well in predicting post-operative complications.[42] Furthermore, the complexity of the Leiden methods was considered as making their clinical use somewhat difficult.[42]

The existing knowledge of risk factors and methods of risk stratification may, in some cases, precipitate interventions for the purpose of reducing operative risk. Impaired pre-operative pulmonary function may, for example, prompt peri-operative strategies including the use of bronchodilators, physiotherapy, glucocorticoid administration, cessation of smoking, avoidance of the long acting neuromuscular blocker pancuronium, and optimisation of post-operative analgesia which may reduce the risk of pulmonary complications, whilst a knowledge of renal impairment may, for example, encourage a heightened awareness of the adequacy of fluid repletion.[46] The suggestion of coronary artery disease may, similarly, prompt treatment strategies with the intent of improving outcome, however there is evidence to suggest that pre-operative cardiac revascularization may not offer significant benefit[28], and the time taken for evaluation and treatment of coronary artery disease before vascular surgery may in fact be harmful.[48] The relatively recent emergence of robust evidence of a reduction in peri-operative cardiac death and non-fatal myocardial infarction associated with the use of beta-adrenoreceptor antagonists (β-blockers) amongst high-risk patients undergoing major vascular surgery[49, 50] has provided an additional, perhaps under-utilised,[51] method of cardiac risk reduction. The presence of non-modifiable risk factors, such as age or gender, clearly does not provide an opportunity for specific interventions to reduce the associated risk. They may, however, prompt the use of an alternative treatment approach, namely endovascular aneurysm repair (EVAR).

It is seems likely that the benefits to be derived from the existing capacity to both stratify and modify risks associated with AAA repair have already been expressed in the reduction in morbidity and mortality seen over recent decades.[14] Achieving

further improvements may require a novel approach to assessing and potentially modifying specific risks which contribute to these two end-points. Assessment of an individual's pre-operative immune status, and their immunological response to AAA surgery is one such potential approach to risk stratification worthy of investigation.

The benefits to be derived from an enhanced ability to stratify an individual's operative risk are also likely to be applicable to those undergoing lower extremity revascularization procedures for artherosclerotic disease of the lower extremity. Symptomatic peripheral vascular disease of the lower limb is a common entity, the frequency of which increases with advancing age.[52] Recent reports derived from populations in the United Kingdom describe prevalences of intermittent claudication amongst elderly men ranging from 4.6%[53] to 7%[54] whilst the incidence of critical limb ischaemia has been estimated by the European Working Group as ranging from 500 to 1000 per million population per year[55] . Whilst the treatment option of surgical revascularization of the lower limb is considered as frequently imposing a lesser degree of physiological stress than that of aortic aneurysm repair[56] the necessity to adequately assess risk remains high, in view of the high burden of co-morbidity, and indeed debility amongst this patient cohort reflected by particularly high 5-year cumulative mortality rates reported to range from 4.8%[57] to 17% [58] for male claudicants rising to 50% for those with critically ischaemic limbs.[59] This degree of pre-operative morbidity may contribute to the 30-day operative mortality rates of 2.3% to 8% [60-62] reported for lower extremity bypass; rates comparable to those for AAA repair. The significance of measures which have the potential to reduce the post-operative morbidity and mortality experienced by this patient group is heightened by the frequency of this procedure in current vascular surgical

practice.[63] Furthermore, the availability of non-surgical treatment options, including angioplasty, sympathectomy or pharmacological measures such as prostaglandins[64], provides an alternative for those deemed unsuitable for surgical revascularization and therefore heightens the obligation to accurately assess surgical risk, and to minimize this risk whenever possible. Whilst it is cannot be disputed that cardiovascular events are the leading cause of post-operative mortality and morbidity after vascular surgery,[65] it is proposed that, as for aortic surgery, immune status, or factors reflective of immunocompetence, are worthy of investigation as indicators of specific risks incurred with lower extremity revascularization surgery.

1.2 Systemic Inflammatory Response Syndrome (SIRS) and Sepsis

The concepts of systemic inflammatory response syndrome (SIRS), sepsis and the adverse immunologically based sequelae which they may precede, namely septic shock, multiple organ dysfunction syndrome (MODS) and multiple organ failure (MOF),[66, 67] usefully categorize the undeniably complex immunological processes that may be experienced by patients subject to injury, including major vascular surgery. These events describe a hierarchical continuum of increased inflammatory response to injury, and inturn, an increasing risk of adverse outcomes associated with immune dysfunction.[66] Whilst this method of classification is not without some criticism,[68] it does permits a comprehensible approach to considering the risk of adverse outcomes. A capacity to predict, and ultimately ameliorate, the post-operative occurrence of SIRS, sepsis and the adverse immunological sequelae which they may precede would assist clinicians in the task of pre-operative risk stratification prior to major vascular surgery and is likely to positively impact upon post-operative morbidity and mortality rates, particularly those pertaining to AAA repair.

1.2.1 Pathophysiology of SIRS

Localized inflammation is the body's innate and initial, non-specific protective response to tissue injury produced by mechanical, chemical or microbial stimuli involving cellular effectors and humoral mediators.[67] The cellular effectors of this highly amplified, but generally tightly controlled inflammatory response are the polymorphonuclear leukocytes (PMNs), monocytes/macrophages and endothelial

cells, whilst amongst the initial pro-inflammatory mediators released into the micro-environment are eicosonadoids, platelet–activating factor (PAF), and a selection of cytokines[69] . The latter are simple polypeptides or glycoproteins, ≤30 kDa in size, with pleiotropic, regulatory effects frequently exerted over cells of the immune system.[70] Tissue insult or injury triggers activation of the leukocyte effectors resulting in increased leukocyte aggregation and tissue infiltration, where the leukocytes undergo a respiratory burst and increase their production of cytokines and other inflammatory mediators.[71, 72] Concomitantly, endothelial cell activation occurs in response to the local milieu of humoral and leukocyte-derived factors, resulting in upregulated expression of several adhesion molecules and receptors on their surface. In addition, activated endothelial cells synthesise and secrete additional cytokines and secondary inflammatory mediators, including prostaglandins, leukotrienes, thromboxanes, PAF, oxygen free radicals, nitric oxide and proteases including cathepsin and elastase, many of which are also generated by leucocytes. The presence of activated endothelial cells and the enhanced cytokine milieu results in activation of the coagulation cascade which, in turn, leads to local thrombosis, thereby minimizing blood loss and walling off injured tissues in an attempt to isolate inflamed areas.[67]

The local pro-inflammatory milieu co-ordinates the elaborate web of events designed to ameliorate tissue injury and limit further damage. To ensure the pro-inflammatory mediators do not become destructive, however, a localized, compensatory anti-inflammatory response ensues, involving, but not limited to interleukin(IL)-4, IL-10, IL-11, IL-13, soluble tumour necrosis factor-alpha (TNF-α) receptors, IL-1 receptor antagonists and transforming growth factors.[69, 73-77] This compensatory response acts

to diminish monocyte major histocompatibilty complex (MHC) class II expression, impair antigen presenting activity, and reduces the ability of cells to produce pro-inflammatory cytokines.[77] These opposing, localized pro- and anti-inflammatory events may therefore achieve the restoration of homeostasis and lead to the resolution of tissue injury without systemic involvement.[69]

Systemic spillover of small quantities of cytokines into the circulation may occur if a critical level of pro-inflammatory mediators is reached at the local site.[78] At this stage, the systemic presence of these mediators is considered an appropriate and beneficial mechanism of controlling an insult not able to be controlled in the microenvironment.[67, 69] The systemic pro-inflammatory mediators recruit PMNs, lymphocytes, platelets and coagulation factors to the local site[79] and, in turn, stimulate a compensatory systemic anti-inflammatory response to down-regulate cytokine production and counteract the effect of cytokines already released thereby keeping the initial inflammatory response in check. Ultimately, pro- and anti-inflammatory events may achieve homeostasis resulting in successful wound healing or resolution of infection. Such events produce few, if any, clinical symptoms or signs, and organ dysfunction is rare.[67, 69, 77]

An alternative outcome to the successful resolution of inflammatory events following the small systemic spillover of mediators, is failure to re-establish homeostasis with the initiation of a massive systemic, typically pro-inflammatory cascade with predominantly destructive rather than protective effects.[67] This pathophysiological process is recognized clinically as the systemic inflammatory response syndrome

(SIRS), and patients at this stage may display symptoms and signs indicative of incipient MODS.

One of the pathophysiological events underlying SIRS, initiated by the flood of inflammatory cytokines, and the numerous humoral cascades triggered by them, is progressive endothelial dysfunction, resulting in increased microvascular permeability and subsequently transudation into the extravascular space and distant end organs.[69, 80-84] Dysregulation of vasodilatory and vasoconstrictive mechanisms is another pathophysiological feature of the SIRS cascade, and results in uncontrolled systemic vasodilatation, which exacerbates transudation, contributes to maldistribution of blood flow[85, 86] and produces a decrease in systemic vascular resistance which may be manifest as hypotension.[67] These phenomena, together with a depression in myocardial contractility, potentially resulting from paracrine nitric oxide production, coronary non-occlusive microvascular damage and myocyte injury,[79] negate the normal homeostatic responses to maintain oxygen delivery and correct the abnormal arteriovenous difference in oxygen content.[87, 88] The cumulative effects of these adverse processes are end-organ hypoperfusion, oedema, initiation of anaerobic metabolism and potentially end-organ dysfunction.[67]

Leukocyte and endothelial cell activation are critical, early events in the physiology of acute inflammation[89] and this process has, in turn, been identified as contributing to the early pathological features of SIRS. Modulation of complementary adhesion molecules on the surface of leucocytes and the endothelium is a key event in leukocyte extravasation from the vascular lumen to interstitial tissues during acute inflammation, in particular, the process of leukocyte margination, rolling, adhesion

to the endothelium and diapedesis through endothelial intercellular junctions and across the basement membrane.[89] The adhesion of large numbers of leucocytes to the vascular endothelium during SIRS has been noted to interrupt microcirculatory flow.[90] Obstruction of the microcirculation during SIRS is exacerbated by excessive microthrombi and platelet sludging,[67, 91] the development of which is triggered by an increasingly pro-coagulant environment coupled to ongoing injury to the vascular endothelium.

The mechanisms by which an originally coagulant neutral environment becomes procoagulant , thereby contributing to occlusion of the microvasculature in SIRS, are multiple, and relate principally to the disturbance or modulation of the endothelium's normal role in regulating intra-vascular coagulation through separation of pro-coagulant pathways, inhibition of pro-coagulant proteins, regulation of fibrinolysis and production of thromboregulatory compounds.[92] Endothelial stimulation or injury, as occurs in SIRS, may induce a forty-fold increase in the endothelium's normally low basal secretion of the procoagulant enzyme, tissue factor.[67] Endothelial damage, induced by activated leucocytes in SIRS, disturbs the endothelium's normal barrier function preventing contact between the intravascular coagulation factor, Factor VII, and subendothelial tissue factor, thereby limiting activation of the extrinsic coagulation pathway and similarly preventing exposure of platelets to pro-agreggant subendothelial constituents including collagen and Von Willenbrand factor.[67, 89] The endothelial damage associated with SIRS may also disturb the capacity of these cells to produce and express the proteoglycan heparin sulphate, which normally serves to localize and enhance the activity of antithrombin III and induce feedback inhibition of the pro-coagulant Factor VIIa-tissue factor complex

through its interaction with factor Xa. Similarly, a procoagulant environment may be generated in SIRS by a disturbance of the normal endothelial function of procoagulant protein inhibition via the autoregulatory protein C system, with deficiencies in the pathway's constituents, protein C, protein S and thrombomodulin, having been suggested.[93-95] The expression of factors including platelet-endothelial cell adhesion molecule and thromboxane by activated endothelial cells may also contribute to intravascular coagulation in SIRS.[67] In addition, the extrinsic pathway of the coagulation cascade may be triggered by TNF-α,[96] one of the most influential mediators implicated in SIRS and sepsis.[67] It is as a consequence of such events that micro-thrombi may interrupt micro-circulatory flow, contributing to maldistribution of blood flow and possibly ischaemia, which may inturn lead to ischaemia-reperfusion injury (IRI).[97] The cumulative effect of these processes is the potential to exacerbate end-organ injury.[67]

The potentially destructive regional and systemic responses in SIRS, namely increased peripheral vasodilation, excessive microvascular permeability, leukocyte and endothelial activation and the associated acceleration in intravascular coagulation, contribute to profound pathophysiological changes in various end-organs and are considered major aetiological factors in the development of septic shock,[67] disseminated intravascular coagulation[98] adult respiratory distress syndrome (ARDS) and other end-organ dysfunction leading to MODS and potentially MOF.[67]

It must be recognized that SIRS, a concept globally adopted by investigators and clinicians[99] since its introduction into common parlance by the 1992 statement

generated by the American College of Chest Physicians (ACCP) and Society of Critical Care Medicine (SCCM) Consensus Conference[100] , describes only the pro-inflammatory sequelae which may result if immunological disequilibrium occurs following the original systemic spillover of cytokines. A state reflecting an overwhelming anti-inflammatory reaction may also arise in response to the initial systemic spillover of inflammatory mediators amongst patients who therefore never manifest an overwhelming pro-inflammatory cascade. This state of anti-inflammatory excess may also develop amongst those who survive an initial pro-inflammatory cascade but whose subsequent compensatory response is greater than biologically required.[69] The resulting state is one of immunosuppression, variously described in the literature as "immune paralysis",[101] a "window of immunodeficiency"[102] and perhaps most commonly the "compensatory anti-inflammatory response syndrome" or CARS.[103] The proponents of this concept suggest that the cellular basis for the development of such an immunosuppressed state may be an apparently inappropriate inhibition of monocyte/macrophage, B- and T-lymphocyte functions by pro-inflammatory mediators, particularly the interleukins. It is the fundamental changes in monocyte expression and function, namely a persistent decrease in the expression of MHC class II molecules[102] and a reduced ability to generate pro-inflammatory cytokines,[102, 104, 105] that has led Bone *et al.* to propose that CARS be defined by a reduction in the expression of monocyte human leukocyte antigen (HLA)-DR to less than 30% combined with a diminished ability of monocytes to produce inflammatory cytokines, such as TNF-α or IL-6.[69] The known capacity of the pro-inflammatory mediators to down-regulate their own synthesis and to enhance the synthesis of natural antagonists may also contribute to the immunosuppression if in excess of the body's requirements.[67] It has been

proposed that it is the state of immunosuppression, synonymous with CARS, that explains the increased susceptibility to infection following haemorrhage, burns and trauma[106-110] and may account for the anergy often found in those subject to thermal or traumatic injury[107] and potentially the anergy in patients with pancreatitis.[111]

In the preceding model of immune responses to injury the patient's clinical outcome is influenced, if not determined, by the results of a battle between pro- and anti – inflammatory reactions, aptly described as the "Ying and Yang" of the inflammatory system, the initial clinical manifestations of which are SIRS, CARS or an intermediary mixed inflammatory response syndrome (MARS).[67] The adverse states of MODS and MOF may be reached by an extreme and persistent immune response that is inappropriate for the patient's biological needs, sometimes referred to as a state of "immunological dissonance".[69] In many patients, it is a persistent overwhelming pro-inflammatory response that underlies the development and progression of MODS and MOF with persistently elevated levels of pro-inflammatory mediators having been associated with increased mortality in patients with SIRS and MODS.[112-115] In others, persistence of the immunosuppression that characterizes CARS, accounts for 'immunological dissonance' and contributes to adverse outcomes with one study demonstrating an 85% mortality rate amongst those patients whose human leukocyte antigen-DR (HLA-DR) expression was less than 30% for more than 3 or 4 days.[102]

Whilst much remains to be understood about SIRS, its associated family of syndromes, and their natural histories, and whilst attempts to modulate the immunological cascades which comprise these syndromes remain in their infancy,

the very definite contribution to post-operative morbidity made by immune dysfunction suggests that a capacity to predict these events prior to their establishment would be a welcome advent to operative risk assessment.

1.2.2 Polymorphonuclear Leukocyte Priming

Integral to the current understanding of the pathophysiology of inflammation is the concept of leukocyte, and more specifically PMN, activation. The key biochemical events to which the rubric 'leukocyte activation' refers, are those of receptor-ligand binding triggering activation of complex intracellular signalling systems, with the resulting biological activities including elaboration of arachidonic acid metabolites, degranulation and secretion of lysozomal enzymes and activation of the oxidative burst, and modulation of leukocyte adhesion molecules.[89, 116]

A more recently appreciated phenomenon is that of 'leukocyte priming'.[89] Cellular priming is generically defined as an altered response to an agonist induced by an antecedent stimulus.[117] The concept of PMN priming refers to the finding that resting neutrophils in the circulation are poorly responsive to many agonists[118] but when exposed to priming agents, the rate and extent of biochemical functions involved in PMN activation, such as the oxidative burst and degranulation, as well as the resulting cellular functions such as phagocytosis, are greatly enhanced when the cell is exposed to a subsequent or associated pro-inflammatory stimulus. Indeed, studies of neutrophil function in unfractionated blood have suggested that, rather than merely enhancing PMN function, priming converts the PMN from a 'non-responder' to a 'responder' status. A variety of agents including certain cytokines, low concentrations of chemoattractants and endotoxin are considered effective PMN

primers. A principal mechanism of priming is thought to be an increase in the number of specific receptors present on the PMN surface, due to the priming agent inducing a rapid mobilization of sub-cellular granules to the plasma membrane.

Quantitative change in receptors present on the PMN is not considered an adequate explanation of the mechanisms underlying PMN priming. An additional mechanism by which priming agents are thought to act, is by inducing qualitative changes in receptors. Such changes may alter the ligand binding properties of the receptor or may allow the receptor to become coupled to a new signal transduction pathway that can lead to cell activation.[116] Exemplifying the latter, are findings by Watson *et al.*[119] These authors demonstrated that, in unprimed neutrophils, the respiratory burst activated by the PMN chemoattractant N-formylmethionine Leucyl-Phenylalanine can occur in the absence of signalling by phospholipase D activation, however, upon priming of dihydronicotinamide adenine dinucleotide (NADPH) oxidase, the key enzyme involved in the respiratory burst, PMN activation becomes heavily dependant on phospholipase D signal transduction.[119]

Multiple authors have conceptualized inflammatory mediated tissue injury, notably MOF and acute lung injury, as a "two-hit" phenomenon in which PMN priming, the first hit, occurs early after injury, creating a vulnerable period of PMN hyperactivity.[120] A subsequent activating stimulus, the second hit, during this period of PMN priming is considered necessary for initiating an intense, inflammatory response that results in PMN-mediated organ injury which culminates in MOF. [121-125] Bone has similarly proposed that this model of priming and subsequent cellular activation may underlie many cases of SIRS and MODS.[69] Whilst Bone suggests

that considerable evidence supports the contention that a single event such as massive infection or injury alone is sufficient to cause these disorders, this author contends that many cases may arise due to a pre-existing state of priming. In contrast to other authors who have identified the priming event as an moderate to large antecedent insult, such as gut ischaemia-reperfusion in a rat model of MOF[123] or physical trauma in patients subsequently developing inflammatory mediated tissue injury,[121, 124, 126] Bone proposes that patients may be primed to developed these inflammatory disorders simply due to a variety of existing conditions such as metabolic, neoplastic, or immunodeficiency disorders; diabetes or cirrhosis and indeed senescence, since these conditions, including advanced age, are associated with abnormal cytokine levels.[69, 127] This author contends that refining the capacity to identify those at risk of SIRS or MODS requires a consideration of the potential for pre-existing priming at a cellular or mediator level.[69]

1.2.3 Definition of Systemic Inflammatory Response Syndrome (SIRS)

A uniform definition of SIRS, enabling the clinical recognition of this inflammatory disorder, was established in 1991 at the ACCP/SCCM Consensus Conference.[100] The resulting consensus statement determined that SIRS should be identified using four clinical criteria, as described in Table 1. The concurrent presence of two or more of these criteria, where the presence of these criteria represent an acute alteration from baseline in the setting of a disorder known to cause endothelial inflammation, and in the absence of any other known cause for such abnormalities, constitutes SIRS.

Table 1. ACCP/SCCM definition of Systemic Inflammatory Response Syndrome (SIRS).[100]

ACCP/SCCM Definition of Systemic Inflammatory Response Syndrome (SIRS)
Two or more of the following criteria: • A temperature of > 38 °C or <36 °C • A heart rate of > 90 beats per minute • A respiratory rate of > 20 breaths per minute, or $PaCO_2$ < 32 mmHg • A white blood cell count of > 12.0 x 10^9/L, <4.0 x 10^9/L , or > 10% immature neutrophils (band forms) *In the setting (or strong suspicion) of a known cause of endothelial inflammation, such as:* • Infection (caused by gram-negative or gram-positive bacteria, viruses, fungi, parasites, yeasts, or other organisms) • Pancreatitis • Ischaemia • Multiple trauma and tissue injury • Haemorrhagic shock • Immune-mediated organ injury • Administration of an exogenous mediator (eg. tumour necrosis factor, interleukin-1, interleukin-2) *In the absence of any other known cause for such clinical abnormalities.*

$PaCO_2$, partial pressure of carbon dioxide in arterial bood.

1.2.4 Definition of Sepsis

The ACCP/SCCM Consensus Conference also addressed the previously haphazard nomenclature in popular usage to describe infectious processes and the systemic response to them. 'Sepsis' was the term designated to SIRS of infectious origin. Sepsis was therefore defined as the systemic inflammatory response to infection, manifested by the same clinical features as those previously defined for SIRS (Table 1). Furthermore, the definition stipulated that it should be determined that these manifestations are a part of the direct systemic response to the presence of an infectious process and that they represent an acute alteration from baseline in the absence of other known causes for such abnormalities.[100] In 2001 the SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference revisited the definitions of sepsis and associated syndromes established in 1992. The consensus definition of infection (Table 2) was essentially the same as that used in the 1992 document, however the definition of sepsis was subject to modification. According to these current guidelines 'sepsis' continues to refer to systemic inflammation in response to infection, however the document explicitly states that infection and therefore sepsis may be diagnosed when the infectious process is strongly suspected even in the absence of microbiological confirmation. In addition, the possible clinical signs of systemic inflammation in response to infection have been significantly extended beyond the original SIRS criteria as evident in Table 2. The definition of SIRS itself, whilst subject to some criticism of it's excessive sensitivity and lack of specificity, remained unchanged by the 2001 consensus document.[99]

Table 2. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference diagnostic criteria for sepsis.[99]

SD, standard deviation; WBC, white blood cell; SBP, systolic blood pressure; MAP, mean arterial blood pressure; SvO_2, mixed venous oxygen saturation; PaO_2, partial pressure of oxygen in arterial blood; FiO_2; inspired oxygen concentration; INR, international normalised ratio; aPTT, activated partial thromboplastin time

†SvO_2 >70% is normal in children (normally, 75-80%), as is a cardiac index 3.5-5.5, therefore neither should be used as signs of sepsis in newborns or children.

‡Diagnostic criteria for sepsis in the paediatric population are signs and symptoms of inflammation plus infection with hyper- or hypothermia (rectal temperature >38.5 ºC or <35 ºC), tachycardia (may be absent in hypothermic patients), and at least one of the following indications of altered organ function: altered mental status, hypoxaemia, increased serum lactate level, or bounding pulses.

SCCM/ESICM/ACCP/ATS/SIS Diagnostic Criteria for Sepsis

Infection, defined as a pathologic process induced by a microorganism, documented or suspected, and some of the following:

General variables

- Fever (core temperature > 38.3 °C)
- Hypothermia (core temperature <36 °C)
- Heart rate > 90 beats per minute or >2 SD above the normal value for age
- Tachypnoea
- Altered mental status
- Significant oedema or positive fluid balance (>20 mL/kg over 24 hours)
- Hyperglycaemia (plasma glucose >120 mg/dL or 7.7mmol/L) in the absence of diabetes

Inflammatory variables

- Leukocytosis (WBC count > 12.0 x 10^9/L)
- Leukopaenia (WBC count < 4.0 x 10^9/L)
- Normal WBC count with > 10% immature forms
- Plasma C-reactive protein >2 SD above the normal value
- Plasma pro-calcitonin > 2 SD above the normal value

Haemodynamic variables

- Arterial hypotension† (SBP <90 mmHg , MAP <70, or a SBP decrease > 40mmHg in adults or <2 SD below normal for age)
- SvO_2 >70%†
- Cardiac index > 3.5 L/min/m^2 †

Organ dysfunction variables

- Arterial hypoxaemia (PaO_2/FiO_2 < 300)
- Acute oliguria (urine output < 0.5 mL/kg/hr or 45mL/hr for at least 2 hours)
- Creatinine increase >0.5mg/dL
- Coagulation abnormalities (INR >1.5 or aPTT > 60 secs)
- Ileus (absent bowel sounds)
- Thrombocytopaenia (platelet count < 100 x 10^9/L)
- Hyperbiliribinaemia (plasma total bilirubin > 4 mg/dL or 70mmol/L)

Tissue perfusion variables

- Hyperlactataemia (> 1 mmol/L)
- Decreased capillary refill or mottling

1.2.5 Relevance of SIRS and Sepsis in Major Vascular Surgery

Despite the vigour with which the SIRS concept has been embraced by both researchers and clinicians,[99] and a clear interest in the systemic inflammation provoked by the combination of surgical trauma and IRI which characterises major arterial surgery, the latter demonstrated by an array of reports examining fluctuations in individual biochemical and immunological inflammatory parameters, reporting of the incidence of SIRS associated with major vascular surgery is remarkably limited. In a study examining the inflammatory response to EVAR compared with the conventional open method of AAA repair, Sweeney *et al.*[128] reported a 12.5% incidence of SIRS following EVAR, whilst SIRS occurred in 33.3% of the cohort undergoing the open approach. The authors noted that the apparent difference in the incidence of SIRS associated with the two methods of repair failed to reach statistical significance.

The only other published data on the incidence of SIRS following major vascular surgery appears in two recent papers published in consecutive years by a research group from the Department of Surgery, University of Leicester.[129, 130] Bown *et al.*,[130] in their report of a prospective study involving 100 consecutive patients undergoing open AAA repair, describe an 89% incidence of SIRS following elective AAA repair, a 92% incidence of SIRS following emergency repair of non-ruptured aneurysms whilst 100% of patients undergoing repair of a ruptured AAA are reported to have experienced SIRS post-operatively. Identical rates of SIRS following elective and emergency repair of ruptured AAA's were reported by Norwood *et al.* the following year in their study involving 151 consecutive patients.

It is implicit in the publication by Norwood *et al.*[129] that the findings are derived from an expansion of the same cohort of patients described by Bown *et al.*,[130] and whilst the consistency of the findings is encouraging, the results cannot be considered to be independent findings. The disparity in the incidence of SIRS described by Sweeney *et al.*[128] and the Leicester researchers may be attributed to the markedly smaller cohort of twenty patients involved in the former study. It would be reasonable to suggest that the higher incidence reported by the Leicester researchers is therefore more reliable, and that consistent with the authors' conclusions, SIRS is indeed a common event following AAA repair. This finding does not, however, negate the potential utility of the SIRS concept when applied to this or other patient populations.

The ACCP/SCCM conference acknowledged the broad, encompassing nature of the definition of SIRS and recommended that it be combined with further risk stratification or probability risk estimation techniques to measure the position of an individual along the continuum of increasing severity of inflammation.[100] Whilst the original consensus document made reference to several existing methods of severity scoring and risk estimation, further efforts to develop scoring and stratification systems relating to the SIRS/MODS continuum were recommended.[100] Whilst designed to address the natural history and epidemiology of SIRS, sepsis, severe sepsis and septic shock rather than establish a scoring system, in a large prospective cohort study of intensive care and ward patients fulfilling SIRS criteria, Rangel-Frausto *et al.* stratified their population according to the number of SIRS criteria met. A progressive increase in the incidence of sepsis development was noted according to the initial number of SIRS criteria fulfilled, whilst the time to development of

sepsis was progressively shorter as more SIRS criteria were met. Similarly, the attack rates of ARDS, disseminated intravascular coagulopathy (DIC), acute renal failure (ARF) and shock increased directly as patients met a greater number of the criteria for SIRS. Finally, there was a highly statistically significant increase in overall mortality when those stratified as initially fulfilling two SIRS criteria were compared with the group without SIRS. This finding was replicated when the group fulfilling three criteria was compared with the group with two SIRS criteria, as it was when the overall mortality amongst those fulfilling all four SIRS criteria was compared with that in the group characterised by three SIRS criteria.[66] These findings imply prognostic value in stratification according to the number of SIRS criteria met at the time of study inclusion. In their study of patients undergoing AAA repair, Norwood *et al.* dichotomised elective patients into those with either high or low SIRS scores according to the 50th centile of the mean SIRS score for the first four post-operative days. Whilst this method of stratification failed to identify a positive correlation between high SIRS scores and adverse outcomes, when patients were dichotomised according to cumulative SIRS scores, calculated by adding the daily score for the first four post-operative days, those with very high cumulative SIRS scores were found to be significantly more likely to die. This finding was interpreted as indicating that high scores for a number of days in the early post-operative period was a significant predictor of death.[129] The limited data available therefore tends to support the original contention that stratifying SIRS according to the severity of inflammation is a useful concept and may be achieved by considering the number of SIRS criteria fulfilled.

Surprisingly few studies in the vascular surgery literature report on the incidence of sepsis following major vascular surgery and, when reported, incidences range considerably. Three relatively large, but retrospective English language studies[15, 40, 42] and one prospective, but comparatively small study,[131] which have documented the incidence of sepsis following elective open AAA repair, describe rates varying from 0.3 to 4%, with variable follow-up periods and a lack of definitions hampering the interpretation of this data. These rates seem remarkably low when data derived from the prospective study by Rangel-Frausto *et al.*,[66] which has clearly employed the term 'sepsis' in accordance with the consensus definitions, is considered in the context of elective AAA repair. Whilst this study involved mixed medical and surgical patients, amongst those initially classified as having SIRS only, rates of progression to culture-negative sepsis ranged from 37% to 46% whilst 32% to 46% progressed to culture- positive sepsis, the variability arising from the number of SIRS criteria initially fulfilled, and with a follow-up not greater than that employed by the aforementioned studies describing sepsis post open AAA repair. If these prognostic rates are applied to the situation of elective AAA repair with its associated high incidence of SIRS as previously discussed, an incidence of sepsis considerably greater than 0.3 to 4% would be expected. Whilst some uncertainty therefore remains about the precise rate of sepsis following elective AAA repair when consensus definitions are employed, it is undoubtedly lower than the rate of SIRS, yet the implications of this diagnosis are of even greater consequence.

The importance of the occurrence of sepsis following AAA repair is highlighted by data documenting its contribution to mortality amongst this patient population. Nine percent of the 30-day mortality rate calculated for the 820 patients undergoing

elective open AAA in the prospective UK Small Aneurysm Trial, was attributed to sepsis, with only cardiac events, pulmonary problems and renal failure causing more post-operative deaths.[29] It is also noteworthy that multiple organ failure, the syndrome viewed as the most severe extreme of the inflammatory continuum, accounted for 6% of 30-day post-operative deaths.[29] Indeed, amongst their considerably smaller patient cohort, Cohen *et al.* attributed 100% of their 4.9% mortality rate to MODS, with sepsis preceding MODS in 20% of cases.[131]

Like AAA repair, reports of the relative importance of sepsis following lower limb revascularization are infrequent with much caution required when interpreting their findings due to a haphazard use of undefined terms relating to sepsis. Among those few reports which appear to use the terminology as it was intended is a study by Ramdev *et al.* who considered outcomes in a moderately sized cohort of dialysis-dependent patients undergoing infra-inguinal revascularization and report a 2% rate of sepsis prior to discharge.[132] Studies by Mertens *et al.* [133] and Chalmers *et al.* [134] have reported on the contribution of sepsis to the high mortality rate amongst patients with documented infra-inguinal graft infections. Mertens et al. describe an 18% mortality rate in their cohort of 67 patients, 58% of which was attributed to sepsis.[133] In a somewhat smaller cohort of 26 consecutive patients with infra-inguinal graft infections, Chalmers et al. noted a 15% mortality rate, 100% of which was caused by methicillin resistant *Staphylococcus aureus* (MRSA) sepsis.[134] Despite the inadequacies of the existing data, the considerable impact of sepsis that appears to be associated with major arterial surgery supports the proposal that a capacity to better identify those at greater risk of this event would be beneficial.

1.3 Polymorphonuclear Leukocyte Integrin and Immunoglobulin G Fc Receptors

PMNs are recognized as playing a pivotal role in SIRS, sepsis and their associated inflammatory states, not only in a protective capacity but, paradoxically, as critical effectors of tissue injury[67] with the same cellular processes underlying their contribution to both host defense and tissue destruction.[135] The critical bridge between cellular and humoral stimuli and leukocyte responses are the receptors borne on their surface. PMN functions in inflammatory and infectious processes are mediated by cell surface receptors belonging to the β_2-subfamily of the integrin superfamily in addition to members of the immunoglobulin (Ig) superfamily, namely receptors for the constant, or Fc, fragment of IgG (FcγR).[116] It is not surprising then that these determinants of PMN function have provoked the interest of those investigating host response to inflammatory and infectious stimuli.

1.3.1 CD11/CD18 Leukocyte Adhesion Molecules

1.3.1.1 Structure and Distribution

The CD11/CD18 leukocyte adhesion molecules are three surface membrane heterodimeric glycoproteins belonging to the β_2-subfamily of the integrin superfamily.[135-137] Each heterodimer consists of one of three homologous but distinct α subunits, namely CD11a, CD11b or CD11c, with apparent molecular masses of 180Kd, 155Kd, and 150Kd respectively; each non-covalently linked with a common β subunit, named CD18, of 94Kd.[135, 136, 138] The resulting $\alpha\beta$ transmembrane complexes are therefore CD11a/CD18, designated LFA-1; CD11b/CD18, alternatively named Mac-1, Mo1 and CR3; and CD11c/CD18, designated LeuM5 or

p150,95.[116, 135] Both sub-units constituting each of the three heterodimers span the leukocyte plasma membrane once, with a short C-terminal cytoplasmic region and a large N-terminal extracellular domain.[135] The expression of the β_2-integrin molecules is restricted to leukocytes, however the heterodimers have distinct leukocyte distributions and their relative abundance varies according to leukocyte type, state of differentiation and activation. Whilst CD11a/CD18 is present on all leukocytes, the expression of both CD11b/CD18 and CD11c/CD18 is more restricted with both antigens normally present on PMNs, monocytes, macrophages and natural killer cells. CD11c/CD18 is also expressed on specific cells of B-lymphocyte lineage including those characterising hairy cell leukaemia in addition to certain cloned cytotoxic T lymphocytes.[135] Importantly, it has been noted that the CD11b/CD18 heterodimer is vastly more abundant than the other two antigens on the surface of activated neutrophils.[139] For this reason the CD11b/CD18 heterodimer, and frequently the CD11b sub-unit in isolation, has been considered a phenotypic marker of PMN activation.[138, 140] It is worthy of note that whilst there is a marked quantitative increase in the surface expression of CD11b/CD18 during priming by translocation of pre-formed pools of receptors to the cell surface in secondary and tertiary PMN granules, there is a body of evidence indicating that additional or alternative qualitative modifications to the CD11/CD18 receptors are required for mediating the various adhesive interactions mediated by these receptors.[116, 135, 141] One mechanism for such qualitative modifications of receptor function appears to be receptor phosphorylation by protein kinase C.[116, 135, 141] Receptor clustering, potentially also mediated in part by phosphorylation, is an additional mechanism that has been proposed to qualitatively regulate receptor function.[116]

1.3.1.2 Function

The function of this group of receptors can broadly be considered to be adhesion-related. Whilst some functions are unique to the CD11b/CD18 heterodimer, there is considerable overlap in the functions of the three CD11/CD18 molecules in leukocytes of the myeloid series as evident in Table 3. For these cell types, all CD11/CD18 receptors contribute to chemotaxis and adhesion to cytokine-activated endothelium.[135] The latter summarises the essential role of these molecules, and CD11b/CD18 in particular, in the transition of the PMN from a state of rolling to firm adhesion to the endothelium[142] and in transendothelial migration of PMNs[143] during the process of extravasation. The ligand most commonly considered to interact with CD11b/CD18 in this process is the inducible antigen, intercellular adhesion molecule-1 (ICAM-1, CD54), however other, as yet unknown ligands may be involved.[135, 144, 145] All CD11/CD18 molecules are also capable of mediating antibody dependant cell mediated cytotoxicity (ADCC).[135, 146]

The CD11b/CD18 heterodimer is considered essential for binding and phagocytosis of particles opsonised by the complement component C3bi in addition to homotypic adhesion [116, 135] and is the only CD11/CD18 molecule capable of stimulating particle-induced PMN oxidative burst and degranulation.[135, 147, 148] The CD11b/CD18 molecule has also been proposed as a signalling partner of the PMN IgG receptor FcγRIIIb (CD16b).[149] The critical role of this group of receptors in contributing to immunological defense mechanisms is highlighted by the clinical manifestations of immunodeficiency present in those patients with an inherited deficiency of CD11/CD18 (Leu-CAM deficiency).[135]

Table 3. Functions mediated by CD11/CD18 in myeloid series leukocytes.[135]

Leukocyte Function	Relevant Heterodimer
Spreading/random migration/chemotaxis	CD11a,b,c/CD18
Adhesion to endothelium	CD11a,b,c/CD18
ADCC	CD11a,b,c/CD18
Binding to C3bi	CD11b,c/CD18
Aggregation	CD11a,b/CD18
Phagocytosis	CD11b/CD18
Particle-induced oxidative burst and degranulation	CD11b/CD18

ADCC, Antibody dependent cell mediated cytotoxicity

1.3.1.3 CD11b and Clinical Outcomes

The concept of pre-operatively predicting post-operative complications induced by an activated immune system using leukocyte immunophenotyping for activation antigens and adhesion molecules is exemplified in a study by Tárnok *et al.*[150] These authors examined the ability of neutrophil, monocyte and eosinophil adhesion molecules and activation markers to pre-operatively predict the occurrence of post-operative edema and effusion (POEE), an inflammatory complication following cardiopulmonary bypass (CPB) surgery, in forty-nine children without other pre-operative evidence of inflammation based on C-reactive protein (CRP) serum concentrations or white blood cell (WBC) count. Of the 122 cell surface marker variables examined, summarized data indicates that CD11a, CD18 and CD45 expression on all three leukocyte types, in addition to CD69 expression on neutrophils and CD11b on monocytes, was found to be significantly higher amongst those children who subsequently developed POEE compared with those who did not. It is notable, however, that use of a single parameter was insufficient for individual risk assessment, but when the most discriminating parameters were combined, two discriminant analysis methods correctly classified the post-operative outcomes of over 89% of patients. These methods were considered to be more useful than surgical data in identifying patients who would later progress to POEE. Based on the most discriminating cell surface markers, the authors suggest that POEE patients may have an allergic or atopic predisposition which when combined with the immune activation induced by surgical trauma and CPB led to POEE through a summative effect.[150]

Several other authors have focussed on the potential relationship between PMN CD11b and outcomes following trauma and surgery. In a small study of patients with severe blunt trauma, Shih *et al.*[151] identified that whilst PMN CD11b expression was elevated following trauma compared with healthy controls, it did not correlate with injury severity as classified by the injury severity score. More importantly, one week following injury CD11b expression remained significantly higher than healthy control values amongst those who had developed an infectious complication, in contrast to un-infected patients whose CD11b expression was similar to that of healthy controls. Whilst no predictive capacity is implied in this study, a relationship between PMN CD11b expression and infectious events is apparent.

In a study involving twenty-eight patients undergoing major abdominal resectional surgery, Wakefield *et al.*[140] examined functional and phenotypic markers of PMN activation, namely hydrogen peroxide production and CD11b expression respectively, amongst patients who developed sepsis compared with those who did not. On the first post-operative day, both mean CD11b expression and hydrogen peroxide production were significantly greater in those patients who subsequently developed post-operative sepsis. Importantly, on the first post-operative day the two groups were clinically indistinguishable with septic complications developing only after the fourth post-operative day. Interestingly, there was no statistical difference between the groups with regard to CD11b expression between the third to tenth post-operative days, despite sepsis having become clinically apparent during this period. The authors conclude that an exaggerated PMN activation response to surgery is an early marker of those destined to develop sepsis.

Foulds *et al.* examined peri-operative PMN activation in a small cohort of twenty patients undergoing thoracoabdominal aortic aneurysm (TAAA) repair and noted that those eleven patients who subsequently developed complications, defined by the presence of renal and/or respiratory failure with paraperesis co-existing in two patients, were distinguished from the uncomplicated group by a significantly higher CD11b expression fifty minutes following the commencement of reperfusion.[152] On the basis of these findings, the authors concluded that intra-operative PMN activation, indicated by increased CD11b expression, is a marker for the development of post-operative complications following TAAA repair and suggested that a greater degree of endotoxin absorption, associated with visceral ischaemia, may account for these findings.

It is notable that the preceding two studies did not suggest a pre-operative difference in CD11b expression between the complicated and uncomplicated groups. In a subsequent publication of findings from fifty-one patients undergoing TAAA repair, which may represent an extension of their previous data set, Foulds *et al.*[153] re-iterate an apparent capacity for CD11b expression to predict the subsequent development of adverse events. Significantly higher CD11b expression occurred amongst those who developed organ failure compared with those who did not, with the difference becoming apparent thirty minutes after aortic clamp removal (reperfusion) and persisting until the first post-operative day. Similarly, the 13.7% of patients who died within twelve hours of the operation demonstrated a significantly higher CD11b expression from thirty minutes after aortic clamp application until the end of the procedure compared to those who survived beyond twelve hours. It is of great interest that the pre-operative level of CD11b expression in the 13.7% of patients

who died intra-operatively or shortly thereafter was also significantly greater than those who survived this period. In an analysis of the sub-group of forty-five patients undergoing TAAA repair without CPB, pre-operative levels of CD11b were found to be significantly higher in those who developed multiple-organ failure compared with those who developed failure of only a single organ. Analysis of data derived from the twelve patients with a Crawford type II TAAA identified a significantly higher pre-operative CD11b expression among those patients who subsequently developed major complications requiring additional post-operative support compared with those who recovered from surgery without major complications. The authors propose that the finding of a greater magnitude of intra-operative CD11b expression amongst those who did not survive the operation coupled with the higher pre-operative expression of this PMN antigen amongst these non-survivors compared with those who survived beyond the first 12 post-operative hours, indicates that the PMN's in the non-survivors existed in an "active" state prior to operative intervention with the subsequent TAAA repair acting as a secondary insult , resulting in an exaggeration of PMN activation in this cohort of patients. The pre-existing state of PMN activation was interpreted by these authors as reflective of the general immunological status of these patients, which rendered them more susceptible to the adverse effects of inflammatory mediators produced by visceral ischaemia-reperfusion. This concept was similarly proposed to account for the capacity of higher pre-operative CD11b expression to predict a greater degree of organ failure amongst the non-CPB subset of patients in addition to the development of major complications amongst those patients with type II TAAA's. The extent of sub-group analysis in this study must, however, raise some concerns about the validity of findings.

1.3.1.4 CD11b and AAA Repair

Only a limited number of studies involving small numbers of patients have examined CD11b expression in AAA surgery and, with the exception of the studies of TAAA patients by Foulds *et al.*[152, 153] who included thirteen patients undergoing AAA repair in a separate control group, and who were unable to identify significant changes in CD11b expression from baseline amongst this group, none of these have attempted to relate this marker to post-operative events. Most recently, Norwood *et al.*[154] identified a significant increase in PMN CD11b expression in femoral vein blood during AAA repair, which was significantly higher than levels of expression of this integrin measured from portal vein blood or systemic blood sampled from the radial artery. The graphical representation of this data in fact appears to indicate a decline in mean CD11b expression with surgery in blood sampled from the latter two sites. It is possible that methodological issues may, in part, account for the findings in this study with the authors alluding to potentially long and seemingly inconsistent periods having elapsed between blood sampling and the labelling of samples with anti-CD11b monoclonal antibody. Barry *et al.*[155] noted an initial rise in CD11b expression in vena caval blood samples following lower limb reperfusion in patients undergoing AAA repair with a simultaneous decrease in integrin expression in systemic arterial blood. Further decreases in CD11b in both vena caval and arterial blood were noted at a later sampling time. These authors also noted the apparently contradictory finding that total neutrophil count and more importantly neutrophil respiratory burst activity increased during the procedure suggesting that an increasing number of more 'active' PMNs were in fact generated by AAA repair. The possibility of allosteric hindrance has been proposed by other authors[154] to account for this incongruity of increasing respiratory burst activity simultaneous with decreasing CD11b activity.

This theory proposes that a falsely low level of CD11b expression was detected due to more active PMNs, that is those with a greater expression of CD11b, binding to ligands such as soluble ICAM-1 present in the sample, rendering the CD11b/CD18 binding domain saturated, thereby preventing binding by the fluorescence-labelled anti-CD11b probes.

Norwood *et al.* further suggest that the apparent disparity between venous and arterial levels of CD11b expression may be the result of pulmonary trapping of activated neutrophils.[154] In an attempt to clarify the issue of tissue sequestration or allosteric hindrance giving rise to inaccurate results Swartbol *et al.* [156] examined the effect of adding plasma from patients undergoing open and endovascular AAA repair to 'normal' donor granulocytes. An upregulation of donor CD11b was noted with the addition of plasma sampled from patients undergoing endovascular repair during the ischaemic phase and following reperfusion. Whilst conclusive evidence of the precise nature and magnitude of perturbations of CD11b expression induced by AAA repair is therefore lacking, due to the limited number of available studies which are hampered further by small patient numbers and potential methodological issues, current findings do, however, support the occurrence of PMN activation with AAA repair and the occurrence of a potentially variable increase in CD11b expression which may be anatomically restricted.

1.3.2 Immunoglobulin G Fc Receptors (FcγR)

Leukocyte FcγR belong to the Ig superfamily and are often referred to as a crucial link between the humoral branch and the effector cells of the immune system.[96, 149, 157] Binding of the constant region of IgG, the dominant antibody class in plasma,[96,

[137] to FcγR induces a plethora of cell type specific pro- and anti-inflammatory functions.[96, 149, 157, 158]

1.3.2.1 FcγR Classification

Leukocyte FcγR constitute a heterogeneous family of membrane bound and soluble proteins. In humans, twelve structurally and biochemically distinct isoforms are recognized and divided into three major classes: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) (Table 4).[96, 149, 157] Despite intra-class heterogeneity, the three classes can be distinguished on the basis of their molecular weight, primary structure, monoclonal antibody reactivity, affinity and specificity for ligands, and cellular distribution pattern.

The twelve leukocyte FcγR isoforms are encoded by eight genes on the long arm of chromosome 1 (1q21-24).[96, 159] The FcγRI (CD64) class is encoded by three genes (FcγRIA, -IB and -IC), generating four different mRNA transcripts, namely FcγRIa, -Ib1, -Ib2 and -Ic.[160] The FcγRIb1 and FcγRIc transcripts do not specify surface expressed receptors but may encode soluble forms of the receptor, whilst FcγRIb2 has been shown to be retained in the endoplasmic reticulum.[161] FcγRIa, the only surface expressed isoform in this class, characterizes this class as the only high-affinity human receptor capable of binding monomeric IgG.[149, 157] Three FcγRII genes (FcγRIIA, - IIB and –IIC) generate six messenger ribonucleic acid (mRNA) transcripts (FcγRIIa1, -IIa2, -IIb1, -IIb2, -IIb3 and –IIc). Members of the FcγRII class are considered low-affinity receptors, due to their low affinity for monomeric IgG, but do interact efficiently with IgG in complexed or aggregated form. This class is further characterized by FcγRIIa being the sole FcγR capable of binding to IgG2,

the main IgG subclass induced in response to bacterial capsular polysaccharide.[149, 157, 162] The FcγRIII class is encoded by two genes (FcγRIIIA and –IIIB) generating two transcripts. The FcγRIIIa isoform has an intermediate affinity for binding monomeric IgG, whilst the –IIIb isoform is considered low-affinity with reference to its monomeric IgG binding capability. Both class III receptors do, however bind IgG immune complexes readily.[96, 149, 157]

1.3.2.2 FcγR Structure and Signalling

The basic structure of FcγR consists of a ligand binding α chain with an extracellular part composed of two or, in the case of FcγRIa, three extracellular Ig-like domains; a transmembrane region; and a cytoplasmic tail. The FcγRIIIb isoform, however, is the only receptor without an intracellular component and instead is anchored in the outer leaflet of the cell membrane via a glycosyl-phosphatidylinositol (GPI) molecule. In order to induce signalling, most FcγR depend on an association with a γ- or ζ-chain homo- or hetero-dimer signaling sub-unit whilst a β-chain may serve as a signal amplifier for FcR γ-chain signaling.[163, 164] These signaling sub-units provide FcγRI and FcγRIII with signaling capacities via immunoreceptor tyrosine-based activation motifs (ITAM) (+) in their intracellular tail. The cytoplasmic tail of FcγRII contains either an ITAM or an immunoreceptor tyrosine-based inhibition motif (ITIM) (-) which induce signaling cascades in the absence of an accessory signaling sub-unit.[96, 149] FcγRIIIb is unique in its method of signal transduction as its lack of a cytoplasmic tail indicates that it has no intrinsic signaling capacity yet nor does it associate with a γ- or ζ-chain sub-unit. Three alternative mechanisms by which FcγRIIIb may transduce signals that have been suggested are by interaction with FcγRIIa, by utilizing CR3 as a signaling partner or finally by localization of FcγRIIIb to

specialized plateaus ('rafts') in the cell membrane which are enriched with accessory signaling molecules, such as Src-like tyrosine kinases, which are subsequently activated.[116] Table 5 summarizes the signaling mechanisms employed by FcγR subclasses.

1.3.2.3 FcγR Cell Distribution and Expression

The cellular distribution of leukocyte FcγR is summarized in Table 5. FcγRI is constitutively expressed on mononuclear phagocytes. Normally FcγRI is present on the surface of a few circulating PMNs but its expression is up-regulated by the cytokines granulocyte-colony stimulating factor (G-CSF)[165] and interferon-γ (IFN-γ), with extreme sensitivity to this cytokine having been demonstrated by Buckle *et al.*[166] Interestingly, *in vitro* study of this receptor by Buckle *et al.,* in small numbers of healthy volunteers, has demonstrated a wide inter-individual variation in the percentage of PMNs able to express FcγRI after a 12-hour incubation period at 37°C in tissue culture medium, as well as intra-individual variability in the inducibilty of PMN FcγRI by cytokines on different occasions. The authors suggest that these findings may demonstrate the influence of the individual's *in vivo* microenvironment on FcγRI expression.[166]

FcγRIIa is expressed on virtually all myeloid cells, including platelets, whilst FcγRIIb expression is restricted to phagocytes and B-lymphocytes. FcγRIIc is expressed on natural killer (NK) cells. FcγRIIIa is present on monocytes, macrophages, NK cells and T lymphocyte subsets. PMNs are the only cell type known to constitutively express FcγRIIIb, however eosinophils can be induced to do so.[96, 149] Similar to their findings relating to FcγRI, Buckle *et al.* have found variation

amongst individuals in the expression of FcγRIIIb on their untreated PMNs in addition to inter-individual variations in the inducibility of this molecule by IFN-γ.[166] A noteworthy feature of PMN FcγRIIIb is the ability of the extracellular portion of FcγRIIIb to be cleaved from its GPI anchor upon cell activation[167] resulting in the formation of the soluble receptor,[168] with surface levels regulated by the balance between rates of receptor shedding and mobilization of pre-formed intra-cellular stores to the cell surface.[169-172]

1.3.2.4 Leukocyte FcγR Function

Leukocyte effector functions triggered by FcγR are diverse .[96] Important leukocyte functions associated with FcγRI include ADCC, phagocytosis, and clearance of immune complexes[173-175] and this FcγR is superior to others in initiating antigen presentation.[176, 177] FcγRIIa is known to potently induce leukocyte functions such as phagocytosis and degranulation, and due to its unique capacity to interact with IgG2, is thought to be crucial for clearance of encapsulated bacteria.[149] FcγRIIIb is thought to play a dominant role in PMN binding, phagocytosis and respiratory burst activation of IgG-opsonized pathogens[172, 178] and is particularly important in activating the secretion of reactive oxidants in response to soluble immune complexes.[172] FcγRs are also thought to play a role in down-modulation of pro-inflammatory responses. FcγRIIb may participate in down-modulation of antibody responses and may have inhibitory effects on monocyte/macrophage functions. Soluble FcγR (sFcγR) , such as sFcγRIIIb secreted by PMNs, may also play a regulatory role by competing for IgG-immune complexes thereby interfering with induction of FcγR-mediated functions.[149]

Table 4. Characteristics of human leukocyte FcγR classes.[96, 149]

	FcγRI (CD64)	FcγRII (CD32)	FcγRIII (CD16)
Chromosomal locus	1q21	1q23-24	1q23-24
Genes	FcγRIA, -B, -C	FcγRIIA, -B, -C	FcγRIIIA, -B
Transcripts	Ia; Ib1; Ib2; Ic	IIa1; IIa2; IIb1; IIb2; IIb3; IIc	IIIa; IIIb
Molecular weight (kDa)	72	40	50-80
Affinity for IgG	High (10^8-10^9/M)	Low (<10^7/M)	IIIa: Intermediate ($3x10^7$/M) IIIb: Low (<10^7/M)

Table 5. Leukocyte FcγR subclass distribution, signaling properties and allotypes.[96, 149]

	FcγRI (CD64)	FcγRII (CD32)			FcγRIII (CD16)	
Receptor class	FcγRIa	FcγRIIa	FcγRIIb	FcγRIIc	FcγIIIa	FcγIIIb
Cellular Distribution	Myeloid cells PMN (G-CSF, IFN-γ induced)	Myeloid cells Plt T (subset) Endothelial cells	Mo Mϕ B	NK	Mo (subset) Mϕ NK T (subset)	PMN Eosinophils (IFN-γ induced)
Signaling motif		ITAM	ITIM	ITAM		
Accessory signaling subunit	γ	γ		ND	B, γ, ζ	CR3, FcγRIIa
Allotype		R131/H131			V158/F158	NA1/NA2

PMN, polymorphonuclear leukocyte; G-CSF, granulocyte-colony stimulating factor; IFN-γ, interferon-γ; Plt, platelet; T, T lymphocyte; Mo, monocyte; Mϕ, macrophage; B, B lymphocyte; NK, natural killer cell; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; ND, not determined.

1.3.2.5 PMN FcγRI (CD64) and FcγRIII (CD16) Expression in Clinical Practice

Several authors have examined modulation of PMN FcγRI as a marker of inflammatory and infective conditions, however their focus has been on the modulations of this receptor in established conditions rather than as a predictor of clinical events. Allen *et al.*[179] found that PMN FcγRI expression usefully distinguished between patients with inflammatory auto-immune disease and those with culture-proven infection. Median PMN FcγRI expression was significantly greater in the group with proven infection compared with that of patients with auto-immune inflammation whilst both of these groups demonstrated a significantly greater level of receptor expression than the control group. A study by Qureshi *et al.*[180] examined PMN FcγRI distribution and expression amongst ICU patients with SIRS compared with non-SIRS ICU patients and healthy controls. The percentage of PMNs expressing FcγRI was found to be significantly higher in SIRS patients than both non-SIRS patients and healthy controls. The median number of FcγRI molecules per PMN was also significantly greater in the cohort with SIRS compared to the healthy control group. Within the group of SIRS patients, the sub-group of patient with culture-proven sepsis manifested both particularly high numbers of FcγRI-bearing PMNs and numbers of FcγRI molecules per PMN compared with SIRS patients without sepsis. Fischer *et al.*[181] contributed to the accumulating evidence of the role of PMN FcγRI modulation in infective disease processes demonstrating significantly higher levels of PMN FcγRI expression amongst ICU patients with septic shock compared with critically ill patients without sepsis. The findings of Layseca-Espinosa *et al.*[182] in their study of PMN FcγRI in neonatal sepsis support the notion of this receptor as a marker of more clinically subtle

infective processes with enhanced expression of CD64 found to be a highly specific marker, albeit with a low sensitivity, for the early diagnosis of this often clinically non-specific illness. These clinical studies attest to the importance of FcγRI in inflammatory disease and suggest it as a useful marker of early or established inflammatory and infective processes, and potentially one with an ability to discriminate between the two disease states. No studies to date have examined the potential prognostic or predictive capacity of PMN FcγRI with respect to susceptibility to inflammatory or infective disease following an inciting stimulus.

Several investigators have examined the effect of stimuli including tissue injury and infective processes on PMN FcγRIIIb expression, with particularly interesting findings, suggestive of a predictive capacity for this receptor, emanating from two studies addressing the potential relationship between surgical injury and PMN expression of FcγRIIIb. In a small study of patients with extensive burns, Shehab *et al.* [183] documented a significant reduction in the expression of PMN FcγRIIIb amongst these patients compared with age-matched healthy controls from the second to the twentieth day following the thermal insult. This reduction was interpreted as the result of PMN activation with subsequent receptor shedding. Wagner *et al.*[184] also demonstrated a significant reduction in the percentage of FcγRIIIb-positive PMNs amongst fourteen patients suffering severe bacterial infections compared with a control group and an even more impressive reduction in the mean level of PMN FcγRIIIb expression. Based on modulations of this and other surface receptors observed following in vitro exposure of PMNs to bacterial lipopolysaccharide, this reduction in PMN FcγRIIIb was able to be attributed to loss of this receptor from the PMN cell surface.

The findings of Wakefied *et al.*[168] , derived from their study of the effect of surgery on PMN FcγRIIIb expression and metabolism are, however, inconsistent with the findings of the preceding studies. Amongst their moderately sized cohort of patients undergoing major abdominal resectional surgery, there was no significant fluctuation in the level of FcγRIIIb surface expression throughout the post-operative course compared to baseline pre-operative levels. These investigators additionally investigated the influence of surgery on the capacity of PMNs to mobilize intracellular stores of FcγRIIIb to the cell surface. Surgery was demonstrated to reduce the amount of this receptor that could be expressed on the cell surface following maximal stimulation suggesting that the effect of surgery was to deplete intracellular stores of FcγRIIIb. Furthermore, treatment of PMNs sampled pre- and post-operatively with the physiological activators phorbol myristate acetate (PMA), TNF, formyl-methionyl-leucyl-phenylalanine (FMLP), and *Escherichia coli* lipopolysaccharide (LPS) serotype O55:B55, demonstrated that surgery induced PMNs to shed FcγRIIIb in response to such activators. The authors' interpretation of these collective findings is that receptor shedding does in fact occur in response to surgical intervention, however cell surface expression appears to be maintained at constant levels due to mobilization of intracellular stores to the cell surface, with these stores subsequently becoming depleted.

One of the most relevant findings highlighted by Wakefied *et al.* [168] was identified by considering levels of PMN FcγRIIIb expression in those who developed post-operative sepsis compared with those who did not develop this complication. FcγRIIIb expression was found to be significantly higher both pre-operatively and throughout the post-operative period amongst the group of patients who subsequently

developed sepsis, the authors suggesting that this receptor may therefore identify a cohort susceptible to sepsis following surgical intervention. Whilst this study was unable to provide a definitive explanation for this finding, the investigators noted that the apparently greater incidence of malignancy in the group developing sepsis may be, in part, responsible for the observation since it is recognized that individuals with malignant disease are at high risk of surgical infections and data from animal studies has indicated a higher level of PMN FcγRIIIb expression in tumour-bearing mice. Attributing the difference in receptor expression and sepsis susceptibility to the distribution of malignant disease between the septic and non-septic groups is hazardous given that, as acknowledged by the authors, the difference in the incidence of malignancy between the two groups was in fact not statistically significant. The lack of a definitive explanation for the empiric observation regarding the level of PMN FcγRIIIb expression and post-surgical development of sepsis does not, however, detract from the potential value of this finding.

The findings of Spark *et al.*[185] , in their study of PMN FcγRIIIb expression and phagocytosis in fifty patients undergoing elective infrarenal AAA repair, parallel those of Wakefield *et al.*[168] . Spark *et al.* also note that a significantly higher level of FcγRIIIb expression pre-, intra- and post-operatively characterizes those patients who develop either SIRS or sepsis post-operatively compared with those who do not experience these post-operative events. In addition to PMN phenotypic differences between these two patient groups, Spark *et al.* also identified differences in PMN behaviour. More specifically, the authors documented inter-group differences in the responses of post-operatively obtained PMNs to in vitro stimulation with respect to receptor expression in addition to differences in numbers of phagocytosing PMNs

and bacteria phagocytosed per cell. The relevance of these PMN receptors to clinical events is therefore apparent with preliminary evidence of a potentially predictive role for these receptors with respect to clinical outcomes.

1.3.2.6 FcγRIIa (CD32a) and FcγRIIIb (CD16b) Polymorphisms

FcγRs exhibit genetically determined polymorphisms. Whilst a number of non-functional polymorphisms, as well as polymorphisms with unknown functional significance have been identified, those receiving most attention are the polymorphisms manifested by the two FcγR constitutively expressed by human PMNs, FcγRIIa and FcγRIIIb, which have been shown to be both functionally significant and relevant to human disease (Table 5).[96, 149]

FcγRIIa manifests a biallelic polymorphism with the alleles, designated FcγRIIa-H131 and -R131, differing by two amino acids in their extra-cellular regions. It is the G to A point mutation in the region specifying its membrane proximal ligand binding domain, causing an arginine (R) to histidine (H) amino acid substitution at position 131 and generating the FcγRIIa-R131 and -H131 allotypes respectively, which critically affects this receptors Ig affinity and specificity.[96, 149, 157, 186-188] The FcγRIIa-H131 allotype displays higher binding efficiency for complexed human IgG2 and IgG3, compared to FcγRIIa-R131.[189] Since FcγRIIa-H131 is the sole leukocyte FcγR known to interact efficiently with human IgG2, the capacity to bind this important IgG subclass is therefore dependant on the individual's FcγRIIa genotype.[96, 149, 157] The functional consequence of this biallelic polymorphism has been proven by studies demonstrating that PMNs from FcγRIIa-R131 homozygous

individuals phagocytose IgG2-opsonized particles far less efficiently than PMNs from FcγRIIa-H131 homozygotes.[190, 191] PMNs from individuals who are heterozygous for this polymorphism have an intermediate phagocytic capacity for IgG2-opsonized bacteria and IgG3-sensitized erythrocytes when compared with the homozygote phenotypes.[157, 192]

FcγRIIIb bears the neutrophil antigen (NA) polymorphism in its membrane distal Ig-like domain. The two isoforms, FcγRIIIb-NA1 and -NA2, differ by four amino acids, two of which alter potential glycosylation sites[159, 190, 193, 194] PMNs obtained from NA1 homozygotes exhibit a higher affinity for immune complexed IgG3[192] in addition to a greater phagocytic capacity for particles bound to a variety of opsonins including pooled human IgG[190] , Ig subclasses IgG1 and IgG3[192] and the FcγRIII-dependant probe concanavalin A[158] , compared to PMNs derived from NA2 homozygotes. Whilst this superior phagocytic capacity of PMNs from NA1 homozygotes compared with NA2 homozygotes has therefore been consistently demonstrated, the mechanism underlying the difference remains undefined. It has, however, been demonstrated to be independent of FcγRIIa phenotype and appears to be IgG-ligand independent.[190, 192]

The identification of functional FcγR polymorphisms has led to intense study of these alternative allotypes as factors which may contribute to susceptibility to, or alter the clinical course of auto-immune or infectious diseases.[96, 149] The finding that the FcγRIIa-H131 allotype is the sole leukocyte receptor capable of efficient interaction with IgG2, combined with the knowledge that IgG2 responses are of particular importance to host defense against encapsulated bacteria, has resulted in

multiple studies investigating the possibility that the FcγRIIa-R131 allotype, and its characteristically inefficient phagocytosis of IgG2-opsonized bacteria, may represent a risk factor for infections by encapsulated bacteria in particular. Studies suggesting that this may indeed be of clinical relevance include that by Sanders et al. [195] which demonstrated a decreased frequency of the FcγRIIa-H131 homozygous genotype amongst Dutch children with *Streptococcus pneumoniae* upper respiratory tract infections, whilst the FcγRIIa-R131 homozygous genotype has been found to be enriched amongst American bacteraemic patients[196]. Furthermore, the FcγRIIa-R131 genotype has been reported to be associated with susceptibility to and severity of meningococcal disease[197-199]. As individuals homozygous for both FcγRIIa-R131 and FcγRIIIb-NA2 have the least efficient FcγR combination on their PMNs,[149] this phenotype was examined by Fijen *et al.*[200] and found to be increased among complement deficient patients with high frequencies of meningococcal disease. The combination of homozygosity for FcγRIIa-R131 and FcγRIIIb-NA2 together with the FcγRIIIa-F158 homozygous genotype has been shown to be increased among Dutch relatives of patients with meningococcal disease.[201] Periodontitis, an infectious disease resulting from the direct effects of anaerobic Gram-negative periodontopathic bacteria combined with specific host inflammatory responses, has been the subject of extensive investigations into its association with FcγR polymorphisms.[96, 149] Studies in Japanese individuals indicate that the FcγRIIIb-NA2 allele is a risk factor for recurrence of adult periodontitis, but not for disease susceptibility[202]; that generalized early onset periodontitis was associated with NA2 homozygosity[203]; that the combination of FcγRIIIa-V158 and FcγRIIIb-NA2 homozygosity is associated with a more severe course of disease[204]; whilst FcγRIIIb-NA1 homozygosity is associated with resistance to periodontitis[205]. Amongst the

associations between FcγR polymorphisms and periodontitis demonstrated in studies involving Dutch patients is the finding that the FcγRIIa-H131 homozygous genotype is associated with more severe disease.[206] There are also suggestions that FcγR polymorphisms may be relevant to viral and parasitic infections.[96, 149] There are no studies examining any potential relationship between FcγR polymorphisms and sepsis *per se*, nor susceptibility to, or severity of, post-operative infective complications.

1.3.3 Influence of Genetic Polymorphisms on Operative Risk and Sepsis

There is an increasingly large body of literature which has looked to genetic variability in an attempt to gain insight as to why the physiologic response to surgery varies among individuals, and furthermore, whether such variability may predict specific adverse peri-operative clinical outcomes. Encouraging data has emerged from these studies suggesting that specific alleles and genotypes may predict pathophysiological response and clinically relevant post-operative adverse events. Continued identification of allotypes and haplotypes predictive of operative outcome has the potential to decrease surgical morbidity and mortality through pre-operative risk assessment and through an enhanced ability to identify subsets of patients likely to benefit from administration of prophylactic therapy.[207]

Studies emerging from a variety of surgical settings have suggested that genotype may significantly influence the degree and severity of the inflammatory response to surgery which may in turn impact upon the occurrence of those adverse post-operative outcomes with an immunological basis.[207] The intense inflammatory

response generated by coronary artery bypass graft (CABG) surgery and CPB has stimulated several investigators to examine the influence of genetic variability in this setting. Shastri *et al.*[208] demonstrated significantly greater protamine-induced complement activation and post-operative pulmonary shunt fraction in patients undergoing CPB-assisted CABG with the complement component C4a null allele.

The overwhelming majority of studies investigating genetic modulation of surgical pathophysiology and outcomes have, however, focused on cytokine genetics given that cytokines play a critical role in orchestrating the inflammatory response to surgery. Even after controlling for possible confounding factors, Brull *et al.*[209] noted significantly higher levels of the pro-inflammatory cytokine interleukin (IL)-6 after CABG with CPB in patients carrying the G-572C allele and in patients homozygous for the G-174C allele. Grocott *et al.*[210] demonstrated that apolipoprotein (APO) E genotype significantly influences the balance of pro-inflammatory IL-1β and its naturally occurring inhibitor IL-1 receptor antagonist (IL-1ra) in response to CPB, whilst increased levels of pro-inflammatory TNF-α and IL-8 have been associated with APO ε4 genotype in patients undergoing CABG. Yende *et al.*[211] examined the influence of three single nucleotide polymorphisms (SNPs), two at TNF loci and at an IL-10 promoter polymorphism, on the outcome of CABG. The G to A substitutions at position -308 within the promoter region of the TNF-α gene and at the +250 site within the first intron of the TNF-β (lymphotoxin α) gene, have been associated with elevated levels of TNF-α[207, 211-214] whilst the G to A substitution at position -1082 within the IL-10 promoter region is associated with lower IL-10 levels[211]. These authors demonstrated that amongst patients undergoing CABG with CPB, those with a TNF +250G/-308G haplotype had a longer duration of mechanical

ventilation than those without this haplotype, whilst the Il-10 -1082 G allele was associated with a higher mortality rate compared to the A allele.

These findings, relating genetic polymorphisms to surgical outcomes, are not limited to the cardiac surgery setting. For example, Carter *et al.*[215] observed that allele 2 at the IL-1ra polymorphic site predicts the development of pouchitis after the surgical treatment of ulcerative colitis by colectomy and ileal pouch-anal anastomosis. Only one study to date has examined genetic variability and its influence on the outcome from AAA repair. Bown *et al.*[216] have recently demonstrated that amongst patients undergoing elective open AAA repair, those possessing a G allele at the IL-6 -174 locus had a significantly higher incidence of organ failure than those who did not, whilst those patients with an A allele at the TNF-α -308 locus had significantly longer critical care stays than those who did not posses this allele. The authors appropriately suggest caution in generalizing the findings given that the same associations were not present in a cohort of patients undergoing emergency repair of ruptured AAAs nor did a particular allele at the loci examined predict more than one of the adverse outcome measures within the elective patient cohort. It is in fact the uncertainty surrounding the validity of these particular findings, when considered in the context of considerable evidence supporting the concept of genetic modulation of post-operative outcome in other surgical settings, which must encourage further investigation of genetic polymorphisms and outcome following major vascular surgery, particularly AAA repair.

Genetic epidemiology studies provide much support for the notion that susceptibility and response to infection have a strong genetic influence. Whilst the ability to

identify the genetic basis of nonmendelian polygenic disorders, such as susceptibilty to sepsis and septic shock, represent a considerable challenge, studies examining candidate genes and their polymorphism have given rise to promising, although sometimes conflicting results.[217]

Polymorphisms of the TNF genes, encoding TNF-α and TNF-β have been implicated in sepsis susceptibility and severity in several studies. In a study examining the role of the bi-allelic polymorphism at position -308 of the TNF-α gene in patients with post-operative sepsis Tang *et al.*[218] noted a higher frequency of the A allele compared in this cohort compared with the general population in addition to a higher mortality rate amongst those with septic shock who carried the A allele compared with those who did not. These findings are strongly supported by Mira *et al.*[219] who found that the A allele occurred more frequently in medical ICU patients with septic shock compared to a control group. These authors also demonstrated this allele to be an independent risk factor for death due to septic shock. Together these studies demonstrate an association of the A allele at the TNF-α -308 locus with a susceptibility to sepsis, septic shock and death due to septic-shock. It is notable however that these findings were not supported in a smaller, but well designed study of post-operative patients with severe sepsis.[220]

Several studies have noted that the bi-allelic restriction site fragment polymorphism in the first intron of the TNF-β gene at position 1064-1069, identified by the restriction fragments TNFB1 and TNFB2, appears to influence sepsis susceptibility and outcome. Majetschak *et al.* [214] noted that homozygosity for the TNFB2 allele conferred a susceptibility to the development of severe sepsis following severe blunt

trauma whilst Stuber *et al.*[212] reported that amongst their cohort of post-operative patients with severe sepsis TNFB2 homozygotes had a significantly higher mortality rate than heterozygotes. Both studies confirmed that amongst individuals with severe sepsis, TNFB2 homozygotes display increased levels of circulating TNF-α. Schlüter *et al.*[221] examined the influence of a SNP (G/C) at position -174 in the IL-6 gene promoter region in a group of post-operative intensive care unit (ICU) patients. Whilst the polymorphism did not affect the incidence of sepsis, amongst those who developed sepsis, the GG homozygous genotype was significantly more frequent amongst survivors compared with those who died. Given that this finding was independent of the systemic IL-6 response, the authors suggest that other genetically linked polymorphism may be the primary cause for the apparent survival advantage.

When the various findings of this array of studies are considered together, the principal implication is that there is an abundance of evidence for a genetic influence on the individual immunological response to stimuli, including microbiological or mechanical insults such as surgery, and this may influence susceptibility and outcome from peri-operative infection.

1.4 Cytokines

1.4.1 Cytokines: An Overview

Cytokines are regulatory proteins whose constitutive production, with notable exceptions, is generally low or absent but are secreted in response to stimuli arising from both exogenous and endogenous challenges to the integrity of the host, by white blood cells and indeed almost all nucleated cells in the body, with pleitotropic regulatory effects on haematopoietic and many other cell types that participate in host defense and repair processes.[70, 222, 223] Most are simple polypeptides or glycoproteins $\leq$ 30 kDa in size with many existing as homodimers or homotrimers with one, IL-12, being a heterodimer. Cytokines function as intercellular messengers, marshalling co-ordinated cellular responses typically by altering rates of cell proliferation, inducing changes in states of cellular differentiation or expression of differentiated cell function and induce such actions by binding to specific high affinity cell surface receptors. Whilst there are several important instances when cytokines do not obey the general rule, it is considered a characteristic feature that contributes to their distinction from hormones, that cytokine production is transient and they act over short distances as autocrine or paracrine signals in local tissues and only in specific instances spill over into the systemic circulation and initiate systemic reactions.[70, 223] Their role as critical mediators in the pathogenesis of SIRS, sepsis and MODS[67, 223] exemplifies such instances. Molecular mechanisms with important functional consequences manifested by this group of proteins also include synergistic and antagonistic effects on cells following exposure to multiple cytokines; cascade effects whereby one cytokine may increase, or decrease the production of another; receptor transmodulation, referring to the modulation by one cytokine of another's receptor expression; and receptor trans-signaling, where one cytokine alters signaling

by receptors for other cytokines or growth factors. Redundancy, referring to the significant overlap in the bioactivity of structurally distinct cytokines, is also a characteristic of this group of proteins.[70, 224] Whilst their biology is therefore complex, and their nomenclature somewhat chaotic, the importance of comprehending and extending the current knowledge of their role in those subject to surgical insults was aptly summarized by Raeburn *et al.* [222] who described surgical patients as "..a stew of pulsating cytokines."

The generation of cytokines in an excessive, uncontrolled manner by stimuli including tissue trauma, haemorrhage, large volume blood transfusion, ischaemia-reperfusion injury[224, 225] and potentially the exposure of usually sterile sites to micro-organisms and the associated adverse clinical consequences which may result, is an issue relevant to most forms of major surgery. The somewhat unique combination and intensity of these insults in patients undergoing major vascular surgery, and open AAA repair in particular, makes the cytokine response amongst this patient cohort particularly noteworthy. The nature and timing of the cytokine response to elective open AAA repair has already gained some attention with the presentation of sometimes conflicting data in the literature. A selection of these studies have examined the relationship of a narrow spectrum of the cytokine response to subsequent clinical outcomes with variable findings.[224] The same concept has been adopted by several investigators and applied to cardiac surgery and trauma patients with promising findings. Identification of a potential pattern of cytokine response which may serve as a marker or predictor of subsequent adverse clinical events necessitates an understanding of the putative mechanisms of cytokine generation in major vascular surgery, the process by which these cytokines may induce tissue

injury of clinical consequence and the basic biology of a selection of the potentially relevant cytokines, including those which have been the focus of much attention and those which have, to date, been the subject of fewer clinical studies, particularly those involving patients undergoing major vascular surgery .

1.4.2 Cytokine Biology

1.4.2.1 Tumour necrosis factor-α (TNF-α)

TNF-α is a pleiotropic pro-inflammatory cytokine with multiple and diverse biological effects and is considered as playing an important role in the host response to stimuli associated with major surgical interventions.[222, 226] This cytokine is synthesized as a 26 kDa transmembrane precursor which undergoes proteolytic cleavage to yield the mature biologically active 17 kDa TNF-α[227] that forms a trimer in solution[228] . Cellular sources of TNF-α are numerous and include both non-immune cells as well as multiple immune cells, including macrophages/monocytes, PMNs, NK cells, B and T cells, basophils, eosinophils and mast cells. An array of factors may account for cell-specific stimulation of TNF-α production. Those most relevant to the surgical setting include bacterial endotoxin (LPS) induction in monocytes, T-cell receptor activation, cross-linking of B cell surface Ig, complement component C5a, the cytokines IL-1 and macrophage inflammatory protein-1α (MIP-1α), leukotrienes, hypoxia, nitric oxide and oxygen free radicals. A diverse range of suppressors of TNF-α expression by macrophages has also been identified. Amongst these are prostaglandin E_2 (PGE_2); the cytokines IFN-α, IFN-γ, IL-4, IL-6, IL-10, and G-CSF; and glucocorticoids.

Localized low level expression of TNF-α participates in beneficial tissue remodeling and host defense, however systemic overproduction, as a response to infection and injury, may result in deleterious effects.[226] Biosynthesis of TNF-α is therefore tightly controlled at many different levels ensuring only scant production of this cytokine in quiescent cells, yet rapid and significant upregulation occurs in response to cellular activation.[229] The *in vivo* cellular responses stimulated by TNF-α amongst immunological and non-immunlogical cell lines encompasses an array of mechanisms, most of which are considered pivotal in the hosts inflammatory and stress response to injury.

The summative effect of the individual cellular responses stimulated by TNF-α accounts for the clinical responses to this cytokine observed *in vivo.* Amongst the central nervous system effects of systemic TNF-α of particular relevance to insults associated with surgical intervention are the production of fever, anorexia and alteration of pituitary hormone secretion. Metabolic manifestations of the cellular effects to TNF-α include net protein and lipid catabolism, the release of stress hormones and insulin resistance. The cardiovascular responses to this cytokine include the cardinal features characterizing systemic inflammation, sepsis and MOF. Rearrangement of the vascular endothelial cytoskeleton with loss of tight junctions and increased cellular permeability induced by TNF-α causes the capillary leakage syndrome. This feature, in addition to TNF-α mediated negative inotropism, decreased peripheral vascular tone and pro-coagulant effects on the vascular endothelium account for several of the hallmarks of these pro-inflammatory states. Indeed, septic shock is one of a number of infectious and pro-inflammatory disease processes in humans in which TNF-α has been directly implicated.[226]

1.4.2.2 Interleukin-1β (IL-1β, IL-1F2)

IL-1β, more recently named IL-1F2 reflecting its membership to the IL-1 ligand superfamily which is composed of three other primary members in addition to six less thoroughly studied ligands,[230] has been considered as the prototypical pro-inflammatory cytokine induced by common surgical stimuli.[222] IL-1β is synthesized as a biologically inactive 30 kDa precursor molecule which is generally cleaved intracellularly by the cysteine protease IL-1β converting enzyme (ICE), or caspase-1, to generate the mature 17.5 kDa form of IL-1β which is subsequently secreted.[222, 230] A variety of proteases commonly found in inflammatory fluids can, however, process the IL-1β precursor extracellularly into its active form. The primary sources of IL-1β are the blood monocyte, tissue macrophages and dendritic cells. B cells and NK cells are also sources as are certain malignant tumour cell lines. The induction of IL-1β production is highly sensitive to stimulation by bacterial products.[230] TNF-α is also known to induce IL-1β expression,[231] whilst *in vitro* studies have led to the recognition that IL-1 itself can stimulate its own expression.[230] As is the case with TNF-α, there is a dissociation between transcription and translation steps of IL-1β synthesis. Stimulants such as complement component C5a, hypoxia, adherence to surfaces, or clotting of blood, whilst inducing mRNA synthesis in monocytes, do not result in significant translation into protein,[232] however the addition of stimulants such as bacterial endotoxin results in augmented translation.[230]

The primary role of Il-1β is to function as a pro-inflammatory cytokine by stimulating the expression of genes associated with inflammation as well as those associated with auto-immune disease. The most salient properties of IL-1β in inflammation are the initiation of cyclooxygenase type 2 (COX-2), type 2

phospholipase and inducible nitric oxide synthase, which accounts for the production of PGE_2, PAF and nitric oxide following IL-1β exposure. An additional pro-inflammatory property of IL-1β is its ability to increase the expression of adhesion molecules such as ICAM-1 on mesenchymal cells and vascular-cell adhesion molecule-1 (VCAM-1) on endothelial cells.[222, 230] The latter property promotes the infiltration of inflammatory and immunocompetent cells into the extravascular space. In addition to its role in promoting inflammation, this cytokine may function as an angiogenic factor, as an adjuvant during antibody production and induces differentiation of bone marrow stem cells into cells of the myeloid series.[230]

1.4.2.3 Interleukin-6 (IL-6)

IL-6 is a glycoprotein which is subject to differential post-translational modifications including N- and O-linked glycosylations and phosphorylations, resulting in a molecular mass ranging from 21 to 28kDa, the prevailing subtype after endotoxin challenge being a 26-kDa protein.[67, 233-235] Like most cytokines, IL-6 is pleiotropic with a wide range of biological activities and is produced by both lymphoid and non-lymphoid cells.[233] It is known to regulate immune reactivity through its action as a B cell stimulatory factor[234] and through its involvement in T cell activation, growth and differentiation[234, 236, 237] . Il-6 is one of several cytokines considered to be haemopoietic growth factors[238] with a stimulatory effect on pluripotent haematopoietic progenitors and megakaryocytes and may play a part in the final maturation of cells of the granulocyte-monocyte lineage.[233]

A particularly important function of IL-6 in the context of acute surgical insults is an apparently causal role in the induction of the hepatic acute-phase response[233] , which

describes the synthesis and secretion of specific proteins by hepatocytes following stressful stimuli[239] . Additional functions of this cytokine of particular relevance to the surgical setting include its role as an essential component of the febrile response to IL-1 and lipopolysaccharide, its capacity to stimulate the secretion of a variety of anterior pituitary hormones including adrenocorticotrophic hormone (ACTH), and a suggested part in the function of macrophages and neutrophils *in vivo*.[233] Its importance in the inflammatory response to injurious stimuli is reflected by its use by some investigators as an index of the magnitude of the systemic inflammation.[222]

Actions of IL-6 extend beyond those directly relevant to the surgical setting and include a possible role in growth of nerve and vascular smooth muscle cells as well as osteoclast development; acting as a growth factor for various cells including keratinocytes and hepatocytes and those constituting plasmacytomas, myelomas, hybridomas, renal cell carcinomas, and Kaposi's sarcoma. Conversely, this cytokine may serve as a growth inhibitor for a number of other carcinoma and leukaemic cell lines.[233] IL-6 production is regulated by a number of signals. Its production in T cells or T-cell clones is induced by T-cell mitogens or antigenic stimulation. LPS enhances IL-6 production in monocytes and fibroblasts. A number of peptide factors including the cytokines IL-1, TNF, IL-2 and IFN-β also induce IL-6 production. In contrast, the cytokines IL-4 and IL-13 inhibit its production in monocytes.[233]

1.4.2.4 Interleukin-10 (IL-10)

IL-10 is a non-covalent homodimer whose importance in the response to surgical stimuli is attributable to its role as a key regulator of immune responses and whose predominant effects result in it being considered an anti-inflammatory, and more

generally, an immunosuppressive cytokine despite possessing some pro-inflammatory and immunostimulatory properties.[222, 240] Il-10 is expressed in a variety of cells including T cells, B cells, monocytes and macrophages, NK cells, keratinocytes, eosinophils, mesangial cells, epithelial cells and tumour cells.

The potent immunosuppressive and anti-inflammatory function of IL-10 may be recognized by its actions on individual cell types and through study of its *in vivo* effects in experimental pro-inflammatory states. Anti-inflammatory and immunosuppressive cellular effects of this cytokine on T helper (T_H) cells include the direct inhibition of production of T_{H1} cytokines as well as IFN-γ, the induction of long term anergy and the production of a negative regulatory T cell subset[241, 242]. An immunosuppressive effect is exerted by IL-10 on B cells by the inhibition of T dependant responses. Negative regulatory effects of IL-10 on monocytes and macrophages include inhibition of their antigen presenting functions; decreased synthesis of cytokines including IL-1α, IL-1β, IL-6, IL-8, IL-12, TNF-α, granulocyte-macrophage colony stimulating factor (GM-CSF) as well as reactive oxygen and nitrogen intermediates; inhibition of both COX-2 expression and IL-1 receptor induction; inhibition of proliferation and promotion of apoptosis of these cells; and decreased expression of several surface receptors involved in immunostimulatory signal transduction. IL-10 induces comparable effects on dendritic cells, reducing synthesis of specific cytokines and reducing the expression of selected receptors, in addition to decreasing the antigen presenting capacity of the dendritic cell.

Notable effects of IL-10 on the PMN include inhibition of mobilization of specific granules to the cell membrane and suppression of superoxide production. The ability of this cytokine to inhibit IFN-γ production by NK cells and the expression of ICAM-1 on activated vascular endothelial cells may also contribute to the anti-inflammatory effect of IL-10.[240]

IL-10 production is regulated by other cytokines. Production of IL-10 by monocytes, T and B cells, NK cells and mast cells may be induced by IL-1, IL-2, Il-3, IL-6, IL-7, IL-12 and IL-15.[243-248] TNF-α, IFN-γ, and transforming growth factor-β (TGF-β) have also be found to induce IL-10 production in specific cell types.[249-254] Conversely IL-4, IL-13 and IFN-γ have been found to exert an inhibitory influence on IL-10 production in activated monocytes,[255, 256] whilst IL-10 may have an autoregulatory role by inhibiting its own production[257].

1.4.2.5 Interleukin-12 (IL-12, IL-12p70)

Receiving far less attention in the surgical literature, despite being considered as one of the cytokines of surgical relevance by Raeburn *et al.* [222], is IL-12. Biologically active 1L-12 is a 70 kDa heterodimeric protein composed of a heavy chain (p40) and a covalently associated light chain (p35), the expression of each sub-unit being independently regulated. Co-expression of both sub-units therefore determines the ability of a given cell type to express and secrete bioactive IL-12. Those cells recognized as having the capacity to secrete bioactive IL-12 are monocytes, macrophages, dendritic cells, neutrophils, endothelial cells and mesoglia.

IL-12 has been considered as playing a crucial role in regulating nearly every aspect of the immune response through its positive impact on cell-mediated immunity, both at the level of its induction as well as effector mechanisms. Specifically, it is the key factor in the induction of protective T_{H1}-type responses by CD4-positive T cells; it promotes the cytolytic activity of T lymphocytes and natural killer cells; it plays a role in B cell survival, differentiation and effector function and modulates antigen presenting cell (APC) function.[258] The capacity of IL-12 to induce the production of TNF-α in addition to IFN-γ, in synergism with IL-18, confers this cytokine with a potent pro-inflammatory potential.[222, 258, 259] Effective induction of bioactive IL-12 is thought to require at least two different signals which may include the interaction of CD40L, expressed by helper T cells, with CD40 on APCs in combination with a co-stimulatory cytokine, the combination of signal induction by both IFN-γ and a bacterial product, or CD40L engagement together with a bacterial product. A range of factors have been demonstrate to powerfully inhibit IL-12 production including IL-10, IFN-γ and glucocorticoids.[258]

1.4.2.6 Chemokines

The chemokines are a burgeoning family of over forty structurally related low molecular weight cytokines, most of which exhibit chemotactic activity for a limited spectrum of leukocytes, thus giving origin to the family's title (*chemo*tactic cyto*kines*).[222, 260-262] Chemokines exert their biological effects, which are now recognized as extending far beyond leukocyte physiology, by binding to and subsequently signaling via specific G protein-coupled cell surface receptors on their target cells.[260-263] Most chemokines have at least four cysteines in highly conserved positions, enabling the classification of these proteins into subfamilies, designated

CXC or α-chemokines, CC or β-chemokines, CXXC or δ-chemokines and C or γ-chemokines respectively, on the basis of the relative position of these cysteine residues. The CXC subfamily is so named as one non-conserved amino acid separates the first two NH_2-terminal cysteines (cysteine-X amino acid-cysteine). This subfamily is subdivided further into ELR^+ and ELR^- CXC chemokines, based on the presence or absence of the tripeptide glutamic acid-leucine-arginine (ELR) amino acid motif immediately preceding the first cysteine residue.[260-263] The latter classification has functional implications with the ELR^+ CXC chemokines binding to CXC receptor 1 (CXCR1) and/or CXCR2 and acting as potent PMN chemoattractants and activators, in contrast to the ELR^- CXC group of chemokines which generally bind to CXCR3-CXCR5 and act primarily on mononuclear leukocytes. [260-263] Members of the second major chemokine subfamily, the CC chemokines are characterized by the first two cysteines being adjacent to one another with no intervening amino acid (cysteine-cysteine). The CXXC subfamily is comprised of only one member, named fraktalkine, or alternatively neurotactin, in which three non-conserved amino acids intervene between the first two cysteine residues. The C chemokine subfamily similarly consists of a single member, lymphotactin, characterized by the lone cysteine residue in the NH_2-terminal domain.[260, 262, 263]

1.4.2.6.1 Interleukin-8 (IL-8, CXCL8)

IL-8, or CXCL8 according to the recently introduced systematic chemokine nomenclature system,[261, 264, 265] is the prototypical CXC chemokine[222, 262] and is considered by Raeburn *et al.*[222] to be one of the surgically relevant cytokines. This ELR^+ CXC chemokine is produced by leukocytic cells, including monocytes, T cells,

PMNs and NK cells; non-leukocytic somatic cells, namely fibroblasts, endothelial cells and epithelial cells; as well as tumour cells.[261, 262, 266, 267] In most cell types, its production is non-constitutive, but is rapidly induced by a variety of stimuli including pro-inflammatory cytokines, such as IL-1 and TNF-α;[268] bacteria;[269] bacterial products;[261] and may be induced by hypoxia[261] . Inhibition of IL-8 gene transcription may be achieved by IFN-γ and glucocorticoids.[261] Differential processing at the NH_2-terminus, largely determined by cell type, yields several truncation analogs of IL-8, with the most abundant naturally occurring form being 72-amino acids in length.[261, 270, 271]

The principal effects of IL-8 on PMNs are those of activation and promotion of transendothelial migration. Activation events stimulated by IL-8 include induction of degranulation and respiratory burst;[267, 272] activation of 5-lipoxygenase with release of leukotriene B_4 and 5-hydroxyeicosatetraenoic acid (5-HETE);[273] in addition to the induction of PAF synthesis by PMNs [274] . Also considered activating events, and critical to the trafficking of PMNs from the vascular to the extravascular compartment during inflammatory processes, are the IL-8 stimulated processes of PMN shedding of L-selectin, quantitative and qualitative up-regulation of the β_2-integrins CD11b/CD18 and CD11c/CD18,[275-277] thereby signaling the conversion of early low-affinity selectin-mediated rolling of PMNs along the endothelium to the high-affinity, integrin-mediated interaction that leads to extravasation [260] . Furthermore, IL-8 promotes chemotaxis by enhancing PMN adhesion to extra-cellular matrix proteins through CD11b/CD18.[275]

Cellular activation and chemotactic effects of IL-8 are not, however, limited to PMNs. IL-8 elicits a weak respiratory burst in monocytes[278] and may have a role in macrophage migration *in vivo*[261] . IL-8 induces effects in basophils including chemotaxis,[279] adherence to endothelial cells,[280] and can induce the release of histamine and leukotrienes from IL-3 pretreated basophils[281] . Chemotaxis by both T-[282] and B-cells[283] also occurs in response to IL-8. The pathological processes in which IL-8 is considered to play a central role are clearly consistent with the cellular effects of this chemokine. Of particular relevance to a variety of surgical settings is the demonstrated involvement of IL-8 in infectious processes.[261] Of perhaps even greater consequence to vascular surgery is the likely involvement of CXC chemokines, including IL-8, in establishing ischaemia-reperfusion injury[261] which is characterized by PMN adhesion to vascular endothelium and tissue infiltration[284, 285] .

1.4.3 Generation of Cytokines in Major Vascular Surgery

1.4.3.1 Ischaemia and Reperfusion Injury (IRI)

The generation of cytokines in response to tissue injury, transient ischaemia and microbiological insults occurs to a variable extent in most major surgical interventions, however the necessary interventions involved in major elective vascular surgery and open aortic surgery in particular provide the relatively unique situation of exposure of patients to unavoidable, intense, yet relatively predictable cytokine-provoking stimuli. The consistent exposure to ischaemia and subsequently IRI associated with open aortic surgery is considered as the major surgical stimulus to cytokine production to which these patients are subjected.[223] Application of vascular clamps during aortic surgery is a predominant cause of tissue ischaemia and IRI initiated in tissues distal to the site of clamping and possibly those supplied by

arterial branches immediately proximal to the clamp,[223] with arterial clamping during lower limb revascularization procedures imparting an analogous insult.

1.4.3.2 The Vascular Endothelium

Damage to the vascular endothelium during IRI may contribute to the generation of cytokines during major vascular surgery. IRI-associated endothelial damage results in PMN adhesion, activation and endothelial transmigration, triggering further tissue damage and impairs nitric oxide production by endothelial cells, further exacerbating endothelial dysfunction and impairing vascular homeostasis. Cytokines are therefore generated directly by the damaged endothelial and vascular smooth muscle cells themselves, and indirectly through the process of PMN activation. Hypoxia itself, independent of subsequent reperfusion events, is also a stimulus for endothelial cytokine production.[223]

1.4.3.3 Lower Limbs and Gastro-intestinal Tract

Both the lower limbs and the gastro-intestinal tract have been implicated as sites of origin of the inflammatory response, and particularly the generation of cytokines, associated with AAA repair.[286] The cytokine profiles generated by isolated limb IRI in humans have not been studied in great detail. Raised IL-1 and TNF-α levels have been observed following tourniquet-induced limb ischaemia[287] whilst IL-6, IL-10 and IL-1 receptor antagonist have also been observed following traumatic and tourniquet-induced limb ischaemia[288] . Intra-operative sampling of femoral vein blood during open AAA repair by Holmberg *et al.*[289] demonstrated significant increases in IL-6, IL-10 and the CC chemokine sub-family member monocyte

chemotactic protein-1 (MCP-1), during the ischaemic phase of surgery, with further increases following reperfusion.

Norwood *et al.*[286] sought to examine the relative contribution made by the gastro-intestinal tract and lower limbs to cytokine production during open AAA repair by comparing IL-6 levels in femoral vein, portal vein and radial artery blood. The finding that IL-6 levels were significantly higher in portal vein samples compared with radial artery samples during ischaemia, and were significantly higher than both femoral vein and radial artery samples during reperfusion, led these authors to conclude that the gastro-intestinal tract was the predominant source of IL-6 and is therefore likely to be responsible for the initiation of the inflammatory response caused by AAA repair. IRI induced by vascular clamping is not, however, considered to be the sole cause of cytokine release from the gastro-intestinal tract during open AAA repair. General handling of the bowel, mesenteric traction and intestinal cooling may also be important contributors to intestinal ischaemia as a consequence of the release of vasoactive agents. which may inturn disrupt mucosal integrity leading to translocation of bacterial endotoxin from the lumen[223, 224, 286] Endotoxaemia, demonstrated after AAA repair and implicated in the aetiology of the inflammatory response, may thus contribute to the observed cytokinaemia.[224, 286] Finally, the release of cytokines from the gastro-intestinal tract may be stimulated indirectly by effects on the lower limbs. White cell activation in the lower limbs following reperfusion may result in plugging of the intestinal vasculature by leukocytes thereby inducing hypoperfusion. In addition, lower limb reperfusion may induce intestinal hypoperfusion by provoking redistribution of blood from the splanchnic circulation.[224]

1.4.3.4 Kidneys

Renal ischaemia and IRI are also believed to contribute to cytokine generation during AAA repair. Whilst supra-renal aortic cross clamping imparts the greatest effects on renal perfusion, infra-renal clamping also causes renal damage and changes in renal perfusion. Elevated tissue levels of IL-1, IL-2, IL-6, TNF-α, TGF-β, and IFN-γ identified in animal studies of renal IRI suggests that the total or partial ischaemic insult to the renal parenchyma and subsequent reperfusion during open AAA repair may contribute to an observed cytokinaemia.[223] Large volume blood transfusion is an additional factor which may contribute to the peri-operative cytokine response pattern.[224]

1.4.3.5 Cytokine Generation in EVAR

The marked difference in operative techniques involved in EVAR compared with open AAA repair imply that different pathophysiological mechanisms may account for the inflammatory response, and therefore cytokine generation, associated with these procedures. Whilst a reduction in tissue injury, blood transfusion and most notably IRI occurs with EVAR compared to open AAA repair, factors which may contribute to the initiation of the inflammatory response to EVAR include cellular interactions with the biomaterials lining the endograft, in addition to manipulations with introducers and catheters resulting in direct endothelial damage or indeed the release of cytokines, such as IL-6, from thrombus within the aneurysmal aorta.[290]

1.4.4 Cytokines and the Prediction of Clinical Outcomes

The concept of using cytokine profiles to predict the occurrence of adverse clinical outcomes is not without precedent. Several authors have identified biologically valid correlates between specific cytokine profiles and subsequent clinical events amongst cohorts including those subject to trauma, cardiac surgery, and indeed major vascular surgery, with several examples of cytokine levels predicting outcome in established pro-inflammatory states such as MOF.

Several authors have investigated the capacity of cytokine profiles to predict clinical events amongst patients undergoing open AAA repair. As with similar studies involving different patient cohorts, non-survival has frequently been selected as a principle measure of clinical outcome. Amongst a small cohort of eleven patients undergoing open AAA repair, selected on the basis of their clinical outcome, Soong *et al.*[291] examined plasma levels of IL-6 and changes in soluble p55TNF receptor levels amongst survivors compared with non-survivors over five post-operative days, with p55TNF receptor levels considered by these authors to be a sensitive marker of the systemic response to endotoxaemia. The longitudinal profile of IL-6 levels appeared to differ between the two patient groups, with significantly greater plasma IL-6 levels apparent amongst non-survivors at 48 and 96 hours post-operatively compared with survivors. Similarly, a significantly greater increase in soluble p55TNF receptor levels was observed at 48 and 72 hours amongst those who subsequently died compared with those who survived.

Roumen *et al.*[225] studied cytokine patterns in a cohort constituted by patients subject to multi-trauma, rupture of AAA's complicated by shock, and elective open AAA

repair. These authors dichotomized the entire cohort according to survival, with elective open AAA patients constituting 14% of non-survivors, enabling a comparison of IL-1β, IL-6 and TNF-α levels over the first 24 hours from admission or skin incision. This comparison identified significantly higher levels of both IL-1β and TNF-α, both on admission/skin incision 6 hours later, with a significant difference also being observed at 24 hours in the case of IL-1β, amongst non-survivors compared with survivors. The trend towards greater IL-6 levels in non-survivors, however, failed to reach statistical significance. Dichotomizing the cohort into those who developed ARDS/ MOF, 30% of whom underwent elective AAA repair, and those who did not, also demonstrated both early and late differences in cytokine profiles. Notably, greater levels of IL-1β were apparent as early as 6 hours post admission/skin incision in the ARDS/MOF cohort, and again on the seventh and ninth days, however this just failed to reach statistical significance. Levels of TNF-α amongst those who developed ARDS/MOF were significantly greater at 6 hours and on the seventh day, whilst significantly greater levels of IL-6 distinguished the ARDS/MOF cohort from the second to eleventh day. Bown et al.[216] examined the same panel of cytokines, in addition to IL-10, at 24 hours post-operatively, amongst a sizeable cohort of patients undergoing open AAA repair, either electively or as an emergency procedure. Amongst patients undergoing elective repair, patients categorized as having longer periods in hospital and longer stays in critical care, both exhibited higher levels of IL-10. Amongst patients undergoing emergency AAA repair, those who developed MOF had significantly higher levels of both IL-6 and TNF-α than those who did not whilst TNF-α levels were significantly higher in those patients who subsequently died. Hamano et al.[292] examined patients with established post-operative MOF, following major cardiac, aortic or peripheral revascularization

surgery, in an effort to identify predictors of survival. These authors noted that levels of IL-8, but not IL-6, were significantly higher amongst non-survivors compared with survivors, both at the onset of MOF and one week later. Froon *et al.* [293] studied patients undergoing elective and emergency repair of AAA's and identified higher peak plasma levels of IL-6, as well as the pro-inflammatory soluble ICAM-1, amongst those who subsequently died. In a study involving twenty patients undergoing elective AAA repair, Baigrie *et al.*[294] also noted a unique IL-6 profile distinguishing those who developed unexpected major complications. IL-6 levels in this group were significantly higher within 6-8 hours of skin incision, and remained elevated for longer, compared to those without major complications. Importantly, these authors note that the rises in IL-6 preceded the onset of major complications by 12-48 hours. Amongst 17 patients undergoing elective AAA repair, Jedynak *et al.*[295] found no relationship between serum IL-12 levels and post-operative course.

Studies focussing exclusively on patients undergoing cardiac surgery with CPB also support the concept that cytokine profiles may predict sub-sets of patients at risk of adverse post-operative events. Ben-Abraham *et al.*[296] and Lotan *et al.*[297] present findings from the same cohort of children undergoing cardiac surgery with CPB, to support this contention, with a particular focus on chemokine profiles. Children requiring post-operative inotropes were distinguished by higher IL-8 levels from the time that CPB commenced until twelve hours post-operatively.[296] Significantly higher IL-8 levels also distinguished those suffering from post-operative tachycardia,[296] however the time at which this difference was detected is unclear. These findings suggest that IL-8 may be an early marker of post-operative morbidity. Children requiring post-operative inotropes also tended to have higher MCP-1 levels

at all sampling times from anaesthetic induction until 24 hours post-operatively, however statistical significance was reached only at the time of CPB commencement, one hour post CPB and 12 hours post-operatively.[297] Patients requiring subsequent surgical interventions also displayed higher MCP-1 levels at all time points, with significance reached at the time of CPB commencement and aortic cross clamping. Levels of RANTES, another member of the CC chemokine sub-family, also distinguished this group of patients, being lower at all time points compared with patients not requiring re-intervention, with statistical significance being reached 1, 12 and 24 hours post-operatively.[297]

In a study examining cytokine profiles amongst patients with established MODS post cardiac surgery with CPB, Slabotzki *et al.* [298] noted that those who subsequently died exhibited significantly higher plasma levels of IL-18, a pro-inflammatory member of the IL-1 cytokine family,[259] on each of the first four post-operative day compared with those who survived. The use of the APACHE II illness severity score only distinguished survivors from non-survivors on the fourth post-operative day.

An alternative methodological approach to identifying correlates between *in vivo* plasma cytokine levels and outcome, but with similar implications, is the identification of correlates with cytokine levels generated *in vitro* by stimulation of isolated leukocytes. Hensler *et al.*[299] applied this methodology in a large study of monocyte IL-12 production amongst patients undergoing major gastro-intestinal surgery. Theses authors demonstrated that amongst those subsequently developing sepsis, stimulated monocyte IL-12 secretion was suppressed prior to surgery and remained low until the onset of sepsis suggesting that reduced IL-12 release

distinguishes a sub-set of patients who are susceptible to the development of sepsis following this surgical insult. Those who developed post-operative sepsis were also characterized by significantly greater levels of stimulated monocyte TNF-α secretion prior to surgery and on the first post-operative day compared with those with an uneventful recovery. Similarly, Weighardt *et al.*[300] investigated associations between levels of cytokines generated by stimulated monocytes and survival amongst patients with established sepsis following major visceral surgery. These authors demonstrated that whilst monocytes from all septic patients exhibited suppressed secretion of IL-12p40, IL-1β, and IL-10 during the early phase of sepsis, survival was characterized by a significant recovery of IL-12p40 and IL-1β production. These findings provide further evidence for the contention that the cytokine response may be prognostic of clinical outcomes.

Considered collectively, these studies imply that profiles of certain cytokines may not only act as markers of established clinical processes but may in fact predict impending adverse events amongst those subject to major surgery. No available studies to date, however, have examined the capacity of a wider spectrum of relevant cytokines to predict the onset of sepsis following major vascular surgery.

1.5 Nutritional Status and Surgical Outcomes

1.5.1 Definitions

Adequate nutritional status is a broad concept inferring a sufficient food intake to meet the requirements for specific nutrients to support optimal health and well-being. Whilst there is no universally accepted definition of malnutrition, this term refers to a continuum of inadequate nutritional status due to insufficient intake or exaggerated substrate loss.[301] The term protein-energy malnutrition (PEM) describes the form most frequently seen in clinical practice, in which there is a deficiency of carbohydrate, proteins and fats in addition to multiple vitamin, mineral, and trace element deficiencies[302] and leads to low body fat-free mass and fat mass[303].

1.5.2 Frequency of Malnutrition

Evidence generated over the past thirty years has documented that PEM, or the possibility of its development, occurs frequently in surgical patients admitted to hospital, with a deterioration of nutritional status occurring over the course of hospitilisation.[304] A study of pre-operative nutritional status amongst surgical patients documented the frequency of impaired nutritional status amongst their cohort of patients undergoing major vascular surgery to be 18%, a prevalence greater than observed amongst patients undergoing abdominal surgery.[305] The finding by Spark *et al.*[306] that patients with chronic critical limb ischaemia exhibited significantly lower values of several nutritional markers compared with general surgical patients supports the contention that impaired nutrition appears to be a relatively frequent characteristic of candidates for major vascular surgery. The pathogenesis of undernutrition in these patients has not been defined, however

contributing factors may include the disease process itself and associated pain and loss of appetite.[304] The frequently elderly nature of vascular surgery patients, in particular, may also be a contributor, with PEM a common finding in geriatric patients due to the concurrency of diseases that may impair nutritional status, an age-associated reduction in appetite, and a reduction in lean body mass even in the absence of overt catabolic illness, which may initiate a cycle of illness and wasting.[307] Peri-operative starvation and the hypermetabolic stress response accompanying both surgical trauma and infective complications may compound the adverse metabolic[302, 308], and inturn, the clinical consequences experienced by such patients.

1.5.3 Malnutrition and Surgical Outcomes

Since Studley's[309] 1936 publication on the relationship between pre-operative weight loss and post-operative mortality, evidence of the relationship between malnutrition and adverse post-operative outcomes has continued to accumulate. Subsequent studies have employed an array of surgical cohorts and markers of nutrition to identify an apparent relationship between poor nutritional status and an increase in post-operative complications and mortality.[304, 310] Particularly relevant are studies which have documented that nutritional status may be prognostic for the occurrence of post-operative infective complications.[304, 310, 311] These adverse effects appear to translate to a prolonged post-operative convalescence, hospital stay and an impaired quality of life.[304]

The causal factors underlying the relationship between poor nutritional status and adverse outcomes have also received considerable attention. Modulation of the

immune response by impaired nutrition is considered to have a particularly important bearing on this relationship,[302] however a depression of the immune response by impaired nutrition has not been the universal finding as might be expected. Moderate to severe protein restriction has indeed been demonstrated to inhibit the development of antibody-producing cells, the production of circulating antibody[312] and secretory IgA and antibody affinity[313] . Other aspects of immune function suggested to be impaired by PEM include phagocytic function, the complement system and notably cytokine production.[313] Conversely T-cell mediated immune responses have been suggested to be preserved, and potentially enhanced in states of protein deficiency[314, 315] Similarly, animal studies of chronic moderate restrictions of energy intake, including both carbohydrates and fat, translate into vigorous immunologic function.[316]

Impairments in gastro-intestinal tract function are considered a consequence of both the global effects of malnutrition and a lack of nutrient contact with the gastro-intestinal mucosa and, like the immune response, are considered relevant to the relationship between malnutrition and surgical outcomes.[302] Impaired function of the gut as a barrier against translocation of bacteria and their toxins is found in malnutrition, particularly when additional factors, many of which are relevant to major vascular surgery, such as trauma, endotoxaemia and reperfusion are present.[302] Animal models have indicated that consequences of disruption of gut barrier function may include sepsis and MOF.[302]

Clinical studies of the effect of nutritional status on the cumulative inflammatory response to surgery are, however, relatively few in number and the validity of

generalizing findings limited by the nature of the surgical cohorts examined. A study by Hatada *et al.* [317] indicated that amongst patients undergoing surgery for colorectal cancer, dichotomized according to blood loss as a marker of surgical stress, those who were diagnosed with PEM on the basis of their creatinine height index, exhibited a significantly higher early post-operative serum Il-6 response. Amongst the sub-set of patients with a lower intra-operative blood loss, significantly higher levels of the chemokine stimulating soluble IL-6 receptor (sIL-6R) characterized the malnourished cohort after post-operative day 3, whilst amongst those with a greater intra-operative blood loss, malnourished patients exhibited significantly lower levels of anti-inflammatory IL-1ra on post-operative days 3 and 7 but a significantly greater percentage of peripheral neutrophils and CRP level on post-operative days 3 and 7 respectively. Collectively these findings were interpreted as indicative of a more marked activation of the pro-inflammatory network associated with a decreased antagonistic reaction amongst malnourished patients. Surprisingly, there was no statistical difference in the rate of septic complications between those with PEM and those considered to be adequately nourished. These authors proposed that the observed association between malnutrition and a more intense pro-inflammatory response to surgery may have a bearing on neutrophil activation and subsequent clinical outcomes amongst those undergoing surgery for colorectal cancer. Comparable conclusions were drawn from a study of liver transplant recipients which reported an involvement of surgically induced malnutrition in an exaggerated post-operative cytokine response. [318]

Evidence obtained from immunonutritional studies supports the suggestion that improved nutritional status may dampen exaggerated inflammatory responses

stimulated by surgery[319] including some evidence to suggest reductions in pro-inflammtory cytokines[320] and SIRS days per patient amongst those with trauma induced MOF[321].

1.6 The Neuroendocrine Response to Surgical Stress

Major surgical interventions represent a significant physiological stress, triggering marked changes in the activities of the hypothalamo-pituitary-adrenocortical (HPA) axis and the autonomic nervous system with measurable fluctuations in their constituent hormones and neuropeptides.[322, 323] These, as well as other neuroendocrine effects, produce changes in cardiovascular, metabolic, immune and haemostatic function,[323] which have evolved as an integral part of the host's homeostatic response to injury[324] . In modern surgical practice, however, where severe physiological disturbances are prevented or rapidly treated, there is some evidence to suggest that these changes may precipitate clinically important complications. Indeed, this realisation has prompted attempts to identify anaesthetic and analgesic regimens which may optimally attenuate the neuroendocrine stress response.[323, 325] Whilst physical injury is typically considered the principal source of the neuroendocrine response to surgery, given that surgery may be viewed as a source of psychological stress,[326, 327] itself a trigger of neuroendocrine modulation,[328] psychological factors may influence the neuroendocrine response to surgical injury.[326, 327]

1.6.1 The Hypothalamo-pituitary-adrenocortical (HPA) Axis

Corticotropin-releasing factor (CRF) and arginine vasopressin (AVP), synthesized in the hypothalamic paraventricular nucleus, and transported down axonal projections to the median eminence, are the principal neuropeptides involved in mediating HPA axis activity. During non-stressful periods, the HPA axis exhibits a strict circadian rhythm, regulated by the suprachiasmatic nucleus. In response to a stressor, CRF and AVP are immediately released from storage vesicles in the median eminence into the

superior hypophyseal artery and are transported down a dense capillary network of portal blood vessels to the anterior pituitary. Here, CRF and AVP act synergistically on corticotroph cells to increase expression of the ACTH precursor pro-opiomelanocortin (POMC), thereby stimulating the release of ACTH into the circulation. ACTH in turn stimulates secretion of the glucocorticoid, cortisol, from zona fasiculata cells of the adrenal cortex.[239, 328]

HPA axis activity is regulated by a complex system of integrated, multi-layered controls (Fig. 1). Amongst these, glucocorticoids exert negative feed-back control over basal and stress-elevated HPA axis activity, the latter via hippocamapal and hypothalamic receptors.[322, 328] Of particular interest, is the bi-directional regulation between the immune system and HPA axis.[329, 330] Pro-inflammatory cytokines, most notably IL-1α, IL-1β, IL-2, IL-6 and TNF-α, exert marked stimulatory influences on the HPA axis,[331] thus provoking the release of glucocorticoids which complete the homeostatic neuroendocrine-immune regulatory loop by modulating immuno-inflammatory activity.[329, 330] This modulating influence, exerts immunosuppressive effects upon many facets of the innate immune response, as well as cellular and humoral acquired immune responses. Current evidence indicates, however, that the effects of glucocorticoids on immune function, and on cytokine production in particular, should be considered as immunomodulatory rather than simply immunosuppressive as physiological levels of glucocorticoids cause a shift in the pattern of cytokine production from a primarily pro-inflammatory to an anti-inflammatory pattern. This occurs by the glucocorticoid mediated inhibition of T_{H1} cytokine production, together with an enhancement of T_{H2} cytokine production; these

two sub-sets of cytokines corresponding roughly to pro- and anti-inflammatory cytokine categories respectively.[329]

1.6.2 The Sympathetic Nervous System

Surgical stress is one of many stressors, both physical and physocological, known to activate the sympathetic nervous system (SNS), resulting in variable increases in the release of mediators of its responses, including the catecholamines adrenaline and noradrenaline.[322, 332] Adrenaline, noradrenaline and dopamine are secreted by the adrenal medulla in response to stimulation by sympathetic preganglionic nerve fibres in the splanchnic nerves. Whilst the catecholamine output of the adrenal vein is principally adrenaline, noradrenaline also enters the circulation from post-ganglionic sympathetic nerve endings.[239, 322]

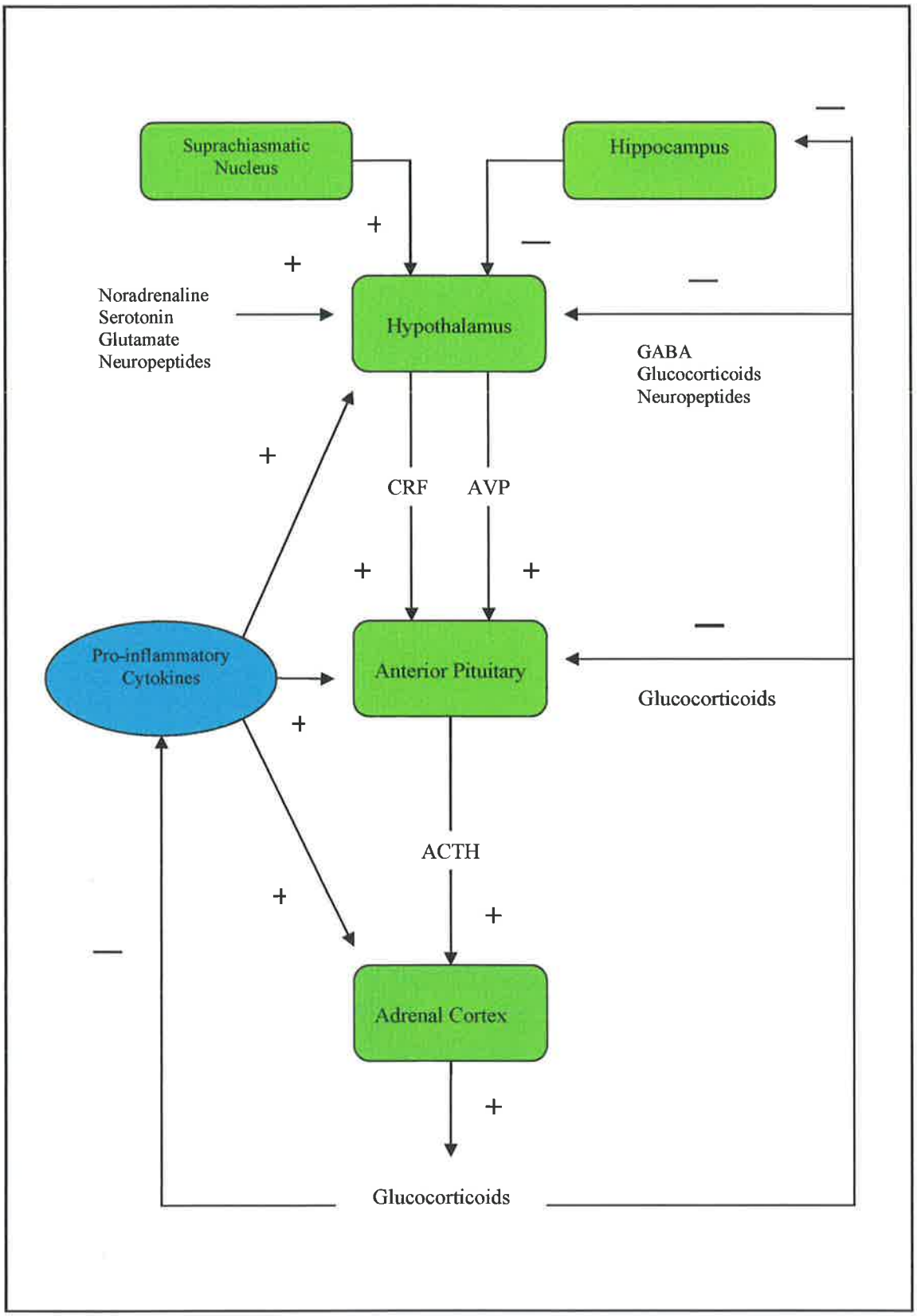
The effects of adrenaline and noradrenaline are multiple and include metabolic effects; a marked influence on the cardiovascular system including inotropic and chronotropic effects, an influence on the state of vasoconstriction, and in turn blood pressure; and a state of heightened alertness is also provoked by catecholamine release.[239] In addition, catecholamines have the potential to influence immuno-inflammatory responses, albeit in what is presumably a far more limited manner than glucocorticoids. Evidence for such a role includes the ability of adrenaline and noradrenaline to inhibit neutrophil chemotaxis *in vitro*,[333] to cause selective suppression of cellular immunity and to induce a shift from T_{H1} to T_{H2}-mediated humoral immunity both *in vitro* and *in vivo*[334]. Furthermore, sympathetic innervation of organs with immune functions, such as the spleen, may also play an important role in the regulation of immunity[329]. *In vivo* studies relating the catecholamine response

to surgical outcomes have suggested a relationship with post-operative haemodynamic and thrombotic outcomes,[335] however a potential relationship with post-operative immuno-inflammatory outcomes has not been investigated to date.

Figure 1. Schematic representation of the Hypothalamo-pituitary-adrenocortical (HPA) axis demonstrating multi-layered and integrated regulation system.[328]

—, inhibitory effect; +, stimulatory effect

ACTH, adrenocorticotropic hormone; AVP, arginine vasopressin; CRF, corticotropin-releasing factor; GABA, gamma-aminobutyrate


Suprachiasmatic Nucleus
Hippocampus
Noradrenaline
Serotonin
Glutamate
Neuropeptides
Hypothalamus
GABA
Glucocorticoids
Neuropeptides
CRF
AVP
Pro-inflammatory Cytokines
Anterior Pituitary
Glucocorticoids
ACTH
Adrenal Cortex
Glucocorticoids

1.7 Psychological Influences on Surgical Response and Outcome

1.7.1 Depression

1.7.1.1 Prevalence of Depressive Disorder

Whilst data on the prevalence of depressive disorder amongst vascular surgery candidates is lacking, general population studies indicate that approximately 10 – 15% of the elderly suffer from depressive complaints warranting intervention,[336] this being the age group most frequently subject to major vascular interventions. The increasing awareness that depression may occur concurrently with other illnesses, and that it may have a negative effect on recovery from those conditions,[337] has led to the study of this disorder amongst specific patient sub-groups, particularly those with cardiac conditions, and of most relevance to the current study, those undergoing CABG surgery. A particularly high prevalence of pre-operative depression, ranging from 15 – 36%, has been reported in studies focusing on those undergoing CABG,[338-341] two of which have examined Australian cohorts[338, 340]. Whether patients facing the prospect of major aortic or peripheral vascular surgery display a similarly high prevalence of depression is unknown.

1.7.1.2 Depression and Adverse Disease Outcomes

Convincing evidence has emerged from studies involving patients with cardiac conditions to suggest that depression may increase the risk of adverse disease outcomes.[338, 339] Much of this data emanates from studies involving patients experiencing myocardial infarction, which have found that a diagnosis of major depression significantly increases the risk of subsequent cardiovascular mortality and

morbidity, independent of disease severity.[342-345] This knowledge, in turn, has prompted several investigators to examine the influence of pre-operative depression on outcomes following CABG surgery. Baker *et al.*[338] reported that the incidence of late mortality was significantly greater amongst those CABG surgery patients displaying pre-operative depressive symptoms compared with those who were not considered depressed. Furthermore, Burg *et al.*[339] found that pre-operative depression independently predicted several measures of post-operative morbidity six months following CABG surgery, namely cardiac-related hospital re-admission, continued surgical pain and failure to return to previous activities. How depression leads to adverse outcomes in cardiac patients remains unclear, although several explanations, both psychosocial and biological, have been proposed.[337]

1.7.1.2.1 Depression and Health-related Quality of Life (HRQoL)

Health-related quality of life (HRQoL) is an outcome measure that is now considered as one of the essential elements of health care evaluation[346] and has been increasingly recognised as an important component of outcome analysis in vascular surgery research[347, 348]. The concept of HRQoL is multi-faceted[349] and refers to how health impacts an individual's ability to function and their perceived well-being in physical, mental and social domains of life.[346] Similar to the relationship demonstrated amongst cardiac cohorts between depression and traditional outcome measures such as medical morbidity and mortality, studies suggest that depression may also be a particularly important predictor of HRQoL.[350] Not only have depressive symptoms following myocardial infarction been shown to predict decreased HRQoL,[351] but several studies suggest amongst cohorts of cardiac surgery have suggested that pre-operative depressive symptoms are associated with poorer

post-operative HRQoL.[350, 352] It is unknown whether depression is a similarly important determinant of HRQoL following major vascular surgery.

1.7.1.3 Depression and Immune Dysregulation

Abundant evidence has accumulated demonstrating that depression and psychological stress are associated with immune dysregulation, and furthermore are associated with an increased susceptibility to infectious diseases.[353-355] An array of studies have documented the suppression of various indices of immune function in depressed patients, including zymosan-induced PMN phagocytosis, mitogen-stimulated lymphocyte proliferation and NK cell activity, supporting the traditional view of depression being associated with features of immunosuppression.[354] More recently, an equally large and convincing body of evidence has arisen to indicate that major depression is, in addition, associated with the activation of various aspects of immune function.[353, 354, 356, 357] The finding of increased concentrations of cytokines amongst depressed individuals, including IL-6, sIL-6R, soluble IL-2 receptor (sIL-2R), IL-1β, IL-1Ra and IFN-γ, have been presented to support this concept. Increased concentrations of complement, various positive acute phase proteins, increased numbers of specific T-and B-cell subsets and enhanced monocyte phagocytosis also indicate the presence of immunological activation in depressed patients.[354] Considered together, the evidence strongly supports the notion of immune dysfunction amongst depressed patients, manifesting as suppression of some immune responses whereas other responses are enhanced.[356] Whether this immune dysregulation increases susceptibility of depressed individuals to immune-related disorders is not yet known.[355] The possibility that it may influence the immunological response to surgical injury has not yet been examined.

1.7.1.4 Neuroendocrine Features of Depression

Hyperactivity of the HPA axis is a frequent finding amongst depressed patients.[353, 354] Elevations in both CRF and cortisol have been reported, suggesting that a defect in the negative feedback of cortisol on CRF secretion exists in these patients.[354] It is also worth noting that the co-existence of elevated circulating pro-inflammatory cytokines and hypercortisolaemia observed in depressed patients is somewhat of a paradox, given the usual inhibitory influence of glucocorticoids on the release of pro-inflammatory cytokines. The absence of the normal inverse relationship between circulating glucocorticoid and pro-inflammatory cytokine concentrations in depressed patients has been attributed to a defect in the inhibitory feedback of glucocorticoids on cytokine secretion by immunocytes.[354] It is unknown whether the HPA axis hyperactivity observed in un-stimulated depressed patients may result in an altered endocrine response to surgical injury.

1.7.2 Anxiety

If the concept that neuro-endocrine responses to surgery may be influenced be psychological variables[327] is to be examined, then anxiety, a state characterized by subjective feelings of tension, nervousness and worry, and objectively by activation of the autonomic nervous system,[358] must be considered as a potential influence on this response. Only one research group appears to have directly examined this concept in a small cohort of patients undergoing major abdominal surgery.[359] Whilst their findings appear to support the notion that the surgical stress response, indicated by plasma cortisol and catecholamine levels, is associated with psychological influences, they are contrary to the intuitive view that pre-operative anxiety should promote a larger endocrine response. Indeed, they suggest that anxiety, or a variable

correlated with it, might protect against a larger stress response.[359, 360] Further investigations of the influence of anxiety on the neuro-endocrine response to surgery are therefore warranted.

1.8 Immune and Neuroendocrine Responses in Open AAA Repair Compared to EVAR

Since the introduction of an endovascular method of AAA repair by Volodos in the Ukraine[361] and Parodi, Palmaz and Barone in Argentina[362] in the early 1990's, commercial development of the technology[363] combined with an increasing body of data to support its use[17] has led to the worldwide diffusion of EVAR[363]. Whilst its use has been tempered by clinical concerns pertaining to endoleak and endotension, combined with the lack of long-term evidence of either graft durability or survival benefits, it is employed by many as a feasible alternative to open AAA for patients whose anatomy is considered suitable.[364] Receiving less attention than the clinical controversies that currently dominate the literature relating to EVAR, is the pathophysiological response triggered by this relatively new technique and its relationship to the short-term morbidity[21, 365] and mortality[17, 363] advantages it confers compared with open repair. Investigations into the immuno-inflammatory response to EVAR compared with open AAA repair have generated sometimes conflicting evidence, whilst few reports comparing the neuroendocrine response to the alternative methods of repair have been generated.

1.8.1 The Immuno-inflammatory Response

A compelling rationale underlies the suggestion that given the clear differences in operative technique between EVAR, advocated as minimally invasive, and open AAA repair, the magnitude or nature of the immuno-inflammatory responses they provoke must also differ. EVAR avoids several pro-inflammatory stimuli necessitated by open transperitoneal AAA repair, namely a large abdominal incision,

mobilization of abdominal viscera, retroperitoneal dissection[366] and notably aortic and iliac-cross clamping with the attendant bowel IRI it provokes[367], although the lower limbs may still be subject to short periods of ischaemia[368]. Whilst the notion that EVAR may therefore involve an attenuated systemic inflammatory response is intuitive and supported by some research[128], evidence to the contrary has also been presented. Inconsistencies between current findings, and the limited range of cytokines examined, indicates that further comparison is warranted.

Several studies examining cytokine responses to EVAR and open AAA repair have provided evidence to support the intuitive hypothesis that open AAA repair provokes a greater pro-inflammatory response. Elmarasy *et al.*[367] noted that the generation of IL-6 was significantly greater during open repair compared with EVAR from two to twenty-four hours post-reperfusion, but no statistical difference was observed between assays at subsequent time points. Similarly, significantly greater mean levels of TNF-α were detected during conventional repair from four to twelve hours post-reperfusion. Boyle *et al.*[366] reported the similar finding of higher median plasma IL-6 levels in association with open repair, reaching statistical significance on the first and second post-operative days. Median plasma levels of TNF-α were also found to be higher in the open cohort compared to those undergoing EVAR, reaching significance at six time points between the start of the procedure and the third post-operative day. IL-1β was also assayed but did not differ between the two methods of AAA repair. Odegard *et al.*[369] also examined IL-6 and TNF-α responses to both methods of repair until the first post-operative morning, however employed a less direct method of comparison. These authors compared the maximal increase in cytokine levels generated by the two alternative methods of repair and noted that the

maximal increase in IL-6 generated by open repair was significantly greater than during EVAR, however no significant fluctuations in TNF-α were associated were either procedure. Swartbol *et al.* [370] also identified significantly higher levels of IL-6 during open AAA repair in contrast to TNF-α levels which were greater in those undergoing EVAR, the latter finding being attributed to the intra-operative hypotension affecting this cohort.

Consistent with the findings of these studies, Rowlands *et al.*[368] reported significantly higher levels of IL-6 resulting from open AAA repair compared with EVAR, the difference between groups being noted at all time points and persisting until 144 hours post-clamp release. These authors also examined IL-8 levels, and whilst the statistical comparison between groups is not clearly reported, graphical representations of the data suggest no obvious difference between groups. Levels of IL-10 generated by the alternative methods of repair were not significantly different. The finding of a significantly higher peak IL-6 level associated with open repair compared with EVAR, reported by Bolke *et al.* [371] , is also consistent with the aforementioned studies. A study by Sweeney et al. [128] is hampered somewhat by a small sample size but is the only report to have used the SIRS classification[100] to compare the inflammatory response to EVAR and open AAA repair. These authors describe a lower incidence of SIRS resulting from EVAR but this apparent difference failed to reach statistical significance.

Using a study design and cohort sizes comparable with others investigating the inflammatory response to the alternative methods of AAA repair, Morikage *et al.* [372] have documented findings which differ somewhat from their peers. Mean plasma IL-

6 levels in the EVAR cohort tended to be greater than those of the open cohort at all time points until the sixth post-operative day, reaching significance on the first post-operative day, despite the cohorts being well matched for co-morbidities, experiencing statistically comparable operative times, and a lower mean blood loss experienced by the EVAR cohort. The finding that IL-8 levels did not differ between the two methods of repair is, however, consistent with other research[368] . The comparison of the inflammatory response to the alternative methods of repair reported by Galle *et al.* [290] , demonstrated a tendency toward higher mean TNF-α and IL-8 levels in the EVAR cohort, however the differences were not statistically significant. IL-6 levels did not differ between the EVAR and open methods of repair. It is notable that the cohort sizes involved in this study are somewhat smaller than those described by other research groups.

1.8.2 The Neuroendocrine Response

Prior to publication of data derived, in part, from the current study[373] only two preceding studies have contrasted the neuroendocrine response experienced as a result of EVAR with that associated with the conventional open approach. Thompson *et al.* [374] examined plasma catecholamine levels during each method of repair, and noted a greater adrenaline response to open AAA repair until the final sampling time, thirty minutes following aortic clamp release or aortic balloon deflation. Mean plasma noradrenaline levels also tended to be higher in the open cohort but this failed to reach statistical significance. In a study involving relatively small patient cohorts, Salartash *et al.*[375] also compared serum catecholamine levels in addition to cortisol and several other measures of the neuro-endocrine response to surgical stress during the first twenty-four hours from the commencement of surgery. Consistent with the

findings of Thompson *et al.* [374] , these authors reported a greater increase in adrenaline levels during open repair compared with EVAR, the difference reaching statistical significance six, eighteen, and twenty-four hours after the baseline pre-operative sample. No statistical difference in noradrenaline levels were detected between patient groups. Cortisol levels were elevated compared with baseline in both groups, but the rise from baseline was significantly greater in the open cohort at only one sampling time, six hours after surgery commenced. Indeed, twenty-four hours after surgery commenced the increase from baseline in the EVAR group appeared to exceed that of the open cohort but this was not statistically significant. In view of the limited nature of the available evidence, further data comparing the neuroendocrine response, particularly cortisol, to the alternative methods of AAA repair is warranted.

1.9 Aims of Current Study

The principle aims of the current study were:

1. To identify early predictors of adverse events, in particular SIRS and sepsis following major vascular surgery by examining the relationship of the following variables with measures of clinical outcomes including, where relevant, SIRS, sepsis and infection, in addition to several measures of general post-operative morbidity:

   - markers of immunological function, namely:
     - pre-operative levels of expression of PMN β_2-integrin, CD11b, and immunoglobulin G Fc receptors, FcγRI (CD64) and FcγRIIIb (CD16b);
     - genotypes of the polymorphic immunoglobulin G Fc receptors, FcγRIIa (CD32), and FcγRIIIb (CD16b);
     - pre-, intra- and early post-operative levels of cytokines TNF-α, IL-1β, IL-6, IL-8 (CXLC8), IL-10 and IL-12p70.
   - markers of neuroendocrine function, namely urinary free cortisol, urinary adrenaline and noradrenaline, assayed peri-operatively.
   - pre-operative nutritional status.
   - presence or absence of pre-operative depression.

2. To examine a potential relationship between pre-operative psychological variables, namely depression and anxiety, and neuroendocrine responses to major vascular surgery.

3. To identify potential differences in neuroendocrine and immunological responses to open AAA repair compared with EVAR, as possible biological mechanisms underlying observed clinical outcomes.

CHAPTER 2

METHODS

2.1 Approval and Informed Consent

The protocol employed in this prospective cohort study was reviewed, approved and monitored by the North Western Adelaide Health Service Ethics of Human Research Committee and the Royal Adelaide Hospital Research Ethics Committee, whose deliberations are guided by the Declaration of Helsinki of 1975, as revised in 1983, and the National Health and Medical Research Council Guidelines on Human Experimentation. Written informed consent to participate in the study was obtained from all subjects, or their next of kin, at the time of recruitment.

2.2 Patients

2.2.1 Inclusion Criteria

Consecutive patients scheduled to undergo elective open aortic aneurysm repair or EVAR by The Queen Elizabeth and Royal Adelaide Hospital Vascular Surgery Units, Adelaide, South Australia, and those scheduled to undergo a major lower limb revascularization procedure at the Queen Elizabeth Hospital between 31st March 2004 and 29th April 2005 were considered for recruitment to this study, having been identified as surgical candidates by a vascular surgeon. Allocation of aortic aneurysm patients to the alternative open or endovascular methods of repair did not, therefore, involve a process of randomisation but was determined by the surgical team prior to study entry. Anatomical suitability for EVAR was a principal determinant of the chosen method of repair, however surgeon and patient preference were also considerations. All study participants underwent pre-operative assessment by a consultant anaesthetist and, in many cases, a general physician or cardiologist, who identified patients as medically fit to proceed with surgery, with or without pre-operative optimisation of co-morbid conditions. There were neither age nor gender specifications for involvement in the study.

2.2.2 Exclusion Criteria

Patients unwilling or unable to give informed consent to participation in the study, including those unable to communicate in English without the assistance of an interpreter, were excluded from involvement. The occurrence of major surgery of any kind, or a minor vascular intervention considered to be associated with IRI, within three months preceding the proposed procedure was a criterion for exclusion

from enrolment in the study. Patients taking immunosuppressive medication, with the exception of corticosteroids, were also excluded.

Due to the frequency of ulceration and gangrene in patients with critically ischaemic lower limbs, who constitute a large proportion of those undergoing revascularization procedures, neither infection nor inflammation *per se*, could be considered as criteria for exclusion. Fulfilment of the criteria for the diagnosis of SIRS (Table 1) or sepsis (Table 2) between the time of recruitment and operative intervention was, however, grounds for exclusion.

Criteria were specified which precluded the measurement or analysis of particular patient measures but not of overall study involvement. These are described in the relevant sections to follow.

2.2.3 Withdrawal Criteria

Major surgical intervention within the study period, subsequent to the principal vascular repair, was established as a protocol violation warranting the withdrawal of such patients from further participation in the study and exclusion of their data from analysis. Subject non-compliance with the study protocol and patient request were also established as grounds for withdrawal from the study.

2.2.4 Open Aortic Aneurysm Repair ('Open') Cohort

2.2.4.1 Inclusions and Exclusions

Retrospective audit of computerised theatre logs (HASS-OT) identified 68 patients who underwent elective open aneurysm repair by the aforementioned vascular units during the specified time period. Of these, 17 (25.0%) were unable to be accessed in sufficient time to offer participation in the study. Of the remaining 51 accessible patients, 15 (22.1%) fulfilled at least one of the exclusion criteria and were therefore either not enrolled or withdrawn from study involvement. Nine (13.2%) of these patients declined the invitation to participate, two (2.9%) patients had experienced major surgery within three months of the proposed date for aneurysm repair, one (1.5%) patient was non-English speaking, one (1.5%) was receiving ongoing immunosuppressive medication for a renal transplant and a further patient (1.5%) was excluded from consideration due to evidence of pre-operative sepsis associated with an existing graft infection. All data pertaining to one (1.5%) recruited patient was excluded from analysis due to an unplanned major operative intervention (partial T9, T10-L1 laminectomy and evacuation of spinal haematoma), necessitated by a complication of epidural insertion, on post-operative day two. The remaining 36 (52.9%) patients constitute the 'open' cohort considered in this study.

2.2.4.2 Patient Characteristics and Aneurysm Morphology

All patients' demographic details and relevant aspects of their medical histories were documented following consent to study involvement and were complemented by current information obtained from their medical records. Burden of comorbidity was recorded using the American Society of Anesthesiologists (ASA) Physical Status

Classification[376] in addition to the Charlson Index,[377] a measure whose concurrent, predictive and construct validity has been established amongst surgical and medical populations as has it's reliability[378] . This index is comprised of 19 weighted conditions, and yields a score generated by summing the weights of those conditions manifested by the individual (Appendix 1, Table 1). The presence and severity of specific comorbid conditions considered most relevant to vascular surgery outcomes, namely diabetes, hypertension, hyperlipidaemia, cardiac, renal, pulmonary and carotid disease, in addition to tobacco use, was documented according to the grading system recommended by the Joint Council of The Society for Vascular Surgery and the North American Chapter of the International Society for Cardiovascular Surgery (SVS/ISCVS)[44] (Appendix 1, Table 2). Aneurysm morphology was determined from pre-operative computerised tomography (CT) imaging.

Thirty-six patients (5 female, 31 male) with a median age (range) of 72 (52 - 81) years constituted this cohort. The demographic and clinical profile of this cohort, including presence of specific comorbid conditions and use of β-adrenoreceptor antagonists (β-blockers), is reported in Table 6. Thirty-four of these patients underwent repair of an AAA. The aortic aneurysms of the remaining two patients in this cohort were thoraco-abdominal (TAAA), the repair of which was planned as endovascular in one case and a hybrid open and endovascular approach in the other. The intra-operative conversion of both cases to an open approach by laparotomy necessitated these patients being classified in the 'open' cohort for comparability of outcome measures. The details of aneurysm morphology, including anatomic site and maximum aneurysm diameter, characterising both the 'open' and the EVAR cohorts, are presented in Table 7.

Table 6. Demographic and clinical characteristics of the 'open' aneurysm repair, EVAR and 'lower limb' revascularisation cohorts.

EVAR, endovascular aneurysm repair; NS, not significant; ASA, American Society of Anaesthesiologists' physical status classification.

Values are expressed as absolute numbers and percentages in parentheses unless otherwise indicated.

†Values expressed are median with range in parentheses; *p* values obtained by pair-wise post hoc analyses using Mann-Whitney U tests following demonstration of statistically significant difference between the three cohorts by Kruskal-Wallis test.

‡Refer to Appendix 1, Table 1 for full details of Charlson Index of Comorbidity scoring system.

§Values are absolute numbers, with percentage in parentheses, of those with the co-morbid or clinical condition. Following initial scoring using the Joint Council of The Society for Vascular Surgery and the North American Chapter of the International Society for Cardiovascular Surgery (SVS/ISVS) grading system for conditions that may modify surgical outcomes,[44] (Appendix 1, Table 2), grades 1, 2 and 3 were then collapsed into one category to indicate the presence of the comorbid condition, whilst grade 0 constituted the category indicative of the absence of the co-morbidity.

¶ Severity did not exceed grade 1 for any subject.

†† Statistical comparison between group values performed using Pearson's chi-square test (2 x 3 or 3 x 3 contingency tables as appropriate).

	Open Aneurysm Repair (*n*=36)	EVAR (*n*=17)	Lower Limb Revascularisation (*n*=17)	*p* value		
				Open vs EVAR	Open vs Lower Limb	EVAR vs Lower Limb
Demographics						
Age (years)[†]	72 (52 - 81)	78 (61 - 86)	62 (41 - 84)	0.004	NS	0.031
Gender (F:M)	5:31	0:17	4:13		NS[††]	
Country of birth						
Australia	20 (55.6%)	10 (58.8%)	12 (70.6%)			
United Kingdom	12 (33.3%)	2 (11.8%)	2 (11.8%)			
Italy	1 (2.8%)	2 (11.8%)	0 (0.0%)			
Greece	1 (2.8%)	0 (0.0%)	0 (0.0%)			
Germany	1 (2.8%)	0 (0.0%)	2 (11.8%)			
Holland	1 (2.8%)	1 (5.9%)	0 (0.0%)			
Poland	0 (0.0%)	1 (5.9%)	1 (5.9%)			
Ukraine	0 (0.0%)	1 (5.9%)	0 (0.0%)			
Marital status						
Married/Defacto	29 (80.6%)	11 (64.7%)	11 (64.7%)			
Separated/Single/ Divorced/Widow	7 (19.4%)	6 (35.3%)	6 (35.3%)			
Living arrangements						
Accompanied	29 (80.6%)	11 (64.7%)	15 (88.2%)			
Alone	7 (19.4%)	6 (35.3%)	2 (11.8%)			
Clinical Characteristics						
ASA						
II	9 (25.0%)	3 (17.6%)	3 (17.6%)			
III	22 (61.1%)	13 (76.5%)	3 (17.6%)		NS[††]	
IV	5 (13.9%)	1 (5.9%)	1 (5.9%)			
Charlson Index score[†‡]	2 (0 - 4)	1 (0 - 3)	3 (1 - 6)	NS	0.012	0.001
Diabetes[§]	6 (16.7%)	3 (17.6%)	9 (52.9%)		0.013[††]	
Hypertension[§]	23 (63.9%)	9 (52.9%)	7 (41.2%)		NS[††]	
Hyperlipidaemia[§]	31 (86.1%)	11 (64.7%)	11 (64.7%)		NS[††]	
Cardiac disease[§]	20 (55.6%)	8 (47.1%)	7 (41.2%)		NS[††]	
Pulmonary disease[§]	18 (50.0%)	10 (58.8%)	5 (29.4%)		NS[††]	
Renal disease[§¶]	11 (30.6%)	2 (11.8%)	2 (11.8%)		NS[††]	
Carotid disease[§]	1 (2.8%)	2 (11.8%)	1 (5.9%)		NS[††]	
Tobacco use[§]	21 (58.3%)	4 (23.5%)	10 (58.8%)		0.043[††]	
Current malignancy	2 (5.6%)	2 (11.8%)	0 (0%)			
β-blocker administration						
Pre-operatively	27 (75.0%)	11 (64.7%)	3 (17.6%)			
Pre-, intra-, or post-operatively	31 (86.1%)	11 (64.7%)	7 (41.2%)		0.003[††]	

Table 7. Aneurysm features and selected operative details for 'open' aneurysm repair and EVAR cohorts.

N/A, not applicable; IIA, internal iliac artery; CIA, common iliac artery; SFA, superficial femoral artery; SMA, superior mesenteric artery; IMA, inferior mesenteric artery; POD 3, post-operative day three.

Values are expressed as absolute numbers with percentages in parentheses, unless otherwise indicated.

†Values expressed as median (range)

‡Excluding thoracoabdominal aneurysm repairs, hence n=34

§ Defined according to Joint Council of The Society for Vascular Surgery and the North American Chapter of the International Society for Cardiovascular Surgery (SVS/ISVS) Ad Hoc Committee on Reporting Standards recommendations.[379]

*Significant difference versus EVAR, p = 0.014 (Mann-Whitney U test)

	Open Aneurysm Repair (n=36)		EVAR (n=17)	
Aneurysm Features				
Anatomic site	Infrarenal Juxtarenal Thoracoabdominal	28 (77.8%) 6 (16.7%) 2 (5.6%)	Infrarenal	17 (100%)
Maximum diameter (cm)[†]	6.0 (4.7 - 10.1)[*]		5.5 (4.3 - 6.5)	
Operative Details				
Operative access	Midline laparotomy Transverse laparotomy	29 (80.6%) 7 (19.4%)	Bilateral groin incisions Percutaneous	16 (94.1%) 1 (5.9%)
Site of proximal arterial control[‡]	Infrarenal Suprarenal	25 (73.5%) 9 (26.5%)	N/A	
Other procedures[§]:				
Adjunctive vascular	Graft limb to left IIA bypass & oversew right IIA	1 (2.8%)	Failed embolisation accessory renal artery (POD 3)	1 (5.9%)
	Subclavian to aortic graft bypass & endograft to aortic bifurcation bypass	1 (2.8%)	Angioplasty & stent insertion	1 (5.9%)
	Oversewing CIA	1 (2.8%)	Coiling IIA	1 (5.9%)
	SFA embolectomy	1 (2.8%)		
	Aorta to SMA bypass	1 (2.8%)		
	Re-implanatation IMA	1 (2.8%)		
Concomitant non-vascular	Ureteric stent insertion	1 (2.8%)	N/A	
	Adhesiolysis	2 (5.6%)		
	Colonic polypectomy	1 (2.8%)		
	Excision Meckel's diverticulum	1 (2.8%)		

2.2.4.3 Anaesthetic Management

Whilst formal standardisation of anaesthesia was not able to be instituted for the purposes of this study, standard regional and institutional practises resulted in considerable homogeneity in the anaesthetic management of study participants. All open aneurysm repairs were performed under a general anaesthetic (GA). Prior to the induction of anaesthesia, epidural catheter insertion was attempted in 27 patients for the purpose of intra-operative and/or post-operative analgesia. A test dose of ropivacaine or bupivacaine in adrenaline was administered in the 26 patients in whom successful insertion occurred. Induction was achieved with propofol and/or midazolam with or without the use of fentanyl/remifentanil/alfentanil. Vecuronium, rocuronium or atracurium was administered to facilitate tracheal intubation. Maintenance of anaesthesia was with inhalational agents in all but three cases, using isoflurane/sevoflurane/desflurane in an oxygen/air mixture, epidural administration of a fentanyl and ropivacaine/bupivacaine cocktail in 18 patients and/or intravenous fentanyl/remifentanil/alfentanil. In the remaining three patients, total intravenous anaesthesia (TIVA) was employed for maintenance, using propofol in combination with remifentanil in two cases, and with an epidural cocktail in another. Additional boluses of a nondepolarizing neuromuscular blocking agent were administered to all patients as required for maintenance of muscle relaxation. Ventilation was adjusted to maintain normocarbia. Of the 35 patients who survived beyond the completion of surgery, 12 (34.3%) were considered suitable for extubation at the conclusion of surgery following reversal of residual relaxants with neostigmine and glycopyrrolate, the cessation of inhalational or intravenous anaesthetic agents and the restoration of spontaneous ventilation. Artificial ventilation was continued in the ICU in the remaining 23 (65.7%) patients.

Ethical principles and practicality prevented standardisation of methods used to maintain intra- or post-operative homeostasis during this study. The methods used intra-operatively and in the ICU; including the administration of crystalloid/colloids, blood products and pharmacotherapy; were therefore at the discretion of the anaesthetist or intensivists respectively, but were prospectively documented. Intra-operative pharmacotherapy to maintain haemodynamic variables within acceptable limits involved the use of noncatecholamine sympathomimetic agents, including phenylephrine, ephedrine and metaraminol, and in some cases the catecholamines adrenaline and noradrenaline for management of hypotension. ICU management of hypotension also involved the use of adrenaline and noradrenaline in addition to dobutamine. Agents used for management of intra-operative hypertension were nitroglycerine, esmolol and metoprolol.

2.2.4.4 Operative Management

Prophylactic antibiotics administered at anaesthetic induction consisted of a first generation cephalosporin +/- gentamicin +/- metronidazole, according to surgeon preference. Further intra-operative doses were administered according to operative duration. All operative procedures considered in this study were undertaken by 16 principal surgeons (nine vascular consultants, three fellows and four trainees). All surgeons operating on those 34 patients in the 'open' cohort with an AAA employed a standard transperitoneal method of repair. The abdominal cavity was accessed via a midline incision in 29 (80.6%) of the 36 patients in the 'open' cohort, and via a transverse incision in the remaining 7 (19.4%) patients. The sites of proximal arterial control in the AAA patients are reported in Table 7. Prior to initial aortic clamping 2500 to 5000 units of unfractionated heparin were administered intravenously (IV)

with repeated boluses of 1000 to 5000 units administered as required. Intravenous protamine was administered prior to closure for reversal of excessive anti-coagulation as indicated by the activated partial thromboplastin time (aPTT). Mannitol was infused in 11 (30.6%) members of this cohort prior to aortic clamping. Twenty-six of the grafts inserted in the 34 AAA patients were gelatin coated Dacron grafts (Gelsoft Plus®, Sulzer Vascutek Ltd., Renfrewshire, Scotland, UK) and eight were polytetrafluoroethylene (PTFE) grafts (Advanta®, Atrium, Hudson, New Hampshire, USA; and GORE-TEX®, W.L. Gore & Associates Inc, Flagstaff, Arizona, USA). Thirteen tube and 21 bifurcated grafts were used in these patients.

The planned endovascular TAAA repair involved insertion of a customised fenestrated thoracic endograft (Cook, Brisbane, Queensland, Australia). This procedure was complicated by the occlusion of the coeliac and superior mesenteric arteries (SMA) by the fenestrated graft, which could not be overcome by endovascular graft manipulation, necessitating a laparotomy to enable an adjunctive aortic-SMA bypass. The planned hybrid open and endovascular approach involved the endovascular deployment of TAG® Thoracic Endoprosthesis in combination with an endograft to aortic bifurcation bypass via an open approach using a GORE-TEX® straight Vascular Graft (both W.L. Gore & Associates Inc, Flagstaff, Arizona, USA) as the principle procedure. This was facilitated by an adjunctive temporary aortic bypass procedure comprised by a coeliac trunk and left renal artery to right common and left external iliac artery bypass using a bifurcated graft, in association with a left subclavian to right limb of bifurcated graft bypass (GORE-TEX® Vascular Grafts, W.L. Gore & Associates Inc, Flagstaff, Arizona, USA).

In the process of these 36 aneurysm repairs, an adjunctive vascular procedure, defined by the SVS/ISCVS Ad Hoc Committee on Reporting Standards as one that aims to augment the principal repair,[379] occurred in six cases. Whilst there were no cases requiring an ancillary vascular procedure, defined as one that does not contribute to the effects of the vascular repair,[379] five cases involved concomitant non-vascular procedures. These additional procedures are detailed in Table 7.

2.2.4.5 Post-operative Management

Those 35 patients who survived the immediate operative period were either transferred directly to the ICU (27 patients, 77.1%) or to a high-dependency unit (HDU) following a period in the surgical recovery room (8 patients, 22.9%). All patients progressed through the HDU prior to being transferred to the vascular surgery ward for ongoing management as their clinical condition improved. Post-operative antibiotic prophylaxis, consisting of a first or third generation cephalosporin, was administered to all patients. Subsequent antibiotic regimens were administered according to the presence of documented or suspected infection. Post-operative analgesia was optimised in all cases, the initial regimen determined by the Acute Pain Service. Twenty-four (70.6%) of the 34 subjects who survived beyond the early post-operative period were initially administered post-operative analgesia via the epidural route, in the form of a fentanyl and bupivacaine/ropivacaine cocktail. The remaining patients were initially administered intravenous opioid analgesia. During the patients' subsequent post-operative course, these regimens were substituted with subcutaneous and oral analgesia. Prophylactic doses of low molecular weight or unfractionated heparin were administered subcutaneously during the post-operative course.

2.2.5 Endovascular Aortic Aneurysm Repair (EVAR) Cohort

2.2.5.1 Inclusions and Exclusions

Retrospective audit identified 27 patients who underwent EVAR as an elective procedure by the specified vascular units during the period of patient recruitment. Two (7.4%) of these 27 patients were inaccessible, preventing an invitation to participate being made, whilst a further four patients (14.8%) declined such an invitation. Two patients (7.4%) were excluded on the basis of an inability to obtain informed consent as a result of being non-English speaking and cognitively impaired, respectively. An additional two patients (7.4%) were excluded, having undergone a major operative procedure and a more minor vascular procedure known to induce IRI respectively, within the three months preceding the proposed EVAR. The remaining 17 patients (63.0%) constitute the EVAR cohort considered in this study.

2.2.5.2 Patient Characteristics and Aneurysm Morphology

Documentation of demographics, clinical details and aneurysm morphology for the EVAR patients involved in this study was undertaken in the same manner as described for the 'open' cohort. Seventeen patients (0 female, 17 male) with a median age (range) of 78 (61 - 86) years constituted this cohort. The demographic and clinical profile of these patients is summarised in Table 6. The anatomical site of aneurysms amongst the EVAR cohort was infrarenal in all cases (Table 7).

2.2.5.3 Anaesthetic Management

Epidural insertion was not considered necessary in any patient undergoing this procedure. As with the 'open' cohort, all EVARs were performed under a GA.

Induction was similarly achieved with propofol and/or midazolam with or without fentanyl/remifentanil administration. Tracheal intubation was then facilitated using non-depolarizing neuromuscular blocking agents as for the 'open' procedures. Inhalational anaesthesia, as previously described but without the addition of epidurals, was employed for maintenance of anaesthesia in all but two cases. The latter two patients received TIVA comprised of propofol and remifentanil. Maintenance of muscle relaxation and acceptable ventilatory parameters were achieved as described for the 'open cohort'. Only two (11.8%) patients in this cohort required mechanical ventilation beyond the conclusion of surgery, whilst the remaining 15 (88.2%) were able to be extubated immediately following surgery. As for the 'open' cohort, agents used to manage intra-operative hypotension, unresponsive to fluid administration, were the noncatecholamine sympathomimetics phenylephrine, ephedrine and metaraminol, and in some cases, the catecholamine adrenaline was employed for this purpose.

2.2.5.4 Operative Management

Operative antibiotic prophylaxis in this patient group was the same as that described for the 'open' cohort. EVAR procedures were undertaken by a principal surgeon in association with an interventional radiologist. Vascular access was obtained via bilateral transverse or longitudinal groin incisions in 16 (94.1%) cases permitting exposure of the common femoral arteries (CFAs) through which endoluminal stents and their delivery system were subsequently introduced. Percutaneous vascular access was achieved in one (5.9%) patient. Two thousand five hundred to 5000 units of unfractionated heparin were administered IV prior to graft deployment with additional boluses administered as required. Eleven of the grafts used in this patient

cohort were Zenith® AAA Endovascular Grafts (Cook, Bloomington, Indiana, USA), four were Excluder® Bifurcated Endoprostheses (W.L. Gore & Associates Inc, Flagstaff, Arizona, USA) and two were Talent® Stent Grafts (Medtronic AVE, Santa Rosa, California, USA). Adjunctive vascular procedures were required in three cases as outlined in Table 7.

2.2.5.5 Post-operative Management

Only one (5.9%) patient in this cohort required direct transfer to the ICU for post-operative management. The remaining 16 (94.1%) patients were managed in the HDU or vascular ward according to their clinical condition following a period in the surgical recovery room. Post-operative antibiotic prophylaxis consisted of a first generation cephalosporin. Additional or subsequent antibiotics were administered according to the presence of documented or suspected infection. Early post-operative analgesia, when required, consisted of IV or subcutaneous opiates. Such regimens were rapidly replaced with oral opiate and/or non-opiate analgesics. Post-operative prophylactic anticoagulants were administered as in the 'open' cohort. One patient received heparin in therapeutic doses during the post-operative period prior to re-introduction of warfarin.

2.2.6 Lower Limb Revascularisation ('Lower Limb') Cohort

2.2.6.1 Inclusions and Exclusions

Retrospective audit identified 47 patients who underwent a major operative lower limb revascularisation by the Queen Elizabeth Hospital Vascular Unit during the specified time period. Twenty-three (49.0%) of these could not be accessed in

sufficient time to offer participation and completion of pre-operative study requirements. Five (10.6%) patients declined the invitation to participate, whilst a further two (4.3%) were non-English speaking and were therefore excluded from further consideration. The remaining 17 (36.2%) patients constitute the 'lower limb' cohort considered in this study.

2.2.6.2 Patient Characteristics and Lower Limb Pathology

Seventeen patients (4 female, 13 male) with a median age (range) of 62 (41 - 84) years constituted this cohort. A profile of their demographic characteristics and co-morbid conditions is presented in Table 6. The indication for operative intervention in all cases was chronic arterial occlusive disease resulting in symptomatic lower limb ischaemia, the severity of which was graded according to SVS/ISCVS recommendations for standardized reporting practices[44] and is reported in Table 8. The underlying pathology in all but one case was atherosclerotic arterial disease, the exception being one case of adventitial cystic disease.

2.2.6.3 Anaesthetic Management

Fifteen (88.2%) of the 17 cases were performed under GA, preceded by epidural catheter insertion on one patient. The method of anaesthetic induction and tracheal intubation was identical to that previously described for the EVAR cohort. Maintenance of anaesthesia was with inhalational agents in 11 patients, as described for the two preceding cohorts, with the adjunctive use of epidural anaesthesia in the one, previously mentioned, case. TIVA, using propofol with or without remifentanil was employed for anaesthetic maintenance in four cases. Only one (5.9%) patient required mechanical ventilation beyond the immediate post-operative period. The

remainder of those who received a GA were extubated at the completion of the operation. Two (11.8%) patients in this cohort underwent the operative procedure with regional anaesthesia, namely spinal anaesthesia consisting of a bupivacaine/fentanyl cocktail, combined with IV propofol sedation.

Methods of managing abnormal intra-operative haemodynamic parameters were highly comparable to those employed for the two cohorts previously discussed. Pharmacotherapy for hypotension included the sympathomimetics phenylephrine, ephedrine, metaraminol and, in one case, the catecholamine noradrenaline. Hypertension and/or tachycardia were managed with IV administration of the β-blockers metoprolol or atenolol. Atropine was employed for the management of intra-operative bradycardia in one case.

2.2.6.4 Pre-operative and Operative Management

Five patients in this cohort received oral or IV antibiotics for a period preceding operative intervention. Intra-operative antibiotic administration, guided when relevant by pre-operative regimens, consisted of either a first generation cephalosporin, a penicillin, quinolone, or glycopeptide +/- gentamicin +/- metronidazole. The operative lower extremity revascularisations performed on the 17 patients in this cohort were categorized according to SVS/ISCVS reporting standards[44] and are reported in Table 8. Standard operative techniques were employed when performing the revascularisation procedures. Prior to vascular clamping, 3000 to 5000 units of unfractionated heparin were administered IV and repeated boluses of 1000 units administered as indicated. Prophylactic mannitol was infused in one patient. Eleven (64.7%) of the revascularisation procedures involved

the exclusive use of autologous vein grafts. Four of the five (29.4%) cases using synthetic grafts exclusively, involved a PTFE graft (Unity Construction®, Sulzer Vascutek Ltd., Renfrewshire, Scotland, UK; GORE-TEX Propaten®, W.L. Gore & Associates Inc, Flagstaff, Arizona, USA; and Advanta®, Atrium, Hudson, New Hampshire, USA) whilst a gelatin coated Dacron graft (Gelsoft Plus®, Sulzer Vascutek Ltd., Renfrewshire, Scotland, UK) was employed in one of these cases. In one (5.9%) case both autologous vein and a synthetic PTFE graft was used to achieve revascularisation. Adjunctive vascular procedures were performed in five cases, whilst three patients required an ancillary vascular procedure at the time of the principle revascularization. These procedures are summarised in Table 8.

2.2.6.5 Post-operative Management

Two (11.8%) of the 17 patients in this cohort were transferred directly to the ICU for post-operative management. The clinical condition of the remaining 15 (88.2%) patients enabled their early post-operative management to occur in the HDU or vascular ward following an initial period in the surgical recovery room. Initial post-operative antibiotic regimens were the same as those employed during the intra-operative period for this cohort. Subsequent regimens were, in some cases, modified according to the presence of documented or suspected infection. Early post-operative analgesia was delivered via the epidural route, in the form of a fentanyl and bupivacaine/ropivacaine cocktail, in one (5.9%) of the 17 patients. The remaining 16 patients (94.1%) were administered IV or subcutaneous opioids or oral analgesia as indicated. Therapeutic doses of heparin were indicated during the post-operative course of four patients (23.5%), whilst the remaining 13 patients (76.5%) received

prophylactic post-operative anticoagulants as described for the preceding two cohorts.

Table 8. Severity of lower limb ischaemia and selected operative details for 'lower limb' revascularisation cohort.

Lower Limb Revascularisation Cohort (n=17)		
Disease Severity		
Chronic limb ischaemia grade†	Grade 0	0 (0%)
	Grade I	6 (35.3%)
	Grade II	4 (23.5%)
	Grade III	7 (41.2%)
Operative Details		
Category of operation	Supra-inguinal revascularisation	2 (11.8%)
	Infra-inguinal revascularisation	15 (88.2%)
Other procedures†:		
Adjunctive vascular	Endarterectomy of CFA/PFA ± SFA± popliteal artery & vein patch	3 (17.6%)
	Aortic endarterectomy	1 (5.9%)
	Re-implantation IMA	1 (5.9%)
Ancillary vascular	Debridement ± split skin graft	2 (11.8%)
	Toe amputation	1 (5.9%)

CFA, common femoral artery; PFA, profunda femoral artery; SFA, superficial femoral artery; IMA, inferior mesenteric artery.

Values are expressed as absolute numbers with percentages in parentheses.

†Categorized according to the Joint Council of The Society for Vascular Surgery and the North American Chapter of the International Society for Cardiovascular Surgery (SVS/ISVS) recommendations for standardised reporting practices.[44, 379]

2.3 Documentation of Operative and Post-operative Clinical Variables

2.3.1 Operative Duration and Duration of Ischaemia

For each subject, operative duration (in minutes) was recorded for the purpose of identifying an association with specific outcome variables. Similarly, the duration of intra-operative ischaemia (in minutes) was documented for the purpose of identifying whether this variable was associated with specific outcomes in the current study. Duration of ischaemia, without reperfusion, was thus determined from operative records and defined as the period commencing from initial occlusion of aortic or peripheral arterial flow until the first occasion of reperfusing the first lower limb for the open AAA and lower limb cohorts. This period was not considered applicable to the EVAR procedure.

2.3.2 Other Clinical Variables

In addition to the variables referred to previously, namely anaesthetic type, categorised as GA or spinal with sedation, and principle route of post-operative analgesia, categorised as epidural or other, other intra- and post-operative variables were prospectively documented for the purpose of description and comparison of cohorts. Documented variables included the estimated intra-operative blood loss (in millilitres, ml); total volume of blood transfused intra-operatively (autologous, donor packed red cells and/or cell-saved blood, in ml); total volume of blood transfused post-operatively (autologous, donor packed red cells and/or cell-saved blood, in ml); and total volume of crystalloid and/or colloid (in ml) administered intra-operatively. Duration of post-operative mechanical ventilation (in minutes) was also recorded.

2.4 Blood Sample Collection and Storage Protocol

Blood samples were drawn from subjects for the purpose of pre-operative measurement of PMN integrin and immunoglobulin receptor expression and genotyping, and for plasma cytokine assays and standard haematology and biochemistry analyses at pre-, intra- and post-operative time points.

A pre-operative peripheral venous blood sample was drawn from all subjects within seven days, and in most cases within 24 hours, of their proposed procedure and immediately aliquoted into two pre-chilled 4ml and 10ml collection tubes on ice and transported to the laboratory. The 4ml collection tube was then stored for a maximum period of 24 hours, at 4°C, until deoxyribonucleic acid (DNA) extraction was performed, typically within two hours of sample collection. Blood contained within the 10 ml tube was used for determination of PMN integrin and immunoglobulin expression using the methodology described below. Plasma was obtained within two hours of venipuncture from the residual blood as described below. An intra-operative blood sample was drawn into 10ml collection tubes from subjects in the 'open' and 'lower limb' cohorts immediately prior to the first occasion of reperfusion of the first lower limb, or immediately prior to endovascular stent deployment for those in the EVAR cohort. These operative stages were considered to most accurately demarcate a time when maximal ischaemia, without reperfusion, had been induced by the three respective operative procedures, and were therefore considered as a comparable sampling time, designated as T_0. Blood drawn at this, and subsequent time points, was kept at room temperature for a maximum of two hours, until plasma was separated from the sample. Subsequent samples were similarly drawn from all patients at 4, 24, 72 and 120 hours following T_0, unless a

subject was discharged prior to the sampling time or refused the intervention. These time points are henceforth designated $T_0 + 4$, $T_0 + 24$, $T_0 + 72$, and $T_0 + 120$ hours, respectively.

All of the aforementioned samples were drawn into collection tubes (Vacuette®, greiner bio-one GmbH, Kremsmünster, Austria) coated with K-ethylenediamine tetra-acetic acid (K-EDTA). The techniques used for sampling of blood at the intra- and post-operative time points were dependent on available vascular access. When *in situ*, upper limb arterial lines were used as the primary source for blood samples, whilst existing central venous lines were a secondary source of blood samples at these time points. On the occasions when blood was drawn through existing vascular access, the initial 3-5ml of blood sampled was discarded in accordance with standard practice in order to avoid potential sample contamination, prior to collection of blood for analysis. Peripheral venipuncture was undertaken when no alternative vascular access was available for blood sampling. Plasma was obtained from blood at all time points by centrifugation (Megafuge 1.0R, Heraeus Instruments, Germany) at room temperature (22°C) for 10 minutes at 2500g. Separated plasma was then pipetted in 0.5ml aliquots into 1.5ml polypropylene microtubes (Sarstedt, Nümbrecht, Germany) and immediately placed into storage at -80°C until batch analysis.

Assessment of the post-operative outcome measures, SIRS, sepsis and APACHE II (acute physiology and chronic health evaluation) score, the details of which are described in sections to follow, on each of the first five post-operative days whilst the patient remained hospitalised, required that parameters measured from complete blood examinations and standard biochemical analysis of blood samples, namely

total WBC count, packed cell volume (PCV), serum sodium, potassium and creatinine, be determined on each of these days. These laboratory data were obtained from analyses performed on blood samples drawn on each of post-operative days (POD) 0, defined as zero to 24 hours post skin closure, POD1 (24 to 48 hours post skin closure), POD2 (48 to 72 hours post skin closure), POD3 (72 to 96 hours post skin closure) and POD4 (96 to 120 hours post skin closure), either in the course of routine clinical care or for the exclusive purposes of the current study. In all cases, blood for haematological studies was collected into 4ml K-EDTA tubes (Vacuette®, greiner bio-one GmbH, Kremsmünster, Austria) whilst samples for biochemical analysis were collected into 10ml lithium heparin tubes (Vacuette®, greiner bio-one GmbH, Kremsmünster, Austria). These haematological and biochemical analyses were performed using automated instruments by the Institute of Medical and Veterinary Science (IMVS) (Woodville South and Adelaide laboratories, South Australia).

2.5 Measurement of Pre-operative PMN CD11b, FcγRI (CD64) and FcγRIIIb (CD16b) Expression

2.5.1 Direct Immunofluorescence Staining

Pre-operative peripheral venous blood samples, drawn into pre-chilled 10ml K-EDTA coated collection tubes as previously described, were kept on ice for exactly 45 minutes following venipuncture, at which time direct immunofluorescence staining was commenced using a methodology adapted from guidelines provided by Serotec (Oxford, UK), for use with their directly conjugated monoclonal antibodies.[380] Previous studies in this laboratory have demonstrated a moderate, gradual increase in measures of CD11b expression, and to a lesser extent CD16b expression, over the course of a given sample standing on ice for one hour post venipuncture, after which measured expression declines (data not shown).The aforementioned 45 minute period standing on ice therefore permitted transport of all samples to the laboratory whilst ensuring standardisation of the small, but measurable effect of *ex vivo* time on PMN surface integrin and immunoglobulin levels. At this time point, 100µl aliquots of the subject's well mixed whole blood sample were pipetted into each of four 5ml plastic test tubes (Selby Biolab, Victoria, Australia). A 10 µl aliquot of R. phycoerythrin (RPE)-conjugated purified mouse IgG1 anti-human CD11b (Serotec, Oxford, UK) was then rapidly added to the whole blood contained within the first of the test tubes. Five µl aliquots of Fluorescein Isothiocyanate Isomer 1 (FITC)-conjugated purified mouse IgG1 anti-human CD16 (Serotec, Oxford, UK), and FITC-conjugated purified mouse IgG1 anti-human CD64 (Serotec, Oxford, UK) were added to blood contained in the second and third test tubes respectively. A 5 µl aliquot of FITC- and RPE- conjugated mouse IgG1 isotype

negative control (Serotec, Oxford, UK) was added to the blood sample contained in the fourth test tube for the purpose of identifying 'background' antibody binding. Prior to their initial use, all directly-conjugated antibody reagents were reconstituted or diluted, where necessary, according to the manufacturer's instructions and thoroughly mixed prior to subsequent usage. Optimal volumes of conjugated antibody reagents added to whole blood samples were determined by a series of titrations, guided by the manufacturer's recommendations, previously undertaken in this laboratory (data not shown). The four test tubes were then agitated to ensure thorough mixing of the contained whole blood and directly-conjugated antibody product and subsequently incubated for 30 minutes at room temperature in the dark. Two ml of Erythrolyse Red Blood Cell Lysing Buffer (Serotec, Oxford, UK), diluted according to the manufacturer's instructions prior to use, was added to the whole blood-monoclonal antibody mixture contained within each tube which was then vortexed to achieve thorough mixing of the contents. Lysis of erythrocytes was thus achieved following incubation of the tubes for 10 minutes at room temperature in the dark. A leucocyte pellet was obtained in each respective tube by centrifugation (Megafuge 1.0R, Heraeus Instruments, Germany) at 500g for 5 minutes at room temperature and the supernatant decanted. The pellets were then washed by resuspension in 2ml of phosphate buffered saline (PBS) containing 20mM glucose (Ajax Chemicals, Auburn, NSW, Australia) plus 1% bovine serum albumin (Sigma Chemical Co Ltd., St Louis, Missouri, USA) (PBSG) added to each tube, and the suspensions centrifuged at 500g for 5 minutes at room temperature. The resulting leucocyte pellets were obtained by decanting and discarding the supernatants, and then resuspended in 300µl of 0.5% paraformaldehyde in PBS. Each tube was then

stored for a maximum of 72 hours in the dark at 4°C, until data was acquired from the samples by flow cytometry.

2.5.2 Flow Cytometry

Flow cytometric analysis of antibody-labelled blood samples was performed within 72 hours of immunofluorescence staining, based on previous work in this laboratory which has demonstrated stability of measures of CD11b and CD16b expression when data acquisition occurs within this time period (data not shown). Immunofluorescence studies were performed using a FACScan flow cytometer, (Becton Dickson Immunocytometry Systems, San José, California, USA) supported by an Apple Macintosh computer using CELLQuest acquisition software (Becton Dickson, San José, California, USA), calibrated prior to each episode of analysis with the use of Flow-Set® Flow Cytometry Calibration Beads (Beckman Coulter Inc., Florida, USA) thereby eliminating variable responses by the flow cytometer over time. Forward angle and 90° light scatter measurements were collected and 10 000 fluorescence (FL1 or FL2) events counted on each sample within a gated region for PMNs. The resulting data was analysed using WinMDI 2.8 software (Scripps Research Institute, http://facs.scripps.edu/software.html) employing a density plot to gate the PMN population by virtue of its light scattering properties. From the density plot, the software was used to generate a frequency histogram of fluorescence events for each analysed sample and, by conversion of the log acquired data to a linear scale, an arithmetic mean fluorescence intensity (MFI) was generated, this value being considered a measure of the level of a receptor's expression. The level of background antibody binding was accounted for by subtracting the MFI measured from a subject's negative control sample from each of the three MFI values measured

from that subject's samples labelled with anti-CD11b, anti-CD16 and anti-CD64 antibodies respectively. The MFI values described henceforth refer to values adjusted for background antibody binding. Reproducibility of data obtained by the methods of immunofluorescence staining and flow cytometric analysis described herein has previously been demonstrated in this laboratory (data not shown).

2.6 Determination of FcγRIIa (CD32a) and FcγRIIIb (CD16b) Genotypes

2.6.1 Isolation of Deoxyribonucleic Acid (DNA) From Whole Blood

Genomic DNA was prepared from pre-operative peripheral venous blood drawn into a 4ml K-EDTA collection tube stored at 4°C until DNA isolation was commenced. This occurred within 24 hours, and in most cases within two hours of venipuncture, thus minimising DNA degradation due to apoptosis. Genomic DNA extraction was performed using the commercially available QIAamp® DNA Mini Kit (Qiagen Inc, Valencia, California, USA) according to the manufacturer's Blood and Body Fluid Spin Protocol. This protocol yields purified DNA in 200μl of elution buffer, which was stored at 4°C until batch DNA amplification by polymerase chain reactions (PCR). Prior to PCR, the concentration and purity of DNA isolated from each patient's blood sample was confirmed by spectrophotometry (SmartSpec® 3000, Biorad, Hercules, California, USA).

2.6.2 FcγRIIa (CD32a) Genotyping

FcγRIIa (CD32a) genotyping was performed using an allele-specific PCR based on the method described by Rodríguez *et al.*[381] Two master mixes, designated 'Hmix' and 'Rmix' according to their CD32a H131- or R131-allele specificity respectively, were prepared to each yield a volume of 20μl per sample for amplification. The composition of each master mix is detailed in Table 9. One hundred ng of each patient's DNA, diluted with distilled water when necessary to yield a volume of 5μl, was then combined with 20μl of both the 'Hmix' and 'Rmix'. Negative controls were

included with each episode of PCR amplification, consisting of 20μl of both master mixes each combined with 5μl of distilled water without the addition of DNA, for the purpose of identifying any master mix contamination. The common CD32a antisense primer, 5′ CAA TTT TGC TGC TAT GGG C 3′, used in this methodology is from an intron shared by the sequences for FcγRIIa, FcγRIIb and FcγRIIc [381] . The two human growth hormone primers (5′ HGH and 3′ HGH) included in the master mixes served as internal positive controls, amplifying a 439-bp (base-pair) fragment of the human growth hormone (HGH) gene.[381]

The PCR was performed in a thermal cycler (MyCycler®, Biorad, Hercules, California, USA) using a protocol consisting of one cycle at 95°C for 10 minutes (initial heat activation); 10 cycles of 96° for 1 minute (denaturation), 54°C for 2 minutes (annealing) and 72°C for1 minute (extension); followed by 22 cycles of 95°C for 1 minute, 54°C for 2 minutes, and 72°C for 1 minute, to enhance the sensitivity; and a final extension step at 72°C for 10 minutes. PCR products were then kept at 4°C until gel electrophoresis was performed. Duplicate PCRs were performed and analysed for most samples to increase the certainty of genotyping.

Ethidium bromide-stained 2% agarose gels were produced for each PCR by dissolving 2 grams of DNA grade agarose (Progen Biosciences, Archerfield BC, QLD, Australia) in 100ml of tris-acetate (TAE) [0.04M TAE, 0.001M ethylenediamine tetraacetic acid (EDTA); Sigma Chemical Co Ltd., St Louis, Missouri, USA)] followed by the addition of 10 μl of 10 mg/ml ethidium bromide (Sigma Chemical Co Ltd., St Louis, Missouri, USA). The resulting mixture was subsequently poured and allowed to solidify. The PCR amplification products,

including 'Hmix' and 'Rmix' negative controls, in addition to a 1kb DNA ladder (Invitrogen Australia Pty. Ltd., Mount Waverley, Victoria, Australia) and a pUC19 ladder (Geneworks, Adelaide, Australia), were separated on the ethidium bromide-stained 2% agarose gel by electrophoresis at 100 Volts. The resulting gel was then visualized under ultra-violet (UV) light using a Quantity One Gel Documentation System (Biorad, Hercules, California, USA) supported by a Dell computer using Quantity One, version 1.0 software (Biorad, Hercules, California, USA). Lanes loaded with PCR products were evaluated for the presence of the 439 bp HGH gene amplification control product, to demonstrate success of the PCR process, and for the presence of a 253bp CD32a gene amplification product. The presence of a CD32a amplification product in only that lane corresponding to either the H131- or R131-specific PCR, therefore indicated homozygosity for that particular allele, whilst visualization of a CD32a amplification product in two lanes, corresponding to the H131- and R131-specific PCRs respectively, indicated heterozygosity of the subject from which the DNA was obtained.

2.6.3 FcγRIIIb (CD16b) Genotyping

FcγRIIIb (CD16b) genotyping was performed using an allele-specific PCR based on the method described by Bux *et al.*[382] In a method comparable to that described for CD32a genotyping, two master mixes, designated 'NA1' and 'NA2' according to their CD16b NA1- or NA2-allele specificity respectively, were prepared to each yield a volume of 20µl per sample for amplification. The composition of each master mix is described in Table 10. One hundred ng of each patient's DNA, diluted with distilled water when necessary to yield a volume of 5µl, was then combined with 20µl of both the 'NA1' and 'NA2' master mixes. Two negative controls were

included with each episode of PCR amplification, comprising 20µl of both master mixes each combined with 5µl of distilled water without the addition of DNA, for the purpose of identifying any master mix contamination. The two HGH primers used in this methodology were identical to those used for CD32a genotyping, and were similarly employed as internal positive controls, their amplification of a 439-bp fragment of the HGH gene confirming the success of each PCR. The CD16b NA1- and NA2-allele specific primers, in addition to the common CD16b antisense primer used in this methodology, bind within the exon EC-1 of the CD16b gene. In addition the allele specific primers discriminate between the CD16b gene and the highly homologous CD16a gene. The NA1-specific primer is situated at position 208-227 and contains a mismatch at position four from the 3′ end in order to prevent mispriming and to enhance specificity. The use of this primer in the NA1-allele specific PCR yields a 141-bp amplification product when this allele is present in a subject's template DNA. The NA2-specific primer is situated at position 130-147 and has a T at the 3′ end and a C seven nucleotides from the 3′ end, these comprising two polymorphic sites. The presence of an NA2 allele in a subject's template DNA results in a 219-bp amplification product from the use of this primer in the NA2-specific PCR. The common CD16b antisense primer is situated at position 331-348.[382]

The PCR was similarly performed in a thermal cycler (MyCycler®, Biorad, Hercules, California, USA) using the following amplification protocol: one cycle at 94°C for 15 minutes (initial heat activation); 30 cycles of 94°C for 1 minute (denaturation), 55°C for 1 minute (annealing) and 72°C for 1 minute (extension); followed by a final extension step of 72°C for 10 minutes. PCR products were again

kept at 4°C until gel electrophoresis was performed. As was the case for CD32 genotyping, duplicate CD16 PCRs were also performed and analysed for most samples to increase the certainty of genotyping.

The amplification products of each CD16b PCR episode, including 'NA1' and 'NA2' negative controls, in addition to the DNA ladders previously described, were separated using gel electrophoresis and visualized under UV light using a methodology identical to that described for CD32a genotyping. With the exception of lanes loaded with negative control samples, the presence of the HGH amplification control band was identified to confirm a successful enzymatic process in each lane loaded with PCR product. NA1 homozygosity was indicated by the presence of a 141-bp product in the lane corresponding to that patient's NA1-specific PCR, combined with the absence of a 219-bp product in the lane loaded with that subject's NA2-specific PCR. Conversely, NA2 homozygosity was indicated by a 219-bp product in the lane loaded with contents of the NA2-specific PCR combined with the absence of a 141-bp product in the lane loaded with that subject's NA-1 specific PCR. Heterozygosity was thus indicated by the presence of both the 141-bp and 219-bp products in the individual lanes loaded with products of that subject's NA1- and NA2-specific PCR, respectively.

Table 9. Master mix composition for FcγRIIa (CD32a) polymerase chain reaction (PCR) based on the method of Rodríguez *et al.*[381]

Stock Solutions	Final Concentration	Master Mix (µl/sample)
10x Hotstar Buffer (10x)†‡		2.5
40mM dNTP§ (10mM each)	200µM each	0.5
5U/µl HotstarTaq DNA Polymerase‡	0.5U	0.1
Distilled water		10.65
10µM 5′ HGH primer¶ 5′ CAG TGC CTT CCC AAC CAT TCC CTT A 3′	0.5µM	1.25
10µM 3′ HGH primer¶ 5′ ATC CAC TCA CGG ATT TCT GTT GTG TTT C 3′	0.5µM	1.25
10µM CD32 antisense primer¶ 5′ CAA TTT TGC TGC TAT GGG C 3′	0.5µM	1.25
Hmix: 10µM H131-specific sense primer¶ 5′ ATC CCA GAA ATT CTC CCA 3′ *OR*	0.5µM	1.25
Rmix: 10µM R131-specific sense primer¶ 5′ ATC CCA GAA ATT CTC CCG 3′	0.5µM	1.25
Total		20µl

dNTP, deoxynucleotide triphosphates; DNA, deoxyribonucleic acid: HGH, human growth hormone.

† Contains 15mM $MgCl_2$

‡(Qiagen Inc., Valencia, California, USA)

§(Promega Corporation, Madison, Wisconsin, USA)

¶(Geneworks, Adelaide, Australia)

Table 10. Master mix composition for FcγRIIIb (CD16b) polymerase chain reaction (PCR) based on the method of Bux *et al.*[382]

Stock Solutions	Final Concentration	Master Mix (µl/sample)
10x Hotstar Buffer (10x)†‡		2.5
40mM dNTP§ (10mM each)	200µM each	0.5
5U/µl HotstarTaq DNA Polymerase‡	0.5U	0.1
Distilled water		10.65
10µM 5′ HGH primer¶ 5′ CAG TGC CTT CCC AAC CAT TCC CTT A 3′	0.5µM	1.25
10µM 3′ HGH primer¶ 5′ ATC CAC TCA CGG ATT TCT GTT GTG TTT C 3′	0.5µM	1.25
10µM CD16 antisense primer¶ 5′ ATG GAC TTC TAG CTG CAC 3′	0.5µM	1.25
NA1 mix: 10µM NA1-specific sense primer¶ 5′ CAG TGG TTT CAC AAT GTG AA 3′ *OR*	0.5µM	1.25
NA2 mix: 10µM NA2-specific sense primer¶ 5′ CAA TGG TAC AGC GTG CTT 3′	0.5µM	1.25
Total		20µl

dNTP, deoxynucleotide triphosphates; DNA, deoxyribonucleic acid: HGH, human growth hormone.

† Contains 15mM $MgCl_2$

‡(Qiagen Inc., Valencia, California, USA)

§(Promega Corporation, Madison, Wisconsin, USA)

¶(Geneworks, Adelaide, Australia

2.7 Plasma Cytokine Assays

Plasma samples from each subject in the 'open' and EVAR cohorts, separated and stored from whole blood drawn pre-operatively, at T_0, T_0 + 4, T_0 + 24, T_0 + 72, and T_0 + 120 hours, as previously described, were thawed immediately prior to commencing cytokine assays. Limitations on the availability of assay kits prevented the measurement of cytokines in plasma obtained from subjects in the 'lower limb' cohort. Furthermore, the limited availability of cytokine kits permitted samples obtained at T_0 + 120 hours to be analysed for only 16 of the subjects undergoing AAA repair. Cytokine data from this time point was therefore excluded from the analyses and discussion presented hereinafter.

Concentrations (pg/ml) of TNF-α, IL-1β, IL-6, IL-8 (CXLC8), IL-10 and IL-12p70 present in the aforementioned plasma samples were determined using the BD® Cytometric Bead Array (CBA) Human Inflammation Kit (BD Biosciences, San Diego, California, USA) in accordance with the manufacturer's instructions. All sample assays were performed using plasma in an undiluted form.

The assay principle of the BD® CBA cytokine kit used in this study enables multiple soluble cytokine analytes to be simultaneously assayed from a single plasma sample. Thus, the Human Inflammation Kit employs six bead populations with distinct fluorescence intensities that have been coated with capture antibodies specific for TNF-α, IL-1β, IL-6, IL-8 (CXLC8), IL-10 and IL-12p70 proteins. The multiple bead populations are thus mixed together to form the BD® CBA. Aliquots of the CBA are then incubated with the individual test plasma samples, and with a series of standard dilutions. The latter are a set of serial dilutions of the recombinant human TNF-α, IL-

1β, IL-6, IL-8 (CXLC8), IL-10 and IL-12p70 proteins, and are required for the purpose of generating standard curves for each respective cytokine. The capture bead and plasma/recombinant protein standards combinations are then washed, centrifuged, and supernatants removed prior to incubation with aliquots of a detection reagent which consists of RPE-conjugated anti-human TNF-α, IL-1β, IL-6, IL-8 (CXLC8), IL-10 and IL-12p70 proteins. The resulting combinations are again washed, centrifuged and their supernatants removed prior to resuspension of the resulting pellet. The result of these steps is the formation of complexes comprised of recombinant or test sample cytokines sandwiched between the specific corresponding capture bead and the corresponding RPE-conjugated detection antibody.

Following preparation of CBA plasma samples and standard serial dilutions, flow cytometry was undertaken to acquire the data in accordance with the manufacturer's instructions, using a BD FACSCalibur® flow cytometer, supported by an Apple Macintosh computer using CELLQuest (Becton Dickson, San José, California, USA) and BD® CBA Software (BD Biosciences, San Diego, California, USA). Briefly, instrument settings were optimised, using the kit's Cytometer Setup Beads protocol, prior to each episode of flow cytometric analysis. Data from the recombinant standard serial dilutions, including a negative control sample, was acquired for the purpose of generating standard curves for each analyte, followed by data acquisition from the prepared plasma assays. BD® CBA Software (BD Biosciences, San Diego, California, USA) was subsequently used to extrapolate individual cytokine concentrations from the prepared samples by comparison against the corresponding standard curve. The individual standard curve range for each cytokine assayed using

the BD® CBA Human Inflammation defined the minimum and maximum quantifiable levels as 20 pg/ml and 5000 pg/ml respectively.

2.8 Determination of Neuroendocrine Response to Surgical Interventions

2.8.1 Twenty-four Hour Urine Collections

The HPA response to surgical intervention was assessed by quantitation of free cortisol in patients' urine, whilst the SNS response was determined by assaying urinary adrenaline and noradrenaline levels. These assays were performed on 24-hour urine saves, collected during three time intervals. A baseline, pre-operative 24-hour urine collection was performed in the week preceding the proposed vascular procedure, a second 24-hour collection was commenced at anaesthetic induction, and a third 24-hour urine save was commenced 72 hours from anaesthetic induction. These three intervals are designated hereinafter as T(pre-op), T(0-24), and T(72-96) respectively; the latter two designations describing the collection interval with reference to anaesthetic induction. Urine saves were collected in containers containing 2.4M hydrochloric acid, as a catecholamine preservative, by subjects and/or nursing staff who were provided with written and verbal instructions on performing the collection. Urinary free cortisol (UFC) and urinary catecholamine assays were performed by the IMVS, Department of Clinical Biochemistry, in the Woodville South and Adelaide laboratories respectively, using established methodologies, employed for commercial application, as outlined below. Incomplete urine saves were not submitted for laboratory analysis. All urinary measures are expressed in nmol/24 hours.

2.8.2 Automated Chemiluminescent Immunoassay for Urinary Free Cortisol (UFC)

An ACS:180® Cortisol Assay (Bayer Healthcare, New York, USA) was employed for measurement of UFC in 24-hour urine collections. Briefly, this automated system performs a competitive direct chemiluminescent immunoassay for determination of cortisol concentrations in patient samples. Thus, during a five minute incubation period at 37°C, cortisol in the patient sample competes with acridium ester-labelled cortisol, contained in a vehicle comprised of buffered saline with sodium salicylate, sodium azide and preservatives, for binding to polyclonal rabbit anti-cortisol antibody bound to monoclonal mouse anti-rabbit IgG antibody, which is covalently coupled to paramagnetic particles in a vehicle of buffered saline, sodium azide and preservatives. The system then magnetically separates the paramagnetic particles and initiates a chemiluminescent reaction by the addition of hydrogen peroxide in an alkaline environment. The amount of relative light units detected by the system is inversely related to the concentration of cortisol in the patient sample, a measure of which is generated by comparison against stored master curve values. Cortisol concentration values generated by the system are converted by the laboratory into nmol/24 hours using a standard formula recommended by the manufacturer.

The ACS:180® Cortisol assay is considered to be highly specific for cortisol, however cross-reactivity by structurally related compounds and pharmaceuticals has been quantitated by the manufacturer.[383] UFC measures from samples excreted by those subjects who were administered pharmaceuticals demonstrated by the manufacturer to have greater than 0.20% cross-reactivity with the ACS:180® Cortisol assay during the collection interval were therefore excluded from analyses.

An additional UFC measure was excluded due to the unexpected administration of a dexamethasone suppression test during the collection interval.

2.8.3 Urinary Catecholamine Assay by High Performance Liquid Chromatography (HPLC)

The urinary catecholamines, adrenaline and noradrenaline, were assayed by HPLC with electrochemical detection according to an established commercial laboratory technique. Briefly, an aliquot of acidified urine was added to a 1M phosphate buffer (pH 7.0) and internal calibrator. The pH was adjusted to pH 6-7 and the mixture poured onto a disposable micro-cation exchange column. The column was then washed with an aliquot of ammonium phosphate buffer and the catecholamines then selectively eluted from the column with ammonium pentaborate. Alumina extraction was then performed by adding activated alumina to a portion of the eluent which had been mixed with sodium metabisulphite and tris buffer. After washing, the catecholamines were removed from the alumina with 0.4M acetic acid. An aliquot of the acetic acid eluent was then used in a HPLC assay. The individual catecholamines were thus separated on a reverse phase column and measured with an electrochemical detector.

2.8.4 Validity of Urinary Measures of Neuroendocrine Response

The validity of employing urinary measures of excreted cortisol and catecholamines as measures of the neuroendocrine stress response is evident from the finding that UFC and urinary catecholamine measures are closely related to their corresponding

serum concentrations.[384, 385] Furthermore, the lack of influence of diurnal patterns of secretion on 24-hour urinary cortisol measurements has resulted in this methodology being considered as an optimal approach to the assessment of cortisol production,[386] and by implication, HPA axis activity.

2.9 Determination of Pre-operative Nutritional Status

Each consenting subject's pre-operative nutritional status was assessed using two distinct methodologies, namely the practitioner-administered Mini Nutrtional Assessment (MNA) tool and body composition analysis by dual energy X-ray absorptiometry (DEXA).

2.9.1 The Mini-Nutritional Assessment (MNA)

Subject's pre-operative nutritional status was assessed, in part, using the MNA administered in the pre-operative period by the study investigator. The MNA is a practitioner-administered tool designed to provide a single, rapid assessment of nutritional status in elderly patients. This 18 item tool is comprised of anthropometric assessments, namely measurements of body mass index (BMI), mid-arm and calf circumference and an assessment of weight loss; a general assessment, consisting of six questions related to lifestyle, mobility and medications; a dietary assessment, consisting of eight questions regarding the details of food and fluid intake and autonomy of feeding; and a self-assessment component, measuring the subject's self-perception of their health and nutrition. An overall score (maximum 30 points) is calculated which classifies the patient as either well nourished (a score of $\geq$ 24 points), at risk of malnutrition, indicating borderline nutritional status (17-23.5 points) or malnourished (<17 points) (Appendix 2, Figure 1).[387-389] With the use of these recommended scoring thresholds, the MNA has been found to have a sensitivity of 96%, a specificity of 98%, and a predictive value of 97%.[388]

The validity of the MNA scale was established by three principal successive studies. The developmental and validation studies involved populations representing a

spectrum of elderly subjects, from the frail, severely malnourished to the very healthy, and established the validity and discriminatory potential of the tool. The third study was used to validate the MNA in a non-institutionalised elderly population in a different cultural context.[390] Inter-observer agreement when using the scale has since been investigated and confirmed its reliability.[388, 391]

The MNA was developed specifically for the evaluation of nutritional status amongst frail, elderly individuals, defined as those with functional impairments, such as mobility, hearing or cognitive disorders, those who live alone, in nursing homes, or who are more than 85 years old but living in the community. Those who developed the tool have, however, also recommended its use amongst hospitalised elderly, and notably amongst those who require surgery.[388] This nutritional assessment tool was therefore considered applicable to major vascular surgery candidates who comprise the cohorts included in the current study.

2.9.2 Dual Energy X-Ray Absorptiometry (DEXA)

Whole body composition analysis was performed pre-operatively by DEXA scanning. Body composition analysis by DEXA is a relatively novel technique which generates regional and total body measures of bone mineral content (BMC), fat-free soft tissue mass (FFST), and fat mass (FM). To perform these measurements, DEXA systems have an X-ray source, which after appropriate filtration, emit two effective photon energy peaks which are transmitted through the patient and subsequently measured by the scanning instrument.[392, 393] When placed on one side of an object, attenuation of the incident photons occurs, the extent of which is related to the thickness, density and chemical composition of the object as well as the energy of the

incident photon. Thus, lean tissue, fat and bone result in different degrees of attenuation of incident photons of a given energy due to differences in their density and chemical composition. Based on this phenomenon, DEXA analysis, which employs a two-compartmental model of body composition, uses an algorithm relating the masses of the two constituent tissues, or compartments, to their relative attenuation of the two DEXA energy peaks, the latter being calculated from the measured intensity of the transmitted photon beam and known mass attenuation coefficients associated with the body tissues in question. Thus, for those body regions where the photon beam passes through bone and overlying soft tissue, a set of two equations, using the appropriate attenuation coefficients, is employed to produce measurements of bone mass and total soft tissue mass. For body regions without bone, the same two-compartmental model, and set of equations but with the appropriate attenuation coefficients, is used to generate measurements for FM and FFST. Generation of the final regional and total body composition values reported following DEXA scanning, that is BMC, FFST, and FM, involves the scanning instrument separating pixels of the scanned image. For whole body scans, approximately 40 to 45% of the pixels are classified as containing bone. The remaining pixels are used to calculate a fat-to-lean ratio. For those scanned regions where the beam has passed through bone, permitting the initial calculation of a bone and overlying total (fat and lean) soft tissue mass only, the latter value is then broken down into FM and FFST values, by applying the fat-to-lean ratio calculated from non-bone pixels in the same scan region.[394]

The relative novelty of this application of DEXA accounts for the relatively limited body of research addressing the validity of the technique. Whilst a high degree of

reproducibility of measurements has been demonstrated, data confirming the absolute accuracy of the technique is less abundant. The lack of verification of total body DEXA measurements by human cadaver analyses is notable in this regard, however animal validation studies have been performed.[393, 394] The accuracy of the technique is further supported by the demonstration of a good agreement between DEXA derived values of BMC, FFST and FM and values derived from the reference norm most often preferred for evaluation and/or calibration of alternate body composition analysis techniques, namely a multi-compartmental model based on elemental neutron activation analysis.[395] The manufacturer of the DEXA instrument may, however, exert a substantial influence on DEXA measurements due to the effect of calibration and the algorithms used.[394] No patient specific factors are known to significantly influence the validity of DEXA derived BMC or FM measurements. Deficiencies or excesses in a subject's state of hydration would be appropriately registered as changes in FFST.[392]

Analysis of consenting subject's pre-operative body composition was thus performed using GE-Lunar Prodigy Vision densitometers (GE Medical Systems Lunar Corporation, Wisconsin, USA) in the Department of Nuclear Medicine and Bone Densitometry, The Royal Adelaide Hospital and in the Osteoporosis Centre, Departments of Endocrinology and Nuclear Medicine, The Queen Elizabeth Hospital. The two densitometers, employed for routine clinical bone densitometry scanning and research purposes, were cross calibrated using a phantom. On those occasions when patients had unavoidably experienced radioisotope administration prior to scanning, at least one week was allowed to elapse prior to DEXA scanning to avoid the possibility of this factor influencing DEXA measurements. When this aim

was unable to be achieved, due to the proximity of the impending operative intervention, affected subject's were excluded from this analysis, but not the study overall. In addition, limitations on access to the densitometers prevented some subjects from undergoing body composition analysis.

Five DEXA derived measures of total body composition, namely fat-free mass (FFM), fat mass (FM), fat-free mass index (FFMI), fat-mass index (FMI), and estimated skeletal muscle mass (SMM), were identified as relevant and potentially useful markers of nutritional status on the basis of findings in the existing literature and were therefore determined for each scanned subject. FM and FFM were selected as markers of nutritional status on the basis of abundant evidence demonstrating loss of these component tissues with undernutrition, and PEM in particular.[303, 396] Total body FM (kg) was obtained directly from DEXA data reports, whilst FFM (kg) was calculated from reported data by summing its constituent components, total BMC and FFST, as described by Hansen *et al.* [397]. It has been suggested that normalisation of individual's FFM and FM measurements for height, to yield the indices FFMI and FMI index respectively, may result in more valid indicators of nutritional status.[398] Modelled after BMI, height normalised indices were therefore calculated, according to the method of VanItallie *et al.*[398], as follows:

FFMI = FFM (kg)/height $(m)^2$,

and

FMI = FM (kg)/ height $(m)^2$

It has been recognised that whilst an individual's FFM is a useful indicator of PEM, this body composition measure is in itself a surrogate marker of that individual's

total skeletal muscle mass.[399] Thus SMM, which does in fact represent a large portion of FFM,[397] is considered a reliable indicator of PEM severity and outcome[400] . This is attributable to skeletal muscle serving as a large and highly metabolically active pool of protein that is responsive to negative energy balances, and furthermore, it's mass can be quantitated.[397, 400] The use of DEXA measurements represents one of the ways in which SMM may be estimated. Thus, if it is assumed that the limb FFST value measured by DEXA closely represent limb SMM, as discussed by Heymsfield *et al.*,[401] then total SMM can be calculated for normal individuals on the basis of the proportion of limb SMM to total SMM (0.75) reported in cadaver studies.[397] Total body SMM (kg) was therefore calculated for scanned subjects from the sum of total arm and total leg FFST values, assuming that this sum represents total limb SMM, and that this inturn represents 75% of total body SMM, using the following formula, as described by Hansen *et al.*[397] :

SMM = 1.333 (arm FFST + leg FFST)

This method of SMM estimation has been validated in a group of healthy older subjects.[397]

2.10 Pre-operative Psychological Assessments

Following successful recruitment and informed consent, which entailed a description of the nature and purpose of the psychological inventories used in the study and confirmation of confidentiality, subjects were asked by the study investigator to independently complete three self-administered inventories according to provided instructions, at a convenient time during the pre-operative period. Cognitively impaired subjects, or those whose level of literacy prevented comprehension of written English to the standard required by the self-administered tools, were identified at this stage and excluded from psychological assessment, but not from the overall study. Completed inventories were collected pre-operatively by the study investigator.

The Beck Depression Inventory-II (BDI-II)[402] and the Center for Epidemiological Studies-Depression Scale (CES-D)[403] constituted two of the pre-operative psychological assessment tools completed by individual subjects for the purpose of identifying depression. This enabled the cohorts to be dichotomised into depressed and non-depressed individuals according to the findings of each instrument. These two screening instruments which differ somewhat in content, were selected and their findings independently assessed to enhance the validity of findings, in view of concerns raised by a pilot study (data not shown) which identified an inexplicably low incidence of depression amongst a comparable cohort when the BDI-II alone was employed. A measure of each subject's trait anxiety was obtained from the third questionnaire completed in the pre-operative period, the Spielberger Trait-Anxiety Scale (STAI Form Y-2).[358]

2.10.1 Measures of Depression

2.10.1.1 Beck Depression Inventory-II (BDI-II)

The BDI-II is a 21-item self-report inventory designed to measure depressive symptoms and severity of depression in persons aged 13 and older, which can be completed in 5 to 10 minutes when self-administered (Appendix 3, Figure 1). The BDI-II represents a substantial modification of the original BDI, and its primary revision, the BDI-IA, such that the assessment of symptoms corresponds to the current Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria[402] thereby improving content validity. The content of this inventory reflects the cognitive, affective, somatic, and vegetative symptoms of depression.[404] BDI items have been found to discriminate between anxiety and depression, and to be more strongly correlated with the latter.[405] Since its initial revision, the BDI has been widely accepted and used for assessing the intensity of depression in psychiatric and normal populations, including older adults,[404] and has an extensive history of use in primary care depression screening research[405] . The BDI-II has been shown to be reliable with high measures of internal consistency and test-retest reliability.[404] Construct and criterion validity have been demonstrated by the developers of this psychometric tool.[402]

Completed BDI-II questionnaires were manually scored according to the inventory's guidelines. The total score range generated by this inventory is zero to 63. According to these guidelines, a total score of zero to 13 is considered to be in the 'minimal range', a score in the range of 14 to 19 indicates mild depression, 20 to 28 indicates moderate depression, whilst severe depression is indicated by a score of 29 to 63.[404] Individuals in each study cohort were therefore categorised into a non-depressed

group, when their BDI-II score was ≤ 13, or a depressed group if their total BDI-II score was >13.

2.10.1.2 Center for Epidemiological Studies-Depression Scale (CES-D)

The CES-D is a 20-item inventory developed for use as a research tool to measure the presence and severity of depressive symptomatology in the general population (Appendix 3, Figure 2).[403] When self-administered it requires approximately 10 minutes to complete. Items assess perceived mood and level of functioning during the preceding week. Its focus on cognitive-affective, rather than physical symptoms of depression has recommended its use in studies involving cardiac patients amongst whom comparable physical symptoms may be due to their medical condition itself rather than any associated psychiatric condition.[337] It is considered a reliable instrument with a high internal consistency. The moderate test-retest correlations reported for this instrument may be expected as the scale measures 'current' level of symptomatology and is therefore sensitive to changes in depressive symptoms. The identification of stronger correlations with shorter test-retest intervals supports this notion. Furthermore, good content and criterion validity are features of this scale, which is both widely recognized[404] and used in studies involving cohorts of cardiac patients[337, 406] and the elderly[407].

CES-D inventories were manually scored according to accompanying guidelines. The possible range of scores is zero to 60. A higher total score reflects greater symptoms of depression, weighted by frequency of occurrence in the preceding week.[404] A total CES-D score of ≥ 16 is the standard cut-off for clinical

depression.[404, 405] A separate dichotomisation of patients in each cohort into depressed and non-depressed groups was therefore performed on the basis of their CES-D score, independent of the findings of the BDI-II. Those with a total score of ≥ 16 were categorised as depressed, whilst those with a score of < 16 were categorised as non-depressed, according to the CES-D.

2.10.2 Measurement of Trait Anxiety

2.10.2.1 Spielberger Trait-Anxiety Scale (STAI Form Y-2)

One of the most widely used measures of anxiety,[408] the Spielberger State-Trait Anxiety Inventory (STAI) for Adults is comprised by two independent self-report scales, STAI Form Y-1 and STAI Form Y-2, for measuring state and trait anxiety respectively.[358] The two scales distinguish between the concepts of personality states and personality traits. In contrast to personality states, which describe often transitory emotional states evoked by an appropriate stimulus, personality traits can be conceptualised as relatively enduring differences among people in their tendencies to perceive the world in a certain way and to react or behave in a particular and predictable manner.[358] Subjects participating in the current study were asked to complete only the trait anxiety scale, STAI Form Y-2, pre-operatively according to instructions provided on the test form. The concept of trait anxiety thus refers to the relatively stable characteristic of individuals' tendency to perceive a stressful situation as dangerous or threatening and reflects the extent to which they respond to such situations with elevations in the intensity of their state anxiety reactions. Trait anxiety may also reflect individual differences in the frequency and intensity with which anxiety states have been manifested in the past, and the probability that state anxiety will be experienced in the future. The stronger an individual's anxiety trait,

the more probable it is that the individual will experience greater state anxiety in response to a threatening situation.[358] In view of the impracticality, and indeed impossibility, of assessing state anxiety at multiple peri-operative time points, trait anxiety was used as a surrogate measure to grade subject's likelihood of manifesting a greater state anxiety in response to events experienced in the peri-operative period.

The STAI was designed for self-administration and each of the component forms can be completed in six to ten minutes. STAI Form Y-2 is a 20-item inventory of statements that assess how a subject generally feels by rating the frequency of their feelings of anxiety on a four-point scale (Appendix 3, Figure 3).[358] Completed forms were manually scored, according to the inventory's guidelines, by adding the weighted scores for each of the twenty constituent items. The total score for both STAI Form Y-1 and STAI Form Y-2 can range from a minimum of 20 to a maximum of 80.[358] The reliability of this tool has been determined in several different cohorts. Moderate to strong test-retest correlations have been identified, depending on the cohort tested, whilst measures of internal consistency for the trait anxiety scale, including those calculated using subjects from the Australian general population, are uniformly high.[358, 408] Evidence of the concurrent, convergent, divergent and construct validity of the STAI scales has also been demonstrated. A feature of the STAI Form Y-2 making its use particularly valid in the current context is that unlike other trait anxiety scales, it does not employ items that may reflect depression rather than anxiety. It is worthy of note that whilst correlations of individual's scores for the two STAI forms support the theory that those high in trait anxiety tend to be higher in state anxiety, a finding with potential implications for the use of STAI Form Y-2 as a surrogate measure in the current study is that the strength

of the correlations depend on the amount and kind of stress under which the state anxiety is measured. This is consistent with the Trait-State Anxiety theory which predicts higher correlations between social and evaluative situations and lower correlations in situations of physical danger.[358]

2.11 Outcome Measures

2.11.1 Systemic Inflammatory Response Syndrome (SIRS) Score and Duration

For the purposes of the current study, SIRS was defined using the only recognized consensus definition, established in 1991 by the ACCP/SCCM Consensus Conference[100], described in detail in Chapter 1. On the basis of the limited published data reporting the frequency of SIRS following AAA repair[128-130], it was anticipated that the use of simply the presence or absence of SIRS would not be a sufficiently meaningful outcome measure. Furthermore, combining a severity scoring system with a broad, encompassing clinical definition such as that established for SIRS for the purpose of providing a measure of the position of an individual along the inflammatory continuum, was vigorously recommended by the ACCP/SCCM Consensus Conference.[100] For this reason, SIRS severity was measured by means of a scoring system and by determining the duration for which SIRS was present in individual patients.

2.11.1.1 SIRS Score

On each of the first five post-operative days, described herein after as POD 0 (zero to 24 hours post skin closure), POD1 (24 to 48 hours post skin closure), POD2 (48 to 72 hours post skin closure), POD3 (72 to 96 hours post skin closure) and POD4 (96 to 120 hours post skin closure), the presence of SIRS was prospectively identified and, when present, the number of SIRS criteria met was used as a weighting of the severity of SIRS on that day. According to the ACCP/SCCM definition[100] (Table 1, Chapter 1) the presence of zero or one criterion does not constitute SIRS, hence a

score of 0 was assigned on days when either zero or only one criterion were met. When either two, three or four criteria were met simultaneously on a given day, a score of 1, 2 or 3 respectively was assigned. Thus, for each of POD 0 to POD4, a score of 0, 1, 2 or 3 was assigned. On any day when SIRS was present, but the number of criteria fulfilled simultaneously fluctuated over the 24-hour period, the score associated with the maximum number of criteria fulfilled simultaneously was assigned for that day. When subjects were discharged before the end of POD4 (120 hours post skin closure), it was considered reasonable to assume that SIRS was no longer present. As a result, any of the first five post-operative days that elapsed following discharge were assigned a score of 0, unless a higher score was necessitated by the number of criteria met during any of the 24-hour period that elapsed prior to discharge. A cumulative SIRS score was determined for each subject by summing the SIRS scores on each of POD 0 to POD4. The possible cumulative SIRS score range was therefore 0 (scores of 0 on each of POD 0 to POD4) to 15 (scores of 3 on each of POD 0 to POD4).

The validity of using SIRS criteria to weight the severity of SIRS is suggested by early research which indicated that an increasing number of SIRS criteria was directly associated with the incidence of subsequent ARDS, DIC, shock and mortality[66] . The use of comparable scoring systems have been previously employed.[129, 409] The system employed in the current study most closely resembles that used by Norwood et al. in a study of the clinical value of the SIRS concept in AAA repair[129] . These authors scored patients from 0 to 4 on each of the post-operative days evaluated, according to the number of SIRS criteria met, and similarly generated cumulative scores by summation of a subject's daily scores. It was found

that elective AAA patients with high cumulative SIRS scores in the first four post-operative days were more likely to die, suggesting the utility of this scoring system as an outcome measure during the early post-operative period.

2.11.1.2 SIRS Duration

An additional, surrogate measure of the severity of an individual's SIRS response was the duration (measured in days), for which SIRS was present during the first five post-operative days. Consecutive and non-consecutive days on which SIRS was diagnosed were tallied to yield the final duration of SIRS for each subject. Once again, SIRS was assumed to be absent during any period within the five day post-operative study interval that elapsed following discharge.

The use of SIRS duration as an outcome measure is conceptually comparable to the frequently used morbidity measures of post-operative length of ICU or hospital stay. Furthermore, the use of SIRS duration as a potentially useful outcome measure is supported by literature which suggests that the persistence of SIRS over time may be a more accurate indicator of a pathologic inflammatory process than brief periods of SIRS, or the occurrence of SIRS *per se*.[130, 410]

2.11.2 Sepsis Occurrence

The current consensus definition of sepsis produced by the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference[99] and detailed in Chapter 1, was employed in the current study. Briefly, these guidelines define sepsis as a systemic inflammatory response in the presence of documented or suspected infection. In accordance with these guidelines, the presence of infection in

the current study was therefore determined by positive microbiological culture or the strong suspicion of invasion by micro-organisms, on the basis of radiological investigations. Pulmonary consolidation, for example, as indicated by X-ray or computed tomography (CT) was therefore considered as evidence of infection, even in the absence of microbiological confirmation. Thus, the presence or absence of sepsis, within 30 post-operative days was documented for each subject. This was achieved by the thorough, prospective evaluation of all available clinical information throughout each subject's hospitalisation. The occurrence of sepsis following discharge was identified retrospectively by examining the details of radiological and/or laboratory data in addition to subject's history of public hospital episodes within this period, using the South Australian public hospital system's OACIS Clinical Care Suite (Dinmar Inc., USA) database. When the suggestion of a septic event was identified from the information contained in this database, subjects' public hospital records were retrieved to confirm or refute the occurrence of sepsis.

2.11.3 Occurrence of Infection

The occurrence of any infection, regardless of the presence or absence of an associated systemic inflammatory response, within 30 days post-operatively was documented for each subject. The definition of infection used for this categorisation was that contained within the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference diagnostic criteria for sepsis[99] (Chapter 1, Table 2), described above. The information required for this categorisation was therefore simply extracted from the data collected for the purpose of identifying the presence or absence of sepsis, as described above. This outcome measure was therefore used

to dichotomise each cohort into those who developed an infection and those who did not.

2.11.4 Measures of General Post-operative Morbidity

2.11.4.1 Occurrence of Moderate/Severe Post-operative Complication(s)

The occurrence of all local and systemic complications within 30 post-operative days was identified using the process described for the identification of sepsis. Complications were documented and their severity scored according to the recommendations of the SVS/ISCVS Ad Hoc Committee on Reporting Standards[44, 411] (Appendix 4, Figure 1). This system involves each complication being assigned a severity score of either 1, denoting a mild complication; 2, to denote a complication of moderate severity; or 3, indicating a severe complication. Patients in each cohort were therefore dichotomised into those who developed one or more moderate/severe (severity score of 2 or 3) complications and those who did not.

2.11.4.2 Maximum APACHE II Score

The APACHE II (Acute Physiology And Chronic Health Evaluation) severity of disease classification system[412] was used as a measure of general post-operative morbidity (Appendix 4, Figure 2). Briefly, APACHE II assigns weighted point-scores according to the degree of derangement of twelve routinely measured physiological variables, age and previous health status to provide a general measure of acute illness severity. This tool generates a total score which may range from 0 to 70. Increasing total scores indicate increased illness severity and have been closely

correlated with the subsequent risk of hospital death. This validity of this index has been demonstrated amongst medical and surgical patients in ICU settings and has been recommended for use as a research tool for the purpose of classifying illness severity.[412]

For each subject, an APACHE II score was thus calculated on each of POD 0 to POD4, whilst subjects remained hospitalised. Each patient's maximum APACHE II score was used as a measure of their post-operative morbidity.

2.11.4.3 ICU and Post-operative Length of Stay (LOS)

ICU LOS (expressed in days) and total post-operative hospital LOS (expressed in days) were documented for each subject and used as additional measures of post-operative morbidity.

2.11.5 Health-related Quality of Life (HRQoL)

2.11.5.1 Medical Outcomes Study 36-Item Short-Form (SF-36) Health Survey

In association with the request to complete the afore-mentioned psychological inventories (Section 2.10), subjects were asked to pre-operatively self-administer the Medical Outcomes Study 36-Item Short-Form (SF-36) Health Survey (Standard English – Australia/New Zealand Version 1.0)[413] (Appendix 4, Figure 3) for the purpose of assessing pre-operative HRQoL. Like the psychological inventories, completed SF-36 surveys were collected by the investigator prior to operative intervention. Post-operative HRQoL was subsequently assessed at one, three and six months following surgical intervention by requesting patients to complete and return

SF-36 surveys which were sent by mail at each of the afore-mentioned assessment intervals.

The SF-36 health survey is a widely used 36-item questionnaire designed to examine perceived health status amongst respondents aged 14 and older. Self-administration of the survey requires approximately 7 to 10 minutes, and is amongst the methods of administration for which the instrument was designed.[346, 413] The instrument measures generic health concepts relevant across age, disease and treatment groups[413] and has been frequently used for the assessment of HRQoL amongst cohorts of AAA patients and those with peripheral vascular disease.[348, 414-417] All but one of the 36 items constituting the SF-36, contribute to one of eight multi-item scales, each of which aggregates two to 10 items. The multi-item scales measure the following eight health concepts:

- Physical Functioning (PF): extent to which health limits physical activities such as self-care, walking, climbing stairs, bending, lifting, and moderate and vigorous exercises.
- Role Functioning - Physical (RP): extent to which physical health interferes with work or other daily activities, including accomplishing less than wanted, limitations in the kind of activities performed, or difficulties in performing activities.
- Bodily Pain (BP): intensity of pain and effect of pain on normal work, both inside and outside the home.
- General Health (GH): personal evaluation of health, including current health, health outlook, and resistance to illness.
- Vitality (VT): feeling energetic versus feeling tired and worn out.

- Social Functioning (SF): extent to which physical health or emotional problems interfere with normal social activities.
- Role Functioning – Emotional (RE): extent to which emotional health interferes with work or other daily activities, including decreased time spent on activities, accomplishing less, and not working as carefully as usual.
- Mental Health (MH): general mental health, including depression, anxiety, behavioural-emotional control, and general positive affect.

Thus, answers to each item are scored to produce raw scale scores for each of the eight health concepts, which are then transformed to a 0 to 100 scale.[413] Scoring algorithms can then be applied to produce two norm-based summary scores, the Physical Component Summary score (PCS) and the Mental Component Summary score (MCS).[418-421] These summary scores reflect the contribution of physical and mental factors respectively, to overall HRQoL[346]. In all cases, higher scores indicate a better HRQoL.[413] SF-36 scoring software was used for scoring surveys in the current study. Only the PCS and MCS scores were employed in analyses of HRQoL. The reliability and validity of this instrument, and its constituent scales, have been extensively reported.[346]

2.12 Documentation of Potential Confounding Clinical Factors

2.12.1 Peri-operative Pharmacotherapy

The peri-operative use of particular pharmacotherapeutic agents was recognised as a potential confounding factor in the analyses examining the influence of other patient characteristics on specific outcome measures. The optimal approach of considering the pre-operative use of these agents as exclusion criteria was not viable given the frequency of their use amongst the population being investigated. Furthermore, the establishment of a protocol of peri-operative care, preventing their use in the study period, was not ethically acceptable. Prospective documentation of their administration, and testing for their influence on relevant outcome measures was therefore undertaken.

Given that one of the ACCP/SCCM Consensus Conference criteria for defining SIRS is a heart rate exceeding 90 beats per minute,[100] (Table 1, Chapter 1) the administration of β-blockers was considered to be a factor with the potential to confound analyses relating to this outcome due to their negative chronotropic effect. Each cohort was thus dichotomised into those subjects who were administered β-blockers either pre-operatively, intra-operatively, or in the first five post-operative days, and into those who did not receive this class of drugs during this period.

In view of the anti-inflammatory and immunosuppressive properties of exogenous corticosteroid medications, the administration of this drug class was considered as being a potential confounding influence on analyses of the outcome measures of

SIRS, sepsis and occurrence of infection. For the purpose of testing this possibility, subjects in each cohort were dichotomised into those subjects who were administered exogenous oral or intravenous corticosteroids either pre-operatively, intra-operatively, or during the first five post-operative days, and into those who were not administered this class of drugs during the aforementioned period.

Whilst there is currently no available literature describing the pharmacological influence of the exogenously administered catecholamines adrenaline and noradrenaline on the urinary measures of the endogenously produced catecholamines, recently published analyses of data derived from a subset of the cohorts within the current study provided statistical evidence for a confounding effect of exogenously administered catecholamines on urinary measures of adrenaline and noradrenaline[373] . In view of this suggested confounding influence, the amount (in milligrams) of both adrenaline and noradrenaline administered during each 24-hour urine collection period was documented for each patient for the purpose of inclusion as a covariate in statistical analyses of urinary catecholamines.

2.13 Statistical Analyses

2.13.1 Overview of Statistical Analyses Performed

Prior to undertaking analyses of variables measured on ordinal or numerical scales, each variable's normality of distribution was determined. The measures of central tendency and dispersion used to describe skewed data are the median and range (minimum to maximum values). Nonparametric tests were employed for analyses involving variables with a skewed distribution, with several exceptions. Log_{10} transformations were applied to normalise skewed cytokine data prior to undertaking regression analyses, as described in section 2.13.2. In addition, neuroendocrine data was log_{10} transformed thus normalising the distributions prior to analyses with parametric tests, thereby enabling potential confounding factors to be entered as covariates, when necessary. Whilst the results of statistical analyses of neuroendocrine data presented herein have therefore been obtained following log transformation, for the purpose of clinical relevance, the measures of central tendency and dispersion presented for the neuroendocrine data refer to absolute, rather than log transformed data. The measures of central tendency and dispersion used to describe normally distributed data, as well as neuroendocrine data, are the mean and standard error of the mean (SEM). Parametric tests were employed for those analyses involving only normally distributed variables, inclusive of those subject to logarithmic transformation.

The relationship between two variables measured on nominal scales was examined using chi-square tests, or Fisher's exact tests in instances of small expected frequencies. Relationships between two variables measured on numerical scales were analysed using Pearson product moment correlations or nonparametric Spearman

rank correlations, as appropriate. Partial correlations were used for this purpose when a 'covariate' was included in the analysis. Comparison of two independent groups with regard to a single variable measured on an ordinal or numerical scale was performed using the un-paired Student's t test or its nonparametric counterpart, the Mann-Whitney U test, as appropriate. For analyses requiring the comparison of two independent groups with regard to a dependent numerical variable, when confounding factors, or covariates, required consideration in the analyses, one-way analysis of variance (ANOVA) was employed. Comparison of the same group of subjects with regard to a single normally distributed, or $\log_{10}$ transformed variable measured at two different time points was achieved using paired t tests, or repeated-measures ANOVA for analyses involving covariates.

One-way ANOVA was also employed for the purpose of comparing three groups with one independent variable, with regard to a dependent variable when the latter variable was normally distributed and the assumption of equal variances was met. In those instances where ANOVA resulted in a significant F test, post hoc comparisons were performed using Scheffe's procedure. When the dependent variable was skewed or the assumption of equal variances was not met, the Kruskal-Wallis test was employed to compare three groups with one independent variable. In those instances where the Kruskal-Wallis test was significant post hoc pair-wise comparisons were performed using the Mann-Whitney U test. Statistical significance was defined as $p<0.05$. Analyses were performed using SPSS, version 10.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA).

2.13.2 Relationship Between Cytokines and Sepsis

For the purpose of examining the relationship between subjects' cytokine profiles and the occurrence of sepsis, for every subject contributing to this data set, data for each cytokine assayed was reduced to three values: the pre-operative concentration, the maximum concentration and the average hourly cytokine concentration. The latter is a derived value obtained from a calculation of the area under the curve (AUC) for each of the six assayed cytokines, for each subject. Each AUC is calculated from a plot of concentrations of the relevant cytokine (y-axis) against sampling time (x-axis), the latter commencing from T_0 and terminating at $T_0 + 72$, that is 72 hours following T_0. The resulting AUC therefore represents total cytokine production over the 72 hour period from T_0. Dividing the AUC by 72, therefore yields a calculated value of the average hourly plasma cytokine concentration.

The capacity of the three values for each cytokine, namely the pre-operative concentration, the maximum concentration and the average hourly cytokine concentration, to predict the occurrence of sepsis was examined using a multiple regression model which took the form:

$$\log[p/(1-p)] = \beta_0 + \beta_1 \log(\text{av. hrly. conc.} +1) + \beta_2 \log(\text{maximum conc.} +1) + \beta_3 \log(\text{pre-op. conc.} +1)$$

where p is the probability of developing post-operative sepsis; β_0 is the constant regression coefficient which denotes the intercept of the regression line; and β_1, β_2, and β_3 are the regression coefficients associated with each of the respective independent variables. As indicated by the equation, logs were taken of the independent variables since they were highly skewed.

CHAPTER 3

RESULTS

3.1 Operative Outcomes

3.1.1 Operative and Post-operative Clinical Variables

For each of the three cohorts considered in this study, descriptive statistics of operative and post-operative clinical data, considered to be general markers of the severity of the operative insult and/or associated morbidity, are presented in Table 11. Results of Kruskal-Wallis tests demonstrated that there were significant differences between the three cohorts with respect to operative duration [$\chi^2_{K\text{-}W}$ (2) = 11.64, p = 0.003], intra-operative blood loss [$\chi^2_{K\text{-}W}$ (2) = 14.06, p = 0.001], total volume of blood transfused intra-operatively [$\chi^2_{K\text{-}W}$ (2) = 51.00, p< 0.0001], volume of blood transfused post-operatively [$\chi^2_{K\text{-}W}$ (2) = 6.84, p = 0.033], and the volume of crystalloid and/or colloid administered intra-operatively [$\chi^2_{K\text{-}W}$ (2) = 41.22, p<0.0001]. In addition, the duration of post-operative artificial ventilation differed significantly between the three groups [$\chi^2_{K\text{-}W}$ (2) = 25.62, p< 0.0001]. Post hoc pair-wise analyses were then performed using Mann-Whitney U tests, to identify which two cohorts differed significantly from each other with respect to each of the afore-mentioned clinical variables. These findings are reported in Table 11.

Table 11. Descriptive statistics and inter-cohort comparisons for selected intra- and post-operative clinical variables characterising the 'open', EVAR, and 'lower limb' cohorts.

Tabulated values are medians and ranges in parentheses.
Number of subjects (*n*) contributing data is indicated for each cohort/clinical variable combination.

†Result of post-hoc pair-wise analysis using Mann-Whitney U test, following demonstration of statistically significant difference between the three cohorts for all tabulated clinical variables by Kruskal-Wallis test.

	Open Aneurysm Repair	EVAR	Lower Limb Revascularisation	*p* value†		
				Open vs EVAR	Open vs Lower Limb	EVAR vs Lower Limb
Intra-operative variables						
Operative duration (mins)	207.5 (99 - 945) (*n*=36)	150 (115 - 235) (*n*=17)	215 (99-507) (*n*=17)	0.001	NS	0.022
Crystalloid/colloid infused (ml)	6000 (3052 - 16000) (*n*=36)	2000 (1000 - 5500) (*n*=17)	3500 (1500 - 6000) (*n*=17)	<0.0001	<0.0001	NS
Blood loss (ml)	2053 (732 - 14000) (*n*=24)	500 (0 - 1000) (*n*=2)	538 (100 - 2150) (*n*=9)	0.038	0.001	NS
Blood transfused intra-op. (ml)	1034.5 (1400 - 7200) (*n*=36)	0 (0 - 0) (*n*=17)	0 (0 - 1421) (*n*=17)	<0.0001	<0.0001	NS
Post-operative variables						
Blood transfused post-op (ml)	0 (0 - 3045) (*n*=35)	0 (0 - 299) (*n*=17)	0 (0 - 833) (*n*=17)	0.019	NS	NS
Duration mechanical ventilation (mins)	455 (0 - 8115) (*n*=35)	0 (0 - 1155) (*n*=17)	215 (90 - 507) (*n*=17)	<0.0001	<0.0001	NS

3.1.2 Outcome Measures of General Post-operative Morbidity and Mortality

Descriptive statistics for each of the principal outcome measures of general post-operative morbidity employed in this study are presented in Table 12 for each of the three cohorts. ICU LOS differed significantly between the three cohorts [$\chi^2_{K\text{-}W}$ (2) = 33.02, p <0.0001], as did the maximum APACHE II score attained [$\chi^2_{K\text{-}W}$ (2) = 26.44, p<0.0001]. A significant difference between the three cohorts was also identified with respect to post-operative hospital LOS [F (2, 65) = 18.95, p<0.0001]. The results of post-hoc comparisons, performed using the Mann-Whitney U test or Scheffe's procedure as appropriate, are presented in Table 12, indicating which particular cohort differed from another, for each of the afore-mentioned outcome measures. This table also reports the incidence of one or more moderate/severe complications, as defined in Chapter 2, Section 2.11.4.1, amongst each cohort. The occurrence of this adverse outcome was significantly associated with the category of vascular intervention, that is open AAA repair, EVAR or lower limb revascularisation, performed (Table 12).

Of potential relevance to the validity of the urinary measures of neuroendocrine response is the observation that no subject experienced renal complications of either a moderate (SVS/ISCVS grade 2) or severe (SVS/ISCVS grade 3) nature. Furthermore, only two members (5.6%) of the 'open' cohort and one member (5.9%) of the 'lower limb' cohort experienced mild (SVS/ISCVS grade 1) post-operative renal insufficiency.

A 30-day mortality rate of 4.3% was recorded amongst the collective cohort (n=70) involved in the current study. Two of the non-surviving subjects were female, had a TAAA and were classified as belonging to the 'open' cohort for the purposes of this study (see Chapter 2, Section 2.2.4.2), however their respective aneurysm repairs were performed by different vascular surgeons, at different institutions. One of these subjects experienced a particularly prolonged procedure, lasting 15 hours and 45 minutes, due to difficulties with deployment of a customised fenestrated endograft which necessitated a laparotomy and an aortic-SMA bypass for management of deployment-induced colonic ischaemia. DIC, associated with massive intra-operative haemorrhage, culminated in death six hours and five minutes following skin closure. The other death within this cohort of patients occurred intra-operatively during a hybrid EVAR/open procedure, eight hours and 58 minutes after commencement of the procedure. Major anastomotic haemorrhage, in the setting of coagulopathy with left ventricular decompensation, resulted in the death of this subject. The deaths of these subjects early in the study therefore diminished the size (n) of the 'open' cohort contributing data to many of the analyses presented herein. A third male subject died 21 days following an open AAA repair as a result of a myocardial infarction, preceded by respiratory failure arising from pneumonia in the setting of severe chronic obstructive pulmonary disease. The occurrence of pneumonia resulted in this subject being categorised as having sepsis.

Table 12. Descriptive statistics and inter-cohort comparisons for principal outcome measures of general post-operative morbidity amongst the 'open', EVAR, and 'lower limb' cohorts.

ICU, intensive care unit; LOS, length of stay.

Number of subjects (*n*) contributing data is indicated for each cohort/clinical variable combination.

†Tabulated data are median and range in parentheses; post-hoc statistical comparison between cohorts performed using Mann-Whitney U tests.

‡Tabulated data are means and SEM in parentheses; post-hoc statistical comparison between cohorts performed using Scheffe's procedures.

§Tabulated data are absolute numbers with percentage of cohort in parentheses; statistical comparison between cohorts performed using Pearson's chi-square test (2 x 3 contingency table).

	Open Aneurysm Repair	EVAR	Lower Limb Revascularisation	*p* value		
				Open vs EVAR	Open vs Lower Limb	EVAR vs Lower Limb
ICU LOS (days)[†]	1.23 (0 - 6.99) (*n*=35)	0 (0 - 0.96) (*n*=17)	0 (0 - 1.19) (*n*=17)	<0.0001	<0.0001	NS
Maximum APACHE II score[†]	17 (10 - 27) (*n*=35)	11 (8 - 18) (*n*=17)	10 (4 - 20) (*n*=17)	<0.0001	<0.0001	NS
Post-operative LOS (days)[‡]	11.12 (0.69) (*n*=34)	4.47 (0.70) (*n*=17)	8.18 (0.88) (*n*=17)	<0.0001	0.031	0.017
One or more moderate/severe complications[§]	27 (75.0%) (*n*=36)	4 (23.5%) (*n*=17)	2 (11.8%) (*n*=17)		0.00001	

3.1.3 Outcome Measures of SIRS, Sepsis and Infection

The incidence of SIRS amongst the surviving members of the 'open', EVAR and 'lower limb' cohorts was 88.2%, 47.1%, and 70.6% respectively (Figure 2). Chi-square analysis (2 x 3 contingency table) confirmed the significant association of SIRS occurrence with procedure type [χ^2 (2) = 9.97, p = 0.007]. Accordingly, cumulative SIRS scores also differed significantly between the three cohorts [$\chi^2_{K\text{-}W}$ (2) = 11.10, p = 0.004]. Figure 3 depicts the cumulative SIRS scores characterising each cohort and reports the findings of post hoc analyses, demonstrating between which cohorts the significant difference in cumulative SIRS scores lay. The cohorts also differed significantly with respect to mean duration of SIRS [F (2, 65) = 7.25, p = 0.001]. Using Scheffe's procedures for post hoc analyses, the 'open' cohort was identified as having a significantly longer mean duration of SIRS than those undergoing EVAR (p = 0.002), whilst the 'lower limb' cohort's mean duration of SIRS did not differ significantly from the other two cohorts (Figure 4).

The incidence of sepsis amongst the 'open', EVAR and 'lower limb' cohorts was 50%, 5.9% and 11.8% respectively (Figure 5). Sepsis occurrence was significantly associated with procedure type [χ^2 (2) = 14.03, p = 0.0009, 2 x 3 contingency table]. Amongst the 17 subjects in the 'open' cohort categorised as having developed sepsis, the aetiology was pulmonary in 16 cases. Two of these subjects fulfilled the criteria for sepsis on the basis of a second source of documented and/or suspected infection, namely post-operative oesophageal candidiasis and a peri-graft collection considered sufficiently suspicious to warrant long-term antibiotic therapy. Post-operative *Escherichia coli* septicaemia accounted for the diagnosis of sepsis in one subject in this cohort. A pulmonary aetiology accounted for all cases of sepsis amongst the

EVAR and 'lower limb' cohorts. It is noteworthy that both subjects categorised as having sepsis in the 'lower limb' cohort underwent supra-inguinal revascularisation procedures.

The incidence of post-operative infection, irrespective of the occurrence of a systemic inflammatory response, was 52.9%, 17.6%, and 35.3% amongst the 'open', EVAR and 'lower limb' cohorts respectively. Chi-square analysis (2 x 3 contingency table) demonstrated an association between the occurrence of a post-operative infection and the type of operative intervention that reached statistical significance [χ^2 (2) = 6.08, p = 0.048]. The excess rate of infection, beyond that described for sepsis, was accounted for amongst the 'open' cohort by an additional case of pneumonia, whilst amongst the EVAR cohort, an additional case of pneumonia in one subject and a superficial wound infection of unspecified microbiological aetiology in another accounted for the infection rate. Amongst the 'lower limb' cohort, an additional four subjects developed post-operative superficial wound infections, two of which were attributed to *Staphylococcus aureus,* being methicillin resistant in one case and methicillin sensitive in the other. Mixed coliforms accounted for one wound infection in this cohort, whilst the aetiological organism(s) were unspecified in the fourth case.

Figure 2. Incidence (%) of post-operative SIRS in the 'open', EVAR and 'lower limb' cohorts.

SIRS incidence was significantly associated with procedure category (cohort) (p = 0.007, 2 x 3 contingency table).

Figure 3. Box and whisker plot of cumulative SIRS scores in the 'open', EVAR and 'lower limb' cohorts.

Plot presents median, first (25%) and third (75%) quartiles, and range of data for each cohort.

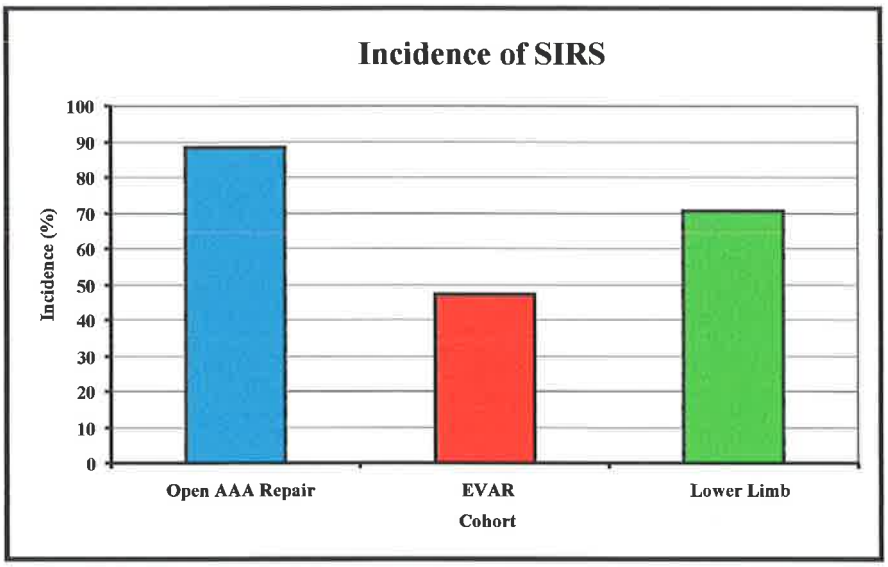
*p = 0.031 (post hoc comparison using Mann-Whitney U test)

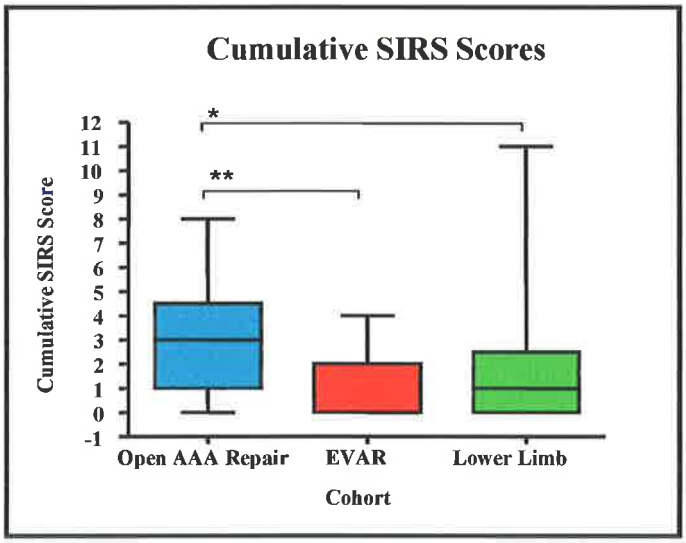
**p = 0.002 (post hoc comparison using Mann-Whitney U test)

Figure 4. Mean duration of SIRS in the 'open', EVAR and 'lower limb' cohorts.

Values are mean (SEM).

*'Open' differs significantly from EVAR cohort, p = 0.002 (post hoc comparison using Scheffe's procedure).


Incidence of SIRS
Incidence (%)
100
90
80
70
60
50
40
30
20
10
0
Open AAA Repair
EVAR
Lower Limb
Cohort


Cumulative SIRS Scores
Cumulative SIRS Score
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
*
**
Open AAA Repair
EVAR
Lower Limb
Cohort

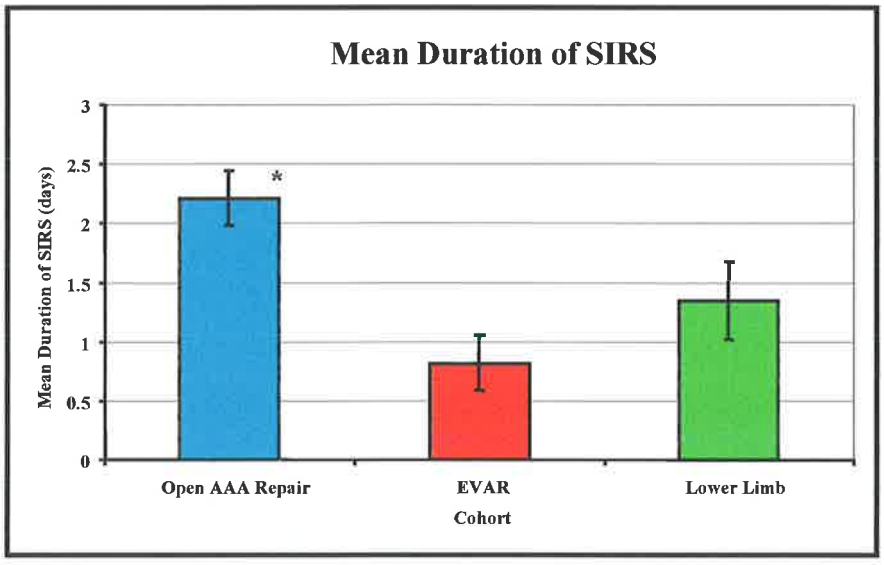
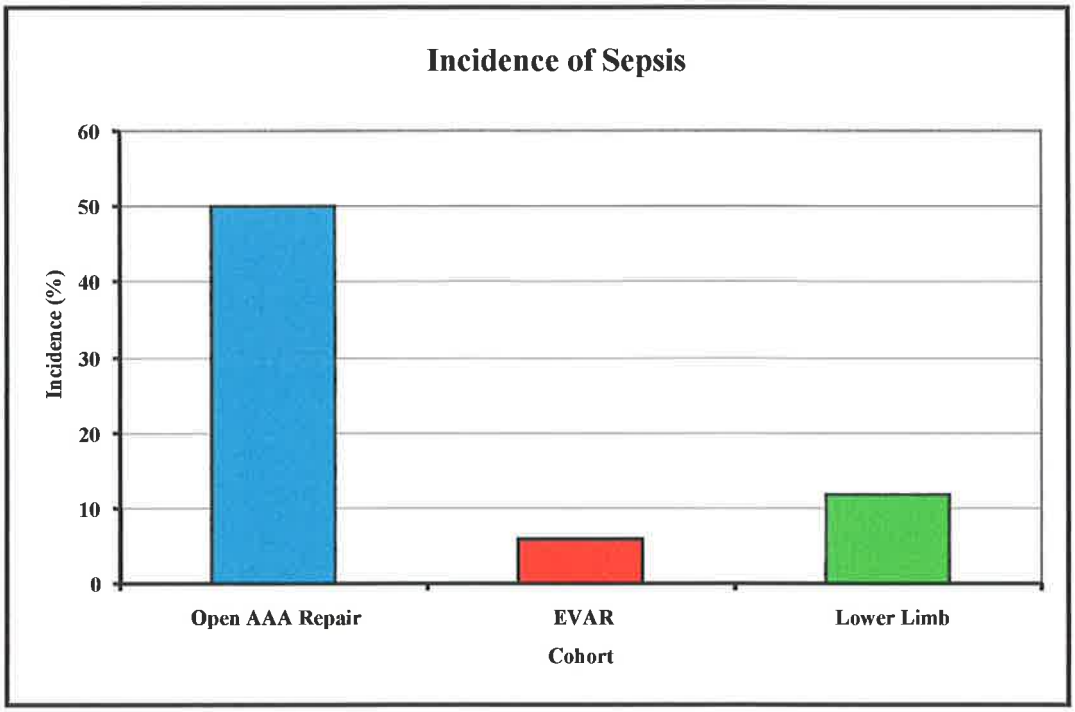

Mean Duration of SIRS
Mean Duration of SIRS (days)
3
2.5
2
1.5
1
0.5
0
*
Open AAA Repair
EVAR
Lower Limb
Cohort

Figure 5. Incidence (%) of post-operative sepsis in the 'open', EVAR and 'lower limb' cohorts.



Incidence of sepsis was significantly associated with procedure category (cohort) (p = 0.0009, 2 x 3 contingency table).

3.1.4 Potential Confounding Factors

3.1.4.1 β-Blocker Administration

The potential influence of β-blocker use, whether administered pre-operatively, intra-operatively, and/or within the first 5 post-operative days, on the classification of presence or absence of SIRS was examined within each of the three operative cohorts. The incidence of administration of this drug class in each cohort is summarised in Table 6, Chapter 1. Statistical analysis demonstrated that within the 'open' cohort, there was no significant effect of β-blocker use on the classification of SIRS as either present or absent ($p = 0.28$, two-tailed Fisher's exact test). The lack of a significant association between β-blocker use and SIRS classification was also apparent for both the EVAR and 'lower limb' cohorts ($p = 0.16$ and $p = 1.00$ respectively, two-tailed Fisher's exact tests). This lack of association was confirmed when all subjects were considered as belonging to one collective cohort [χ^2 (1) = 0.07, p = 0.79].

3.1.4.2 Corticosteroid Administration

Oral or IV corticosteroids use, whether pre-operatively, intra-operatively, and/or within the five post-operative days, occurred amongst four (11.8%) of the surviving members of the 'open' cohort, only two (11.8%) of the 'lower limb' cohort, whilst no member of the EVAR cohort received this medication during the relevant period. The influence of oral or IV corticosteroids on the presence or absence of SIRS, sepsis within 30 post-operative days, and on the presence or absence of infection within 30 post-operative days was therefore examined for the 'open AAA' and 'lower limb' cohorts. Amongst the 'open AAA' cohort, statistical analysis (two-tailed Fisher's

exact tests) demonstrated no relationship between corticosteroid use and the presence or absence of SIRS (p = 1.00), the development of sepsis (p = 0.10) or on the occurrence of infection (p =0.11). Statistical analysis (two-tailed Fisher's exact tests) similarly demonstrated the lack of an influence of corticosteroid use on the occurrence of SIRS (p = 1.00), sepsis (p = 0.23), or infection (p = 0.11) amongst the 'lower limb' cohort.

3.2 Operative Duration and Duration of Ischaemia - Association with Clinical Outcomes

Analyses of the relationships between the two operative variables, 'operative duration' and 'duration of ischaemia', and clinical outcome measures were performed by separately considering the 'open', EVAR and 'lower limb' cohorts.

3.2.1 Associations with SIRS

As reported in Table 13, the correlations between 'operative duration' and both cumulative SIRS score and SIRS duration were weak and not statistically significant. Similarly, the correlations between 'duration of ischaemia' and measures of SIRS severity within the 'open' cohort were weak and non-significant (Table 13).

3.2.3 Associations with Sepsis

As detailed in Table 14, neither the duration of operative procedures nor the duration of ischaemia differed significantly between those who developed sepsis and those who did not.

3.2.3 Associations with Measures of General Post-operative Morbidity

Amongst the 'open' cohort, there was a moderate, positive and statistically significant association between 'operative duration' and the maximum APACHE II score attained. Within this cohort, the weak, positive associations between this operative variable and the outcome measures of ICU LOS and post-operative LOS

approached but did not reach significance. Within the EVAR cohort, however, the positive association between 'operative duration' and post-operative LOS was moderate and significant, whilst the association between 'operative duration' and both the maximum APACHE II score and ICU LOS were weak and not statistically significant. Weak and non-significant associations were also demonstrated between 'operative duration' and all of the aforementioned measures of general post-operative morbidity within the 'lower limb' cohort (Table 13). It is apparent from Table 14 that the duration of the operative intervention failed to distinguish between those subjects who subsequently developed one or more moderate/severe complications and those who did not develop this adverse post-operative outcome within each of the cohorts.

Amongst the 'open' cohort, 'duration of ischaemia' was moderately, positively and significantly associated with both the maximum APACHE II score and post-operative LOS, however, the positive correlation between this operative variable and ICU LOS was weak and non-significant (Table 13). The mean 'duration of ischaemia' did not differ between those who developed one or more moderate/severe complications, and those who did not (Table 14).

Table 13. Correlations between operative variables and measures of SIRS severity and general post-operative morbidity within the 'open', EVAR and 'lower limb' cohorts.

ICU, intensive care unit; LOS, length of stay; mins; minutes

Tabulated values are Spearman rank correlations (r_s), unless otherwise indicated, with associated statistical significance (p value) in parentheses. The actual p value is reported only when the correlation was statistically significant or approached significance at the 0.05 level (two-tailed).

The number of subjects contributing data (n) is reported for each correlation.

Insufficient data on 'duration of ischaemia' was available for meaningful correlations within the 'lower limb' cohort.

†Pearson product moment correlations (r) with statistical significance (p value) in parentheses.

	Cumulative SIRS Score	SIRS Duration (days)	ICU LOS (days)	Maximum APACHE II Score	Post-operative LOS (days)
Operative Duration (mins)					
Open	0.141 (NS) (*n*=34)	0.179 (NS) (*n*=34)	0.322 (0.059) (*n*=35)	0.404 (0.016) (*n*=35)	0.307 (0.077) (*n*=34)
EVAR	0.222 (NS) (*n*=17)	0.248 (NS) (*n*=17)	–0.128 (NS) (*n*=17)	0.002 (NS) (*n*=17)	0.502 (0.040) (*n*=17)
Lower Limb	0.059 (NS) (*n*=17)	0.119 (NS) (*n*=17)	0.201 (NS) (*n*=17)	0.324 (NS) (*n*=17)	0.316 (NS) (*n*=17)
Duration of Ischaemia (mins)					
Open	0.255 (NS) (*n*=29)	0.225 (NS)† (*n*=29)	0.267 (NS) (*n*=29)	0.412 (0.026)† (*n*=29)	0.483 (0.008)† (*n*=29)

Table 14. Association between operative variables and both the development of sepsis and the occurrence of one or more moderate/severe complications within the 'open', EVAR, and 'lower limb' cohorts.

	Sepsis			≥1 Moderate/Severe Complications		
	Present	Absent	*p* value	Present	Absent	*p* value
Operative Duration (mins)†						
Open	200 (99 - 455) (*n*=17)	205 (137 - 350) (*n*=17)	NS	210 (99 - 945) (*n*=27)	190 (137 - 455) (*n*=9)	NS
EVAR	180 (180 - 180) (*n*=1)	150 (115 - 235) (*n*=16)	NS	160 (150 - 180) (*n*=4)	145 (115 - 235) (*n*=13)	NS
Lower Limb	270 (210 - 330) (*n*=2)	215 (90 - 507) (*n*=15)	NS	230 (210 - 250) (*n*=2)	215 (90 - 507) (*n*=15)	NS
Duration of Ischaemia (mins)‡						
Open	59.69 (5.48) (*n*=16)	58.15 (4.89) (*n*=13)	NS	58.70 (4.47) (*n*=23)	60.00 (4.43) (*n*=7)	NS

mins, minutes

Number of subjects contributing data to each group (*n*) is reported.

† Tabulated values are medians and range in parentheses; statistical difference between groups analysed using Mann Whitney U test.

‡ Tabulated values are means and SEM in parentheses; statistical difference between groups analysed using Student's t test.

3.3 Immunological Parameters

3.3.1 Pre-operative PMN CD11b, FcγRI (CD64) and FcγRIIIb (CD16b) Expression and Associations with Clinical Outcomes

Figure 6 presents representative density plots, depicting size and granularity of cell populations, and associated histograms of fluorescence events, generated by WinMDI 2.8 following flow cytometry analysis of suspended leukocyte pellets labelled with mouse anti-human CD11b: RPE, mouse anti-human CD64: FITC, mouse anti-human CD16: FITC and mouse IgG1: FITC/RPE negative control respectively.

Analyses of PMN expression of immunoglobulin G Fc receptors and the β_2-integrin sub-unit, CD11b, were performed by separately considering the 'open', EVAR and 'lower limb' cohorts.

Figure 6. Representative WinMDI 2.8 density plots and corresponding frequency histograms of fluorescence (FL1 or FL2) events measuring mean fluorescence intensity (MFI) for CD11b, FcγRI (CD64), FcγRIIIb (CD16b) and the negative control for a single subject.

MFI, mean fluorescence intensity

Manually gated PMN region is shown on each density plot which depicts size and granularity of cell population.

A. CD11b density plot

B. CD11b frequency histogram of fluorescence (FL2) events

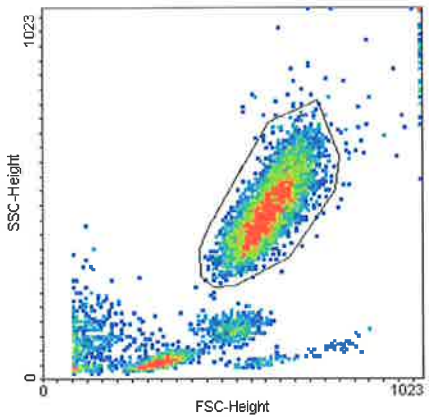
C. FcγRI (CD64) density plot

D. FcγRI (CD64) frequency histogram of fluorescence (FL1) events

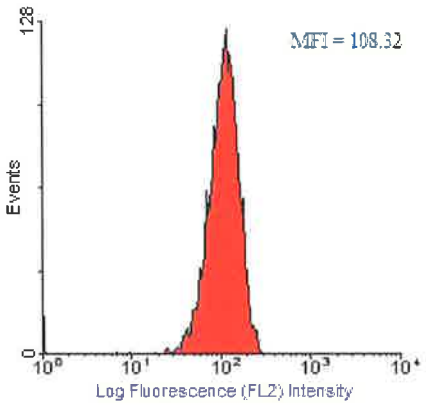
E. FcγRIIIb (CD16b) density plot

F. FcγRIIIb (CD16b) frequency histogram of fluorescence (FL1) events

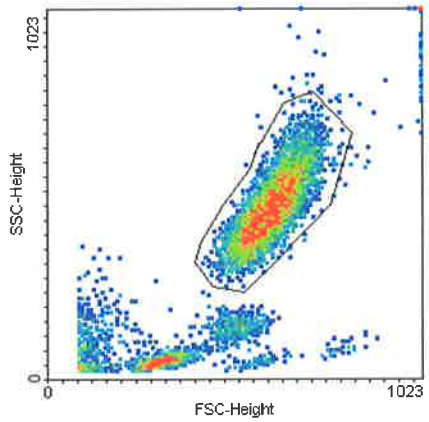
A.


1023
SSC-Height
0
0
FSC-Height
1023

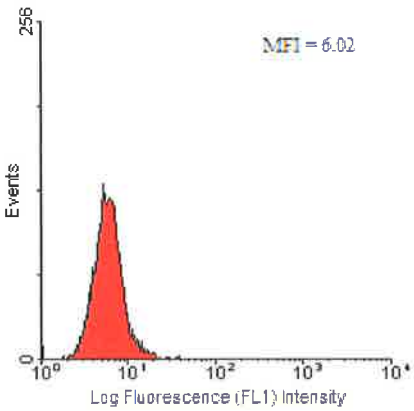
B.


128
MFI = 108.32
Events
0
10^0
10^1
10^2
10^3
10^4
Log Fluorescence (FL2) Intensity

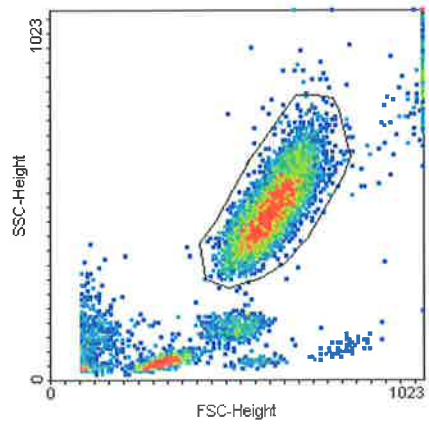
C.


1023
SSC-Height
0
0
FSC-Height
1023

D.


256
MFI = 6.02
Events
0
10^0
10^1
10^2
10^3
10^4
Log Fluorescence (FL1) Intensity

E.


1023
SSC-Height
0
0
FSC-Height
1023

F.

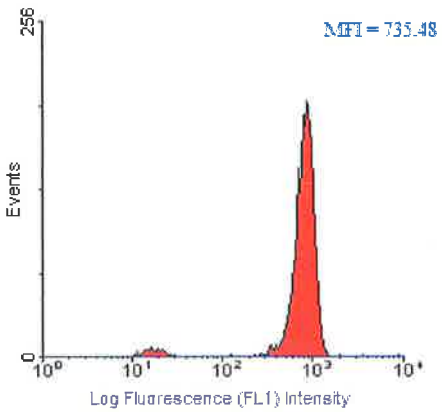

256
MFI = 735.48
Events
0
10^0
10^1
10^2
10^3
10^4
Log Fluorescence (FL1) Intensity

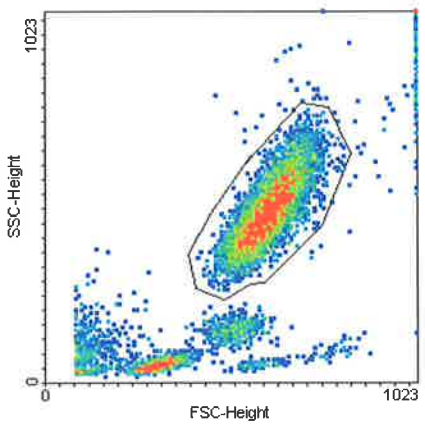
Figure 6. Continued.

G. Negative control density plot

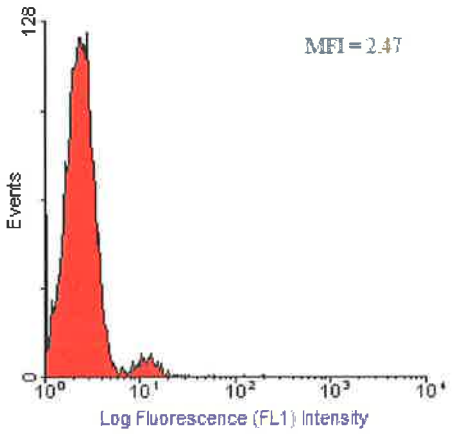
H. Negative control frequency histogram of fluorescence (FL1) events

I. Negative control frequency histogram of fluorescence (FL2) events

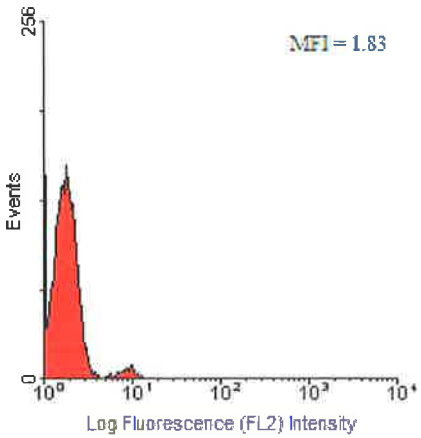
G.


1023
SSC-Height
0
0
1023
FSC-Height

II.


128
MFI = 2.47
Events
0
10⁰
10¹
10²
10³
10⁴
Log Fluorescence (FL1) Intensity

I.


256
MFI = 1.83
Events
0
10⁰
10¹
10²
10³
10⁴
Log Fluorescence (FL2) Intensity

3.3.1.1 Associations with SIRS

As detailed in Table 15, there was a moderate, positive correlation between pre-operative CD11b expression and cumulative SIRS score within the EVAR cohort. It is also noteworthy that within this cohort there was a positive, moderate correlation between CD11b expression and SIRS duration which approached, but did not reach statistical significance. The correlations between these same variables within the 'open' and 'lower limb' cohorts were, however, weak and clearly not statistically significant. The correlations for pre-operative expression of FcγRI (CD64) and FcγRIIIb (CD16b) with measures of SIRS severity were similarly weak and non-significant amongst all operative cohorts (Table 15).

3.3.1.2 Associations with Sepsis

Table 16 reports that there were no significant differences in pre-operative PMN expression of either CD11b, FcγRI (CD64), or FcγRIIIb (CD16b) amongst those who developed sepsis compared with those who did not develop this complication, within any of the operative cohorts.

Table 15. Pearson product moment (r) and Spearman rank correlations (r_s) correlations for pre-operative expression (MFI) of selected polymorphonuclear leukocyte integrin and immunoglobulin G Fc receptors (FcγR) with measures of post-operative SIRS severity within the 'open, EVAR and 'lower limb' cohorts.

	CD11b	CD64 (FcγRI)	CD16b (FcγRIIIb)
Cumulative SIRS Score			
Open	r_s = 0.218 (NS) (n=31)	r_s = –0.082 (NS) (n=24)	r_s = –0.006 (NS) (n=31)
EVAR	r_s = 0.539 (0.047) (n=14)	r_s = –0.266 (NS) (n=14)	r_s = –0.111 (NS) (n=15)
Lower Limb	r_s = –0.021 (NS) (n=16)	r_s = 0.031 (NS) (n=12)	r_s = 0.189 (NS) (n=16)
SIRS Duration (days)			
Open	r = 0.110 (NS) (n=31)	r_s = –0.024 (NS) (n=24)	r = 0.015 (NS) (n=31)
EVAR	r = 0.491 (0.075) (n=14)	r_s = –0.308 (NS) (n=14)	r = –0.147 (NS) (n=15)
Lower Limb	r = 0.119 (NS) (n=16)	r_s = –0.036 (NS) (n=12)	r = –0.039 (NS) (n=16)

The number of subjects contributing data (n) is reported for each correlation.

The actual p value is reported only when the correlation was statistically significant or approached significance at the 0.05 level (two-tailed).

Table 16. Comparison of pre-operative expression (MFI) of selected polymorphonuclear leukocyte integrin and immunoglobulin G Fc receptors (FcγR) between subjects who developed sepsis and those who did not, within the 'open', EVAR and 'lower limb' cohorts.

NS, not significant

The number of subjects contributing data (*n*) is reported for each group.

Actual *p* values are not reported as differences between group values did not approach significance at the 0.05 level.

Data was insufficient for meaningful comparison of CD11b expression amongst members of the EVAR cohort.

†Tabulated values are means and SEM in parentheses; statistical difference between 'sepsis-present' and 'sepsis-absent' groups analysed using Student's t test.

‡Tabulated values are medians and range in parentheses; statistical difference between 'sepsis-present' and 'sepsis-absent' groups analysed using Mann Whitney U test.

	Sepsis		
	Present	Absent	*p* value
CD11b[†]			
Open	94.58 (8.70) (*n*=16)	82.98 (4.670) (*n*=15)	NS
Lower Limb	97.74 (15.13) (*n*=2)	82.68 (6.26) (*n*=14)	NS
CD64 (FcγRI)[‡]			
Open	4.35 (2.25 - 8.92) (*n*=14)	3.76 (3.09 - 6.11) (*n*=10)	NS
EVAR	3.68 (3.68 - 3.68) (*n*=1)	4.21 (3.02 - 7.14) (*n*=13)	NS
Lower Limb	5.22 (2.79 - 7.64) (*n*=2)	5.09 (3.14 - 17.66) (*n*=10)	NS
CD16b (FcγRIIIb)[†]			
Open	564.82 (43.55) (*n*=16)	522.33 (43.55) (*n*=15)	NS
EVAR	519.41 (*n*=1)	543.16 (27.66) (*n*=14)	NS
Lower Limb	570.80 (32.81) (*n*=2)	637.66 (28.82) (*n*=14)	NS

3.3.2 FcγR Genotypes and Associations with Clinical Outcomes

Sample size considerations prohibited FcγR genotype analyses being performed separately for each operative cohort. Subjects were therefore considered as belonging to a single, collective cohort for the purpose of presentation and analysis of data pertaining to FcγR genotypes.

3.3.2.1 FcγRIIa (CD32a) Genotypes and Association with Clinical Outcome

Representative results from gel electrophoresis of the allele-specific PCR for CD32a genotyping of four patients, run in duplicate, are shown in Figure 7. Determination of CD32a genotype was not possible for 12 of the 70 patients comprising the cumulative cohort involved in the study due to operative intervention prior to establishment of this PCR technique. The distribution of CD32a genotypes (R131/R131 : H131/H131 : H131/R131) amongst the remaining cohort of 58 patients, inclusive of non-survivors, was 21% : 24 % : 55%. Deviation of the observed genotype distribution from that expected amongst a population in Hardy-Weinberg equilibrium was statistically insignificant [χ^2 (1) = 0.64, $0.25<p<0.50$]. That is to say, this data followed Hardy Weinberg's equilibrium.

Included in Table 17 are CD32a genotype frequencies amongst those who developed sepsis and those who did not within the collective cohort (n = 56), excluding non-survivors. There was no statistically significant association between CD32a genotype and the development of sepsis [χ^2 (2) = 1.38, p = 0.50]. In particular, there was no

trend towards a greater frequency of sepsis amongst CD32a R131 homozygotes. Table 17 also reports the distribution of CD32a genotypes amongst those who developed an infectious process compared with those who did not within the collective cohort (n = 56). Once again, there was no significant association between CD32a genotype and the development of infection [χ^2 (2) = 1.17, p = 0.557]. Specifically, this data demonstrates no trend towards a greater frequency of infection amongst CD32 R131 homozygotes.

Figure 7. Gel electrophoresis for FcγRIIa (CD32a) genotyping by sequence specific polymerase chain reaction (PCR-SSP).

Representative results from four subjects in duplicate. Upper panel depicts wells loaded with H131-specific PCR products. Lower panel depicts wells loaded with R131-specific PCR products. For any given lane, allocated to an individual subject, presence of a 253 base pair (bp) CD32a gene amplification product in both the upper panel, corresponding to the H131-specific PCR, and lower panel, corresponding to the R131-specific PCR, indicates CD32a heterozygosity. Lanes containing a CD32a gene amplification product in only the upper panel or the lower panel indicate homozygosity for the H131, or R131 allele respectively. Lane allocations and CD32a genotypes are as follows:

Lane 1: 1kb DNA ladder (upper and lower panels)
Lane 2: pUC 19 DNA ladder (upper and lower panels)
Lane 3: 'Hmix' negative control ('Hmix' without DNA, upper panel); 'Rmix' negative control ('Rmix' without DNA, lower panel)
Lane 4: Subject A - H131/R131 heterozygote
Lane 5: Subject A in duplicate
Lane 6: Subject B - H131/R131 heterozygote
Lane 7: Subject B in duplicate
Lane 8: Subject C - H131 homozygote
Lane 9: Subject C in duplicate
Lane 10: Subject D - H131/R131 heterozygote
Lane 11: Subject D in duplicate.

The combination of absence of the 439 bp human growth hormone (HGH) gene amplification product from lane 3 (negative controls, upper and lower panels) and presence of the product in lanes 4 to 11 (upper and lower panels) indicates success of the PCR process.

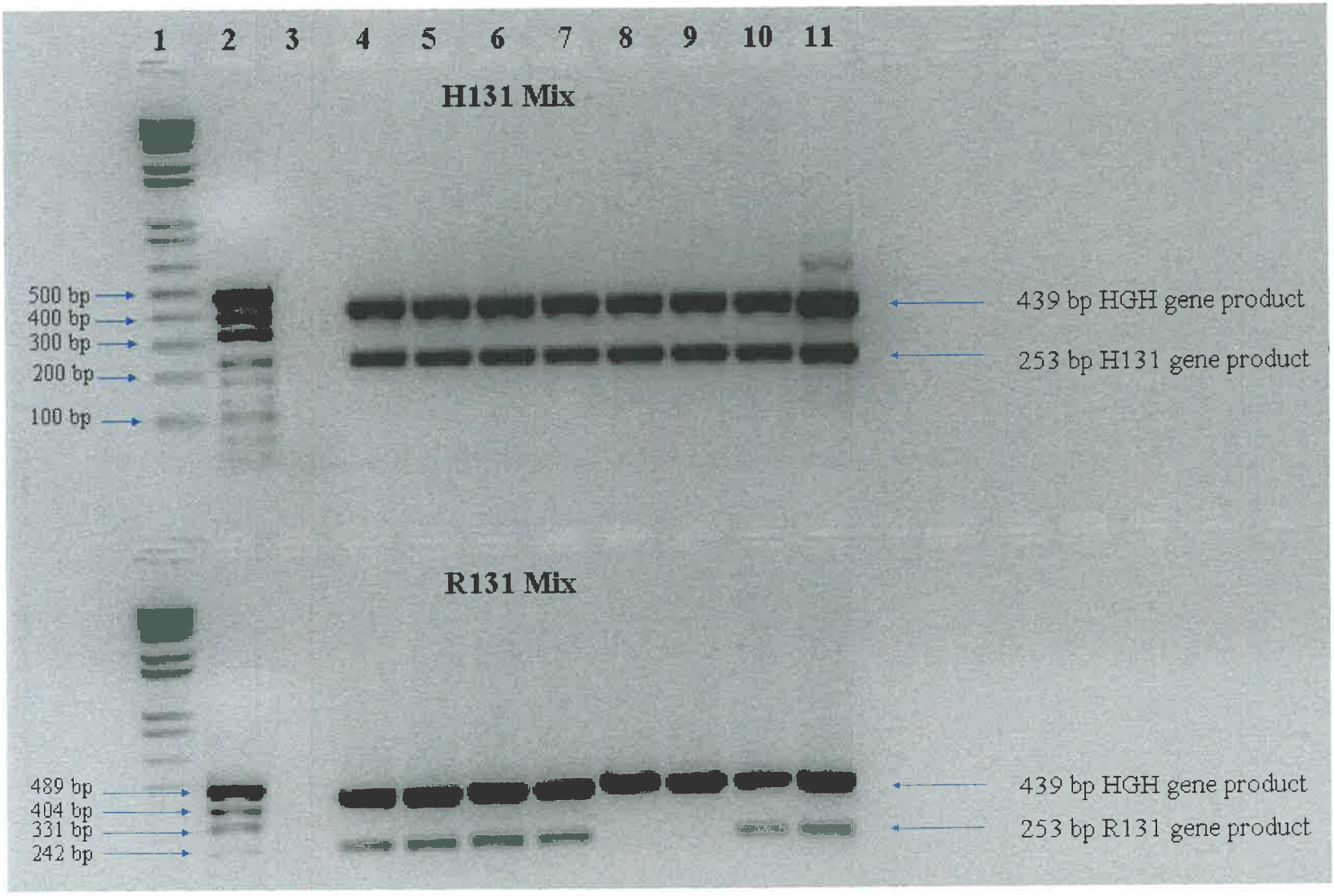

1 2 3 4 5 6 7 8 9 10 11
H131 Mix
500 bp
400 bp
300 bp
200 bp
100 bp
439 bp HGH gene product
253 bp H131 gene product
R131 Mix
489 bp
404 bp
331 bp
242 bp
439 bp HGH gene product
253 bp R131 gene product

Table 17. FcγRIIa (CD32a) genotype distributions according to the presence or absence of post-operative sepsis, and the presence or absence of post-operative infection within the collective cohort.

	FcγRIIa (CD32a) Genotypes (n = 56)			
	R131/R131	H131/H131	H131/R131	p value
Post-operative sepsis				
Present	3 (5.4%)	3 (5.4%)	12 (21.4%)	0.50†
Absent	9 (16.1%)	10 (17.9%)	19 (33.9%)	
Post-operative infection				
Present	5 (21.4%)	4 (7.1%)	15 (26.8%)	0.56†
Absent	7 (12.5%)	9 (16.1%)	16 (28.6%)	

Tabulated values are absolute subject numbers with percentage of total collective cohort (n=56) in parentheses.

†Statistical association between post-operative outcome and FcγRIIa genotype determined using Pearson's chi-square test (2 x 3 contingency table).

3.3.2.2 FcγRIIIb (CD16b) Genotypes and Association with Clinical Outcome

Representative results from gel electrophoresis of the allele-specific PCR for CD16b genotyping of four patients, run in duplicate, are shown in Figure 8. Determination of CD16b genotype was not possible for four of the 70 patients comprising the cumulative cohort involved in the study due to operative intervention prior to establishment of this PCR technique. The distribution of CD16b genotypes (NA1/NA1 : NA2/NA2 : NA1/NA2) amongst the remaining cohort of 66 patients, inclusive of non-survivors, was 17% : 33 % : 50%. This genotype distribution did not differ significantly from that predicted by the Hardy Weinberg equilibrium [χ^2 (1) = 0.054, $0.75<p<0.90$]. That is to say, this data followed Hardy Weinberg's equilibrium.

Table 18 reports CD16b genotype distributions according to the presence or absence of sepsis, and the presence or absence of infection within the collective cohort (n = 64). This data revealed no statistical association between CD16b genotype and the occurrence of either sepsis [χ^2 (2) = 1.66, p = 0.44] or post-operative infection [χ^2 (2) = 0.98, p = 0.61]. More specifically, genotype distributions did not reveal a trend towards a greater incidence of either post-operative sepsis or infection amongst NA2 homozygotes.

Figure 8. Gel electrophoresis for FcγRIIIb (CD16b) genotyping by sequence specific polymerase chain reaction (PCR-SSP).

Representative results from four subjects, in duplicate. Upper panel depicts wells loaded with NA1-specific PCR products. Lower panel depicts wells loaded with NA2-specific PCR products. For any given lane, allocated to an individual subject, presence of both the 141 base pair (bp) NA1-allele amplification product in the upper panel and the 219 bp NA2-allele amplification product in the lower panel indicates CD16b heterozygosity. Lanes containing a CD16b gene amplification product in only the upper panel or the lower panel indicate homozygosity for the NA1, or the NA2 allele respectively. Lane allocations and CD16b genotypes are as follows:

Lane 1: 1kb DNA ladder (upper and lower panels)
Lane 2: pUC 19 DNA ladder (upper and lower panels)
Lane 3: Blank lane
Lane 4: 'NA1 mix' negative control ('NA1 mix' without DNA, upper panel); 'NA2 mix' negative control ('NA2 mix' without DNA, lower panel)
Lane 5: Subject a - NA2 homozygote
Lane 6: Subject a in duplicate
Lane 7: Subject b - NA2 homozygote
Lane 8: Subject b in duplicate
Lane 9: Subject c - NA1 homozygote
Lane 10: Subject c in duplicate
Lane 11: Subject d - NA1/NA2 heterozygote
Lane 12: Subject d in duplicate

The combination of absence of the 439 bp human growth hormone (HGH) gene amplification product from lane 4 (negative controls, upper and lower panels) and presence of the product in lanes 5 to 12 (upper and lower panels) indicates success of the PCR process.

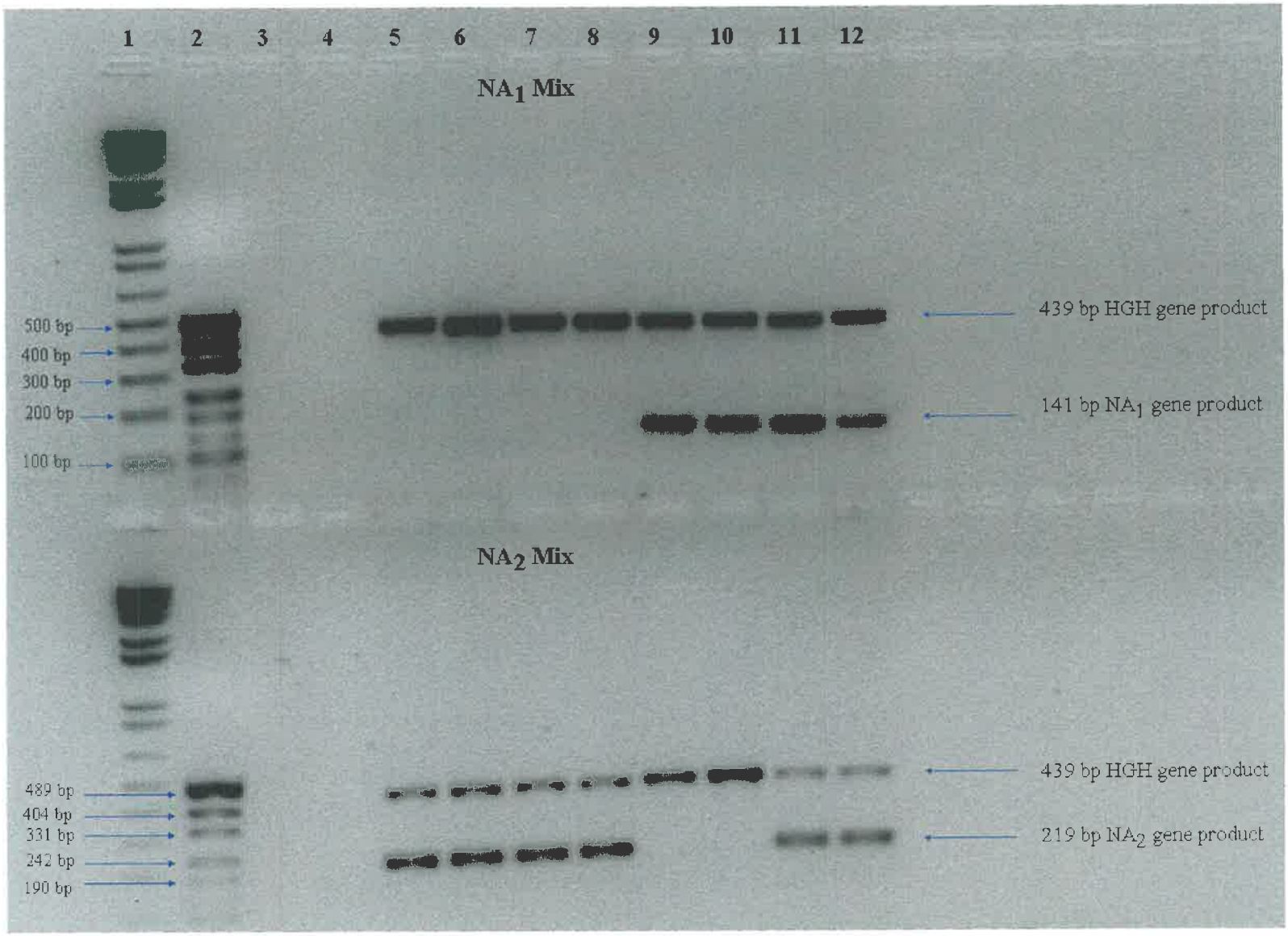

1 2 3 4 5 6 7 8 9 10 11 12
NA1 Mix
500 bp
400 bp
300 bp
200 bp
100 bp
439 bp HGH gene product
141 bp NA1 gene product
NA2 Mix
489 bp
404 bp
331 bp
242 bp
190 bp
439 bp HGH gene product
219 bp NA2 gene product

Table 18. FcγRIIIb (CD16b) genotype distributions according to the presence or absence of post-operative sepsis, and the presence or absence of post-operative infection within the collective cohort.

	FcγRIIIb (CD16b) Genotypes (n = 64)			
	NA1/NA1	NA2/NA2	NA1/NA2	p value
Post-operative sepsis				
Present	3 (4.7%)	8 (12.5%)	7 (10.9%)	0.44†
Absent	8 (12.5%)	13 (20.3%)	25 (39.1%)	
Post-operative infection				
Present	4 (6.3%)	10 (15.6%)	11 (17.2%)	0.61†
Absent	7 (10.9%)	11 (17.2%)	21 (32.8%)	

Tabulated values are absolute subject numbers with percentage of total collective cohort (n=64) in parentheses.

†Statistical association between post-operative outcome and FcγRIIIb genotype determined using Pearson's chi-square test (2 x 3 contingency table).

3.3.3 Plasma Cytokines

3.3.3.1 Cytokine Responses to Open AAA Repair Compared to EVAR

Figures 9 to 14 present plasma concentrations of the cytokines, TNF-α, IL-1β, IL-6, IL-10, IL-12p70 and IL-8, amongst members of the 'open' cohort compared with the EVAR cohort at each of the assayed time points, namely pre-operatively,T_0, $T_0 + 4$, $T_0 + 24$, and $T_0 + 72$, in the form of back-to-back histograms.

Due to the highly skewed nature of this data, cytokine concentrations for the two cohorts, at each of the aforementioned time points, were compared using the Mann-Whitney U test (Table 19 to Table 24). In summary, there were no statistically significant differences in concentrations of TNF-α, IL-1β or IL-12p70, at any of the assayed time points, between the 'open' and EVAR cohorts. (Tables 19, 20 and 23) Whilst the two cohorts' pre-operative concentrations of IL-6 were comparable, concentrations of this cytokine were significantly higher in the 'open' cohort compared with the EVAR cohort at T_0 ($p < 0.001$) and $T_0 + 4$ ($p < 0.001$). Elevated median concentrations of IL-6 at $T_0 + 24$ and $T_0 + 72$ were apparent amongst both cohorts, however the difference between the cohorts was not statistically significant at either time point (Table 21). Similarly, pre-operative IL-10 concentrations did not differ significantly between the cohorts. Plasma IL-10 concentrations were significantly higher in the 'open' cohort at T_0 ($p = 0.02$), $T_0 + 4$ ($p = 0.005$), and $T_0 + 24$ ($p = 0.02$) compared to those of the EVAR group. Concentrations of IL-10 at $T_0 + 72$ did not differ between the two operative groups. (Table 22) Whilst both cohorts exhibited raised median IL-8 concentrations pre-operatively, these concentrations did

not differ significantly. Significantly greater concentrations of this cytokine were associated with open AAA repair, compared to EVAR at T_0 ($p = 0.03$) and $T_0 + 4$ ($p = 0.001$), however the difference did not reach significance at $T_0 + 24$ ($p = 0.07$). Raised median concentrations of IL-8 persisted in both cohorts at $T_0 + 72$, but did not differ significantly between the cohorts. (Table 24)

Figure 9. TNF-α plasma concentrations (pg/ml) amongst members of the 'open AAA' cohort compared with those of the EVAR cohort at each assayed time point.

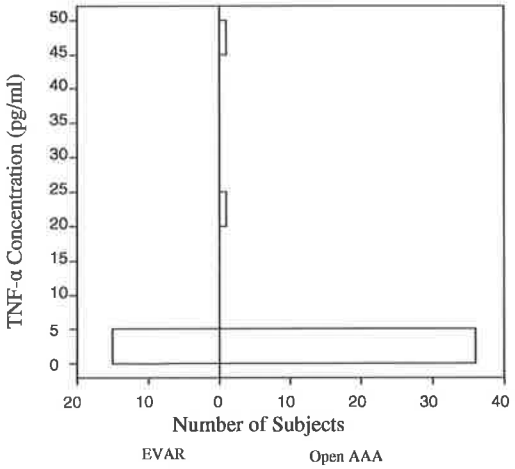
T_0: Immediately prior to reperfusion of first lower limb in 'open AAA' cohort and immediately prior to endograft deployment in EVAR cohort (time of maximal ischaemia without reperfusion).

$T_0 + 4$: four hours post T_0.

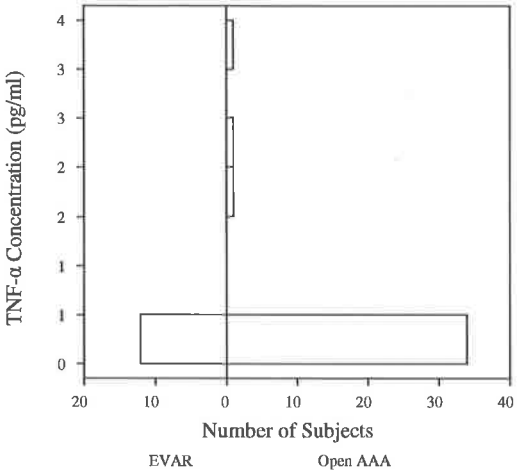
$T_0 + 24$: twenty-four hours post T_0.

$T_0 + 72$: seventy-two hours post T_0.

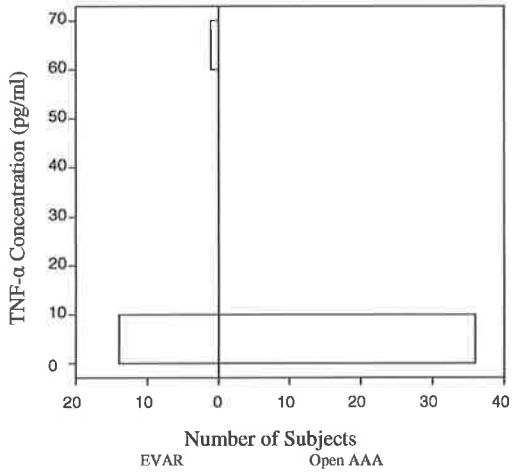
TNF-α Pre-operatively



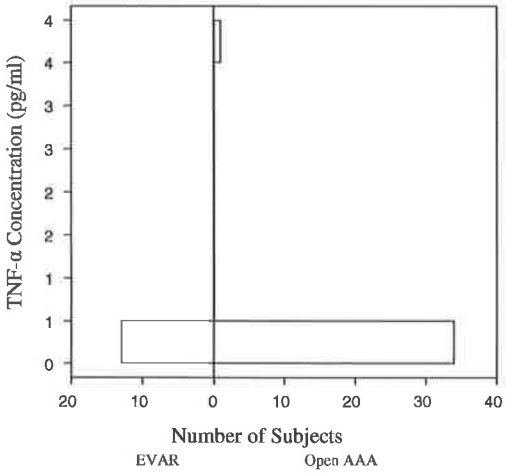
TNF-α at T_0



TNF-α at T_0+4



TNF-α at T_0+24



TNF-α at T_0+72

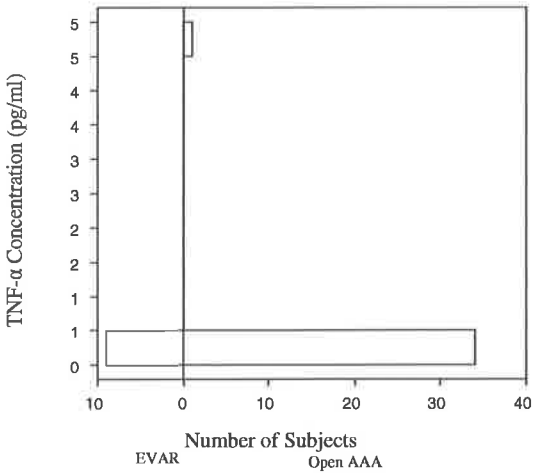


Table 19. Median TNF-α concentrations for 'open' cohort compared with EVAR cohort at pre-, intra- and post-operative time points.

TNF-α Sampling Time	Open AAA Repair	EVAR	*p* value†
Pre-operative	0 (0 - 46.50) (*n*=38)	0 (0 - 0) (*n*=15)	0.28 (NS)
T_0	0 (0 - 3.25) (*n*=37)	0 (0 - 0) (*n*=12)	0.33 (NS)
$T_0 + 4$	0 (0 - 3.15) (*n*=36)	0 (0 - 62.90) (*n*=15)	0.73 (NS)
$T_0 + 24$	0 (0 - 3.75) (*n*=35)	0 (0 - 0) (*n*=13)	0.57 (NS)
$T_0 + 72$	0 (0 - 4.79) (*n*=35)	0 (0 - 0) (*n*=9)	0.65 (NS)

NS, Not significant

T_0, immediately prior to reperfusion of first lower limb in open AAA repair or immediately prior to endovascular stent deployment in EVAR (time of maximal ischaemia).

$T_0 + 4$, four hours following T_0.

$T_0 + 24$, 24 hours following T_0.

$T_0 + 72$, 72 hours following T_0.

Tabulated values are medians and ranges of concentrations (pg/ml) in parentheses. Number of subjects (*n*) contributing data is indicated for each time point.

†Statistical difference between cohorts calculated using Mann-Whitney U test.

Figure 10. IL-1β plasma concentrations (pg/ml) amongst members of the 'open AAA' cohort compared with those of the EVAR cohort at each assayed time point.

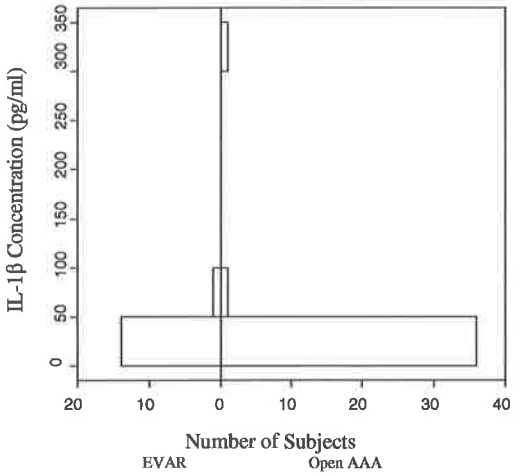
T_0: Immediately prior to reperfusion of first lower limb in 'open AAA' cohort and immediately prior to endograft deployment in EVAR cohort (time of maximal ischaemia without reperfusion).

$T_0 + 4$: four hours post T_0.

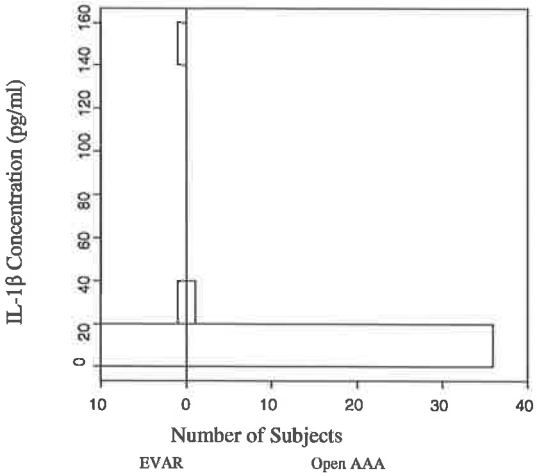
$T_0 + 24$: twenty-four hours post T_0.

$T_0 + 72$: seventy-two hours post T_0.

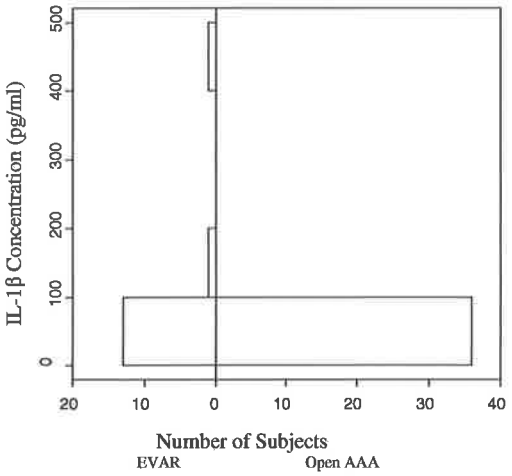
IL-1β Pre-operatively



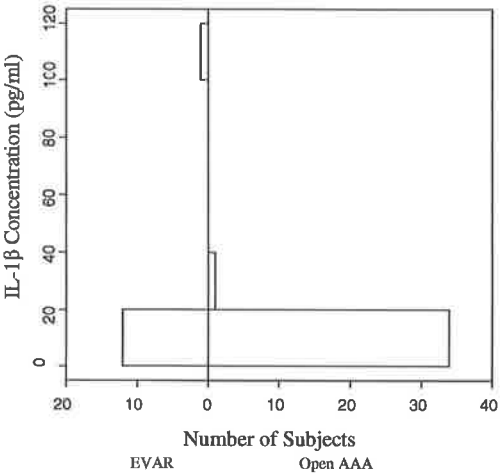
IL-1β at T_0



IL-1β at T_0+4



IL-1β at T_0+24



IL-1β at T_0+72

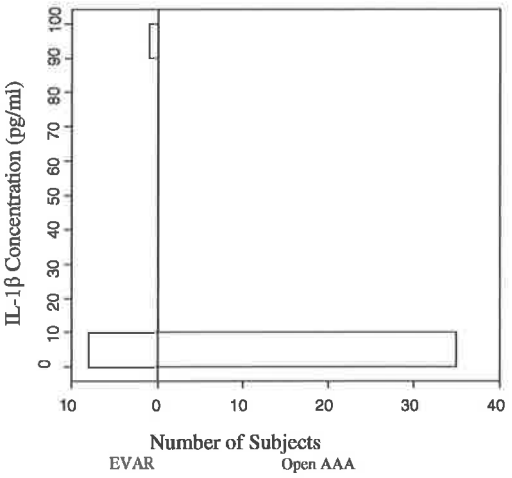


Table 20. Median IL-1β concentrations for 'open' cohort compared with EVAR cohort at pre-, intra- and post-operative time points.

IL-1β Sampling Time	Open AAA Repair	EVAR	*p* value†
Pre-operative	0 (0 - 317.11) (*n*=38)	0 (0 - 93.62) (*n*=15)	0.91 (NS)
T_0	0 (0 - 37.94) (*n*=37)	0 (0 - 150.90) (*n*=12)	0.53 (NS)
$T_0 + 4$	0 (0 - 37.60) (*n*=36)	0 (0 - 154.80) (*n*=15)	0.92 (NS)
$T_0 + 24$	0 (0 - 35.58) (*n*=35)	0 (0 - 104.20) (*n*=13)	0.12 (NS)
$T_0 + 72$	0 (0 - 8.13) (*n*=35)	0 (0 - 95.20) (*n*=9)	0.30 (NS)

NS, Not significant

T_0, immediately prior to reperfusion of first lower limb in open AAA repair or immediately prior to endovascular stent deployment in EVAR (time of maximal ischaemia).

$T_0 + 4$, four hours following T_0.

$T_0 + 24$, 24 hours following T_0.

$T_0 + 72$, 72 hours following T_0.

Tabulated values are medians and ranges of concentrations (pg/ml) in parentheses. Number of subjects (*n*) contributing data is indicated for each time point.

†Statistical difference between cohorts calculated using Mann-Whitney U test.

Figure 11. IL-6 plasma concentrations (pg/ml) amongst members of the 'open AAA' cohort compared with those of the EVAR cohort at each assayed time point.

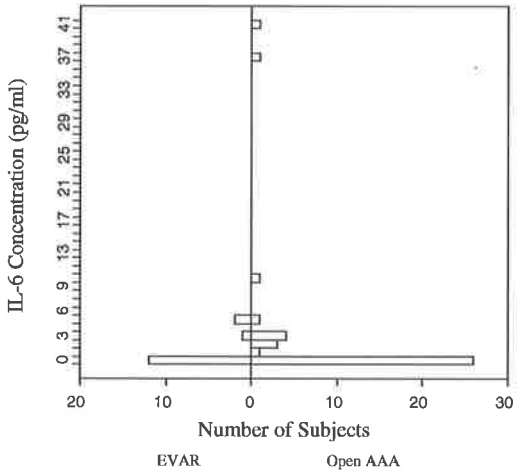
T_0: Immediately prior to reperfusion of first lower limb in 'open AAA' cohort and immediately prior to endograft deployment in EVAR cohort (time of maximal ischaemia without reperfusion).

$T_0 + 4$: four hours post T_0.

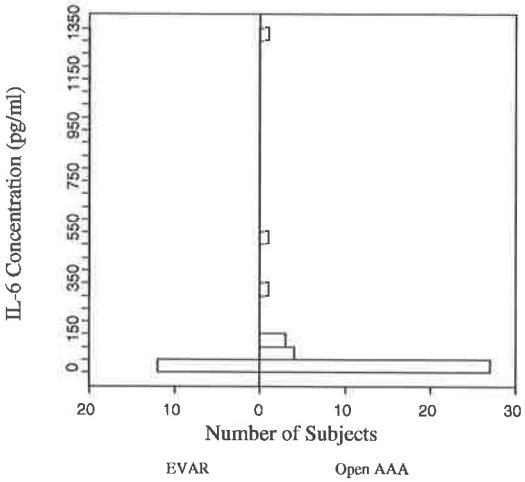
$T_0 + 24$: twenty-four hours post T_0.

$T_0 + 72$: seventy-two hours post T_0.

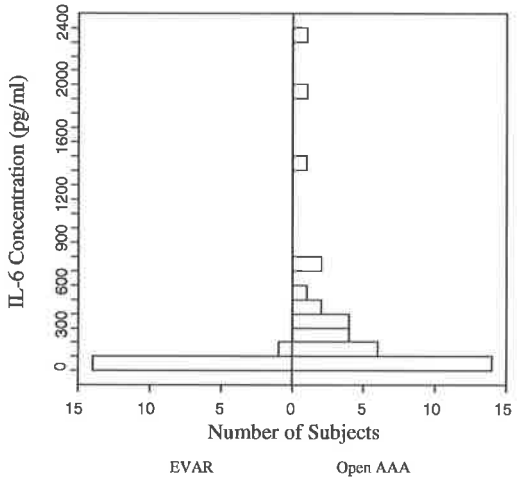
IL-6 Pre-operatively



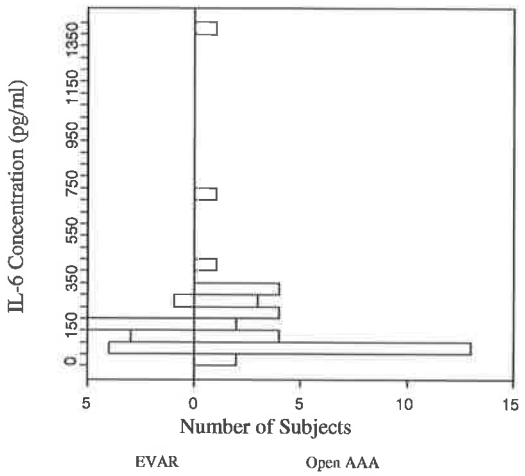
IL-6 at T_0



IL-6 at T_0+4



IL-6 at T_0+24



IL-6 at T_0+72

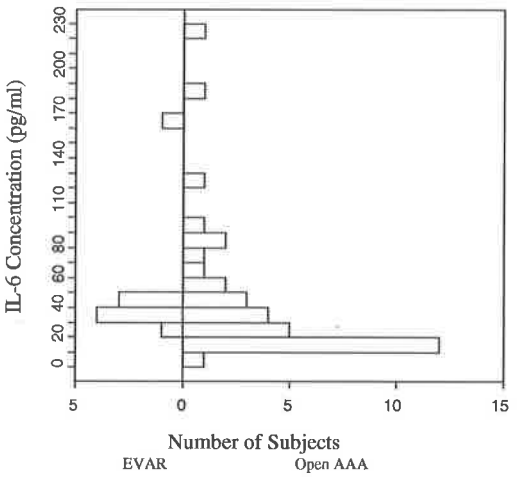


Table 21. Median IL-6 concentrations for 'open' cohort compared with EVAR cohort at pre-, intra- and post-operative time points.

IL-6 Sampling Time	Open AAA Repair	EVAR	*p* value†
Pre-operative	0 (0 - 41.50) (*n*=38)	0 (0 - 5.95) (*n*=15)	0.51 (NS)
T_0	23.10 (0 - 1329.20) (*n*=37)	0 (0 - 33.00) (*n*=12)	<0.001
$T_0 + 4$	164.30 (10.86 - 2377.50) (*n*=36)	29.40 (5.81 - 106.60) (*n*=15)	<0.001
$T_0 + 24$	120.40 (29.24 - 1417.70) (*n*=35)	142.70 (54.39 - 274.47) (*n*=13)	0.98 (NS)
$T_0 + 72$	30.29 (7.10 - 221.70) (*n*=35)	39.7 (24.50 - 165.86) (*n*=9)	0.18 (NS)

NS, Not significant

T_0, immediately prior to reperfusion of first lower limb in open AAA repair or immediately prior to endovascular stent deployment in EVAR (time of maximal ischaemia).

$T_0 + 4$, four hours following T_0.

$T_0 + 24$, 24 hours following T_0.

$T_0 + 72$, 72 hours following T_0.

Tabulated values are medians and ranges of concentrations (pg/ml) in parentheses. Number of subjects (*n*) contributing data is indicated for each time point.

†Statistical difference between cohorts calculated using Mann-Whitney U test.

Figure 12. IL-10 plasma concentrations (pg/ml) amongst members of the 'open AAA' cohort compared with those of the EVAR cohort at each assayed time point.

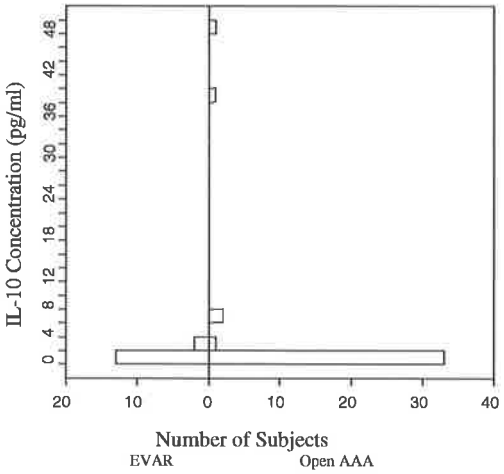
T_0: Immediately prior to reperfusion of first lower limb in 'open AAA' cohort and immediately prior to endograft deployment in EVAR cohort (time of maximal ischaemia without reperfusion).

$T_0 + 4$: four hours post T_0.

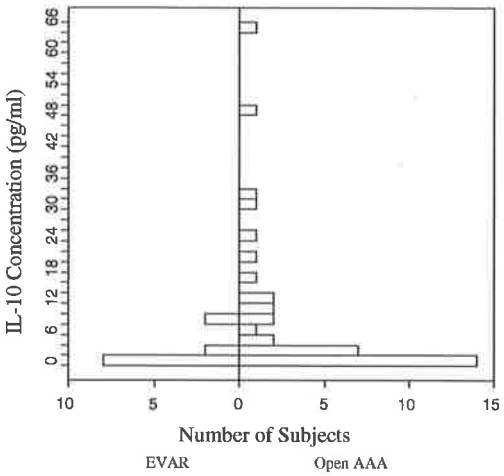
$T_0 + 24$: twenty-four hours post T_0.

$T_0 + 72$: seventy-two hours post T_0.

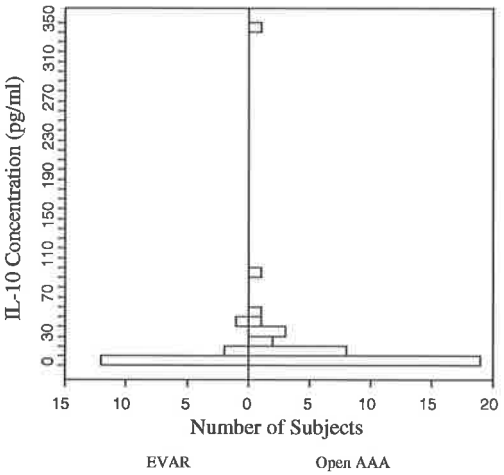
IL-10 Pre-operatively



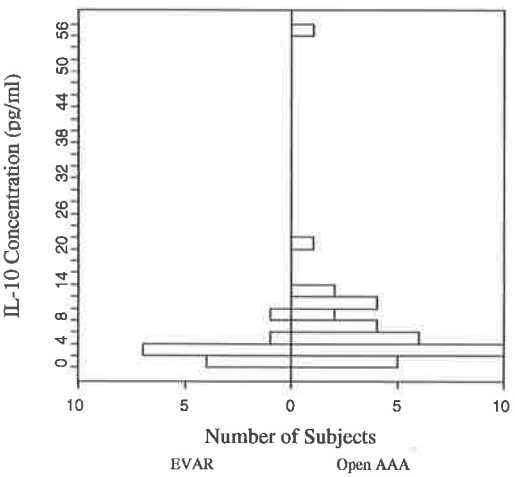
IL-10 at T_0



IL-10 at T_0+4



IL-10 at T_0+24



IL-10 at T_0+72

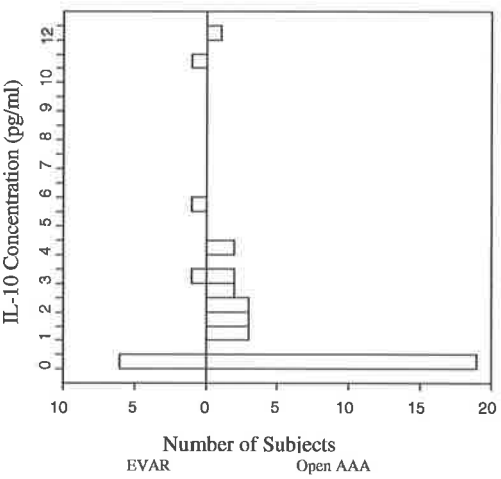


Table 22. Median IL-10 concentrations for 'open' cohort compared with EVAR cohort at pre-, intra- and post-operative time points.

IL-10 Sampling Time	Open AAA Repair	EVAR	*p* value†
Pre-operative	0 (0 - 49.56) (*n*=38)	0 (0 - 3.90) (*n*=15)	0.61 (NS)
T_0	3.40 (0 - 65.10) (*n*=37)	0 (0 - 9.60) (*n*=12)	0.02
$T_0 + 4$	7.85 (0 - 347.10) (*n*=36)	0 (0 - 46.50) (*n*=15)	0.005
$T_0 + 24$	4.50 (0 - 57.63) (*n*=35)	2.27 (0 - 8.80) (*n*=13)	0.02
$T_0 + 72$	0 (0 - 11.60) (*n*=35)	0 (0 - 10.7) (*n*=9)	0.99 (NS)

NS, Not significant

T_0, immediately prior to reperfusion of first lower limb in open AAA repair or immediately prior to endovascular stent deployment in EVAR (time of maximal ischaemia).

$T_0 + 4$, four hours following T_0.

$T_0 + 24$, 24 hours following T_0.

$T_0 + 72$, 72 hours following T_0.

Tabulated values are medians and ranges of concentrations (pg/ml) in parentheses. Number of subjects (*n*) contributing data is indicated for each time point.

†Statistical difference between cohorts calculated using Mann-Whitney U test.

Figure 13. IL-12p70 plasma concentrations (pg/ml) amongst members of the 'open AAA' cohort compared with those of the EVAR cohort at each assayed time points.

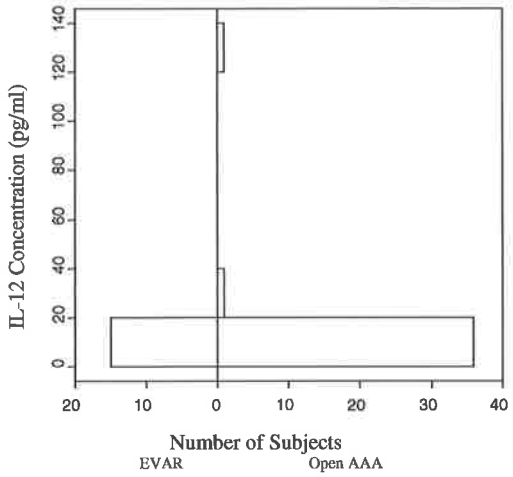
T_0: Immediately prior to reperfusion of first lower limb in 'open AAA' cohort and immediately prior to endograft deployment in EVAR cohort (time of maximal ischaemia without reperfusion).

$T_0 + 4$: four hours post T_0.

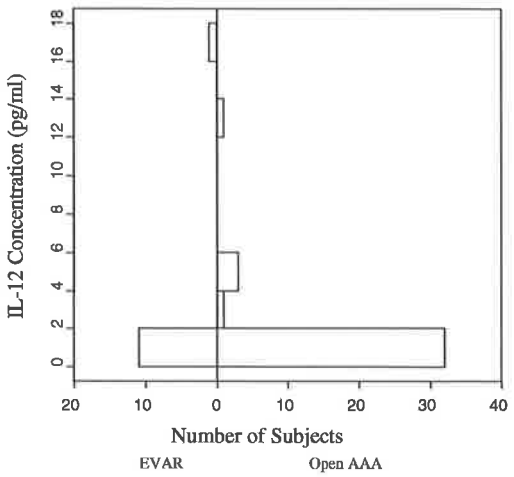
$T_0 + 24$: twenty-four hours post T_0.

$T_0 + 72$: seventy-two hours post T_0.

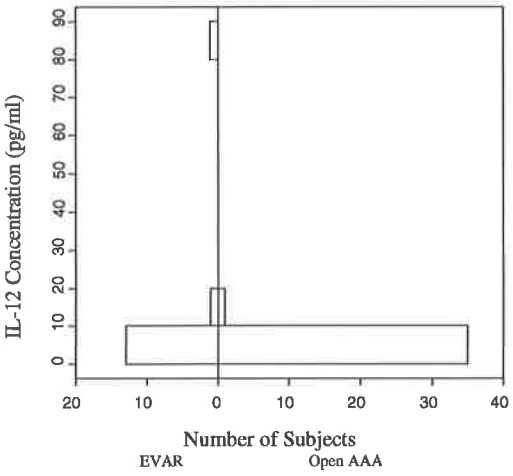
IL-12p70 Pre-operatively



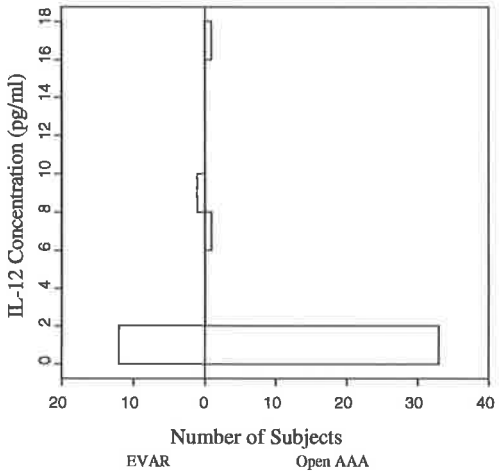
IL-12p70 at T_0



IL-12p70 at T_0+4



IL-12p70 at T_0+24



IL-12p70 at T_0+72

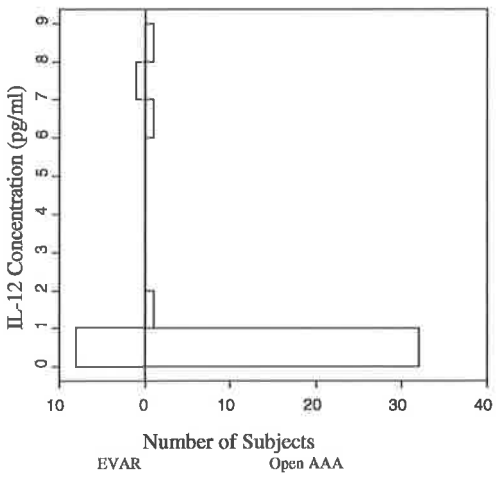


Table 23. Median IL-12p70 concentrations for 'open' cohort compared with EVAR cohort at pre-, intra- and post-operative time points.

IL-12p70 Sampling Time	Open AAA Repair	EVAR	*p* value†
Pre-operative	0 (0 - 120.30) (*n*=38)	0 (0 - 0) (*n*=15)	0.20 (NS)
T_0	0 (0 - 12.24) (*n*=37)	0 (0 - 17.40) (*n*=12)	0.73 (NS)
$T_0 + 4$	0 (0 - 13.86) (*n*=36)	0 (0 - 89.7) (*n*=15)	0.92 (NS)
$T_0 + 24$	0 (0 - 16.92) (*n*=35)	0 (0 - 9.20) (*n*=13)	0.96 (NS)
$T_0 + 72$	0 (0 - 8.81) (*n*=35)	0 (0 - 0) (*n*=9)	0.82 (NS)

NS, Not significant

T_0, immediately prior to reperfusion of first lower limb in open AAA repair or immediately prior to endovascular stent deployment in EVAR (time of maximal ischaemia).

$T_0 + 4$, four hours following T_0.

$T_0 + 24$, 24 hours following T_0.

$T_0 + 72$, 72 hours following T_0.

Tabulated values are medians and ranges of concentrations (pg/ml) in parentheses. Number of subjects (*n*) contributing data is indicated for each time point.

†Statistical difference between cohorts calculated using Mann-Whitney U test.

Figure 14. IL-8 plasma concentrations (pg/ml) amongst members of the 'open AAA' cohort compared with those of the EVAR cohort at each assayed time point.

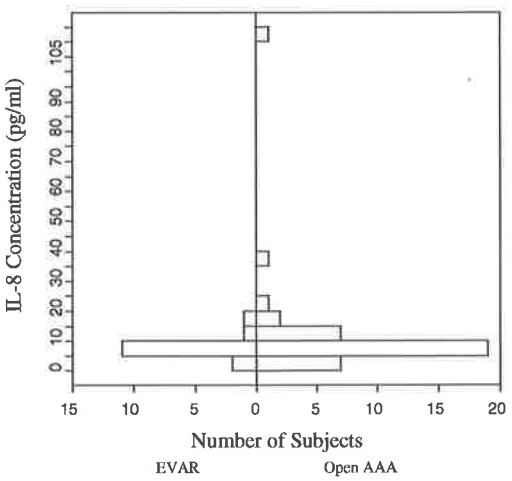
T_0: Immediately prior to reperfusion of first lower limb in 'open AAA' cohort and immediately prior to endograft deployment in EVAR cohort (time of maximal ischaemia without reperfusion).

$T_0 + 4$: four hours post T_0.

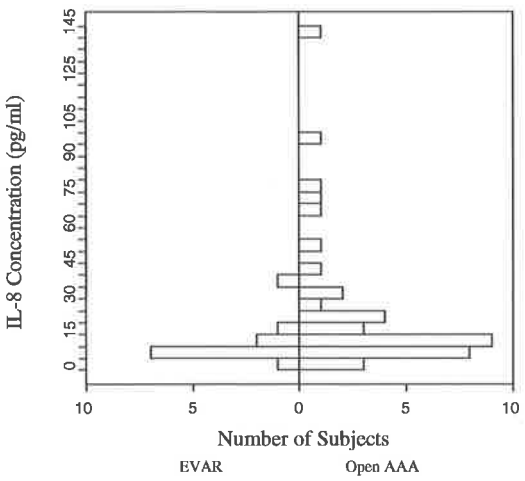
$T_0 + 24$: twenty-four hours post T_0.

$T_0 + 72$: seventy-two hours post T_0.

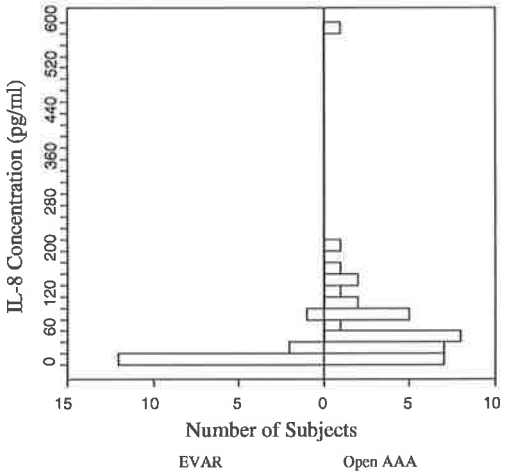
IL-8 Pre-operatively



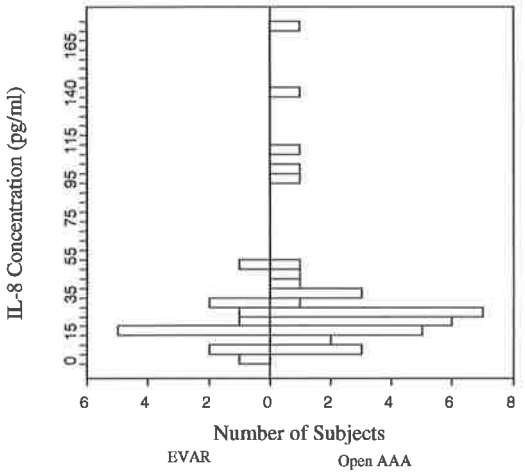
IL-8 at T_0



IL-8 at T_0+4



IL-8 at T_0+24



IL-8 at T_0+72

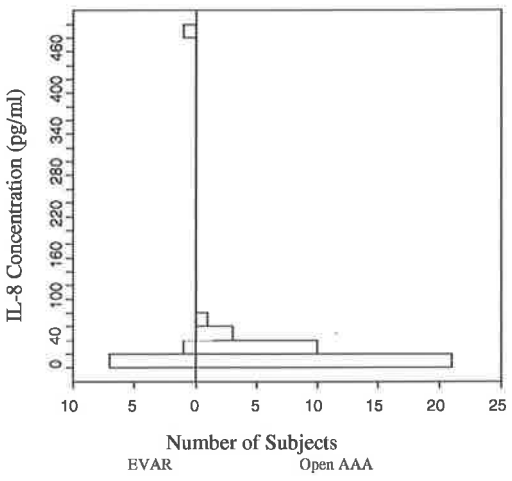


Table 24. Median IL-8 concentrations for 'open' cohort compared with EVAR cohort at pre-, intra- and post-operative time points.

IL-8 Sampling Time	Open AAA Repair	EVAR	*p* value†
Pre-operative	8.0 (0 - 113.87) (*n*=38)	9.1 (1.7 - 17.59) (*n*=15)	0.90 (NS)
T_0	14.41 (3.00 - 143.90) (*n*=37)	8.95 (0 - 35.4) (*n*=12)	0.03
$T_0 + 4$	49.90 (8.55 - 596.01) (*n*=36)	11.4 (5.20 - 91.80) (*n*=15)	<0.001
$T_0 + 24$	26.14 (8.02 - 175.20) (*n*=35)	18.20 (3.60 - 50.03) (*n*=13)	0.07 (NS)
$T_0 + 72$	15.70 (0 - 68.10) (*n*=35)	13.38 (4.6 - 492.77) (*n*=9)	0.51 (NS)

NS, Not significant

T_0, immediately prior to reperfusion of first lower limb in open AAA repair or immediately prior to endovascular stent deployment in EVAR (time of maximal ischaemia).

$T_0 + 4$, four hours following T_0.

$T_0 + 24$, 24 hours following T_0.

$T_0 + 72$, 72 hours following T_0.

Tabulated values are medians and ranges of concentrations (pg/ml) in parentheses. Number of subjects (*n*) contributing data is indicated for each time point.

†Statistical difference between cohorts calculated using Mann-Whitney U test.

3.3.3.2 Prediction of Sepsis from Plasma Cytokine Values

For the purpose of analysing the capacity of plasma cytokine values to predict the development of sepsis, all subjects contributing data to this analysis (n = 51) were considered as a single collective cohort. Using the multiple regression model discussed previously (Chapter 2, Section 2.13.2), logistic regressions were initially fitted separately for each cytokine. Non-significant terms were eliminated, and the resulting regressions are shown in Table 25. Null deviance equalled 68.3, and the residual deviance reported in Table 25 is that remaining after fitting the terms shown. This table also reports the Akaike information criteria (AIC) associated with each regression model. The lowest AIC was obtained for the IL-10 regression model, indicating that of the models specified, this should be the preferred model.

When terms for all cytokines were included in the regression, the only term that remained statistically significant was that of the average hourly concentration of IL-10 [log(av. hrly. conc. IL-10 + 1)]. That is, the other terms added no extra information over and above the average hourly IL-10 concentration term. The constant regression coefficient (β_0) for the model is -2.582 (standard error = 0.871) and the regression coefficient (β_1) for [log(av. hrly. conc. IL-10 + 1)] is 1.159 (standard error = 0.465).

The implications of the regression model are demonstrated by considering Figure 15, which shows the probability of sepsis predicted by the model as a function of the average hourly IL-10 concentration. This plot shows the tendency of those with high hourly IL-10 concentrations to develop sepsis, but demonstrates that for an individual patient, prediction cannot be done with great confidence. As a measure of

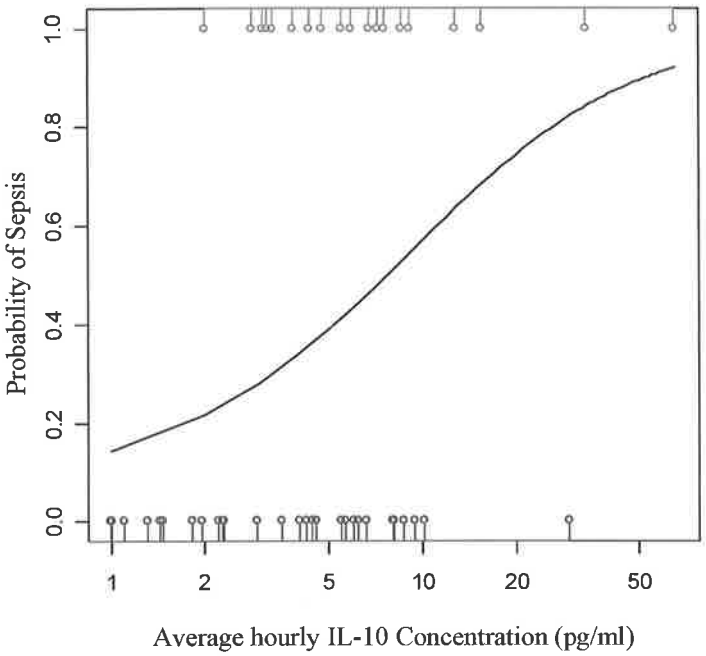
model prediction, Nagelkerke's R^2 value is 0.214. The R^2 index ranges from zero to one, where zero indicates random predictions and one indicates perfect predictions. This R^2 value reinforces the observation that this model cannot predict sepsis occurrence for an individual patient with great confidence.

Table 25. Significant independent variable terms identified by logistic regression model examining relationship between plasma cytokine values and occurrence of post-operative sepsis.

Cytokine	Significant Terms	Akaike Information Criterion	Residual Deviance	Degrees of Freedom	χ^2 Significance (p value)
IL-10	log(av. hrly. conc. + 1)	62.9	58.9	50	0.002
IL-8	log(maximum conc. + 1)	66.7	62.7	50	0.019
IL-6	log(maximum conc. + 1)	66.6	62.6	50	0.018

av, average; hrly, hourly; conc, concentration

Figure 15. Graphical representation of the probability of post-operative sepsis predicted by logistic regression model as a function of average hourly IL-10 concentration including data on which the model is based.



Open red circles (along top) plot average hourly IL-10 concentrations of each subject who developed post-operative sepsis.

Open blue circles (along base) plot average hourly IL-10 concentrations of each subject who did not develop post-operative sepsis.

3.4 Pre-operative Nutritional Status and Associations with Clinical Outcome

Analyses of nutritional data were performed by separately considering the 'open', EVAR and 'lower limb' cohorts.

3.4.1 Body Mass Index (BMI) and DEXA Derived Measures of Body Composition

3.4.1.1 Associations with SIRS

The anthropometric measure of BMI is both a component of the MNA and is reported by DEXA. The associations between this measure of nutrition and measures of SIRS are reported in Table 26. In summary, within the 'lower limb' cohort, there was a moderate, negative and statistically significant association between BMI and both cumulative SIRS score and SIRS duration. The correlations between BMI and measures of SIRS within the 'open' and EVAR cohorts were weak and not statistically significant.

Table 26 also reports the correlations between each of the five DEXA derived measures of body composition with SIRS outcomes. In summary, within the 'open' cohort, there was a moderate, negative and significant association between FFM and cumulative SIRS score. The moderate, negative association between FFM and SIRS duration within this cohort, however, just failed to reach statistical significance. Within the EVAR cohort, the moderate, negative association between FFM and SIRS duration was statistically significant. Within the 'lower limb' cohort, there was a moderate, negative association between FM and cumulative SIRS score which

approached, but did not reach significance. Moderate, negative and statistically significant associations were demonstrated between SMM and both cumulative SIRS score and SIRS duration within the 'open' cohort. The remainder of the associations between the five DEXA derived measures of body composition and SIRS outcomes were weak or moderate, and clearly not statistically significant.

3.4.1.2 Associations with Sepsis

Table 27 reports that there were no significant differences between mean values of BMI amongst those who developed sepsis compared with those who did not, within any of the operative cohorts. Similarly, mean values for each of the DEXA derived measures of body composition did not differ significantly between those who developed sepsis and those who did not (Table 27).

3.4.1.3 Associations with Measures of General Post-operative Morbidity

None of the associations between BMI and measures of general post-operative morbidity reached statistical significance, however amongst the 'open' cohort a weak, negative association with post-operative LOS approached significance (Table 26), whilst amongst the 'lower limb' cohort there was a trend, towards a lower mean BMI amongst those who developed one or more moderate/severe complications compared with those who did not, which approached significance (Table 27).

Tables 26 and 27 report the associations between DEXA derived measures of body composition and all measures of general post-operative morbidity. Amongst the

'open' cohort, FFM was moderately, negatively and significantly associated with ICU LOS. The strength and significance of the association persisted when this body composition measure was indexed for height (FFMI). A moderate, negative and significant association was also demonstrated between SMM and ICU LOS within the 'open' cohort. All other correlations between measures of body composition were weak to moderate and not statistically significant. A trend, which failed to reach significance, towards a lower mean SMM amongst those with one or more moderate/severe complications compared to subjects without this adverse outcome was observed amongst the 'open' cohort. All other mean values of body composition measures were statistically comparable between those with one or more moderate/severe complications and those without this adverse outcome.

Table 26. Pearson product moment (r) and Spearman rank correlations (r_s) for body mass index and five DEXA-derived measures of total body composition with measures of SIRS severity and general post-operative morbidity within the 'open', EVAR, and 'lower limb' cohorts.

ICU, intensive care unit; LOS, length of stay; BMI, body mass index; FFM, fat free mass; FFMI, fat free mass index; FM; fat mass; FMI, fat mass index; SMM, estimated skeletal muscle mass; NS, not significant.

Tabulated values are Pearson product moment (r) or Spearman rank correlations (r_s) as indicated by the column heading with associated statistical significance (p value) in parentheses. The actual p value is reported only when the correlation was statistically significant or approached significance at the 0.05 level (two-tailed).
The number of subjects contributing data to each correlation (n) is reported in row headings.

†(n=33)

	Cumulative SIRS Score (r_s)	SIRS Duration (days) (r)	ICU LOS (days) (r_s)	Maximum APACHE II Score (r)	Post-operative LOS (days) (r)
BMI (kg/m^2)					
Open (n=32)	−0.144 (NS)	−0.249 (NS)	−0.013 (NS)†	−0.259 (NS)†	−0.323 (0.072)
EVAR (n=16)	−0.249 (NS)	−0.236 (NS)	0.420 (NS)	0.264 (NS)	−0.127 (NS)
Lower Limb (n=14)	−0.545 (0.044)	−0.626 (0.017)	−0.332 (NS)	0.127 (NS)	−0.199 (NS)
FFM (kg)					
Open (n=18)	−0.472 (0.048)	−0.452 (0.060)	−0.661 (0.003)	−0.284 (NS)	−0.260 (NS)
EVAR (n=13)	−0.465 (NS)	−0.577 (0.039)	0.154 (NS)	−0.003 (NS)	−0.226 (NS)
Lower Limb (n=12)	−0.090 (NS)	−0.253 (NS)	−0.011 (NS)	−0.277 (NS)	−0.111 (NS)
FFMI (kg/m^2)					
Open (n=18)	−0.336 (NS)	−0.329 (NS)	−0.544 (0.020)	−0.156 (NS)	−0.256 (NS)
EVAR (n=13)	0.267 (NS)	−0.057 (NS)	0.309 (NS)	0.511 (0.074)	−0.153 (NS)
Lower Limb (n=12)	−0.238 (NS)	−0.484 (NS)	−0.151 (NS)	−0.183 (NS)	−0.100 (NS)
FM (kg)					
Open (n=18)	−0.063 (NS)	−0.158 (NS)	−0.074 (NS)	0.114 (NS)	−0.158 (NS)
EVAR (n=13)	−0.132 (NS)	−0.116 (NS)	0.463 (NS)	−0.075 (NS)	0.186 (NS)
Lower Limb (n=12)	−0.525 (0.079)	−0.376 (NS)	−0.124 (NS)	0.163 (NS)	−0.217 (NS)
FMI (kg/m^2)					
Open (n=18)	−0.018 (NS)	−0.051 (NS)	−0.032 (NS)	0.177 (NS)	−0.092 (NS)
EVAR (n=13)	0.069 (NS)	−0.009 (NS)	0.463 (NS)	0.033 (NS)	0.190 (NS)
Lower Limb (n=12)	−0.360 (NS)	−0.309 (NS)	−0.016 (NS)	0.217 (NS)	−0.141 (NS)
SMM (kg)					
Open (n=17)	−0.546 (0.023)	−0.607 (0.010)	−0.649 (0.005)	−0.332 (NS)	−0.420 (NS)
EVAR (n=13)	−0.318 (NS)	−0.424 (NS)	0.309 (NS)	−0.031 (NS)	−0.077 (NS)
Lower Limb (n=12)	0.022 (NS)	−0.163 (NS)	−0.011 (NS)	−0.289 (NS)	−0.266 (NS)

Table 27. Association of mean values of body mass index and five DEXA-derived measures of total body composition with the development of sepsis and the occurrence of one or more moderate/severe complications within the 'open', EVAR, and 'lower limb' cohorts.

BMI, body mass index; FFM, fat free mass; FFMI, fat free mass index; FM; fat mass; FMI, fat mass index; SMM, estimated skeletal muscle mass; NS, not significant.

Tabulated values are mean (SEM).

Statistical difference between group means analysed using Student's t test (two-tailed). The actual *p* value is reported only when the difference between group means approached significance at the 0.05 level.

The number of subjects contributing data (*n*) is reported for each group.

	Sepsis			≥1 Moderate/Severe Complications		
	Present	Absent	*p* value	Present	Absent	*p* value
BMI (kg/m^2)						
Open	26.6 (1.15) (*n*=17)	28.2 (0.69) (*n*=15)	NS	26.4 (0.98) (*n*=26)	28.0 (0.94) (*n*=8)	NS
EVAR	25.0 (*n*=1)	28.5 (1.27) (*n*=15)	NS	29.5 (2.36) (*n*=3)	28.0 (1.41) (*n*=13)	NS
Lower Limb	22.2 (2.15) (*n*=2)	26.0 (0.91) (*n*=12)	NS	21.6 (1.55) (*n*=2)	26.1 (0.89) (*n*=12)	0.071
FFM (kg)						
Open	53.01 (3.24) (*n*=10)	57.89 (3.55) (*n*=8)	NS	51.94 (3.08) (*n*=14)	59.99 (3.29) (*n*=5)	NS
EVAR	51.28 (*n*=1)	59.70 (1.50) (*n*=12)	NS	57.61 (3.41) (*n*=3)	59.49 (1.78) (*n*=10)	NS
Lower Limb	48.38 (13.26) (*n*=2)	53.52 (2.51) (*n*=10)	NS	44.29 (9.17) (*n*=2)	54.33 (2.63) (*n*=10)	NS
FFMI (kg/m^2)						
Open	19.27 (0.77) (*n*=10)	19.51 (0.49) (*n*=8)	NS	18.89 (0.67) (*n*=14)	19.75 (0.46) (*n*=5)	NS
EVAR	17.75 (*n*=1)	20.14 (0.59) (*n*=12)	NS	19.67 (0.96) (*n*=3)	20.05 (0.71) (*n*=10)	NS
Lower Limb	16.66 (2.59) (n=2)	18.60 (0.54) (*n*=10)	NS	16.40 (2.33) (*n*=2)	18.65 (0.55) (*n*=10)	NS
FM (kg)						
Open	22.29 (3.50) (*n*=10)	22.78 (2.21) (*n*=8)	NS	22.57 (2.79) (n=14)	19.97 (1.75) (*n*=5)	NS
EVAR	20.60 (*n*=1)	27.04 (3.16) (*n*=12)	NS	29.32 (4.97) (*n*=3)	25.71 (3.62) (*n*=10)	NS
Lower Limb	16.38 (0.42) (*n*=2)	18.38 (2.17) (*n*=10)	NS	15.42 (0.54) (*n*=2)	18.57 (2.14) (*n*=10)	NS
FMI (kg/m^2)						
Open	8.20 (1.32) (*n*=10)	7.84 (0.82) (*n*=8)	NS	8.26 (1.00) (*n*=14)	6.73 (0.91) (*n*=5)	NS
EVAR	7.05 (*n*=1)	9.10 (1.07) (*n*=12)	NS	9.94 (1.55) (*n*=3)	8.64 (1.23) (*n*=10)	NS
Lower Limb	5.82 (0.58) (*n*=2)	6.55 (0.58) (*n*=10)	NS	5.81 (0.60) (*n*=2)	6.56 (0.91) (*n*=10)	NS
SMM (kg)						
Open	26.49 (1.73) (*n*=9)	31.48 (2.37) (*n*=8)	NS	26.25 (1.88) (*n*=13)	32.82 (2.29) (*n*=5)	0.069
EVAR	26.83 (*n*=1)	31.86 (0.96) (n=12)	NS	31.23 (2.29) (*n*=3)	31.54 (1.12) (*n*=10)	NS
Lower Limb	26.03 (8.30) (*n*=2)	27.77 (1.49) (*n*=10)	NS	22.32 (4.58) (*n*=2)	28.51 (1.62) (*n*=10)	NS

3.4.2 Nutritional Status Classified by Mini Nutritional Assessment (MNA)

Classification of nutritional status using the MNA tool was achieved for most, but not all subjects recruited to the current study, due to the inclusion of this assessment tool in the study protocol after the operative intervention was performed for several subjects. Of the 31 subjects in the 'open' cohort whose nutritional status was classified using the MNA, 24 (77.4%) were classified as 'well-nourished', 6 (19.4%) were classified as 'at risk of malnutrition', and only one (3.2%) was categorised as being 'malnourished'. Since the latter category was represented by a single subject who died shortly after skin closure as described in Section 3.1.2, and for whom the majority of outcome variables were not meaningful, this category was not included in the statistical analyses. It is noteworthy, nonetheless, that this subject was the only individual in the current study to be classified as malnourished, and subsequently died. Indeed, all three subjects who died within the study period were classified as either 'at risk of malnutrition' or 'malnourished'. The nutritional status of sixteen of the EVAR patients was classified using the MNA. Of these subjects, 15 (93.8%) were classified as 'well nourished' and only one was classified as being 'at risk of malnutrition'. All members of the 'lower limb' cohort were assessed using the MNA tool. Nine subjects (52.9%) were categorised as 'well nourished' whilst the remaining 8 (47.1%) were considered to be 'at risk of malnutrition' according to the MNA.

3.4.2.1 Associations with SIRS

As reported in Table 28, neither cumulative SIRS scores nor SIRS duration differed significantly between those categorised as 'at risk of malnutrition' and those categorised as 'well nourished' by the MNA.

3.4.2.2 Associations with Sepsis

Table 29 reports the lack of association between MNA classification and the occurrence of post-operative sepsis within all operative categories.

3.4.2.3 Associations with Measures of General Post-operative Morbidity

It is evident from the results presented in Table 28 and 29, that the only measure of general morbidity that differed significantly between the MNA categories was the APACHE II score, which was significantly higher amongst those 'at risk of malnutrition' compared with 'well nourished' subjects within the EVAR cohort (Table 28).

Table 28. Comparison of measures of SIRS severity and general post-operative morbidity between subjects classified by nutritional status, according to the Mini Nutritional Assessment (MNA)[390].

ICU, Intensive care unit; LOS, length of stay; NS, not significant.

The number of subjects contributing data (*n*) is reported for each group.

The actual *p* value is reported only when the statistical difference between group values was significant at the 0.05 level.

†Tabulated values are medians and range in parentheses; statistical difference between 'at risk of malnutrition' and 'well nourished' groups analysed using Mann-Whitney U test.

‡Tabulated values are means and SEM in parentheses; statistical difference between 'at risk of malnutrition' and 'well nourished' groups analysed using Student's t test.

	At Risk of Malnutrition	Well Nourished	*p* value
Cumulative SIRS Score[†]			
Open	3 (1 - 7) (*n*=5)	3 (0 - 8) (*n*=24)	NS
EVAR	3 (3 - 3) (*n*=1)	0 (0 - 4) (*n*=15)	NS
Lower Limb	1.5 (0 - 11) (*n*=8)	1 (0 - 3) (*n*=9)	NS
SIRS Duration (days)[‡]			
Open	2.60 (0.51) (*n*=5)	2.25 (0.28) (*n*=24)	NS
EVAR	2.00 (*n*=1)	0.73 (0.25) (*n*=15)	NS
Lower Limb	9.13 (1.88) (*n*=8)	0.89 (0.35) (*n*=9)	NS
ICU LOS (days) [†]			
Open	1.85 (0 - 3.18) (*n*=6)	2.05 (0 - 6.99) (*n*=24)	NS
EVAR	0 (*n*=1)	0 (0 - 0.96) (*n*=15)	NS
Lower Limb	0 (0 - 1.19) (*n*=8)	0 (0 - 0) (*n*=9)	NS
Maximum APACHE II Score[‡]			
Open	17.33 (2.42) (*n*=6)	17.17 (0.96) (*n*=24)	NS
EVAR	18.00 (*n*=1)	11.20 (0.54) (*n*=15)	0.007
Lower Limb	9.13 (1.37) (*n*=8)	10.56 (1.72) (*n*=9)	NS
Post-operative LOS (days) [‡]			
Open	12.20 (2.71) (*n*=5)	10.92 (0.67) (*n*=24)	NS
EVAR	4.00 (*n*=1)	3.87 (0.42) (*n*=15)	NS
Lower Limb	7.38 (1.34) (*n*=8)	8.89 (1.17) (*n*=9)	NS

Table 29. Associations between nutritional status, classified according to the Mini Nutritional Assessment (MNA)[390], and the development of post-operative sepsis and one or more moderate/severe complications within the 'open', EVAR and 'lower limb' cohorts.

NS, not significant

Tabulated values are absolute numbers and percentage of each operative cohort in parentheses.

Statistical association between nutritional status and each post-operative outcome (sepsis and one or more moderate/severe complications) determined by Fisher's exact test.

	Sepsis			≥1 Moderate/Severe Complications		
	Present	Absent	*p* value	Present	Absent	*p* value
Open						
At risk of malnutrition	3 (10.3%)	2 (6.9%)	NS	6 (19.4%)	1 (3.2%)	NS
Well nourished	12 (41.4%)	12 (41.4%)		19 (61.3%)	5 (16.1%)	
EVAR						
At risk of malnutrition	0 (0%)	1 (6.3%)	NS	0 (0%)	1 (6.3%)	NS
Well nourished	1 (6.3%)	14 (93.3%)		3 (18.8%)	12 (75.0%)	
Lower Limb						
At risk of malnutrition	2 (11.8%)	6 (35.3%)	NS	1 (5.9%)	7 (41.2%)	NS
Well nourished	0 (0%)	9 (52.9%)		1 (5.9%)	8(47.1%)	

3.5 Neuroendocrine Responses to Surgical Intervention

3.5.1 Reporting of Data and Statistical Considerations

As previously described (Chapter 2, Section 2.13.1), the reported results of statistical analyses involving neuroendocrine data were obtained following $\log_{10}$ transformation of that data. For the purposes of clinical relevance, however, the descriptive statistics (mean and SEM) for neuroendocrine data are presented as absolute (untransformed) values (nmol/24hours).

No subject received exogenous catecholamines during either the T(pre-op) or the T(72-96) urine collection intervals, hence exogenous catecholamine administration did not confound analyses of urinary adrenaline or noradrenaline excretion at these time points. The potential confounding effect of exogenous catecholamine administration on urinary measures of adrenaline and noradrenaline during the T(0-24) interval was controlled for by entering the amounts of adrenaline or noradrenaline administered exogenously during this interval as a covariate in all analyses of T(0-24) urinary adrenaline or noradrenaline excretion respectively, unless otherwise indicated.

For the purpose of describing the patterns of neuroendocrine response within each operative cohort over the course of time (Section 3.5.2), for each respective neuroendocrine measure, mean urinary levels at each time interval were compared with one another using paired Student's t tests, or repeated measures ANOVA when the analysis required the involvement of exogenous catecholamine administration as a covariate. Whilst the use of these analyses is statistically appropriate and necessary for the current purpose of comparing data from the same subjects at two time points,

it is necessary to be aware that when applied to data sets such as these where various data points are missing or excluded, the number of subjects contributing data to each comparison is less than the total cohort and varies for each pair-wise comparison. Furthermore, when this effect is combined with the absence of data due to patient discharge, particularly by T(72-96) within the EVAR cohort, the result is the involvement of particularly small subject numbers in specific analyses of that data. This, in turn, prevented the inclusion of exogenous catecholamine administration as a covariate in analyses of the time course of adrenaline and noradrenaline responses for the EVAR and 'lower limb' cohorts. Exogenous catecholamine administration was, however, either extremely unlikely to have confounded these particular analyses, or was in fact not a relevant consideration, as discussed further in Section 3.5.2. Results of analyses involving particularly small subject numbers are reported, however the potential implications of the sample size should be recognised.

3.5.2 Time Course of Neuroendocrine Responses

3.5.2.1 Urinary Free Cortisol (UFC)

3.5.2.1.1 Open AAA Repair

Amongst the 'open' cohort, there was a significant increase in UFC excretion (nmol/24 hours) from T(pre-op) (mean 382.52, SEM 20.70) to T(0-24) (mean 1393.76, SEM 170.67), [t (20) = −10.81, p <0.0001, n = 21). By T(72-96) a slight reduction in UFC excretion was apparent (mean 1222.37, SEM 78.59) compared with T(0-24) (mean 1527.52, SEM 162.96) but this trend was not statistically significant [t (26) = 1.42, p = 0.17, n = 27). Indeed, UFC at T(72-96) (mean 1153.05,

SEM = 80.74) remained significantly elevated above pre-operative UFC excretion (mean 384.55, SEM = 20.32), [t (19) = −10.90, p <0.0001, n = 20].

3.5.2.1.2 EVAR

Amongst those undergoing EVAR, mean UFC excretion (nmol/24 hours) during T(0-24) was significantly greater than during the pre-operative period [T(0-24): mean 1252.33, SEM 163.35 vs. T(pre-op): mean 351.17, SEM 63.50], [t (5) = −7.36, p = 0.001, n = 6]. As observed amongst the 'open' cohort, the trend towards a decrease in UFC excretion at T(72-96) from the T(0-24) interval [T(0-24): mean 1094.00, SEM 107.16 vs. T(72-96) mean 644.00, SEM 143.04] did not reach statistical significance [t (2) = 2.83, p = 0.11, n = 3].

3.5.2.1.3 Lower Limb Revascularisation

Amongst the 'lower limb' cohort, mean UFC excretion (nmol/24 hours) increased significantly during T(0-24) from baseline levels [T(pre-op): mean 442.20, SEM 77.81 vs T(0-24): mean 1329.50, SEM 236.23], [t (9) = −3.72, p = 0.005, n = 10]. There was a subsequent significant fall in UFC excretion from T(0-24) (mean 1173.89, SEM 230.74) to levels at T(72-96) (mean 603.56, SEM 84.86), [t (8) = 3.84, p = 0.005, n = 9] which did not differ significantly from initial pre-operative values [T(pre-op): mean 398.14, SEM 77.73 vs. T(72-96): mean 538.43, SEM 65.82], [t (6) = −1.62, p = 0.16, n = 7].

3.5.2.2 Urinary Adrenaline Excretion

3.5.2.2.1 Open AAA Repair

Controlling for the effect of exogenous adrenaline administration, there was a significant increase in urinary adrenaline excretion from the initial mean pre-operative level (mean 37.61, SEM 8.32) to that during the T(0-24) period (mean 232.17, SEM 96.03), [F (2, 20) = 14.15, p = 0.001, n = 23]. Once again, controlling for adrenaline administration in analyses, there was a subsequent significant decrease in mean adrenaline excretion from T(0-24) to levels at T(72-96) [T(0-24): mean 170.60, SEM 73.41 vs T(72-96): mean 117.40, SEM 23.67], [F (2, 27) = 8.75, p = 0.006, n = 30], which were comparable to pre-operative levels [T(pre-op): mean 38.45, SEM 8.65 vs. T(72-96): mean 121.32, SEM 31.58], [F(2, 19) = 0.11, p = 0.74, n = 22].

3.5.2.2.2 EVAR

As previously alluded to in Section 3.5.1, analyses examining the changes in adrenaline excretion over time within the EVAR cohort could not control for exogenous adrenaline administration, due to the small sample size involved. On the basis of the non-significant correlation between adrenaline administration and urinary adrenaline excretion during the T(0-24) period (r = –0.51, p = 0.089, n = 12) it is, in fact, unlikely that adrenaline administration exerted any measurable confounding effect on these analyses. The available data demonstrated a significant rise in adrenaline excretion (nmol/24 hours) from T(pre-op) (mean 40.43, SEM 11.46) to T(0-24) (mean 88.00, SEM 29.03) [t (6) = –2.85, p = 0.029, n = 7]. As discussed in Section 3.5.1, whilst several limitations impact upon analyses of trends in adrenaline excretion involving the T(72-96) interval, the available data suggested

that mean urinary levels at T(72-96) did not differ significantly from those at the preceding assay interval [T(0-24): mean 50.75, SEM 19.96 vs. T(72-96): mean 28.25, SEM 1.44], [t (3) = 1.23, $p = 0.31$, $n = 3$].

3.5.2.2.3 Lower Limb Revascularisation

No patient within the 'lower limb' cohort received exogenous adrenaline hence this was not a relevant consideration in the following analyses which demonstrated that apparent fluctuations in adrenaline excretion over time failed to reach statistical significance. Thus, the suggested trend towards a greater excretion of this catecholamine at T(0-24) compared with baseline values was not significant [T(pre-op): mean 25.73, SEM 4.55, T(0-24): mean 47.18, SEM 8.96], [t (10) = −1.88, p = 0.090, n = 11]. Similarly, the trend towards a further rise from T(0-24) to T(72-96) [T(0-24): mean 48.55, SEM 8.41 vs. T(72-96): mean 80.11, SEM 26.18] was not statistically significant [t (10) = –0.096, $p = 0.93$, $n = 11$].

3.5.2.3 Urinary Noradrenaline Excretion

3.5.2.3.1 Open AAA Repair

Once exogenous noradrenaline administration was controlled for in analyses examining the pattern of peri-operative urinary noradrenaline excretion, the suggested modest fluctuations were found not be statistically significant. Thus, the trend towards a greater noradrenaline response during T(0-24) compared with T(pre-op) [T(pre-op): mean 230.61, SEM 18.76 vs. T(0-24): mean 315.00, SEM 60.06] was not significant [F (2, 20) = 3.33, $p = 0.082$, $n = 23$]. The suggestion of a further rise in urinary noradrenaline from T(0-24) (mean 328.10, SEM 53.59) to T(72-96) (mean

415.17, SEM 48.77) was also demonstrated to be non-significant [F (2, 27) = 0.52, p = 0.48, n = 30].

3.5.2.3.2 EVAR

As no patients within the EVAR cohort were administered noradrenaline during the T(0-24) period, this factor was not a relevant consideration in the analyses examining trends in noradrenaline excretion over time. As for the 'open' cohort, the modest fluctuations in the urinary excretion noradrenaline between assay intervals were found to be statistically non-significant. Thus, whilst there was a trend towards a modest rise in urinary noradrenaline from T(pre-op) (mean 298.43, SEM 46.23) to T(0-24) (mean 321.86, SEM 32.37) this was not a statistically significant difference [t (6) = –0.74, p = 0.49, n = 7]. The suggested subsequent decrease at T(72-96) compared to T(0-24) [T(0-24): mean 287.75, SEM 31.16 vs. T(72-96): mean 258.50, SEM 20.91] was similarly non-significant [t (3) = 0.55, p = 0.62, n = 4].

3.5.2.3.3 Lower Limb Revascularisation

Whilst exogenous noradrenaline excretion was not able to be controlled for in analyses examining the trend in urinary noradrenaline levels over time within the 'lower limb' cohort, as described in Section 3.5.1, only one subject was administered this inotrope during the T(0-24) period. It is therefore not surprising that the Pearson correlation between exogenous noradrenaline administration and urinary noradrenaline levels during this time interval was weak and non-significant (r = 0.10, p = 0.71, n = 16), indicating that the following analyses of noradrenaline excretion within the 'lower limb' cohort were extremely unlikely to have been confounded by the administration of inotropes. There was a significant rise in noradrenaline

excretion from T(pre-op) (mean 222.27, SEM 26.48) to T(0-24) (mean 390.64, SEM 53.99), [t (10) = −3.44, p = 0.006, n = 11]. The suggestion of a further rise at T(72-96) was, however, found to be non-significant [T(0-24): mean 370.82, SEM 57.78 vs. T(72-96): 422.91, SEM 110.75], [t (10) = −0.26, p = 0.80, n = 11].

3.5.3 Neuroendocrine Responses in Open AAA Repair Compared to EVAR

3.5.3.1 Urinary Free Cortisol (UFC)

Figure 16 presents mean (SEM) UFC excretion amongst the 'open' cohort compared to the EVAR cohort at each time point in graphical form. It is apparent that UFC did not differ significantly between the two cohorts at either T(pre-op), [t (10.95) = 0.15, p = 0.89], or at T(0-24), [t (40) = 0.30, p = 0.77], but was significantly greater at T(72-96) amongst the 'open' cohort compared with the EVAR cohort [t (31) = −3.73, p = 0.001].

3.5.3.2 Urinary Adrenaline Excretion

Figure 17 compares mean (SEM) urinary adrenaline excretion amongst the two cohorts in a graphical form. The two cohorts did not differ significantly from one another with respect to urinary adrenaline excretion at T(pre-op) [t (33) = 0.43, p = 0.67], nor was there a significant difference at T(0-24) when the analysis controlled for exogenous adrenaline administration [F (2,42) = 0.065, p = 0.80]. As was observed for UFC, adrenaline excretion at T(72-96) was significantly greater amongst the 'open' cohort compared to those who had undergone EVAR [t (32.9) = −5.25, p <0.0001].

3.5.3.3 Urinary Noradrenaline Excretion

Baseline noradrenaline values tended to be higher amongst the EVAR cohort compared with the 'open' cohort, however this difference was not statistically significant [t (33) = 1.94, p = 0.061]. Controlling for exogenous noradrenaline administration in the analysis, a significantly greater excretion of this catecholamine was apparent amongst the EVAR cohort compared to those who underwent open AAA repair, during the T(0-24) interval [F(2,43) = 6.60, p = 0.014]. At T(72-96) the cohorts no longer differed significantly with respect to noradrenaline excretion [t (33) = –0.507, p = 0.23]. These comparisons are presented graphically using absolute (untransformed) mean values in Figure 18.

Figure 16. Mean urinary free cortisol (UFC) excretion at three 24-hour assay intervals for the 'open' and EVAR cohorts.

Data presented are the mean (SEM) as absolute (untransformed) values (nmol/24 hours).

T(pre-op): Pre-operative twenty-four hour assay interval.

T(0-24): Twenty-four hour assay interval commencing at anaesthetic induction.

T(72-96): Twenty-four hour assay interval commencing 72 hours from anaesthetic induction.

Number of subjects (*n*) from each cohort contributing data at each time point is as follows:

Open AAA repair: T(pre-op), $n = 24$; T(0-24), $n = 31$; T(72-96), $n = 29$

EVAR: T(pre-op), $n = 10$; T(0-24), $n = 11$; T(72-96), $n = 4$

*Open vs. EVAR, $p = 0.001$ (two-tailed Student's t test).

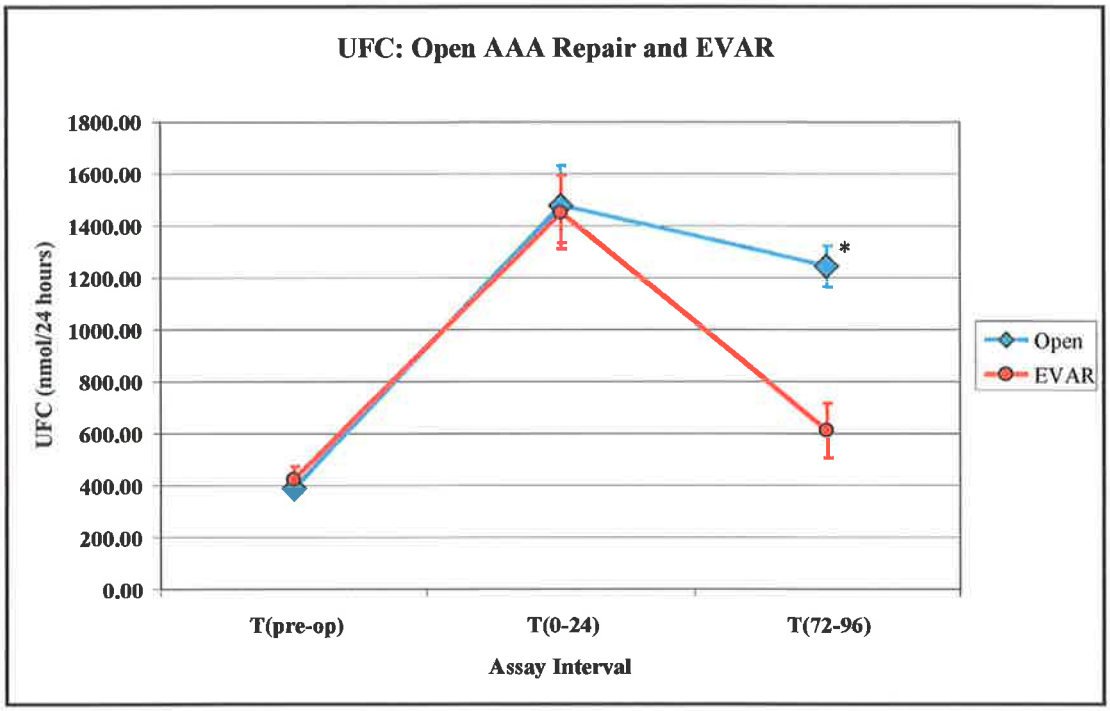

UFC: Open AAA Repair and EVAR
1800.00
1600.00
1400.00
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
UFC (nmol/24 hours)
*
Open
EVAR
T(pre-op)
T(0-24)
T(72-96)
Assay Interval

Figure 17. Mean urinary adrenaline excretion at three 24-hour assay intervals for the 'open' and EVAR cohorts.

Data presented are the mean (SEM) as absolute (untransformed) values (nmol/24 hours).

T(pre-op): Pre-operative twenty-four hour assay interval.

T(0-24): Twenty-four hour assay interval commencing at anaesthetic induction.

T(72-96): Twenty-four hour assay interval commencing 72 hours from anaesthetic induction.

Number of subjects (*n*) from each cohort contributing data at each time point is as follows:

Open AAA repair: T(pre-op), $n = 25$; T(0-24), $n = 33$; T(72-96), $n = 31$

EVAR: T(pre-op), $n = 10$; T(0-24), $n = 12$; T(72-96), $n = 4$

*Open vs. EVAR, $p < 0.0001$ (two-tailed Student's t test).

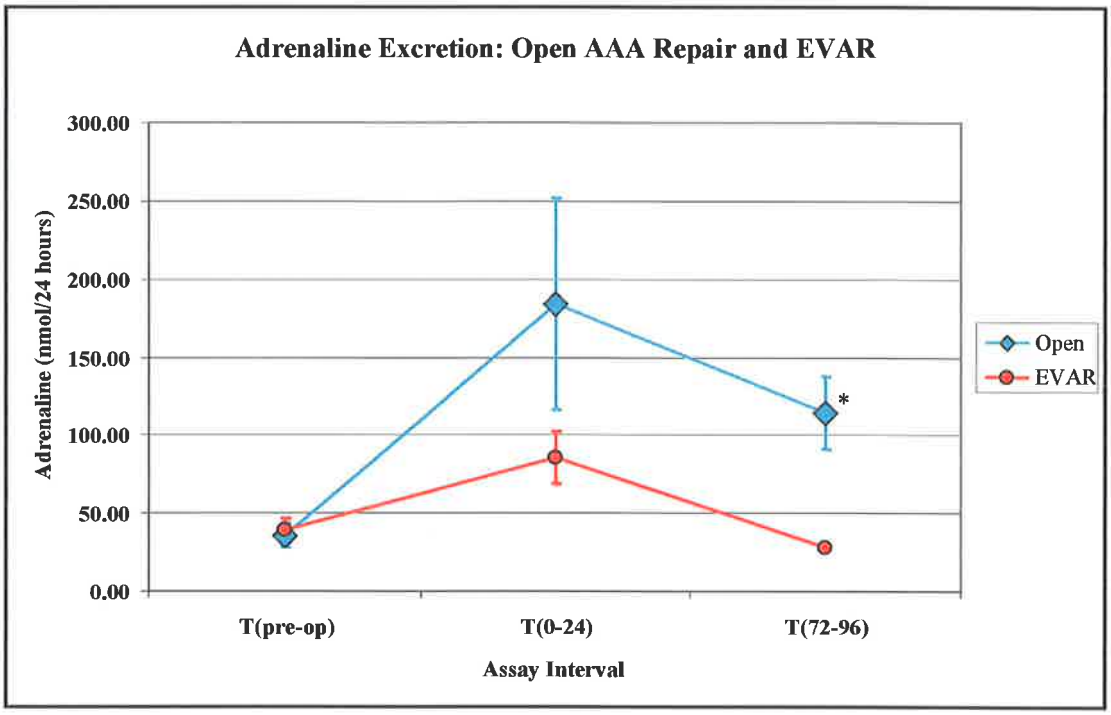

Adrenaline Excretion: Open AAA Repair and EVAR
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Adrenaline (nmol/24 hours)
*
Open
EVAR
T(pre-op)
T(0-24)
T(72-96)
Assay Interval

Figure 18. Mean urinary noradrenaline excretion at three 24-hour assay intervals for the 'open' and EVAR cohorts.

Data presented are the mean (SEM) as absolute (untransformed) values (nmol/24 hours).

T(pre-op): Pre-operative twenty-four hour assay interval.

T(0-24): Twenty-four hour assay interval commencing at anaesthetic induction.

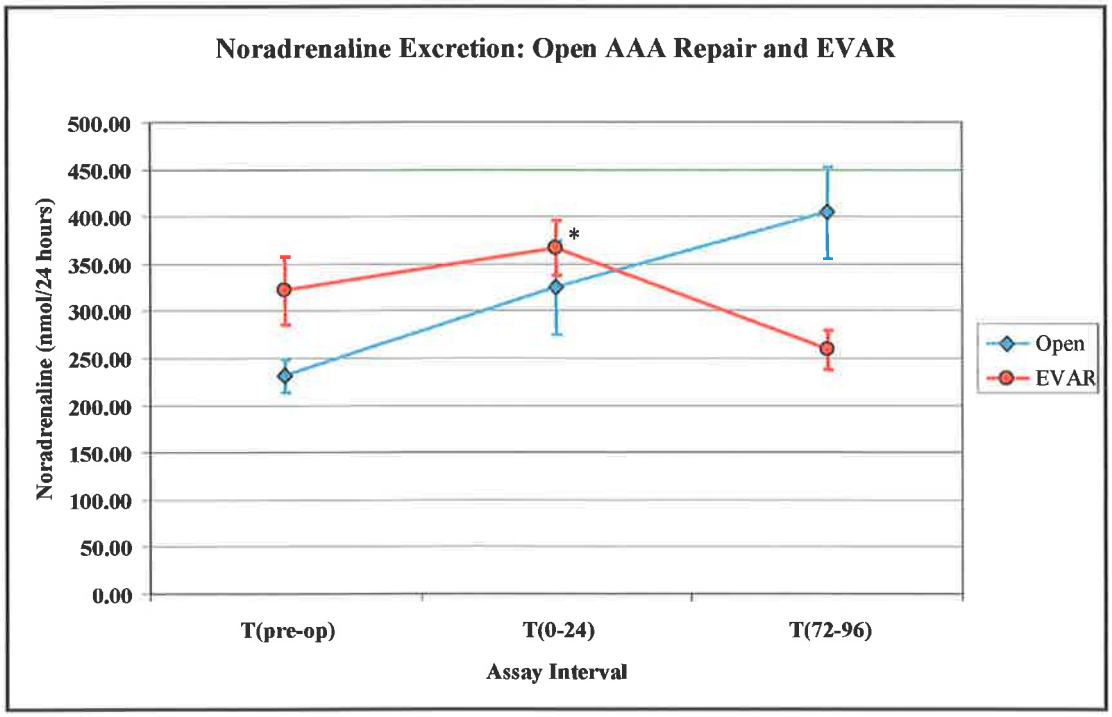
T(72-96): Twenty-four hour assay interval commencing 72 hours from anaesthetic induction.

Number of subjects (*n*) from each cohort contributing data at each time point is as follows:

Open AAA repair: T(pre-op), $n = 25$; T(0-24), $n = 33$; T(72-96), $n = 31$

EVAR: T(pre-op), $n = 10$; T(0-24), $n = 12$; T(72-96), $n = 4$

*EVAR vs. open, $p = 0.014$ (one-way ANOVA, controlling for volume of exogenous noradrenaline administration).


Noradrenaline Excretion: Open AAA Repair and EVAR
Noradrenaline (nmol/24 hours)
500.00
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
*
T(pre-op)
T(0-24)
T(72-96)
Assay Interval
Open
EVAR

3.5.3.4 Post-operative Analgesia

The route of post-operative analgesia, in particular the administration of analgesics via an epidural route in contrast to alternative routes, was considered a factor which may have exerted an influence on the observed neuroendocrine stress response and, in turn, potentially confounded comparisons of neuroendocrine data between operative cohorts. In view of this possibility, the 'open' and EVAR cohorts were dichotomised according to the route of post-operative analgesia administration which was categorised as 'epidural' or 'other'. As previously described (Chapter 2, Sections 2.2.4.5 and 2.2.5.5) the incidence of epidural use amongst those 34 subjects in the 'open' cohort who survived beyond the early post-operative period was 70.6%, compared to 0% of the 17 subjects constituting the EVAR cohort. Chi-square analysis confirmed that the significant association between epidural use and the operative cohort [χ^2 (1) = 22.67, $p < 0.0001$], providing statistical support for the notion that this factor may have confounded the comparison of neuroendocrine responses between the operative cohorts at T(0-24) and/or T(72-96).

3.5.4 Associations Between Neuroendocrine Responses and Clinical Outcome

Analyses of the relationship between neuroendocrine responses and clinical outcome measures were performed by considering all subjects as belonging to a single, collective cohort.

3.5.4.1 Associations with SIRS

Correlation coefficients, and the associated statistical significance, for the associations between measures of the neuroendocrine response at the three assayed time intervals and measures of SIRS severity, are detailed in Table 30. In summary, there was a positive, moderate and highly statistically significant correlation between UFC at T(72-96) and both cumulative SIRS score and SIRS duration. There was, similarly, a positive, moderate and highly significant correlation between adrenaline excretion at T(72-96) and both cumulative SIRS score and SIRS duration. The remaining associations between neuroendocrine measures, including UFC, adrenaline and noradrenaline excretion assayed at the various time intervals, and SIRS outcomes were weak and clearly not significant.

3.5.4.2 Associations with Sepsis

Table 31 presents mean (SEM) values of UFC, adrenaline and noradrenaline excretion amongst those who developed post-operative sepsis compared with those did not develop this complication. In summary, both mean UFC and urinary adrenaline excretion at T(72-96) were significantly greater amongst subjects classified as having post-operative sepsis compared with those without sepsis. At the preceding two assay intervals, however, these neuroendocrine measures did not differ significantly between those who did and did not develop sepsis. Mean urinary noradrenaline excretion, at all assay intervals, failed to distinguish between subjects who did and did not develop sepsis.

3.5.4.3 Associations with Measures of General Post-operative Morbidity

The relationship between the magnitude of neuroendocrine responses at various assay intervals and measures of general post-operative morbidity are presented in Tables 30 and 31. It is apparent that there was a moderate, positive and highly significant correlation between UFC at T(72-96) and the maximum APACHE II score attained (Table 30). Furthermore, a significantly greater mean UFC response during the T(72-96) interval distinguished those who developed one or more moderate/severe complications from those who did not. When adjusted for the amounts of exogenous noradrenaline administered during the T(0-24) interval, mean urinary noradrenaline excretion during this assay interval was, surprisingly, greater amongst subjects who were free of one or more moderate/severe complications compared to those who did experience one or more moderate/severe complications. It is worth noting that the statistical significance of this difference (p = 0.046) was marginal.

Table 30. Correlations between neuroendocrine responses and measures of SIRS severity and general post-operative morbidity within the collective cohort.

LOS, length of stay; UFC, urinary free cortisol; nmol/24 hrs, nanomol per 24 hours; NS, not significant

T(pre-op): Pre-operative twenty-four hour assay interval.

T(0-24): Twenty-four hour assay interval commencing at anaesthetic induction.

T(72-96): Twenty-four hour assay interval commencing 72 hours from anaesthetic induction.

All correlations were performed following $\log_{10}$ transformation of neuroendocrine data. Tabulated correlations are Pearson product moment correlations, except where indicated, with associated statistical significance (*p* value) in parentheses. The actual *p* value is reported only when the correlation was statistically significant or approached significance at the 0.05 level (two-tailed).

The number of subjects contributing data to each correlation (*n*) is reported in row headings.

†Spearman rank correlations.

‡Corresponding row values are partial correlations, with amounts of exogenous adrenaline administered during the urine collection/assay interval entered as a covariate.

§Corresponding row values are partial correlations, with amounts of exogenous noradrenaline administered during the urine collection/assay interval entered as a covariate.

	Cumulative SIRS Score	SIRS Duration (days)	Maximum APACHE II Score	Post-operative LOS (days)
UFC (nmol/24 hrs)				
T(pre-op) (*n*=43)	–0.064 (NS)†	0.059 (NS)	0.053 (NS)	0.132 (NS)
T(0-24) (*n*=57)	–0.017 (NS) †	0.000 (NS)	0.180 (NS)	0.186 (NS)
T(72-96) (*n*=43)	0.413 (0.006) †	0.405 (0.007)	0.580 (<0.0001)	0.220 (NS)
Adrenaline (nmol/24 hrs)				
T(pre-op) (*n*=45)	–0.018 (NS) †	0.010 (NS)	–0.051 (NS)	–0.055 (NS)
T(0-24)‡ (*n*=58)	0.191 (NS)	0.207 (NS)	0.187 (NS)	0.118 (NS)
T(72-96) (*n*=47)	0.403 (0.005) †	0.458 (0.001)	0.120 (NS)	0.157 (NS)
Noradrenaline (nmol/24 hrs)				
T(pre-op) (*n*=45)	–0.154 (NS) †	–0.149 (NS)	–0.134 (NS)	–0.046 (NS)
T(0-24)§ (*n*=58)	–0.193 (NS)	–0.197 (NS)	–0.178 (NS)	–0.203 (NS)
T(72-96) (*n*=47)	0.122 (NS) †	0.234 (NS)	–0.077 (NS)	0.043 (NS)

Table 31. Association between magnitude of neuroendocrine responses and the development of both sepsis and the occurrence of one or more moderate/severe complications amongst the collective cohort.

UFC, urinary free cortisol; nmol/24 hrs, nanomol per 24 hours; NS, not significant

T(pre-op): Pre-operative twenty-four hour assay interval.

T(0-24): Twenty-four hour assay interval commencing at anaesthetic induction.

T(72-96): Twenty-four hour assay interval commencing 72 hours from anaesthetic induction.

Tabulated data are mean (SEM), presented as absolute (untransformed) values. All statistical analyses were performed following $\log_{10}$ transformation of neuroendocrine data. Statistical difference between group means analysed using Student's t test (two-tailed), except where indicated. The actual p value is reported only when the difference between group means was statistically significant, or approached significance, at the 0.05 level.

The number of subjects contributing data (n) is reported for each group.

†Difference between group means determined by one-way ANOVA, with amounts of exogenous adrenaline administered during the urine collection/assay interval entered as a covariate.

‡Difference between group means determined by one-way ANOVA, with amounts of exogenous noradrenaline administered during the urine collection/assay interval entered as a covariate.

	Sepsis			≥1 Moderate/Severe Complications		
	Present	Absent	*p* value	Present	Absent	*p* value
UFC (nmol/24 hrs)						
T(pre-op)	394.21 (46.28) (*n*=14)	416.90 (26.96) (*n*=29)	NS	382.61 (32.96) (*n*=23)	433.33 (31.64) (*n*=21)	NS
T(0-24)	1427.47 (140.45) (*n*=17)	1388.95 (121.94) (*n*=40)	NS	1365.48 (149.02) (*n*=27)	1431.90 (121.89) (*n*=30)	NS
T(72-96)	1242.2 (133.47) (*n*=15)	927.32 (77.60) (*n*=28)	0.046	1231.70 (94.51) (*n*=23)	813.45 (86.92) (*n*=20)	0.001
Adrenaline (nmol/24 hrs)						
T(pre-op)	44.33 (12.60) (*n*=15)	29.33 (3.32) (*n*=30)	NS	35.84 (8.17) (*n*=25)	31.52 (3.57) (*n*=21)	NS
T(0-24)	243.53 (13.90) (*n*=19)	78.00 (14.27) (*n*=42)	NS†	185.63 (74.95) (*n*=30)	75.29 (8.87) (*n*=31)	NS†
T(72-96)	136.56 (30.97) (*n*=18)	67.45 (17.59) (*n*=29)	0.023	122.85 (26.98) (*n*=27)	54.85 (9.42) (*n*=20)	0.063
Noradrenaline (nmol/24 hrs)						
T(pre-op)	236.00 (24.34) (*n*=15)	257.97 (18.97) (*n*=30)	NS	31.76 (19.34) (*n*=25)	268.76 (22.39) (*n*=21)	NS
T(0-24)	414.05 (76.38) (*n*=19)	296.83 (24.64) (*n*=42)	NS‡	319.73 (52.47) (*n*=30)	346.52 (29.58) (*n*=31)	0.046‡
T(72-96)	509.61 (89.37) (*n*=18)	323.97 (31.30) (*n*=29)	NS	405.33 (53.88) (*n*=27)	381.20 (64.43) (*n*=20)	NS

3.6 Psychological Measures and Influence on Surgical Response and Outcome

3.6.1 Pre-operative Depression

3.6.1.1 Incidence of Pre-operative Depression

3.6.1.1.1 Classification by BDI-II

Valid BDI-II scores were available for twenty-seven subjects within the 'open' cohort, of whom four (14.8%) were classified as being depressed pre-operatively. Two (18.2%) of the 11 subjects within the EVAR cohort with valid BDI-II scores were categorised as being depressed. Only four subjects within the 'lower limb' cohort could be classified by the BDI-II, of whom two (50%) were categorised as being depressed. The overall pre-operative incidence of depression amongst study participants, when considered as belonging to a single collective cohort, was therefore 19.0%, according to BDI-II classifications.

3.6.1.1.2 Classification by CES-D

Twenty-three members of the 'open' cohort were able to be classified by the CES-D, of whom six (26.1%) were categorised as being depressed pre-operatively. Three (33.3%) of the nine EVAR subjects with valid CES-D scores were considered depressed, whilst three (42.9%) of the seven subjects within the 'lower limb' cohort for whom CES-D categorisation was possible were classified as being depressed using this inventory. Considering subjects as belonging to a single collective cohort, the CES-D thus yielded an overall incidence of pre-operative depression of 30.8%, which was therefore somewhat greater than detected by the BDI-II.

Where possible, analyses relating to pre-operative depression were performed considering subjects as belonging to the three separate operative cohorts, however, several analyses have considered study participants as a single collective cohort in view of the modest number of subjects able to be classified by either the BDI-II or CES-D.

3.6.1.2 Depression and Measures of General Post-operative Morbidity

Both the BDI-II and CES-D categorisation of the presence or absence of pre-operative depression were considered in analyses examining the relationship between this variable and the two measures of post-operative morbidity, post-operative LOS and the occurrence of one or more moderate/severe complications. Furthermore, analyses were performed both considering each of the three operative cohorts separately, in addition to considering subjects as a single collective cohort. As detailed in Table 32, there were no significant differences within the separate operative cohorts, or the collective cohort, in mean post-operative LOS between those classified as being depressed compared with subjects not considered depressed when subjects were classified by either the BDI-II or the CES-D. There was similarly no association within the separate operative cohorts or the collective cohort, between the presence or absence of pre-operative depression, when categorised by either the BDI-II or the CES-D, and the occurrence of one or more moderate/severe complications (Table 33).

Table 32. Comparison of mean post-operative length of stay (LOS) between subjects classified as 'depressed' or 'not depressed' by BDI-II and CES-D within the 'open', EVAR, 'lower limb' and collective cohorts.

	BDI-II			CES-D		
	Depressed	Not Depressed	*p* value	Depressed	Not Depressed	*p* value
Post-operative LOS (days)						
Open	12.00 (1.78) (*n*=4)	11.45 (0.95) (*n*=22)	NS	11.00 (1.52) (*n*=5)	11.12 (1.10) (*n*=17)	NS
EVAR	2.50 (0.50) (*n*=2)	5.44 (1.21) (*n*=9)	NS	3.00 (0.41) (*n*=4)	4.83 (0.79) (*n*=6)	NS
Lower Limb	5.00 (0.00) (*n*=2)	8.50 (1.50) (*n*=2)	NS	6.00 (1.00) (*n*=3)	11.50 (1.85) (*n*=4)	NS
All Subjects	7.87 (1.80) (*n*=8)	9.64 (0.85) (*n*=33)	NS	7.08 (1.23) (*n*=12)	9.78 (0.91) (*n*=27)	NS

BDI-II, Beck Depression Inventory-II; CES-D, Center for Epidemiological Studies-Depression Scale; LOS, length of stay; NS, not significant.

Tabulated values are means and SEM in parentheses. Statistical difference between 'depressed' and 'not depressed' groups analysed using Student's t test.

The actual *p* value is reported only when the statistical difference between group values was significant at the 0.05 level.

The number of subjects contributing data (*n*) is reported for each group.

Table 33. Association between presence of pre-operative depression, identified by BDI-II and CES-D, and occurrence of one or more moderate/severe post-operative complications within the 'open', EVAR, 'lower limb' and collective cohorts.

BDI-II, Beck Depression Inventory-II; CES-D, Center for Epidemiological Studies-Depression Scale; NS, not significant.

Tabulated values are absolute numbers and percentage of each cohort in parentheses. For each cohort, statistical association between BDI-II or CES-D classification of depression and occurrence of one or more moderate/severe complications determined by Fisher's exact test.

≥1 Mod/Severe Complications	BDI-II			CES-D		
	Depressed	Not Depressed	*p* value	Depressed	Not Depressed	*p* value
Open						
Present	4 (14.8%)	17 (63.0%)	NS	6 (26.1%)	12 (52.2%)	NS
Absent	0 (0%)	6 (22.2%)		0 (0%)	5 (21.7%)	
EVAR						
Present	0 (0%)	4 (36.4%)	NS	0 (0%)	2 (20.0%)	NS
Absent	2 (18.2%)	5 (45.5%)		4 (40.0%)	4 (40%)	
Lower Limb						
Present	0 (0%)	1 (25.0%)	NS	0 (0%)	2 (28.6%)	NS
Absent	2 (50.0%)	1 (25.0%)		3 (42.9%)	2 (28.6%)	
All Subjects						
Present	4 (9.5%)	22 (52.4%)	NS	6 (15.0%)	16 (40.0%)	NS
Absent	4 (9.5%)	12 (28.6%)		7 (17.5%)	11 (27.5%)	

3.6.1.3 Depression and Health-related Quality of Life

For the purposes of analysing the relationship between the presence or absence of pre-operative depression and peri-operative HRQoL, measured by the SF-36 PCS and MCS, subjects were considered as belonging to one collective cohort. The lack of a significant difference in HRQoL between the three operative cohorts, demonstrated using Student's t tests, suggests that the approach of analysing all patients collectively was indeed statistically sound, and was unlikely to have been confounded by subject's having undergone one of three categories of operative intervention, namely open AAA repair, EVAR and lower limb revascularisation.

In addition to considering the potential confounding influence of 'operative category/cohort' on analyses of the relationship between HRQoL and depression, several other potentially confounding variables were considered. Student's t tests demonstrated that there was no significant difference between male and female subjects with respect to HRQoL, suggesting that gender would not confound subsequent analyses. Pearson product moment correlations were performed to identify whether analyses of the relationship between pre-operative depression and peri-operative HRQoL may have been confounded by subjects' age or burden of comorbidity as rated by the Charlson Index score. For those analyses involving post-operative HRQoL as the dependent variable, subjects' pre-operative HRQoL and post-operative LOS were also considered as potential confounding factors. Those variables that were moderately and significantly correlated with SF-36 HRQoL PCS or MCS, at either of the four specific assessment time points, are listed in Table 34, and were interpreted as factors likely to confound analyses of the relationship between depression and the relevant HRQoL measure. These likely confounding

variables were therefore entered as 'independent variables', or 'covariates', in the ANOVAs performed to examine the relationship between the relevant HRQoL measure and depression. Table 34 presents mean SF-36 PCS and MCS measures amongst depressed and non-depressed patients and the results of comparison of the group means using ANOVA, controlling for the listed confounding factors. These analyses were separately performed for the BDI-II and CES-D categorisation of the presence or absence of pre-operative depression.

As reported in Table 34, controlling for subjects' age in the analysis, the mean pre-operative SF-36 MCS was significantly lower amongst depressed subjects, indicating that, as might be expected, the mental health aspect of HRQoL was poorer amongst these subjects compared with subjects categorised as not depressed. This significant difference was apparent for both the BDI-II and CES-D categorisations, however, the BDI-II categorisation suggested a more highly significant difference than the CES-D categorisation. There was no significant difference between depressed and non-depressed subjects with regard to the physical aspect of their pre-operative HRQoL, measured by the SF-36 PCS when the Charlson Index score was used to control for the burden of subjects' comorbidities. One month post-operatively, the pre-operative difference in the mental health aspect of HRQoL between depressed and non-depressed subjects was no longer apparent when confounding variables were controlled for in the analysis. Controlling for the confounding influence of pre-operative SF-36 MCS, depressed subjects identified using the BDI-II were once again found to have a significantly lower mean SF-36 MCS three months post-operatively compared to non-depressed subjects. Using the CES-D categorisation, however, no significant difference between the groups was apparent. In considering

the findings relating to HRQoL at 6 months post-operatively, the relatively small numbers of patients constituting the non-depressed, and particularly the depressed groups, must be appreciated. At this time point, controlling for the confounding effect of subjects' pre-operative SF-36 PCS, the mean SF-36 PCS was significantly lower amongst depressed patients compared with non-depressed patients, categorised using the BDI-II. When the CES-D findings were used to examine the relationship with SF-36 PCS at 6 months, a trend towards a lower score was evident amongst depressed subjects compared to those considered not to be depressed, but this failed to reach statistical significance. Using the BDI-II categorisation of depression and controlling for the confounding effect of pre-operative SF-36 MCS, ANOVA suggested a significantly greater six month SF-36 MCS amongst depressed subjects. This unexpected and paradoxical finding amongst the particularly small cohort of subjects at this time point, was not replicated when the CES-D categorisation of depression was employed. Employing this depression inventory, there was no difference between depressed and non-depressed subjects with respect to the SF-36 MCS at six months (Table 34).

Table 34. Comparison of HRQoL, measured by the SF-36 Physical Component Summary score (PCS) and Mental Component Summary score (MCS) at four peri-operative time points, between subjects classified as 'depressed' or 'not depressed' by BDI-II and CES-D for the collective cohort.

HRQoL, Health-related Quality of Life; SF-36, Medical Outcomes Study 36-Item Short-Form Health Survey; BDI-II, Beck Depression Inventory-II; CES-D, Center for Epidemiological Studies-Depression Scale; NS, not significant.

†Relevant independent variables identified as moderate and significant correlates of a specific dependent variable (SF-36 HRQoL MCS or PCS), using Pearson product moment correlations, were considered as potential confounding factors in the relationship between that dependent variable and depression. These potential confounding factors are tabulated and were entered as covariates in the relevant ANOVA performed to analyse differences in mean SF-36 HRQOL MCS or PCS between 'depressed' and 'not depressed' groups.

‡Tabulated data are mean (SEM) of the dependent variable (PCS or MCS, as indicated in the row heading).

Difference between group means determined by ANOVA, controlling for potential confounding factors.

The actual p value is reported only when the statistical difference between group values was statistically significant, or approached significance at the 0.05 level.

The number of subjects contributing data (n) is reported for each group or correlation.

Dependent Variable: SF-36 HRQoL	Significant Independent Variables* (Covariates)	Dependent-Independent Variable Correlation† (*p* value)	BDI-II‡			CES-D‡		
			Depressed	Not Depressed	*p* value	Depressed	Not Depressed	*p* value
Pre-operative								
PCS	Charlson Index Score	−0.451 (0.007) (*n*=35)	39.43 (4.59) (*n*=6)	39.88 (2.15) (*n*=25)	NS	40.43 (3.96) (*n*=10)	40.13 (2.10) (*n*=23)	NS
MCS	Subjects' age	0.506 (0.002) (*n*=35)	31.69 (3.03) (*n*=6)	52.25 (1.55) (*n*=25)	<0.0001	40.82 (4.02) (*n*=10)	50.56 (1.84) (*n*=23)	0.041
1 Month								
PCS	Pre-op. SF-36 PCS	0.604 (0.001) (*n*=27)						
	Charlson Index Score	−0.381 (0.034) (*n*=31)	37.94 (5.86) (*n*=5)	32.93 (2.57) (*n*=18)	NS	41.29 (4.47) (*n*=7)	32.31 (2.46) (*n*=18)	NS
	Post-operative LOS	−0.447 (0.012) (*n*=31)						
MCS	Pre-op. SF-36 MCS	0.633 (<0.0001) (*n*=27)	32.54 (4.15) (*n*=5)	49.44 (2.12) (*n*=18)	NS	42.85 (3.940 (*n*=7)	47.48 (2.65) (*n*=18)	NS
3 Months								
PCS	Pre-op. SF-36 PCS	0.768 (<0.0001) (*n*=21)						
	Charlson Index Score	−0.476 (0.025) (*n*=22)	36.40 (4.55) (*n*=4)	38.32 (2.64) (*n*=15)	NS	40.47 (5.94) (*n*=5)	39.59 (2.72) (*n*=15)	NS
MCS	Pre-op. SF-36 MCS	0.714 (<0.0001) (*n*=21)	34.38 (6.23) (*n*=4)	55.31 (1.32) (*n*=15)	0.044	45.04 (4.52) (*n*=5)	53.35 (2.82) (*n*=15)	NS
6 Months								
PCS	Pre-op. SF-36 PCS	0.733 (0.025) (*n*=9)	26.34 (*n*=1)	41.35 (5.34) (*n*=6)	0.017	34.91 (6.23) (*n*=4)	48.85 (6.42) (*n*=4)	0.065
MCS	Pre-op. SF-36 MCS	−0.669 (0.049) (*n*=9)	56.97 (*n*=1)	54.36 (3.29) (*n*=6)	0.023	54.22 (2.42) (*n*=4)	55.51 (5.43) (*n*=4)	NS

3.6.1.4 Depression and Post-operative SIRS

Both the BDI-II and CES-D categorisation of the presence or absence of pre-operative depression were once again considered separately in analyses examining the relationship between this variable and the two measures of post-operative SIRS severity, cumulative SIRS score and SIRS duration. Like the analyses examining depression and general post-operative morbidity described in Section 3.6.1.2, the potential relationship between pre-operative depression and SIRS outcomes were analysed separately for each of the three operative cohorts in addition to analysing the potential relationship within the collective cohort (Table 35). Within the relatively small EVAR cohort, mean SIRS duration was found to be significantly longer amongst those subjects considered not to be depressed compared with those classified as having depression, according to the BDI-II. Classification of depression status with the CES-D, however, failed to identify this significant difference. No other significant differences between depressed and non-depressed subjects, with respect to either mean SIRS duration or cumulative SIRS score, were identified (Table 35).

3.6.1.5 Depression and Post-operative Sepsis

Table 36 details the lack of a significant association between the presence or absence of pre-operative depression, when classified either by the BDI-II or the CES-D, and the occurrence of post-operative sepsis within either the 'open', EVAR, or the 'lower limb' cohorts. The suggested lack of a significant association between the presence or absence of pre-operative depression and the development of post-operative sepsis was also apparent when the analysis was performed by considering all subjects within a single, larger cohort.

Table 35. Comparison of measures of SIRS severity between subjects classified as 'depressed' or 'not depressed' by BDI-II and CES-D within the 'open', EVAR, 'lower limb' and collective cohorts.

BDI-II, Beck Depression Inventory-II; CES-D, Center for Epidemiological Studies-Depression Scale; NS, not significant.

The number of subjects contributing data (*n*) is reported for each group.
The actual *p* value is reported only when the difference between group means was statistically significant at the 0.05 level.

†Tabulated values are medians and range in parentheses; statistical difference between 'depressed' and 'not depressed' groups analysed using Mann-Whitney U test.

‡Tabulated values are means and SEM in parentheses; statistical difference between 'depressed' and 'not depressed' groups analysed using Student's t test.

	BDI-II			CES-D		
	Depressed	Not Depressed	*p* value	Depressed	Not Depressed	*p* value
Cumulative† SIRS Score						
Open	1.5 (1 - 6) (*n*=4)	2.5 (0 - 8) (*n*=22)	NS	3 (1 - 8) (*n*=5)	2 (0 - 7) (*n*=17)	NS
EVAR	0 (0 - 0) (*n*=2)	1 (0 - 3) (*n*=9)	NS	1.5 (0 - 3) (*n*=4)	0 (0 - 2) (*n*=6)	NS
Lower Limb	1.5 (1 - 2) (*n*=2)	6 (1 - 11) (*n*=2)	NS	1 (0 - 2) (*n*=3)	2 (1 - 11) (*n*=4)	NS
All Subjects	1 (0 - 6) (*n*=8)	2 (0 - 11) (*n*=33)	NS	1.5 (0 - 8) (*n*=12)	2 (0 - 11) (*n*=27)	NS
SIRS Duration‡ (days)						
Open	1.75 (0.48) (*n*=4)	2.14 (0.31) (*n*=22)	NS	2.20 (0.58) (*n*=5)	2.18 (0.35) (*n*=17)	NS
EVAR	0.00 (0.00) (*n*=2)	1.00 (0.33) (*n*=9)	0.017	1.00 (0.58) (*n*=4)	0.67 (0.42) (*n*=6)	NS
Lower Limb	1.50 (0.50) (*n*=2)	3.00 (2.00) (*n*=2)	NS	1.00 (0.58) (*n*=3)	2.25 (0.95) (*n*=4)	NS
All Subjects	1.25 (0.37) (*n*=8)	1.88 (0.26) (*n*=33)	NS	1.50 (0.36) (*n*=12)	1.85 (0.29) (*n*=27)	NS

Table 36. Association between presence of pre-operative depression, identified by BDI-II and CES-D, and occurrence of sepsis within the 'open', EVAR, 'lower limb' and collective cohorts.

BDI-II, Beck Depression Inventory-II; CES-D, Center for Epidemiological Studies-Depression Scale; NS, not significant; N/A, not applicable.

Tabulated values are absolute numbers and percentage of each cohort in parentheses. For each cohort, statistical association between BDI-II or CES-D classification of depression and occurrence of sepsis determined by Fisher's exact test.

	BDI-II			CES-D		
	Depressed	Not Depressed	*p* value	Depressed	Not Depressed	*p* value
Open						
Sepsis Present	1 (3.8%)	12 (46.2%)	NS	3 (13.6%)	9 (40.9%)	NS
Sepsis Absent	3 (11.5%)	10 (38.5%)		2 (9.1%)	8 (36.4%)	
EVAR						
Sepsis Present	0 (0%)	1 (9.1%)	NS	0 (0%)	4 (40%)	N/A
Sepsis Absent	2 (18.2%)	8 (72.7%)		0 (0%)	6 (60%)	
Lower Limb						
Sepsis Present	0 (0%)	1 (25%)	NS	0 (0%)	1 (14.3%)	NS
Sepsis Absent	2 (50%)	1 (25%)		3 (42.9%)	3 (42.9%)	
All Subjects						
Sepsis Present	1 (2.4%)	14 (34.1%)	NS	3 (7.7%)	10 (25.6%)	NS
Sepsis Absent	7 (17.1%)	19 (46.3%)		9 (23.1%)	17 (43.6%)	

3.6.1.6 Depression and the Neuroendocrine Response to Surgery

Both the BDI-II and CES-D findings were, once again, separately considered in analyses comparing mean UFC excretion, at the three 24-hour assay intervals, between subjects classified as being depressed compared with those considered not to be depressed within the collective cohort. A significantly greater UFC excretion at T(0-24) appeared to characterise subjects who were not considered depressed compared with depressed subjects, when the BDI-II findings were employed (Table 37). This difference was not replicated when the CES-D categorisation was employed in the analysis of UFC excretion at the T(0-24) interval. It is apparent from Table 37 that there were no differences between depressed and non-depressed subjects with respect to mean UFC excretion at either T(pre-op) or T(72-96) when either the BDI-II or the CES-D findings were employed.

3.6.2 Pre-operative Trait Anxiety

3.6.2.1 Association with Neuroendocrine Responses

Correlations between Spielberger Trait Anxiety Scale (STAI Form Y-2) scores and urinary measures of the HPA axis and SNS responses, namely UFC, adrenaline and noradrenaline excretion, at each of the three assay intervals were determined by considering subjects as constituting a single collective cohort and are presented in Table 38. There was a positive, moderate and statistically significant correlation between noradrenaline excretion at T(pre-op) and trait anxiety score. All other correlations between neuroendocrine measures and trait anxiety scores were weak and not statistically significant (Table 38).

Table 37. Comparison of mean urinary free cortisol (UFC) excretion, at three 24-hour assay intervals, between subjects classified as 'depressed' or 'not depressed' by BDI-II and CES-D within the collective cohort.

	BDI-II			CES-D		
	Depressed	Not Depressed	*p* value	Depressed	Not Depressed	*p* value
UFC (nmol/24 hrs)						
T(pre-op)	382.86 (53.41) (*n*=7)	394.04 (33.23) (*n*=23)	NS	388.67 (48.83) (*n*=9)	413.31 (183.53) (*n*=16)	NS
T(0-24)	980.50 (164.73) (*n*=8)	1401.03 (123.62) (*n*=29)	0.035	1187.00 (165.01) (*n*=9)	1425.57 (165.25) (*n*=23)	NS
T(72-96)	734.33 (256.57) (*n*=3)	1107.64 (91.72) (*n*=25)	NS	1139.71 (268.52) (*n*=7)	967.22 (79.07) (*n*=18)	NS

BDI-II, Beck Depression Inventory-II; CES-D, Center for Epidemiological Studies-Depression Scale; nmol/24 hrs, nanomol per 24 hours; NS, not significant.

T(pre-op): Pre-operative twenty-four hour assay interval.

T(0-24): Twenty-four hour assay interval commencing at anaesthetic induction.

T(72-96): Twenty-four hour assay interval commencing 72 hours from anaesthetic induction.

Tabulated data are mean (SEM), presented as absolute (untransformed) values. Statistical difference between group means analysed using Student's t test (two-tailed) following $\log_{10}$ transformation of UFC data. The actual *p* value is reported only when the difference between group means was statistically significant at the 0.05 level.

The number of subjects contributing data (*n*) is reported for each group.

Table 38. Correlations between Spielberger Trait Anxiety Scale (STAI Form Y-2) score and neuroendocrine measures at three 24-hour assay intervals for the collective cohort.

UFC, urinary free cortisol; nmol/24 hrs, nanomol per 24 hours; NS, not significant.

T(pre-op): Pre-operative twenty-four hour assay interval.

T(0-24): Twenty-four hour assay interval commencing at anaesthetic induction.

T(72-96): Twenty-four hour assay interval commencing 72 hours from anaesthetic induction.

All correlations were performed following $\log_{10}$ transformation of neuroendocrine data. Tabulated correlations are Pearson product moment correlations, except where indicated, with associated statistical significance (p value) in parentheses. The actual p value is reported only when the correlation was statistically significant at the 0.05 level (two-tailed).

The number of subjects contributing data to each correlation (n) is reported.

†Partial correlation, with amounts of exogenous adrenaline administered during the urine collection/assay interval entered as a covariate.

‡Partial correlation, with amounts of exogenous noradrenaline administered during the urine collection/assay interval entered as a covariate.

	Trait Anxiety
UFC (nmol/24 hrs)	
T(pre-op)	0.095 (NS) ($n = 25$)
T(0-24)	−0.109 (NS) ($n = 33$)
T(72-96)	−0.086 (NS) ($n = 25$)
Adrenaline (nmol/24 hrs)	
T(pre-op)	0.125 (NS) ($n = 27$)
T(0-24)	0.143 (NS)† ($n = 33$)
T(72-96)	0.166 (NS) ($n = 29$)
Noradrenaline (nmol/24 hrs)	
T(pre-op)	0.419 (0.030) ($n = 27$)
T(0-24)	−0.062 (NS)‡ ($n = 33$)
T(72-96)	0.030 (NS) ($n = 29$)

CHAPTER 4

DISCUSSION

4.1 General Limitations of Current Study

The current study has several general limitations which are relevant to the interpretation of many of the findings presented herein. Given the potential of these limitations to have a bearing on many of the conclusions presented in subsequent sections, a discussion of these limitations from the outset is considered prudent. Additional limitations, relevant only to specific data sets, are presented in the appropriate sections to follow.

4.1.1 Sample Size

The importance of power analysis prior to study commencement cannot be over emphasised,[422] however the relative absence of reliable estimates for most of the variables considered in this study made this process largely invalid. Furthermore, when considering the issue of sample size with respect to the current study, the finite and indeed relatively modest size of the population being sampled must be acknowledged. When combined with the practicalities of subject recruitment, including inaccessibility to potential subjects and the refusal of study participation by others, the resulting cohort size, whilst potentially smaller than statistically optimal, reflected the combination of actual operative activity and the realities of clinical research. Regardless of clinical reality, however, the influence of sample size on the probability of a type I and type II error[422] must be acknowledged. For this reason, operative cohorts were combined when necessary and acceptable, to maximize power of the analyses. Furthermore, cautious interpretations and conclusions have been drawn when small sample sizes were involved.

4.1.2 Inclusion Criteria

A conceivable criticism of the current study may be that the inclusion criteria were overly permissive, resulting in the 'open' and 'lower limb' cohorts, in particular, being somewhat heterogeneous with regard to the surgical interventions experienced by their constituent members. Indeed, the 'open' cohort included not only subjects with abdominal aortic aneurysms, but included those with thoracoabdominal aneurysms, whilst members of the 'lower limb' cohort were subject to a variety of both infra- and supra-inguinal revascularisation procedures. Whilst the recruitment of such subjects was conceivably over-inclusive, limiting the range of acceptable interventions would have resulted in an unacceptable reduction in the size of the population being sampled, and therefore the size of the recruited cohort. It may, however, be argued that the potential confounding effect introduced by the heterogeneity of the cohorts was, in fact, taken into account by the measurement of 'operative duration' and 'duration of ischaemia' and by the subsequent analyses of their relationship to outcome variables. Furthermore, it is proposed that the inclusion of a variety of operative procedures in the 'lower limb' cohort, in particular, increases the capacity to generalise the findings to a wider patient population than if only one type of lower limb revascularisation had been considered.

4.1.3 Exclusion Criteria

Intimately related to the issue of sample size, is that of the stringency of exclusion criteria that may be reasonably adopted. Ideally, subjects' manifesting any factor known to alter the immune response, and in turn the possibility of developing SIRS or sepsis in response to a given insult, would be excluded from a study with the current aims; such stringent criteria would, however, render the current study

virtually implausible due to restrictions that would be imposed on the sample size. Thus, whilst exclusion of subjects' exhibiting symptoms or signs of an inflammatory or infectious process within a given pre-operative period would have been desirable, this was not feasible for the reasons described in Chapter 2, Section 2.2.2. It seems likely that the most significant impact of this factor would have been on data arising from the 'lower limb' cohort, whilst data arising from the 'open' and EVAR cohorts is less likely to have been confounded by such an influence.

The known association of cellular immunodeficiency with human cancer[423] suggests that the presence of malignancy may also, ideally, have been considered a criterion for exclusion. The theoretical advantage of excluding subjects' exhibiting this potentially confounding influence was foregone in view of the possible impact upon sample size. On balance, given that malignancy affected only 5.7% of the collective cohort at the time of recruitment, in addition to the type and stage of those malignancies,[423] it seems unlikely that, if this factor was indeed biologically relevant, it would have had any marked influence on the findings.

In view of the immunosuppressive effect of pharmacological doses of corticosteroids,[329] it would similarly have been optimal to exclude subjects' administered this drug class, either pre-operatively, intra-operatively or within the post-operative study period. It may reasonably have been hypothesised that subjects administered these agents may have had a lower incidence of SIRS, and a greater incidence of infection. Intuitively, it may also have been anticipated that subjects using corticosteroids may have had a greater incidence of sepsis, however, a plausible argument also exists for anticipating a lower incidence of sepsis amongst

subjects administered these agents, due to the potential suppression of the pro-inflammatory systemic response to invading micro-organisms. Once again, however, given the potential impact upon the sample size of the current study, corticosteroid administration was not considered a feasible exclusion criterion. Ultimately, it was encouraging to note that there was no statistically significant relationship between corticosteroid use and the occurrence of either SIRS, infection or sepsis, hence it is particularly unlikely that corticosteroid use confounded analyses involving these outcome measures in the current study. As previously described (Chapter 2, Section 2.8.2), the potential effect of corticosteroid pharmacotherapy on UFC measures was managed by excluding affected urine samples from immunoassay.

4.1.4 Standardisation of Clinical Protocols

Whilst the establishment of strict protocols standardising subjects' care during the intra- and post-operative period is likely to have enabled the minimisation of potential confounding factors and increased the reliability of the study findings, this would not have been ethically acceptable given the frequent need to individualise management to achieve optimal anaesthetic and surgical care. This potential inter-subject variability in management may involve an array of factors including pharmacotherapy, fluid management, and the duration of mechanical ventilation, in addition to the undertaking of various adjunctive vascular, ancillary vascular and concomitant non-vascular procedures at the time of the principal procedure for some subjects. Furthermore, controlling for all possible confounding variables by statistical means is not feasible. The presentation of descriptive statistics for an array of variables considered to have a potential impact on the outcomes in this study is, however, some recompense for the inability to standardise these factors.

Whilst the lack of a formal protocol governing the intra- and post-operative management of study participants may therefore be considered a shortcoming of this study, it is necessary to acknowledge that regional, and particularly local practices are relatively homogeneous, hence minimising the potential impact of non-standardisation, for at least some variables such as anaesthetic techniques. Indeed, this is perhaps more appropriately considered a limitation of observational cohort studies in general, reflected by the level of evidence with which they are associated, rather than being a specific weakness of the current study.

4.2 Consensus Definitions of SIRS and Sepsis

4.2.1 Definition of SIRS

Despite the extent to which the SIRS concept has been adopted by clinicians and investigators[99] since its formal inception in 1992,[100] valid concerns have been expressed in the literature contending that it is both insufficiently specific and excessively sensitive[68, 99, 424] and suggestions made to ameliorate the apparent short-comings[129] . It was for these reasons that the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference revisited the concept of SIRS and its definition and, whilst the validity of the concept was reaffirmed, the previously cited deficiencies were acknowledged. Despite this acknowledgment, however, the participants were unable to propose a more satisfactory biochemical, immunological or clinical diagnostic alternative.[99] Until a consensus can be reached on a more suitable approach to identifying or measuring this inflammatory event, the 1992 definition continues to be used, with an awareness of its inherent weaknesses. Some concerns regarding the potential excessive sensitivity of the SIRS definition in the current study may, however, be allayed by the reassuring observation that the use of β-blockers did not influence classification of the presence or absence of SIRS within the collective cohort, nor was there an influence when subjects were considered as belonging to three separate operative cohorts, despite the effect of this drug class on heart rate, one of the SIRS criteria.

Whilst the validity of their arguments cannot be denied, the critics of SIRS and its definition frequently overlook the recommendation made in the 1992 consensus document, that the newly proposed SIRS definition should, in view of it relatively broad and encompassing nature, be combined with a severity scoring system or

probability risk estimation technique in order to measure the position of an individual patient along the continuum of inflammatory severity.[100] The frequency with which SIRS occurred in the current study, particularly amongst the 'open' cohort, indicates that gradation of the severity of SIRS is not only desirable but essential for the SIRS concept to be usefully applied in the research setting. Furthermore, it is proposed that the use of severity scoring systems in the current study, such as the 'cumulative SIRS score' and, perhaps to a lesser extent 'SIRS duration', goes some way to address the excessive sensitivity of the 1992 SIRS definition by adding the dimensions of a weighting system and/or a time scale, thereby enhancing its discriminatory potential. It is thus suggested that those subjects who may have been erroneously identified as having SIRS, in whom the identified physiological derangement was due to another overlooked factor causing a mild or transient physiological disturbance, would attract a much lower 'cumulative SIRS score' and 'SIRS duration' than those who experience persistent or severe systemic inflammation. It must be acknowledged, nonetheless, that a fundamental change to the 1992 consensus for defining and diagnosing SIRS, which is less inclusive and allows the identification of systemic inflammation with greater specificity, would increase the value and utility of the SIRS concept, and the conclusions of research, such as the current study, which are based upon it.

4.2.2 Definition of Sepsis

The revised definition and criteria for the diagnosis of sepsis that emerged from the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference represents a marked improvement of that established in 1992, from both clinical and research perspectives. As described in Chapter 1, Section 1.2.4, one of the most

notable aspects of the 2001 revision is the explicit statement that infection, and accordingly sepsis, may be diagnosed when an infectious process is strongly suspected even without microbiological confirmation.[99] This modification was an acknowledgement of the clinical reality that a positive microbiological culture may be obtained in less than 50% of patients believed to have sepsis on clinical grounds.[66, 68] From the perspective of diagnosing sepsis in observational research, such as the current study, employment of the 2001 criteria is likely to achieve a reduction in the rate of false-negative diagnoses that were undoubtedly a consequence of the 1992 definition of sepsis, and a rate of sepsis that more closely approximates clinical reality. It must also be acknowledged, however, that use of the 2001 criteria for diagnosing sepsis permits greater scope for false-positive diagnoses than the previous definition which required the presence of a 'confirmed infectious process'[100] . The possible false-positive diagnosis of subjects may, in turn, have hindered the capacity to identify a true association between this outcome measure and variables examined in the current study.

4.3 Incidence of Clinical Outcomes in Current Study

The validity and capacity to generalise the findings of the current study are dependent, in part, on the occurrence of the various outcome measures being consistent with those observed in current vascular practice.

4.3.1 SIRS

Whilst the relative dearth of published data on the incidence of this event amongst patients undergoing major vascular surgery, discussed in Chapter 1, Section 1.2.5, makes it difficult to conclude with great certainty that the findings derived from the current patient cohort are representative of that which might be observed in the population, it is reassuringly apparent that the 88.2% incidence of SIRS amongst the 'open' cohort is highly comparable with the recently described 89% incidence observed by the University of Leicester research group.[129, 130] Similar, reliable comparative data does not exist for the EVAR or 'lower limb' cohorts however it is entirely conceivable that the apparent validity of SIRS classification observed for the 'open' cohort may extend to all operative cohorts in the current study. It is acknowledged, nonetheless, that the smaller size of the EVAR and 'lower limb' cohorts is associated with an increased risk of having identified a non-representative incidence of SIRS for these operative procedures.

4.3.2 Sepsis

As discussed in Chapter 1, Section 1.2.5, not only is there a scarcity of published data on the incidence of sepsis following major vascular surgery, but the variable follow-up periods and lack of definitions which characterises this data, makes direct

comparison with the rates of sepsis identified in the current study fraught with hazards. Given the rates of SIRS affecting subjects in the current study, the documented incidence of sepsis occurrence within the three cohorts are not unreasonable in view of the rate of progression from SIRS to both culture-negative and culture-positive sepsis described by Rangel-Frausto *et al.*[66] (Chapter 1, Section 1.2.5).

4.3.3 General Post-operative Morbidity and Mortality

Of the measures of general post-operative morbidity employed in the current study, ICU and post-operative hospital LOS are amongst the more frequently described variables in current vascular literature, particularly that focussing on open AAA repair and EVAR. Thus, the descriptive statistics presented for these outcome measures are entirely consistent with recently published data, considered representative of international standards, for these patient populations.[17, 21] The 30-day mortality rate observed amongst the current cohort is similarly consistent with accepted standards.[12, 13, 16]

4.4 Rationale for Separation of Study Cohort

There is an intuitive clinical awareness that the three principal operative categories included in the current study differ with respect to a number of operative and post-operative variables in addition to their clinical outcomes. Statistical analyses confirmed that the operative cohorts did indeed differ with regard to multiple intra- and post-operative variables and measures of general post-operative morbidity (Chapter 3, Sections 3.1.1 and 3.1.2). These differences were frequently observed between the 'open' and EVAR cohorts and between the 'open' and 'lower limb' cohorts and may be considered to reflect the severity of the operative insult. Considered collectively, these differences thus indicate that, as might seem intuitive, open AAA repair induced a more severe surgical insult than either EVAR or lower limb revascularisation, whilst the magnitude of the insult associated with the latter two procedures appears to be relatively comparable. Inter-cohort differences in measures of SIRS severity, sepsis and post-operative infection were similarly demonstrated when subjects were considered as constituting three distinct cohorts (Chapter 3, Section 3.1.3). It is a general principle that cohorts should be maximally homogenous, except with regard to the variables of interest, in order to minimise potential confounding effects. The particular importance of adhering to this principle in studies examining SIRS, thereby avoiding erroneous conclusions, has been recently emphasised by Norwood *et al.*[129] . It was therefore considered desirable to separately consider the three operative cohorts for subsequent data analysis, where relevant and permitted by sample size considerations, thus eliminating the possibility of these analyses being confounded by the aforementioned factors. As suggested in Section 4.1.1, however, the pooling of cohorts was considered a statistical necessity for several analyses in view of sample size considerations.

4.5 Operative Variables - Relationship with Clinical Outcomes

In a study pursuing novel approaches to the early prediction of adverse operative outcomes, in particular SIRS and sepsis, it was considered necessary to establish from the outset whether several key operative variables, namely the duration of the operation and the duration of intra-operative ischaemia without reperfusion, may in fact have the capacity to independently predict the occurrence of these outcomes. Not only might such a finding explain the potential lack of relationship between operative outcomes and the other peri-operative variables examined, but would tend to recommend against further attempts to identify novel factors, particularly pre-operative patient characteristics, to predict these adverse outcomes.

4.5.1 SIRS

The intense ischaemia and subsequently IRI to which patients undergoing major vascular surgery, in particular open AAA repair, are subjected is undoubtedly a major stimulus for the activation of inflammatory pathways[223] . Furthermore, duration of ischaemia has been directly correlated not only with the production of cytokines including IL-6 during both open AAA repair[289] and EVAR[425] in addition to IL-10[289] and MCP-1 in open AAA repair[426] , but has also been found to be a correlate of the magnitude of intra- and early post-operative PMN activation, indicated by CD11b expression, in TAAA repair[153] . The lack of relationship between duration of ischaemia and measures of SIRS severity identified in the current study might therefore appear inconsistent with the clear biological contribution of intra-operative ischaemia and IRI to inflammatory events. It is conceivable that the

documented ischaemic duration may have been an insufficiently accurate measure of the magnitude of the actual ischaemic insult or IRI given that events such as clamp re-application were not accounted for in this measure. This, in turn, may have prevented the identification of a true relationship between the variables. It is more likely, however, that the inability to identify a relationship between duration of ischaemia and measures of SIRS severity is attributable to a more fundamental issue, with implications for many of the analyses involving SIRS as an outcome measure. This issue is that of the multi-factorial aetiology of SIRS[224, 225] which, combined with its complex and dynamic nature,[69] may have obscured the identification of a statistical relationship with individual stimuli or their surrogate markers. This issue is similarly likely to underlie the lack of a statistical relationship between operative duration and SIRS outcomes identified in the current study. If the finding of a lack of association between these key operative variables and SIRS outcomes is accepted as being valid, this might be considered as ratification of the need for novel predictors of these inflammatory outcomes.

4.5.2 Sepsis

It has been established that sepsis, like SIRS, is a complex and dynamic clinical syndrome, the occurrence and course of which must result from the interplay of a multitude of variables, including clinical, immunological and microbiological factors.[424] In view of this, the lack of any demonstrated relationship between the operative variables, 'duration of ischaemia' and 'operative duration', and the subsequent development of sepsis, cannot be considered to exclude the possibility that these variables are a component of the aetio-pathogenesis of sepsis, but rather this finding indicates that their individual role is insufficient to permit the

identification of a statistical relationship with sepsis occurrence. Ultimately, however, the inability of these operative variables to differentiate between those who progressed to sepsis and those who did not further validates the pursuit of novel subject-specific characteristics which may render individuals susceptible to sepsis.

4.5.3 Measures of General Post-operative Morbidity

Given that 'operative duration' may be considered a surrogate measure of the magnitude of the overall surgical insult, and that the maximum APACHE II score results in a direct measure of the magnitude of post-operative physiological derangement, the observation that those with a longer operative duration experienced a higher maximum APACHE II score is perhaps not surprising, whilst the finding that a longer 'operative duration' was associated with a longer post-operative LOS amongst the EVAR cohort is similarly intuitive. The statistical confirmation of these clinically intuitive relationships supports the validity of the measured variables.

4.6 Immunological Parameters

4.6.1 Pre-operative PMN CD11b, FcγRI (CD64) and FcγRIIIb (CD16b) - Relationship with Clinical Outcome

4.6.1.1 PMN CD11b

The observations relating to CD11b and SIRS outcomes are the most notable arising from the investigation of the relationship between pre-operative PMN expression of both integrin and immunoglobulin Fc receptors and post-operative inflammatory outcomes. Analyses indicated that a higher pre-operative expression of the PMN activation marker, CD11b, was associated with a higher post-operative cumulative SIRS score and tended to be associated with longer duration of SIRS amongst those in the EVAR cohort. If this is accepted as a valid observation it implies that those individuals who's PMNs existed in an already activated or primed state pre-operatively were subject to more severe, and perhaps more prolonged SIRS. As initially suggested by Foulds *et al.*[153] in their attempt to account for their comparable observations of a greater pre-operative CD11b expression amongst patients undergoing TAAA who died intra-operatively or shortly thereafter compared to those who did not, those who developed MOF compared to those who manifested failure of only a single organ, in addition to those who experienced major post-operative complications compared to those who did not, it is possible that amongst patients with a pre-existing state of PMN activation, indicated by a higher CD11b expression, intra-operative inflammatory stimuli serve as a secondary insult, resulting in an exaggeration of PMN activation thus fuelling the inflammatory cascade, translating to more severe or prolonged post-operative SIRS.

The apparent relationship between CD11b and post-operative SIRS documented in the current study is, as previously indicated, biologically plausible and particularly encouraging, however it is acknowledged that some caution is warranted in the interpretation of these results. It is conceded that the relationship between CD11b and SIRS duration failed to reach statistical significance. Furthermore, the small cohort size raises some concern about the heightened probability of a type I statistical error. The lack of a similar association between CD11b and measures of SIRS amongst the 'lower limb' and particularly the 'open' cohort might be considered as supporting the notion that the findings arising from the EVAR cohort were associated with a type I error. Conversely, it might be argued that these operative interventions are sufficiently distinct, both clinically and biologically, that findings in one cohort should not be expected to be replicated in another. It is conceivable, for example, that the greater ischaemic insult associated with open AAA repair compared to EVAR may nullify any pre-existing PMN activation, thereby eradicating a potential relationship between this pre-operative variable and post-operative outcomes. Finally, Rosenbloom *et al.*[427] have proposed that daily fluctuations in CD11b expression may occur in the absence of an obvious causative factor. Given that the current study's protocol did not require that blood sampling, for the purpose of determining CD11b expression, occur immediately prior to anaesthetic induction but permitted a small degree of variability in the precise pre-operative sampling time, if CD11b expression is indeed labile, it is possible that measured CD11b expression differed slightly from subjects' levels at the commencement of surgery, potentially invalidating the proposed biological basis of the statistical relationship.

The inability of pre-operative CD11b to distinguish between those who developed sepsis and those who did not implies that, if this factor does in fact play a biological role in the complex aetio-pathogenesis of sepsis, it is of an insufficient magnitude to be independently identified in the circumstances of the current study.

4.6.1.2 PMN FcγRI (CD64)

A particularly striking feature of the CD64 MFI data set, which can be appreciated from the tabulated data ranges (maximum and minimum values), is the lack of variance or spread of data about the median value. That is to say, pre-operative CD64 expression was highly comparable amongst subjects, not only within the separate operative cohorts, but throughout the study population. Furthermore, whilst comparison of subjects' MFI data with a control group is not within the scope of the current study, it is reasonable to suggest that, overall, CD64 expression was low, given that CD64 MFI values were not markedly greater than the reported representative FL1 negative control MFI. In view of this low and particularly homogenous level of expression of PMN CD64 throughout the operative cohorts, this variable clearly could not be expected to have any great discriminatory potential. This was confirmed by statistical analyses demonstrating the absence of a relationship with measures of SIRS or the occurrence of sepsis.

4.6.1.3 PMN FcγRIIIb (CD16b)

Unlike PMN CD64, CD16b expression levels varied considerably between individuals, reflected by the tabulated SEM for the CD16b MFI data. This is consistent with literature reporting considerable variation in levels of expression of this receptor amongst healthy volunteers[166] . Nonetheless, it is apparent that the

expression of this receptor demonstrated no relationship with measures of post-operative SIRS or the occurrence of sepsis. This is, perhaps, surprising given the two previously discussed reports (Chapter 1, Section 1.3.2.5) documenting a higher level of pre-operative CD16b expression amongst subjects who subsequently developed these adverse outcomes.[168, 185] Several factors may account for this inconsistency. Like the current study, both of the aforementioned studies involved relatively small patient numbers, hence all are subject to the similar probability of erroneously accepting or rejecting the null hypothesis. Furthermore, the study by Wakefield *et al.*[168] examined subjects undergoing major gastro-intestinal surgery rather than major vascular surgery, and employed a definition of sepsis which differed somewhat from the consensus definition employed in the current study. These differences are in themselves sufficient to account for the discrepancy between the studies findings. Whilst Spark *et al.*[185] did involve subjects undergoing open AAA repair, the group of subjects characterised by a higher pre-operative CD16b expression appears to be heterogenous with regard to the inflammatory outcome they experienced which is imprecisely described as 'SIRS or sepsis', raising the question of whether these findings can be reliably compared with those of the current study. Thus, whilst the current data suggests that pre-operative CD16b levels do not have a measurable influence on either post-operative SIRS or sepsis, and although valid explanations can account for the inconsistencies with published findings, an expansion of the current cohort may be warranted before a definitive conclusion regarding CD16b is made.

4.6.2 FcγR Genotypes and Clinical Outcomes

The observation that the distribution of both the CD32a and CD16b genotypes amongst the current study's Caucasian cohort followed Hardy Weinberg's equilibrium goes some way to indicate that the sample represented the genetic distributions that might be expected in the wider population. The recognition that the CD32a distribution observed in this study was, in fact, highly consistent with published reports involving generally large Caucasian cohorts, including a recent meta-analysis and, perhaps more relevant to the current study, a report of the CD32a genotype distribution amongst a moderately sized group of Australian Red Cross blood donors,[162, 199, 428-431] provides even more convincing evidence that the current cohort was genetically representative of the wider Caucasian population with respect to this FcγR polymorphism. Whilst the observed distribution of CD16b genotypes varied slightly from that previously identified by this laboratory amongst a similarly sized (n = 51) cohort of Australian Red Cross donors (NA1/NA1, 6.0% : NA2/NA2, 55.0% : NA1/NA2, 39%), it was not dissimilar to the distributions reported by studies involving considerably larger Caucasian cohorts[382, 428, 429, 431-434], implying that the current cohort could be considered as representative of the population with respect to the CD16b polymorphism.

Several biological explanations may account for a lack of association between the genotypes under investigation and the occurrence of either infection or sepsis following major vascular surgery. As described in Chapter 1, Section 1.3.2.6, the functional relevance of the CD32a polymorphism in infectious disease is principally attributed to the fact that CD32a-H131 represents the sole human FcγR capable of interacting with IgG2[149]. Given that this IgG sub-class is thought to be particularly

crucial for immunity against encapsulated bacteria,[149] , it is not surprising that a large proportion of the published clinical studies investigating CD32a-R131 associated susceptibility to infection have focussed on infections with a single, specific encapsulated bacteria as the established aetiological agent[96, 149] . Similarly, studies reporting CD16b-NA2 as a risk factor for infection have typically examined diseases caused by a single, specified organism, thought to trigger phagocytosis as a principle host defence mechanism[96, 149] . Multiple organisms, dependent on a variety of virulence factors and therefore stimulating a similar variety of host defences, are likely to have given rise to the cases of infection and sepsis in the current study, and to post-operative infection and sepsis in actual vascular practice. It might therefore be argued that the relatively restricted immunological consequences of the CD32a and CD16b gene polymorphisms are unlikely to induce susceptibility to such microbiologically heterogeneous post-operative events and may therefore account for current study's negative findings.

Before it is accepted that neither CD32a nor CD16b genotype bears any relationship to the occurrence of infection or sepsis following major vascular surgery, however, several other factors that may have adversely influenced the reported analyses require consideration. The relatively small size of the individual operative cohorts prohibited analyses of the relationship between genotype and clinical outcome being performed separately for the three cohorts. The approach of considering subjects within a single collective cohort for these analyses, whilst unavoidable, is considered sub-optimal due to the associated risk of introducing confounding factors, as discussed in Section 4.4. Furthermore, even after pooling of subjects was performed to increase the sample size, the probability that the analysis was underpowered is not

insignificant. As discussed by Ziegeler *et al.*,[207] gene association studies, such as this, must be sufficiently powered to account for genetic admixture, that is the inclusion of subjects originating from many distinct genetic backgrounds, within the study population. Whilst all subjects recruited to the current study were Caucasian, genetic admixture could not be avoided due to the genetically heterogeneous nature of the Australian population and therefore the population sampled by the current study. Ziegeler *et al.*[207] note the particular importance of this issue in studies examining complex polygenic diseases and recommend caution in the interpretation of negative findings arising from association studies examining 'only several hundred patients of high genetic admixture'. The current study, with its modest sample size, element of genetic admixture and use of the complex and presumably non-mendelian polygenic disorder of sepsis susceptibility[217] as an end-point, is therefore subject to these cautions.

4.6.3 Cytokine Responses in Major Vascular Surgery

4.6.3.1 Cytokine Levels Amongst Collective Aortic Aneurysm Repair Cohort

Before addressing the findings regarding the value of cytokines in the prediction of post-operative sepsis, the basic fluctuations, or lack thereof, in systemic levels of the assayed cytokines requires brief consideration.

It is apparent from both the graphical presentation and the descriptive statistics of assayed TNF-α concentrations, that amongst the collective cohort of patients undergoing aortic aneurysm repair, whether by an open technique or EVAR, very

few subjects displayed measurable levels of this cytokine at any of the assayed time points. Whilst it is considered to be amongst the most surgically relevant cytokines,[222] and is one of the most frequently assayed cytokines in studies examining the inflammatory response to AAA repair, there is, in fact, great variability in the literature in the reported capacity to detect circulating TNF-α and, when detected, there is a similarly large degree of variability in the reported magnitude of the TNF-α response[224] . Amongst the explanations previously proposed for these apparently contradictory findings, and particularly for the inability to detect circulating TNF-α, is the use of inaccurate assay methods[224] . Syk *et al.*[425] were, however, able to definitively exclude this as a possible explanation for the absence of TNF-α detected amongst their EVAR cohort. It has also been suggested that a lack of detectable TNF-α may be attributable to *in vivo* antagonism of this cytokine by soluble TNF-α receptors.[224] It is, however, the observation by Swartbol *et al.*[224] that plasma levels of TNF-α peak and decline rapidly following the provoking stimulus, and such a transient fluctuation may therefore be missed without intensive blood sampling intra-operatively and within the first 24 hours post-operatively, that is most likely to account for the overall lack of TNF-α observed in the current study.

As for TNF-α, detectable fluctuations of IL-1β were absent amongst the majority of subjects undergoing AAA repair, despite this cytokine being considered as one of the prototypical pro-inflammatory cytokines induced by common surgical stimuli[222] and multiple reports of elevated levels associated with open AAA repair and EVAR[224] . Once again, several explanations may account for this apparent inconsistency. Amongst these is the suggestion by Baigrie *et al.*[294] that the overflow of IL-1β into the systemic circulation in fact occurs less consistently than other pro-inflammatory

cytokines, such as IL-6. Particularly relevant to the findings of the current study is the observation by these, and other authors, that peak IL-1β concentrations associated with major surgical insults are both early and transient and may only be detected by intense intra-operative blood sampling.[224, 294] As suggested for TNF-α findings, it is therefore possible that the frequency of blood sampling in the current study was insufficient to detect actual elevations in IL-1β.

Whilst IL-12 has also been nominated as a surgically relevant cytokine[222] , it has been infrequently assayed in the relevant vascular surgery literature. The reasons for the relatively infrequent occurrence of measurable IL-12 elevations observed in the current study are therefore less certain, but may include methodological factors. Jedynak *et al.*[295] reported that fluctuations in systemic IL-12 concentrations did occur amongst their relatively small cohort of patients undergoing AAA repair and concentrations were significantly higher than amongst a control group. Possible explanations for the apparent discrepancy between these findings and those of the current study include differing blood sampling times and Jedynak *et al.*'s use of a reportedly highly sensitive enzyme-linked immunosorbent assay (ELISA) technique.[295]

Whilst the time course of cytokine fluctuations was not evaluated statistically, it is notable that the peak levels of all assayed cytokines tended to occur at the $T_0 + 4$ time point. This is consistent with the predominant stimuli for cytokine production during the operative and peri-operative period having been the cumulative surgical insult, intra-operative ischaemia and, perhaps more importantly, IRI.

4.6.3.2 Prediction of Sepsis from Plasma Cytokine Values

Of the cytokines for which notable elevations in plasma concentrations were observed to have occurred in response to surgery, namely IL-6, IL-8 and IL-10, multiple regression demonstrated the tendency for maximum concentrations of IL-6 and IL-8, in addition to the average hourly IL-10 concentrations, to predict the occurrence of sepsis amongst the collective cohort, however, the latter was identified as being the most valuable for this purpose. When considered in the context of sepsis pathogenesis, the statistical tendency for greater average IL-10 concentrations to predict sepsis occurrence within the study cohort may be interpreted in several ways. Firstly, greater average hourly concentrations of the anti-inflammatory cytokine, IL-10, may be considered a response to, and therefore reflective of a more intense pro-inflammatory cascade, which differentiates those developing or manifesting sepsis from patients who do not progress to sepsis. Alternatively, it is proposed that greater average hourly IL-10 concentrations may characterise patients succumbing to overwhelming CARS who are therefore subject to the associated immunological anergy and susceptibility to infection[106-111] and ultimately the risk of sepsis.

Whilst the relationship between IL-10 and sepsis contributes to the body of knowledge about sepsis susceptibility and pathogenesis and emphasises the significance of the anti-inflammatory response in surgical outcomes, the inability of cytokine concentrations to predict sepsis occurrence for the individual indicates that these findings are unlikely to translate to a useful clinical tool. A cautionary warning regarding the potential statistical implications of pooling two operative cohorts (Section 4.4) does, however, apply to the regression analyses and their interpretation.

4.7 Pre-operative Nutritional Status and Relationship to Clinical Outcomes

4.7.1 Pre-operative Nutritional Status

Amongst the measures employed in this study, the MNA together with the commonly used anthropometric measure of BMI, whilst not without their individual limitations, serve as useful indicators of the overall nutritional status of the study cohort. In view of previous reports of the considerable frequency of impaired nutritional status amongst hospitalised surgical patients,[304] and particularly the 18% incidence of impaired nutrition documented by Warnold *et al.*[305] amongst patients undergoing major vascular surgery, it is perhaps surprising that, amongst all subjects whose nutritional status was classified using the MNA, only one was identified as being 'malnourished'. Whilst the use of the MNA may be subject to some criticism, including its application to several subjects who, in view of their relative youth and/or freedom from functional impairment, could not be considered to be amongst the frail, elderly population for whom the tool was designed[389] , its indication that global nutritional status was adequate amongst a surprisingly large proportion of the collective cohort, is strongly supported by the reported BMI descriptive statistics.

Whilst comparison with published rates of malnutrition cannot be performed with any precision due to the variety of measurement tools employed and the associated variability in the definitions used in the existing literature, it is reasonable to suggest that poor nutrition was not as prevalent as might have been predicted. Several explanations for this observation are proposed. It is suggested that involvement of only elective patients, and hence the exclusion of those undergoing emergency

vascular interventions or non-surgical management of vascular pathology, may result in recruitment from a population of patients who have already been selected on the basis of a satisfactory general medical status, of which nutritional adequacy is an integral part. Furthermore, selection bias may have generated data indicative of a better nutritional status than actually present in the target population. For example, inability to obtain informed consent as a consequence of cognitive impairment, a factor known to be associated with malnutrition,[387] may have contributed to the under-representation of malnourished individuals. A similar selection bias may have occurred as a consequence of the conceivable association between refusal to participate and a greater burden of co-morbidity, which is, in turn, suggestive of a greater risk of malnutrition[387].

A final point to appreciate, before considering the analyses of nutritional data, is that the early death of two subjects within the 'open' cohort, whose nutritional status was classified as 'malnourished' and 'at risk of malnutrition' respectively, resulted in an inability to include their nutritional data in relevant analyses due to the lack of associated outcome measures. This therefore resulted in a further reduction in data representative of poorer nutritional status in analyses of the 'open' cohort.

4.7.2 BMI and DEXA Derived Measures of Nutritional Status - Relationship to Clinical Outcome

4.7.2.1 SIRS

Of the five DEXA derived measures of body composition analysed, two indices, namely fat free mass (FFM) and estimated skeletal muscle mass (SMM), in addition

to the anthropometric measure of BMI, demonstrated noteworthy associations with measures of post-operative SIRS. Amongst the 'lower limb' cohort it was notable that a lower BMI was associated with both a higher SIRS score and a longer duration of SIRS, however, this relationship was not replicated amongst the 'open' or EVAR cohorts. It should be noted that the correlations performed for the latter two cohorts involved larger data sets, and may therefore be considered as statistically more reliable than those of the 'lower limb' cohort.

Similar to the aforementioned BMI findings, amongst those within the 'open' cohort, a lower pre-operative FFM was associated with a higher post-operative SIRS score, and tended to be associated with a longer duration of SIRS. Whilst the latter association just failed to reach statistical significance within the 'open' cohort, this same relationship was statistically significant within the EVAR cohort. When FFM was indexed for subjects' height to yield the variable FFMI, the strength and statistical significance of the previously observed associations with SIRS outcomes were no longer apparent. Given that FFM is, in effect, a surrogate marker of the individual's total skeletal muscle mass[399] it is reassuring that a lower SMM was similarly, and significantly associated with both a higher post-operative SIRS score and a longer duration of post-operative SIRS within the 'open' cohort. It is interesting that no relationship between fat mass and measures of SIRS outcomes were apparent, despite the biological basis of the hypothesised relationship being the same as for both FFM and SMM. This, however, by no means invalidates the findings relating to the latter two indices of body composition.

These findings indicate that amongst those subject to major vascular surgery, particularly open AAA repair, a lower pre-operative lean body mass, and perhaps more precisely a lower total skeletal muscle mass, is associated with the development of more severe post-operative SIRS. Stated conversely, a greater lean or skeletal muscle mass appears to protect against the development of a more severe systemic inflammatory response to major vascular surgery. As SMM is considered a reliable indicator of PEM[400] the aforementioned relationship implies that those with PEM, identified by a particularly low SMM, are more likely to develop severe post-operative SIRS than well nourished individuals, who necessarily have a higher SMM. Whilst the findings relating to BMI are perhaps less reliable than those pertaining to FFM and SMM, they are consistent with the notion that poorer nutrition may be related to a more intense inflammatory response to surgery. Collectively, these findings are consistent with the conclusions drawn from both the limited number of clinical studies examining the effect of nutritional status on the inflammatory response to surgery, which have reported a more marked pro-inflammatory response amongst malnourished individuals,[317, 318] and from immunonutritional studies which have identified a dampening of surgically stimulated inflammatory responses by an improved nutritional status[319-321].

4.7.2.2 Measures of General Post-operative Morbidity

As described in Chapter 1, Section 1.5.3, there is a relative abundance of literature documenting the relationship between poor nutrition and a variety of adverse post-operative outcomes. Examining the relationships between nutritional indices and adverse post-operative events was therefore not intended to be conceptually novel. Rather, the identification of trends and significant associations between the various

markers of post-operative morbidity and the DEXA-derived measures of FFM, FFMI, and SMM, in particular, reinforces the validity of the suggestion that, like BMI, these relatively novel measures of body composition do demonstrate sound, and biologically relevant relationships with surgical outcomes.

4.7.2.3 Sepsis

The biological basis for the postulate that poorer nutritional status may increase the risk of sepsis following major vascular surgery would seem to have a particularly strong biological basis, which includes the extensively documented association between poor nutrition and post-operative infection[304] . The findings that malnutrition impairs the gut's function as a barrier against translocation of bacteria and their toxins, particularly in the presence of insults known to be associated with major vascular surgery, and that disruption of gut barrier function may, in turn, lead to sepsis and MOF[302] are also strongly in favour of identification of a relationship between poor nutrition and the development of sepsis in the current study. Yet, this was not the case, as septic and non-septic individuals were unable to be differentiated using BMI and body composition indices as markers of nutrition. This finding is surprisingly consistent with that of Hatada *et al.*[317] who noted that the incidence of post-operative sepsis did not differ significantly between well nourished and malnourished individuals undergoing surgical management of colorectal cancer. It therefore seems likely that whilst nutritional status may play some role in post-operative sepsis pathogenesis, it may not be a straight-forward relationship, nor one which can be demonstrated clinically, given the complexity of both the immunological perturbations associated with differing nutritional states[312-316] and the aetiopathogenesis of sepsis.

4.7.3 MNA Classification of Nutritional Status - Relationship to Clinical Outcome

In view of the preceding evidence, that specific pre-operative nutritional indices do have a relationship with post-operative outcomes, namely measures of SIRS severity and selected measures of general morbidity following major vascular surgery, despite the apparent lack of relationship between these outcome measures, in particular SIRS, and nutritional status classified by the MNA it cannot be concluded that pre-operative nutrition is unrelated to these outcomes. Rather, it is suggested that the lack of relationship must be attributed to the nature of the MNA itself. It is most likely that the MNA's broad categorisation of subjects, whilst clearly desirable in a clinical setting, fails to identify with sufficient detail, those specific aspects of subjects' nutritional status that relate to the severity of post-operative inflammatory events, and the development of specific post-operative morbidities. The single positive finding arising from analyses of MNA data, that those 'at risk of malnutrition' within the EVAR cohort manifested a higher maximum APACHE II score than well nourished subjects, whilst entirely consistent with the exiting literature, is not considered statistically reliable given that there was only one subject within the aforementioned nutritional category.

Whilst the reliability of the analyses examining the relationship between MNA classification of nutrition and sepsis is adversely affected by the particularly small cohort sizes, in light of the preceding discussions regarding both nutritional status and the likelihood of identifying a statistical relationship with sepsis occurrence (Section 4.7.2.3) and the limitations of the MNA in the current setting, it is proposed that expansion of the cohort would, in fact, confirm the current findings.

4.8 Neuroendocrine Response to Surgical Intervention

4.8.1 Potential Limitations of Neuroendocrine Analyses

It may be apparent that the validity of the data obtained from neuroendocrine assays, and therefore the findings drawn from them could, conceivably, have been adversely affected by several factors. These are therefore considered before addressing the findings arising from analyses of this data.

4.8.1.1 Administration of Exogenous Catecholamines

As discussed in Chapter 2, Section 2.12.1, the pharmacological effect of exogenous catecholamine administration on measures of urinary adrenaline and noradrenaline determined by HPLC is currently not documented. The possibility that exogenously administered adrenaline and noradrenaline may be excreted in subjects' urine and subsequently measured by the HPLC assay cannot therefore be excluded. If this were the case, urinary adrenaline and noradrenaline measures would no longer reflect the physiological stress response alone, but would reflect a combination of pharmacological therapy together with the subjects' innate stress response. Given that a protocol preventing administration of exogenous catecholamines was not considered ethically acceptable, and exclusion of patients who were administered these agents was prohibited by the frequency of their use in major vascular surgery, the alternative approach of statistically controlling for the potential effect of this factor on neuroendocrine analyses was adopted. As noted in Chapter 3, Section 3.5.1, this was achieved by entering the volume of exogenous adrenaline or noradrenaline administered as a covariate in relevant neuroendocrine analyses. It is undeniable that, if exogenous catecholamine administration did, in fact, influence urinary

neuroendocrine measures, statistical manipulations could not fully compensate for such a methodological problem.

4.8.1.2 Renal Function

As indicated in Chapter 2, Section 2.8.4, the value of urinary measures of excreted cortisol and catecholamines in the current study is dependant on the observation that the measured values are closely related to their corresponding serum concentrations[384, 385]. It is reasonable to suggest that this relationship may be altered in the presence of a particular degree of renal dysfunction, and therefore urinary excretion of cortisol or catecholamines may no longer strictly reflect neuroendocrine responses. This is, however, unlikely to have occurred to any notable extent in the current study given both the limited severity of pre-operative renal disease amongst the collective cohort (Chapter 2, Section 2.2, Table 6) and the limited occurrence and severity of renal dysfunction as a post-operative complication (Chapter 3, Section 3.1.2).

4.8.2 Time Course of Neuroendocrine Responses

4.8.2.1 Urinary Free Cortisol (UFC)

The observed rise in UFC from the pre-operative assay period to T(0-24) within all operative cohorts reflects the known stimulatory effect of surgical trauma on the HPA axis[322] and is consistent with the elevations in plasma cortisol that have been observed at comparable post-operative time points in amongst mixed and general surgical cohorts subject to either moderate[435] or severe surgical trauma[436]. Published data on neuroendocrine responses at later post-operative time points are relatively

lacking, particularly following major vascular surgery, hence the findings arising from the current study at T(72-96) are a somewhat more novel contribution to current knowledge. It is notable that whilst all cohorts demonstrated a trend towards a decrease in UFC at this time, this was significant only in the 'lower limb' cohort, and indeed levels at T(72-96) approached pre-operative, baseline values in the latter cohort. This is perhaps a more sensitive indicator of the relative degree of surgical trauma induced by the different vascular interventions than intra- and post-operative clinical variables, and implies a more rapid physiological recovery following lower limb revascularisation than occurs following either method of AAA repair. The limitations associated with the EVAR data at T(72-96) (Chapter 3, Section 3.5.1) notwithstanding, it is interesting to consider that, on the basis of cortisol responses, which are considered to correlate well with the degree of surgical trauma,[322] a significant physiological stress response persisted at a time when many EVAR patients are discharged.

4.8.2.2 Adrenaline

The apparent rise in urinary adrenaline levels from baseline values to those observed at T(0-24), although failing to reach statistical significance among the 'lower limb' cohort, is similarly consistent with the known stimulatory effect of surgery on the SNS.[322] The suggested decrease in adrenaline levels by T(72-96), which reached significance in the 'open' cohort but admittedly not within the EVAR cohort, implies that the SNS, and particularly its adrenomedullary component, may return to normal baseline function following surgical insults of this magnitude, more rapidly than the HPA axis. The overall lack of statistical significance associated with the fluctuations in adrenaline over time within the 'lower limb' cohort, together with the observation,

not subject to statistical analysis, that lower limb revascularisation gave rise to generally lower levels of adrenaline than AAA repair, may be interpreted as indicating that this was a weaker, and more variable stimulus for adrenaline secretion than AAA repair, regardless of whether by an open or endovascular approach.

4.8.2.3 Noradrenaline

The overall lack of statistically significant noradrenaline fluctuations, with the exception of the significant rise from baseline to T(0-24) with the 'lower limb' cohort, suggests that this catecholamine may be a less sensitive, and therefore useful marker of the stress response to surgery than either cortisol or adrenaline. It is notable, nonetheless, that within both the 'open' and 'lower limb' cohorts, following an initial rise at T(0-24), a further rise in noradrenaline levels occurred at T(72-96). Whilst the lack of statistical significance associated with these trends cannot be dismissed, the apparent persistent rise in noradrenaline is not entirely surprising in view of findings which have noted elevations in noradrenaline that are more sustained than the rise in adrenaline following traumatic[437] and surgical injury[435].

4.8.3 Association Between Neuroendocrine Responses and Clinical Outcome

4.8.3.1 SIRS

It is notable that there was a direct and highly statistically significant association between HPA axis activity at T(72-96) and measures of SIRS severity within the collective cohort. This relationship may be interpreted in several ways. The more simplistic and perhaps more likely explanation for the relationship is that greater

HPA axis activity, and in turn greater cortisol excretion, reflects the greater degree of physiological stress experienced by subjects with more severe inflammatory responses. Subtly different from this, is the possibility that, given the known stimulatory effect of pro-inflammatory cytokines on the HPA axis[331] , the higher levels of pro-inflammatory cytokines possessed by those with more severe SIRS may stimulate the secretion and therefore excretion of greater amounts of cortisol. The finding that a greater adrenaline response at T(72-96) was directly and significantly associated with both a greater cumulative SIRS score and more prolonged SIRS is, however, inconsistent with the immunosuppressive and anti-inflammatory properties attributed to catecholamines in the literature[334] . It is therefore most likely that the relationship demonstrated in the current study simply reflects the greater physiological stress, and therefore greater degree of adrenomedullary secretion of adrenaline, that occurred amongst subjects who experienced more severe SIRS. If it is accepted that noradrenaline is a less sensitive indicator of surgical stress than either cortisol or adrenaline, as previously suggested (Section 4.8.2.3), then it is not surprising that a relationship between this catecholamine and SIRS severity did not exist. Regardless of the biological mechanisms underlying the observed relationships between SIRS severity and levels of either cortisol or adrenaline, the time point at which the relationships were observed does not support the notion that neuroendocrine responses may be clinically useful predictors of SIRS, but rather they may simply reflect the presence or severity of inflammatory events.

4.8.3.2 Sepsis

Analyses of the relationship between neuroendocrine responses and sepsis within the collective cohort demonstrated that those who developed post-operative sepsis were

characterised by greater cortisol and adrenaline responses at the T(72-96) time point. It is almost certainly not coincidental that these mediators of the stress response, and the time point at which they were assayed, are the same as those found to be associated with SIRS severity. Once again several biological mechanisms are proposed for the observed relationships with sepsis occurrence. A plausible explanation for the observed relationship between cortisol and sepsis occurrence is that greater glucocorticoid levels, reflecting a more intense HPA axis response to the surgical insult, may have exerted an immunosuppressive effect[329, 334] which, in turn, may have induced sepsis susceptibility amongst these individuals. In the case of adrenaline, it may be similarly suggested that a greater adrenaline response to surgery induced sepsis susceptibility as a consequence of this catecholamine's capacity to suppress cellular immunity and promote a switch to a predominantly anti-inflammatory humoral immune response[334] . There is, in fact, considerable evidence supporting this notion that exaggerated SNS and HPA axis activity, arising from physical trauma, or indeed psychological stress, may result in susceptibility to infectious processes.[334] It must be acknowledged, however, that as this explanation implies causation of sepsis by heightened glucocorticoid and catecholamine levels, it would be more plausible if the relationship had involved UFC and adrenaline assayed at the earlier T(0-24) time point. As suggested for SIRS severity, it is therefore most likely that the occurrence of both greater cortisol and adrenaline responses amongst those who developed sepsis simply reflects the greater degree of physiological stress, and in turn HPA axis and SNS stimulation being experienced by septic compared with non-septic patients.

4.8.3.3 Measures of General Post-operative Morbidity

The observation that a greater cortisol response at T(72-96) was directly and significantly associated with the maximum APACHE II score, together with the finding that greater levels of this glucocorticoid assayed at the T(72-96) interval distinguished subjects who developed one or more moderate/severe complications from those who did not, supports the notion that a more intense HPA axis response at this post-operative time point simply reflects the greater physiological derangement being experienced by subjects. These findings therefore render the preceding alternative explanations, that the relationships between SIRS or sepsis and mediators of the stress response were a consequence of bi-directional modulation between the neuroendocrine axes and the immune system, far less likely. Whilst the statistical significance associated with the finding that greater noradrenaline levels at T(0-24) were experience by those who were free of one or more moderate/severe post-operative complications compared to those who did experience this adverse outcome was marginal, a biologically viable explanation can account for this finding. It is plausible that, in contrast to the earlier findings involving neuroendocrine responses at a later post-operative time point, a greater catecholamine response at the earlier T(0-24) interval may reflect the fact that neuroendocrine responses evolved as an integral part of the hosts ability to achieve homeostasis following injury[324] . Thus catecholamine secretion of an adequate, but not excessive magnitude at this assay interval may therefore have contributed to the achievement of a satisfactory resolution to physiological disturbance, hence reducing the likelihood of subsequent morbidity. If this is indeed the case, noradrenaline responses during this interval may have some capacity to predict general post-operative morbidity.

4.9 Psychological Measures and Influence on Surgical Response and Outcomes

4.9.1 Incidence of Pre-operative Depression

It is apparent that the use of the CES-D indicated that pre-operative depression was present amongst a somewhat greater proportion of the collective cohort than suggested by the BDI-II inventory. This discrepancy was unexpected, since concordance between the inventories has been previously demonstrated,[405] and the reason for it is uncertain. Indeed, it would seem more likely for the BDI-II to indicate a greater incidence of depression amongst a surgical cohort given that the BDI has been criticised for having items that are confounded by the physical sequelae associated with physical disability or disease[404] and may therefore give rise to false-positive classifications amongst such a population, in contrast to the CES-D which has been widely used and validated in cohorts of medical patients and the elderly[404]. It has, however, been suggested that the CES-D may not be specific for depression, but may be a measure of general distress.[404] If this were the case, an inflated incidence of depression could conceivably arise from the use of this inventory in a cohort of patients facing the potentially distressing prospect of impending major vascular surgery.

Whilst there is a degree of uncertainty about the true incidence of depression in the current study, due to the discrepancy between the rates suggested by the two depression inventories, it is likely to have been in the range of 19.0% to 30.8%, as indicated by the BDI-II and CES-D respectively. This is somewhat greater than the incidence of depression warranting intervention observed amongst elderly members

of the general population[336] , but is highly comparable to published rates of depression observed amongst patients undergoing CABG[338-341] .

It is unlikely that the aforementioned degree of uncertainty regarding the precise incidence of pre-operative depression in the current study markedly affected the conclusions able to be drawn from analyses involving this variable since all relevant analyses were performed using both the BDI-II and the CES-D findings. It is worth noting that standard cut-off scores, designed to identify even mildly depressed individuals,[405] were used in the current study. The use of more stringent cut-off scores may have reduced the probable inclusion of false-positives in the group of subjects categorised as 'depressed' and may, in turn, have altered the results of analyses involving this variable.

4.9.2 Depression and General Post-operative Morbidity

Employing post-operative LOS and the occurrence of one or more moderate/severe complications as markers of early post-operative morbidity, the current study found no evidence to support the hypothesis that pre-operative depression is associated with greater general post-operative morbidity. These results are therefore at variance with findings emanating from studies amongst cardiac patients,[342-345] and particularly those derived from cohorts of patients undergoing CABG surgery[338, 339] which have demonstrated that depression increases the risk of not only subsequent morbidity but also mortality. It must be noted, however, that these studies examined morbidity and mortality no earlier than 6 months following the relevant cardiac event or intervention, whereas the current study's outcome measures of morbidity were limited to the very early post-operative period. It is therefore possible that

insufficient time was allowed to elapse for a relationship between pre-operative depression and post-operative morbidity to manifest. It is, however, possible that the mechanisms underlying the previously documented relationship between depression and morbidity, which remain uncertain,[337] may not generalise to the cohort of vascular surgery patients examined in the current study. For example, it has been postulated that the relationship documented amongst cardiac patients may exist as depression may result in poor compliance with treatment recommendations, continued smoking, low motivation for positive lifestyle change and potentially serotonin-mediated disturbances in platelet function which, in turn, may all contribute to a greater rate of morbidity and mortality amongst patients with cardiac disease.[337] These factors are far less relevant to subjects undergoing AAA repair. Whilst these biological mechanisms may be more relevant to subjects undergoing lower limb revascularisation for atherosclerotic peripheral vascular disease, it should be recognised that these patients constituted a relatively small proportion of the current study cohort. It is therefore possible that a relationship between pre-operative depression and post-operative morbidity may become evident amongst an expanded 'lower limb' cohort.

4.9.3 Depression and Health-related Quality of Life

The finding that the mental health aspect of HRQoL was poorer amongst depressed patients pre-operatively was not unexpected and was recently demonstrated by Cheok *et al.*[337] amongst a large cohort of cardiac patients. In contrast to the findings of these authors, however, the physical component of pre-operative HRQoL was not affected by the presence or absence of depression in the current study. It is reassuring

that analyses using the two respective depression inventories yielded consistent findings regarding the relationship with pre-operative HRQoL.

It is notable that analyses involving the BDI-II and CES-D respectively, both demonstrated that at one month post-operatively, pre-operative depression was no longer related to the mental component of HRQoL. Given the absence of a pre-operative relationship between depression and the physical component of HRQoL, it is not surprising such a relationship was similarly absent at both one and three months post-operatively.

Employing the BDI-II categorisation of subjects, by three months post-operatively, pre-operative depression was once again associated with a poorer mental health component of HRQoL. It is apparent that the analysis employing the CES-D categorisations failed to demonstrate this relationship. The possibility that the use of the CES-D resulted in more false-positive diagnoses of pre-operative depression than the BDI-II, in association with the smaller sample size involved in analyses at this time point may account for this finding.

In view of the particularly small sample size involved in analyses of HRQoL six months post-operatively, the results of these analyses must be interpreted cautiously. Whilst the observation that subjects categorised as depressed by the BDI-II experienced a poorer physical component of HRQoL at 6 months is not unreasonable, and a comparable trend was indeed apparent when the CES-D classification of depression was employed, a larger sample size is required to confirm these findings. It is, nonetheless, conceivable that non-depressed individuals interpret

their physical recovery, and associated quality of life, more positively than depressed individuals. The seemingly paradoxical finding that depressed patients experienced a better HRQoL at six months post-operatively, from the perspective of mental health, seems unlikely. The fact that this relationship was demonstrated only by the analysis of BDI-II data makes it particularly questionable.

It would not be incorrect to suggest that these findings indicate that patients who are depressed pre-operatively experience a poorer mental health aspect of HRQoL three months post-operatively. This is similar to the conclusion reached by Mayou *et al.* [351] from their study examining depression and anxiety as predictors of outcome in MI patients in which they suggest that baseline depression predicted poorer HRQoL at three and twelve months following subjects' cardiac event and baseline psychological evaluation. It is suggested that a more accurate interpretation of the collective findings regarding pre-operative depression and the mental aspect of HRQoL in the current study is that these two factors are indeed intimately related, as indicated by analyses of pre-operative data, however operative intervention, and perhaps the distress associated with it, results in the mental aspect of HRQoL experienced by all subjects at one month post-operatively being comparable, regardless of the presence or absence of depression pre-operatively. By three months post-operatively, it is likely that patients had recovered sufficiently from the operative intervention to permit the re-emergence of the pre-operative relationship between depression and HRQoL.

4.9.4 Depression and Post-operative SIRS and Sepsis

As previously discussed, (Chapter 1, Section 1.7.1.3) the proposal that pre-operative depression my influence the occurrence or severity of surgical outcomes, such as SIRS or sepsis, which are explicitly related to immune function is based on abundant evidence that depression and psychological stress are associated with immune dysfunction and an increased susceptibility to infectious disease[353-355] . In the current study, however, there was no demonstrable relationship between pre-operative depression and post-operative sepsis and the only evidence to support the notion of a relationship between pre-operative depression and post-operative SIRS, was the finding that non-depressed subjects undergoing EVAR experienced a longer duration of SIRS than their depressed counterparts. The reliability of the positive finding within the EVAR cohort is somewhat questionable given that it was derived from a particularly small sample size, was not replicated by the analysis based on the CES-D categorisation of depression status, and was not observed amongst the 'open' or 'lower limb' cohorts. When considering the reason for the lack of a demonstrable relationship between depression and the aforementioned measures of post-operative outcome, it must be appreciated that the known relationship between depression and immune function is by no means straight-forward, as some aspects of immunity are suppressed amongst depressed individuals whereas other components of the immune response are enhanced[356] . When the effect of surgery, with its associated complex effects on immunity, is added to this relationship, it is perhaps not surprising that no clear, overwhelming relationship was identified with outcome measures whose pathogeneses are themselves complex.

4.9.5 Depression and Neuroendocrine Responses to Surgery

It is accepted that HPA axis hyperactivity occurs frequently amongst depressed individuals,[353, 354] and manifests as elevations in both CRF and cortisol[354] . Of particular relevance to the current study is the observation that not only has hypercortisolaemia been demonstrated amongst depressed individuals[354] but 24-hour urinary cortisol excretion has also been reported to be both significantly correlated with depressive symptoms, measured by BDI scores, and significantly greater amongst those who have clinically significant depression compared to non-depressed individuals[438] . It is perhaps surprising then that baseline, pre-operative UFC did not differ between depressed and non-depressed individuals in the current study. It was even more unexpected, and in fact seems paradoxical, that levels of UFC at the T(0-24) assay interval were found to be greater amongst non-depressed subjects compared to those who were classified as depressed pre-operatively by the BDI-II. Given that this difference was not demonstrated by the analysis based upon the CES-D classifications of depression, and furthermore, that these analyses pooled data from the three operative categories and may therefore have been confounded by this effect, the value of this finding seems questionable. Greater certainty regarding the issue of whether depression influences the HPA-axis response to vascular surgery may be achieved by expansion of the operative cohorts and separation of these cohorts for the purpose of data analysis.

4.9.6 Pre-operative Trait Anxiety and Neuroendocrine Response to Surgery

The finding that trait anxiety was directly and significantly associated with pre-operative noradrenaline levels is consistent with the relationship identified amongst healthy individuals[438] and replicates the results of Salmon *et al.*[359] derived from their comparably sized cohort of subjects undergoing major abdominal surgery. The positive correlation between trait anxiety and post-operative levels of noradrenaline observed by Salmon *et al.* [359] was not, however, apparent in the current study. Indeed, the current study failed to demonstrate any association between subjects' trait anxiety and neuroendocrine responses to surgery. This is at variance with the small body of literature which suggests that there is a significant inverse association between pre-operative trait anxiety and levels of intra-operative adrenaline[359] as well as post-operative levels of both adrenaline and cortisol[327, 359] amongst cohorts undergoing major abdominal or minor colorectal surgery. These same researcher groups have also reported a similar inverse relationship between pre-operative state anxiety and post-operative levels of both cortisol[327, 439] and adrenaline[439] . It is possible that the approach of pooling data from the three operative categories, which have been demonstrated to be associated with differing degrees of surgical trauma, may have introduced a confounding effect which, in turn, contributed to the failure to demonstrate the hypothesised relationship between pre-operative anxiety and neuroendocrine responses to surgery. The discrepancy between the findings of the current study and the aforementioned publications may, however, be attributable to methodological differences. Whilst reports of a relationship between anxiety and neuroendocrine responses to surgery have been based on plasma cortisol and catecholamine levels sampled at specified post-operative intervals,[327, 359] which have

the capacity to detect transient fluctuations in the assayed hormone, the correlations in the current study were based on urinary measures of cumulative HPA axis and SNS responses over 24 hours. It is therefore possible that the current methodology was not sufficiently sensitive to demonstrate the putative relationship between trait anxiety and neuroendocrine responses to major vascular surgery.

4.10 Immunological and Neuroendocrine Responses in Open AAA Repair Compared to EVAR

4.10.1 Comparability of 'Open' and EVAR cohorts

In order to reliably compare open AAA repair and EVAR, with regard to the immunological and neuroendocrine responses associated with the respective operative interventions, it is a general principle that the cohorts should be comparable with respect to all variables other than those that are the subject of analysis. Prior to examining differences demonstrated between the cohorts, a consideration of factors which may have confounded the statistical comparisons and potentially compromised the capacity to attribute observed differences to the nature of the operative procedures is therefore warranted.

It is apparent that subjects constituting the EVAR cohort were significantly older than those undergoing open AAA repair. Given that the phenomenon of human immunesenescence has been widely established[440] and may include alterations in cytokine production,[441] this could, theoretically, have confounded inter-cohort comparisons of SIRS occurrence and severity and cytokine responses. Furthermore, in view of some evidence of HPA axis activation[442] and adrenal hyperfunction amongst the elderly[443] it is reasonable to suggest that age may have influenced subjects' physiological stress response and therefore influenced the ability to reliably compare the neuroendocrine response stimulated by the alternative methods of AAA repair. Aneurysm morphology also differed somewhat between the cohorts. Thus, the greater maximum aneurysm diameter characterising the 'open' cohort could conceivably have influenced the immunological and, potentially, the neuroendocrine

comparisons between the cohorts given that aneurysm diameter may influence the extent of dissection during open AAA repair, whilst the postulated contribution of thrombus to cytokine release during EVAR[290] may imply that thrombus and, in turn, aneurysm size may be relevant to the responses to this method of AAA repair. Furthermore, the inclusion of subjects' in the 'open' cohort with juxtarenal AAAs and TAAAs, as well as those with infrarenal AAAs, of which the EVAR cohort was exclusively comprised, may have similarly confounded the comparison of the operative techniques. It is also apparent that concomitant non-vascular procedures occurred in 13.9% of the 'open' cohort but were not required by any member of the EVAR cohort. Whilst these additional procedures may, theoretically, have contributed to both the immunological and neuroendocrine response attributed to the open method of repair, it should be noted that all concomitant non-vascular procedures were minor interventions; hence the physiological response they induced was likely to have been minimal. Importantly, the two operative cohorts were highly comparable with respect to the burden of comorbidity, as indicated by the Charlson Index and ASA scores, in addition to the incidence of specific comorbid conditions.

The limitation associated with the inability to standardise clinical protocols, including anaesthetic and analgesic techniques, has been previously mentioned (Section 4.1.4). Given that the anaesthetic modification of the neuroendocrine response to surgery has received particular attention in the literature,[322, 324] it was considered prudent to re-visit the topic of non-standardisation of anaesthesia and analgesia in the context of factors which may have confounded comparison of the neuroendocrine responses to the alternative methods of AAA repair. Of particular note was the marked difference between the 'open' and EVAR groups in the extent

to which the epidural route of anaesthesia and analgesia was employed. Indeed, when the rate of post-operative analgesic administration via the epidural route amongst the two groups was compared statistically, a significant association was demonstrated between the category of operative procedure and epidural use. This may be relevant to the comparison of measured neuroendocrine responses given that afferent neural blockade by epidural administration of local anaesthetics and opiates may decrease the endocrine stress response to surgical intervention[324] . The latter effect is in itself the source of extensive research and appears to be influenced by a number of variables including the type and level of surgery.[322, 324] It is somewhat reassuring that a recent randomised, albeit modestly sized trial, investigated patients undergoing uncomplicated open infrarenal AAA repair and demonstrated no difference in cortisol, adrenaline or noradrenaline responses between those randomised to a GA followed by post-operative patient-controlled analgesia with intravenous morphine and those subjects who received a GA combined with intra-operative epidural bupivacaine followed by post-operative analgesia with epidural morphine.[444] In the specific circumstances of the current study, the differential use of epidurals may not, therefore, have induced a measurable difference in the neuroendocrine response between the two cohorts.

4.10.2 The Immuno-inflammatory Response in Open AAA Repair Compared to EVAR

4.10.2.1 Cytokine Responses

In view of the overall lack of fluctuation in assayed levels of TNF-α, IL-1β and IL-12, discussed in Section 4.6.3.1, it is not surprising that these cytokines did not

distinguish between the alternative methods of AAA repair. The current study has, however, provided further evidence to support the most consistent finding in the current literature comparing the inflammatory response to the alternative methods of repair, that greater IL-6 levels are provoked by the open approach.[366-371] It is notable that this difference was observed only at T_0 and $T_0 + 4$, which strongly supports the notion that this difference is a consequence of the nature of the operative intervention, and particularly the greater ischaemia and IRI associated with open AAA repair compared to EVAR.[367] It is particularly interesting that the current study demonstrated that open AAA repair does induce significantly higher levels of IL-8, up to fours hours following maximal ischaemia, in addition to significantly greater levels of the anti-inflammatory cytokine IL-10 up to 24 hours following maximal intra-operative ischaemia, compared to EVAR. This is in contrast to the limited number of published studies which failed to identify any significant difference between the two methods of repair, with respect to levels of these cytokines.[290, 368]

4.10.2.2 SIRS

The current study's finding that post-operative SIRS occurred more frequently and was a more severe and prolonged event after open AAA repair compared to EVAR tends to confirm the trend observed by Sweeney *et al.*[128] and provides further support for the contention that open AAA repair is associated with a greater inflammatory insult than EVAR.

4.10.3 The Neuroendocrine Response in Open AAA Repair Compared to EVAR

The current finding that open AAA repair induced significantly greater levels of cortisol and adrenaline at T(72-96) than EVAR is consistent with the conclusion reached by Thompson *et al.*[374] and Salartash *et al.* [375] that open AAA repair serves as a greater stimulus for release of these stress hormones than EVAR. It is notable, however, that these studies, and therefore the differences they identified, were limited to the intra- and very early post-operative period in contrast to the current study which detected a difference between the operative groups only during a later post-operative period. This discrepancy may be associated with the fact that, unlike plasma assays, the methodology employed in the current study does not have the capacity to detect or, therefore, permit comparison of transient fluctuations in hormone levels. It is suggested that the seemingly inconsistent finding that noradrenaline levels were greater amongst the EVAR cohort at T(0-24) may have arisen as a consequence of the apparently anomalous occurrence of greater pre-operative noradrenaline levels amongst the EVAR cohort. This would seem particularly likely given that both Thompson *et al.*[374] and Salartash *et al.*[375] found no significant differences in noradrenaline levels stimulated by the alternate methods of repair.

In view of the post-operative time point at which neuroendocrine responses distinguished between the 'open' and EVAR cohorts, it cannot yet be suggested that these differing levels of neuroendocrine axis activity and secretion are causal of the differences in clinical morbidity associated with open AAA repair compared to EVAR. Plasma cortisol and catecholamine assays in the intra- and early post-

operative period may provide evidence to support the notion of a causal relationship. Based on current findings, it can be stated that greater HPA axis and SNS activity amongst those undergoing open AAA repair appears to reflect the greater morbidity experienced by these patients compared to those undergoing EVAR.

CHAPTER 5

CONCLUSIONS

AND

FUTURE DIRECTIONS

5.1 SIRS and Sepsis - Value and Limitations

The current study has been largely dedicated to examining the outcomes of SIRS and sepsis following major vascular surgery. These are considered as both biologically important and clinically valuable concepts in vascular surgical practice. These attributes are necessary but not sufficient to enable these outcome measures to be usefully employed in either clinical or research settings. It is imperative for research, and is highly desirable for clinical practice, that these clinical events can be defined and diagnosed with a high degree of both sensitivity and specificity. This is an as yet unresolved limitation of these outcomes, in particular SIRS, and therefore any clinical research of which they are the focus.

There is a general consensus in the current literature that whilst conceptually valid, the SIRS definition lacks specificity and is excessively sensitive.[68, 99, 424] It is suggested that whilst the use of a severity scoring system and assessment of SIRS duration in the current study enhanced the discriminatory capacity of the SIRS definition, the ultimate resolution of its current shortcomings may require the pursuit of a novel definition based on biochemical and/or immunologic criteria, either used alone[99] or in combination with clinical features.

Whilst the revision of the consensus definition of sepsis in 2001[99] represented a significant improvement upon the capacity for diagnosing sepsis bestowed by the original 1992 definition[100], the emphasis was explicitly on facilitating the diagnosis in a clinical setting rather than ensuring suitability for research purposes[99]. The consequence is a set of diagnostic criteria which may be over inclusive when applied rigidly in the research setting. Further refinement of the criteria is, however, unlikely

to yield great benefits. Benefits, both for clinical and research applications, are likely to arise if the recommendation by the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference that a staging system for sepsis should be developed, which stratifies patients on the basis of their *P*redisposing conditions, the nature and extent of the *I*nfecting insult, the nature and magnitude of the host *R*esponse, and the degree of concomitant *O*rgan dysfunction, which they therefore designated the 'PIRO' system for staging sepsis,[99] is pursued. Indeed, the current study can be viewed as an attempt to do just this, through its attempt to identify novel factors which may predispose to sepsis.

A final point made by Levy *et al.*,[99] which is relevant to the current attempt to identify factors which may predispose to sepsis, and which may have contributed to the current study's negative results, is that not only is there likely to be a complex interaction between multiple factors that predispose to the onset and progression of sepsis, but the predisposition induced by an individual factor may be complex. Thus, an individual factor, such as a genetic polymorphism, for example, may result in a more aggressive inflammatory response to an invading microorganism, which may decrease an individual's risk of infection, but may increase the risk of an overly exuberant and potentially harmful inflammatory response if infection does eventuate.[99] An awareness of this complexity is necessary for the planning of future research into factors that may influence sepsis occurrence and outcome.

5.2 Immunological Parameters

5.2.1 PMN Integrin and Immunglobulin G Fc Receptors

Amongst the more encouraging findings arising from the investigation of potential predictors of post-operative SIRS and sepsis, was the significant association between pre-operative CD11b and post-operative SIRS severity. Given that this relationship appeared to be limited to the modestly sized EVAR cohort and that the study protocol permitted some variation in the pre-operative time point at which potentially labile CD11b levels were assayed, confirmation of the current findings through further investigations, which may include an expansion of the EVAR cohort and blood sampling immediately prior to anaesthetic induction, are recommended. Despite the inability to demonstrate a relationship between pre-operative CD16b and pot-operative SIRS or sepsis, the discrepancy between this finding and limited published findings indicates that a larger cohort of subjects is required before a definitive conclusion regarding CD16b can be reached. In contrast, pre-operative CD64 levels appear highly unlikely to be of any value in prediction of these post-operative events.

The aforementioned negative findings should not discourage further forays into the examination of the capacity of cell surface antigens to predict adverse post-operative events. This is reinforced by the findings of Tarnok *et al.*[150] who investigated 122 granulocyte and monocyte cell surface activation antigens and adhesion molecules to identify only 20 that distinguished between patients with post-operative edema and effusion (POEE) and controls, and furthermore suggested the need for a panel of the most discriminative parameters to predict POEE in individual patients, using relatively complex discriminant analysis methods. The prediction of SIRS, and

potentially sepsis, following major vascular surgery may similarly require the investigation of an array of leukocyte cell surface molecules to identify a panel of molecules which may contribute to these events by a summative effect. Intra-operative and early post-operative levels of these leukocyte cell surface molecules would be worthy of inclusion in future investigations. It is evident from the study by Tarnok *et al.*[150] that advanced statistical methods may be required in future investigations examining the predictive value of panels of leukocyte cell surface molecules.

5.2.2 FcγR Genotypes

Whilst the current study demonstrated no evidence of an association between either CD32a or CD16b genotypes and the development of infection or sepsis following major vascular surgery, it is essential to appreciate the methodological limitations which may have influenced these analyses. The most notable limitations were the possibility that the analyses were not sufficiently powered to detect potential associations, particularly due to the effect of genetic admixture, in addition to the possibility that confounding factors were introduced by pooling the 'open', EVAR and 'lower limb' cohorts into a single collective cohort for these analyses. Nonetheless, when these negative findings are considered in the context of the known restricted immunological consequences of CD32a and CD16b polymorphisms it raises some doubt about the likelihood of these genetic polymorphisms having the capacity to predict the microbiologically heterogeneous and immunologically complex events of post-operative infection and sepsis, in particular. Despite this conclusion, sound evidence has been identified in the current literature for a genetic susceptibility to both sepsis[217] and specific peri-operative risks and outcomes,

including inflammatory events[207] . Continued research into the identification of allotypes and haplotypes which may aid in the prediction of these post-operative adverse events, and potentially decrease surgical morbidity and mortality, is therefore vigorously endorsed.

5.2.3 Cytokines

Elevations in IL-6, IL-8 and IL-10 were observed to have occurred in response to AAA repair. Methodological limitations may have contributed to elevations in TNF-α and IL-1β being infrequently observed in the current study. It is suggested that further investigation of IL-12 responses to AAA repair are required before it can be concluded with confidence that this cytokine has minimal relevance to major vascular surgery.

The tendency for IL-6, IL-8, and particularly IL-10 levels to predict sepsis amongst those undergoing AAA repair, emphasises the pathogenetic role played by these cytokines in sepsis development. The limited capacity to reliably predict this adverse event from IL-10 levels for any individual, however, adds to the ever increasing awareness that sepsis is a highly complex, dynamic process which may not be possible to predict on the basis of single parameters.

5.3 Pre-operative Nutritional Status

Whilst the MNA was not found to be a particularly useful tool for assessment of nutritional status for research purposes, it suggested a surprising degree of nutritional adequacy amongst the current cohort. Whilst this may, in part, reflect selection bias, a more optimistic interpretation of this finding is that nutritional status, perhaps as a component of patient's general medical status, already serves as a necessary criterion for selecting patients who are most suitable for major vascular surgical interventions.

A significant inverse association between the DEXA derived body composition measures, FFM and SMM, and post-operative SIRS severity was identified by this study, indicating that a lower pre-operative lean body mass and, perhaps more precisely a lower total skeletal muscle mass, may predispose to more severe SIRS following major vascular interventions. Whilst body composition analysis by DEXA is not yet employed beyond the research setting, these findings strongly support the suggestion that patients with PEM may be at particular risk of more severe post-operative SIRS and, potentially, it's associated adverse inflammatory sequelae. It is suggested that the inability to identify an association between nutritional markers and post-operative sepsis does not discount the potential role of nutritional status in sepsis susceptibility, rather it may reflect the fact that the relationship is complex and not readily identified in a clinical cohort study comprised of a modest number of subjects.

5.4 Neuroendocrine Responses to Surgical Intervention

Unlike cortisol and adrenaline, noradrenaline did not appear to be a useful marker of the physiological stress response to surgical stimuli. Accordingly, greater post-operative cortisol and adrenaline responses were found to be significantly associated with a more severe post-operative systemic inflammatory response in addition to the occurrence of post-operative sepsis. It is conceivable that these associations arose as a consequence of the bi-directional regulation that exists between the immune and neuroendocrine axes[322, 329] , however, an explanation that more fully accounts for these findings is that the HPA axis and SNS responses simply reflected the degree of physiological derangement experienced by those with either more severe SIRS or sepsis. These findings do not, therefore, support the hypothesis that neuroendocrine responses may predict post-operative inflammatory events, but rather they may simply reflect their occurrence and severity. The potential methodological limitations associated with the neuroendocrine data in this study, notably the inclusion of subjects administered exogenous catecholamines and the inability to detect transient fluctuations in hormone levels that may be observed by assaying plasma levels of these hormones, should be appreciated when considering these findings.

5.5 Psychological Measures and Influence on Surgical Response and Outcome

5.5.1 Pre-operative Depression and Post-operative Outcomes

This study has highlighted the frequency of depression amongst vascular surgery patients, which appears to exceed reported rates amongst elderly members of the general population[336], but is comparable to reported rates amongst CABG patients[338-341], which seem to have attracted far greater attention in the surgical literature.

The current study did not identify the relationship between pre-operative depression and post-operative general morbidity described amongst patients undergoing CABG surgery[338, 339]. It is proposed that the biological basis of this relationship may not generalise to those undergoing AAA repair, but may be applicable to patients undergoing lower limb revascularisation. Further investigation of this relationship, involving an expanded cohort of patients undergoing lower limb revascularisation and the consideration of markers of morbidity at later post-operative time points than could be addressed in this study, would be worthwhile.

The current investigation confirmed the relationship between pre-operative depression and a poorer pre-operative mental component of HRQoL that might seem intuitive. Consideration of the effect of surgical intervention on this relationship indicated that in the early peri-operative period, depression was no longer an important determinant of HRQoL, however the relationship appeared to re-emerge three months post-operatively. Thus, it is suggested that the effect of surgery on HRQoL within one month of surgery may negate the influence of depression on

HRQoL, whilst recovery from surgery permits the re-emergence of the pre-operative relationship. The suggestion that pre-operatively depressed patients may experience a poorer physical component of HRQoL six months post-operatively requires a larger sample size for confirmation.

Whilst depressed individuals are known to manifest an array of immunological pertubations[353, 354, 356, 357] and appear to have an increased susceptibility to disease,[353] no convincing relationship between pre-operative depression and the development of either post-operative SIRS or sepsis was evident in this study. In view of these results, it has been suggested that a clear and straight-forward relationship between depression and these surgical outcomes may not, in fact, exist given that the immunological dysfunction associated with the proposed predictive variable is complex,[356] the surgical intervention is in itself a cause of considerable immunological derangement, and the outcomes themselves have a complex pathogenesis. It is similarly feasible that the intense immunological disturbances associated with major vascular surgery may simply negate any pre-existing immunological effects of depression. A clinically relevant relationship between pre-operative depression and the post-operative outcomes of SIRS and sepsis therefore seems unlikely.

5.5.2 Pre-operative Depression and Neuroendocrine Responses to Surgery

The hypothesis that depression may predispose to an exaggerated cortisol response to surgery, as a consequence of the known un-stimulated hyperactivity of the HPA axis in these individuals[353, 354] was not supported by the findings of the current study.

Indeed, it is of some concern that baseline UFC levels demonstrated no difference in HPA axis activity between depressed and non-depressed individuals. Prior to the definitive rejection of the alternative hypothesis, an increased sample size is therefore recommended as is the separation of the three operative cohorts for subsequent analyses of the expanded data set.

5.5.3 Pre-operative Trait Anxiety and Neuroendocrine Responses to Surgery

Whilst a positive and significant relationship between trait anxiety and pre-operative noradrenaline excretion was consistent with the small body of relevant existing literature[359, 438] the apparent absence of a demonstrable relationship with the neuroendocrine responses to surgical intervention was not. It is proposed, once again, that discounting the existence of a relationship between the variables in question may be premature before potential methodological issues have been addressed, particularly the recommendation of separating the operative cohorts for data analysis for the purpose of eliminating potential confounding factors.

5.6 Immunological and Neuroendocrine Responses in Open AAA Repair Compared to EVAR

5.6.1 The Immuno-inflammatory Response

The current study has provided overwhelming evidence that the open approach to AAA repair serves as a greater stimulus for inflammatory events that EVAR. This was evident from the significantly greater levels of IL-6, IL-8 and IL-10 generated in response to open AAA repair compared to EVAR. The time points at which these differences were observed strongly supports the notion that the differing degrees of ischaemia and IRI induced by the respective methods of repair[367] may largely account for the findings. Indeed, not only was the difference confirmed at an immunological level, but the greater inflammatory response associated with the open approach was clinically evident in the greater frequency, severity and duration of SIRS identified in this cohort compared to those undergoing EVAR.

5.6.2 The Neuroendocrine Response

This investigation similarly provided convincing evidence that open AAA repair is associated with a greater degree of HPA axis and SNS stimulation than EVAR. It was notable, however, that a significant difference was apparent only at the later T(72-96) time interval. Published results, although limited, suggest that significant differences may exist in the intra- and very early post-operative period.[374, 375] The influence of exogenous catecholamine administration may, in part, have contributed to this discrepancy as may the method of assaying excreted hormones rather than plasma levels. In view of the time point at which the differences in neuroendocrine response were observed, it cannot yet be concluded that these are causal of the

differences in morbidity associated with the alternate methods of AAA repair, but may be considered as reflective of this clinical difference.

5.7 Concluding Remarks

The current investigation of parameters which may serve as early predictors of adverse events, in particular SIRS and sepsis, following major vascular surgery identified a higher pre-operative expression of CD11b as a potential indicator of more severe post-operative SIRS, and therefore worthy of further investigation whilst a lower pre-operative FFM or SMM was found to be associated with more severe and prolonged SIRS. Levels of IL-6, IL-8, and particularly IL-10 may predict sepsis amongst a large patient cohort, however, these cytokines do not appear to reliably predict this outcome for the individual patient, implying their lack of suitability as predictive tools in the clinical setting. HPA axis and SNS activity appears to reflect rather than predict post-operative SIRS and sepsis. Sepsis, in particular, emerged as an event which may be particularly difficult to predict as a consequence of its complex pathogenesis, which may result in any given, potentially predictive parameter imposing a different risk for the components of infection, inflammatory response and subsequent organ dysfunction. Nonetheless, the current findings should serve to encourage further research, addressing the limitations identified in this study, into factors which may predispose to adverse inflammatory sequelae following major surgery.

Methodological limitations may have impeded the ability to identify a relationship between pre-operative psychological state and the neuroendocrine response to vascular surgery. Further investigation is warranted prior to reaching definitive conclusions regarding this relationship.

Robust evidence of a greater inflammatory response to open AAA repair compared with EVAR has emerged from this investigation, thus clarifying occasionally contradictory findings in the published literature on this topic.

APPENDICES

Appendix 1: Comorbidity Scoring

Table 1. The Charlson Index, a weighted index of comorbidity.[377]

ECG, electrocardiograph; PND, paroxysmal nocturnal dyspnoea; CVA, cerebrovascular accident; TIA, transient ischaemic attack; AML, acute myelogenous leukaemia; CML, chronic myelogenous leukaemia; ALL, acute lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; NHL, non-Hodgkin's lymphoma ; AIDS, acquired immunodeficiency syndrome

†Assigned weight for each condition that a patient has, the sum of which equals the total score. For example: congestive cardiac failure (1) + chronic pulmonary disease (1) + diabetes with end organ damage (2) = total score (4).

Conditions	Assigned weight[†]
Myocardial infarction • Hospitalized & ECG +/- enzyme changes Congestive cardiac failure • Exertional or PND with response to digoxin, diuretics or afterload reducing agents Peripheral vascular disease • Claudicants, bypass for arterial insufficiency, presence of gangrene, acute arterial insufficiency, untreated thoracic or abdominal aortic aneurysm ≥ 6cm Cerebrovascular disease • CVA or TIA Dementia • Chronic cognitive deficit Chronic pulmonary disease Connective tissue disease Peptic ulcer disease Mild liver disease • Cirrhosis without portal hypertension or chronic hepatitis Diabetes without end organ damage	1
Dense hemiplegia or paraplegia Moderate or severe renal disease • Moderate: creatinine > 0.264mmol/L • Severe: dialysis, transplanted, or presence of uraemia Diabetes with end organ damage Leukaemia • AML, CML, ALL, CLL or polycythaemia rubra vera Lymphoma • Hodgkin's lymphoma, NHL, lymphosarcoma, Waldenstrom's macroglobulinaemia, myeloma and other lymphomas Non-metastatic solid tumour • No documented metastases but initially treated within last 5 years	2
Moderate or severe liver disease • Moderate: cirrhosis with portal hypertension , without bleeding • Severe: cirrhosis with portal hypertension and a history of variceal bleeding	3
Metastatic solid tumour AIDS	6

Table 2. Joint Council of The Society for Vascular Surgery and the North American Chapter of the International Society for Cardiovascular Surgery (SVS/ISVS) grading system for conditions that may modify surgical outcomes.[44]

pck/day, packet per day; LDL, low density lipoprotein; ECG, electrocardiograph; MI, myocardial infarction; CCF, congestive cardiac failure; CXR, chest x-ray; PFTs, pulmonary function tests; VC, vital capacity; FEV_1, forced expiatory volume in one second; $PaCO_2$, partial pressure of carbon dioxide in arterial blood; O_2, oxygen

	0	1	2	3
Diabetes	None	Adult onset, controlled by diet or oral agents	Adult onset, insulin controlled	Juvenile onset
Tobacco Use	None or none for last 10 years	None current, but smoked in last 10 years	Current (includes abstinence < 1 year), < 1 pck/day	Current ≥ 1 pck/day
Hypertension	None (diastolic usually < 90mmHg)	Controlled (diastolic usually < 90 mmHg) with single drug	Controlled with two drugs	Requires > 2 drugs or uncontrolled
Hyperlipidaemia	Cholesterol (LDL and total) and triglyceride levels within normal limits for age	Mild elevation, readily controllable by diet	Moderate elevation requiring strict dietary control	Elevation requiring strict dietary and drug control
Cardiac Status	Asymptomatic, with normal ECG	Asymptomatic, but either remote MI by history (> 6 months), occult MI by ECG, or fixed defect on dipyridamole thallium or similar scan	Any 1 of the following: stable angina; no angina but significant reversible perfusion defect on dypridamole thallium scan; significant silent ischaemia (≥1% of time) on Holter monitoring; ejection fraction 25% to 45%; controlled ectopy or asymptomatic arrhythmia; history of CCF that is now well compensated	Any 1 of the following: unstable angina, symptomatic or poorly controlled ectopy/arrhythmia (chronic/recurrent); poorly compensated or recurrent CCF; ejection fraction < 25%; MI within 6 months
Carotid Disease	No symptoms, no evidence of disease	Asymptomatic but with evidence of disease determined by duplex scan or other accepted non-invasive test or arteriogram	Transient or temporary stroke	Completed stroke with permanent neurologic deficit or acute stroke
Renal Status ***(refers to stable levels, not transient drops or elevations in response to IV medication, hydration or contrast media)***	No known renal disease, normal serum creatinine level	Moderately elevated creatinine level as high as 0.210 mmol/L	Creatinine level, 0.220-0.520 mmol/L	Creatinine level > 0.530 mmol/L or on dialysis or with kidney transplant
Pulmonary Status	Asymptomatic, normal CXR, PFTs within 20% of predicted	Asymptomatic or mild dyspnoea on exertion, mild chronic parenchymal x-ray changes, PFTs 65% to 80% of predicted	Between 1 and 3	VC < 1.85 L, FEV_1 < 1.2 L or < 35% of predicted, maximal voluntary ventilation 50% of predicted, $PaCO_2$ >45 mmHg, supplemental O_2 use medically necessary, or pulmonary hypertension

Appendix 2: Mini Nutritional Assessment

Figure 1. The Mini Nutritional Assessment (MNA).[390]

The total assessment score is obtained by adding the individual scores, recorded in the boxes. This total assessment score is then compared with the Malnutrition Indicator Score to classify the subject's nutritional status.

MINI NUTRITIONAL ASSESSMENT (MNA™)

Name:..... ID#..... Weight, kg..... Height, cm: ..... Date: .....

Anthropometric Assessment

		Points
1.	Body Mass Index (BMI) (weight in kg)/ (height in m)^2 a. BMI < 19 = 0 points b. BMI 19 to < 21 = 1 point c. BMI 21 to < 23 = 2 points d. BMI ≥ 23 = 3 points	☐
2.	Mid-arm circumference (MAC) in cm a. MAC < 21 = 0.0 points b. MAC 21 ≤ 22 = 0.5 points c. MAC > 22 = 1.0 points	☐.☐
3.	Calf circumference (CC) in cm a. CC < 31 = 0 points b. CC ≥ 31 = 1 point	☐
4.	Weight loss during last 3 months a. Weight loss > 3kg = 0 points b. Does not know = 1 point c. Weight loss 1-3kg = 2 points d. No weight loss = 3 points	☐

General assessment

6.	Lives independently (not in a nursing home or hospital) a. No = 0 points b. Yes = 1 points	☐
7.	Takes more than 3 prescription drugs per day a. Yes = 0 points b. No = 1 point	☐
8.	Has suffered psychological stress or acute disease in the past 3 months a. Yes = 0 points b. No = 2 points	☐
9.	Mobility a. Bed or chair bound = 0 points b. Able to get out of bed/chair but does not go out = 1 point c. Goes out = 2 points	☐
10.	Neuropsychological problems a. Severe dementia or depression = 0 points b. Mild dementia = 1 point c. No psychological problems = 2 points	☐
11.	Pressure sores or skin ulcers a. Yes = 0 points b. No = 1 point	☐

Dietary Assessment

12.	How many full meals does the patient eat daily a. 1 meal = 0 points b. 2 meals = 1 point c. 3 meals = 2 points	☐

		Points
13.	Selected consumption markers for protein intake • At least one serving of dairy products (milk, cheese, yoghurt) per day? Yes ☐ No ☐ • Two or more sevings of legumes or eggs per week Yes ☐ No ☐ • Meat fish or poultry everyday? Yes ☐ No ☐ a. If 0 or 1 yes = 0.0 points b. If 2 yes = 0.5 points c. If 3 yes = 1.0 points	☐.☐
13.	Consume 2 or more servings of fruits or vegetables per day? a. No = 0 points b. Yes = 1 point	☐
14.	Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing or swallowing difficulties? a. Severe loss of appetite = 0 points b. Moderate loss of appetite = 1 point c. No loss of appetite = 2 points	☐
15.	How much fluid (water, juice, coffee, tea, milk….) is consumed per day? (1 cup = 8 oz) a. Less than 3 cups = 0.0 points b. 3 to 5 cups = 0.5 points c. More than 5 cups = 1.0 points	☐.☐
16.	Mode of feeding a. Unable to eat without assistance = 0 points b. Self-feed with some difficulty = 1 point c. Self- feed without any problem = 2 point	☐

Self Assessment

17.	Do they view themselves as having nutritional problems? a. Major malnutrition = 0 points b. Does not know or moderate malnutrition = 1 point c. No nutritional problem = 2 points	☐
18.	In comparison with other people of the same age, how do they consider their health status? a. Not as good = 0.0 points b. Does not know = 0.5 points c. As good = 1.0 points d. Better = 2.0 points	☐

ASSESSMENT TOTAL (max. 30 points): ☐☐.☐

Malnutrition Indicator Score		
≥24 points	well-nourished	☐
17 to 23.5 points	at risk of malnutrition	☐
< 17 points	malnourished	☐

Appendix 3: Psychological Questionnaires

Figure 1. The Beck Depression Inventory-II (BDI-II)[402] test form.

For each of the 21 constituent items, symptom severity is rated on a four-point scale from 0 (not at all) to 3 (extreme form of each symptom). The total score is obtained by summing the selected severity ratings for each item. A score > 13 indicates depression.

Beck Depression Inventory-II (BDI-II)

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the *one statement* in each group that best describes the way you have been feeling during the *past two weeks, including today*. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
- 0 I do not feel sad.
- 1 I feel sad much of the time.
- 2 I am sad all the time.
- 3 I am so sad or unhappy that I can't stand it.

2. Pessimism
- 0 I am not discouraged about my future
- 1 I feel more discouraged about my future than I used to be.
- 2 I do not expect things to work out for me.
- 3 I feel my future is hopeless and will only get worse.

3. Past Failure
- 0 I do not feel like a failure.
- 1 I have failed more than I should have.
- 2 As I look back, I see a lot of failures.
- 3 I feel I am a total failure as a person.

4. Loss of Pleasure
- 0 I get as much pleasure as I ever did from the things I enjoy.
- 1 I don't enjoy things as much as I used to.
- 2 I get very little pleasure from the things I used to.
- 3 I can't get any pleasure from the things I used to.

5. Guity Feelings
- 0 I don't feel particularly guilty.
- 1 I feel guilty over many things that I have done or should have done.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6. Punishment Feelings
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7. Self-Dislike
- 0 I feel the same about myself as ever.
- 1 I feel I may be punished.
- 2 I am disappointed in myself.
- 3 I dislike myself.

8. Self-Criticalness
- 0 I don't criticize or blame myself as much as usual.
- 1 I am more critical of myself than I used to be.
- 2 I criticize myself for all of my faults.
- 3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10. Crying
- 0 I don't cry anymore than I used to.
- 1 I cry more than I used to.
- 2 I cry over every little thing.
- 3 I feel like crying but I can't.

11. Agitation
- 0 I am no more restless or wound up than usual.
- 1 I feel more restless or wound up than usual.
- 2 I am so restless or agitated that it's hard to stay still.
- 3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making an decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.

1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.

2a I sleep a lot more than usual.
2c I sleep a lot less than usual.

3a I sleep most of the day.
3b I wake up 1-2 hours early and can't get back to sleep.

17. **Irritability**

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.

2a My appetite is much less than before.
2c My appetite is much greater than usual.

3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

Figure 2. The Center for Epidemiological Studies-Depression Scale (CES-D)[403] test form.

For each of the 20 constituent items, symptom frequency is rated on a four-point scale from 0, to indicate rarely or none of the time (less than 1 day), to 3, to indicate most or all of the time (5 to 7 days). Items are summed to obtain a total score, using the 0 to 3 scores selected by the subject for each item. Four items (4, 8, 12, and 16) are worded in a positive direction to reduce tendency towards response bias and are therefore reversed scored. A total CES-D score of $\geq$ 16 indicates depression.

Center For Epidemiologic Studies Depression (CES-D) Scale

Circle the number for each statement which best describes how often you felt or behaved this way *during the past week.*

During the past week.....	Rarely or none of the time (less than 1 day)	Some or a little of the time (1 to 2 days)	Occasionally or a moderate amount of time (3 to 4 days)	Most or all of the time (5 to 7 days)
1. I was bothered by things that don't usually bother me.	0	1	2	3
2. I did not feel like eating; my appetite was poor.	0	1	2	3
3. I felt that I could not shake off the blues even with help from my family or friends.	0	1	2	3
4. I felt that I was just as good as other people.	0	1	2	3
5. I had trouble keeping my mind on what I was doing.	0	1	2	3
6. I felt depressed.	0	1	2	3
7. I felt that everything I did was an effort.	0	1	2	3
8. I felt hopeful about the future.	0	1	2	3
9. I thought my life had been a failure.	0	1	2	3
10. I felt tearful.	0	1	2	3
11. My sleep was restless.	0	1	2	3
12. I was happy.	0	1	2	3
13. I talked less than usual.	0	1	2	3
14. I felt lonely.	0	1	2	3
15. People were unfriendly.	0	1	2	3
16. I enjoyed life.	0	1	2	3
17. I had crying spells.	0	1	2	3
18. I felt sad.	0	1	2	3
19. I felt that people disliked me.	0	1	2	3
20. I could not 'get going'.	0	1	2	3

Figure 3. The Spielberger State-Trait Anxiety Inventory : Trait Anxiety Scale test form (STAI Form Y-2).[358]

For each of the 20 items, subject's are asked to indicate how they generally feel by rating the frequency of their feelings of anxiety on a four-point scale ranging from 1 (almost never) to 4 (almost always). A rating of 4 indicates the presence of a high level of anxiety for eleven of the items on the scale, whereas a high rating for the remaining nine items (items 1, 3, 6, 7, 10, 13, 14, 16, 19) indicates the absence of anxiety. The scoring weights for the nine 'anxiety-absent' items are reversed, thus responses marked 1, 2, 3 or 4 are scored 4, 3, 2 or 1 respectively. A total score is obtained by adding the weighted scores for each of the twenty items. A higher total score therefore indicates greater trait anxiety.

Spielberger Trait-Anxiety Self-Evaluation Questionnaire
(STAI Form Y-2)

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel.

	Almost never	Sometimes	Often	Almost always
1. I feel pleasant	1	2	3	4
2. I feel nervous and restless	1	2	3	4
3. I feel satisfied with myself	1	2	3	4
4. I wish I could be as happy as others seem to be	1	2	3	4
5. I feel like a failure	1	2	3	4
6. I feel rested	1	2	3	4
7. I am "calm, cool and collected"	1	2	3	4
8. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
9. I worry too much over something that really doesn't matter	1	2	3	4
10. I am happy	1	2	3	4
11. I have disturbing thoughts	1	2	3	4
12. I lack self-confidence	1	2	3	4
13. I feel secure	1	2	3	4
14. I make decisions easily	1	2	3	4
15. I feel inadequate	1	2	3	4
16. I am content	1	2	3	4
17. Some unimportant thought runs through my mind and bother me	1	2	3	4
18. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
19. I am a steady person	1	2	3	4
20. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Appendix 4: Post-operative Outcome Scoring

Figure 1. Abridged system for reporting complications including grading of severity recommended by the Ad Hoc Committee for Standardised Reporting Practices in Vascular Surgery of the Society of Vascular Surgery/International Society for Cardiovascular Surgery (SVS/ISCVS).[44, 411]

AAA, abdominal aortic aneurysm

†Gradings of severity are as follows: 1=mild, 2=moderate, 3=severe.

‡Severity grading based on the SVS/ISCVS scale for reporting change in clinical status following treatment for peripheral arterial occlusive disease.[44] According to this scale a grading of +1 defines the following change in clinical status:

minimally improved: greater than 0.10 increase in ankle brachial index but no categorical improvement or vice versa (that is, upward categorical shift without an increase in ankle brachial index of more than 0.10).

Complication	Severity†
Systemic /remote	
Cardiac:	1=little/no haemodynamic consequence
Ectopic/arrhythmia	2=symptomatic/required treatment
Congestive failure	3=cardiac arrest/fatal
Myocardial infarction	
Stroke/Transient ischaemic attack	1=transient ischaemic attack/temporary deficit
	2=permanent deficit
	3=fatal
Deep venous thrombosis	1=hospitalization not prolonged
Suspected	2=treatment prolonged hospitalization
Confirmed	3=required operation
Pulmonary embolism	1=mild, required antithrombotic drugs
Suspected	2=serious, required resuscitation
Confirmed	3=severe, required embolectomy or fatal
Pulmonary	1=prompt recovery with medical treatment
Atelectasis	2=prolonged hospitalization or intravenous antibiotics
Pneumonia	3=prolonged intubation, tracheostomy, deterioration in
Adult respiratory distress syndrome	pulmonary function, oxygen dependence, or fatal outcome
Renal insufficiency	1=transient, not requiring dialysis
Contrast-media induced	2=transient, required dialysis
Thromboembolic	3= permanent (dialysis, transplant, death)
Ischemic (acute tubular necrosis)	
Obstructive	
Coagulation complications (including drug-induced)	1=resolving without treatment
Spontaneous haemorrhage	2=requiring drug therapy
Thrombocytopaenia	3=requiring operation or fatal
Thrombosis from antithrombin III, protein C or S deficiency	
Local/vascular	
Anastomotic haemorrhage	1=observed
External bleeding	2=required aspiration, drainage
Internal (haematoma)	3=required anastomotic repair, revision
Graft thrombosis	1=not corrected
Early	2=required revision or redo
Cause found	3=limb loss (unexpected tissue loss)
Cause not found	
Unsatisfactory haemodynamic result (despite patency)	1= >+1‡ (but less than expected)
Insufficient inflow	2= +1‡
Insufficient outflow	3= < +1‡
'Steal'	
Unexpected tissue loss/amputation	1=minor tissue loss without amputation
	2=minor amputation
	3=major amputation
Atherothrombembolism	1=without tissue loss
	2=with minor tissue loss/amputation
	3=with major tissue loss/amputation
Colon ischaemia	1=not requiring operation
	2=colon resection or colostomy
	3=fatal
Spinal cord ischaemia	1=transient
	2=minor permanent deficit
	3=major permanent deficit

Local/nonvascular	
Non-infectious wound fluid accumulation Haematoma Seroma Lymphocoele	1=observed, resolved 2=aspirated 3=surgical drainage
Wound infections Superficial Deep Exposed/contaminated graft	1=treated with antibiotics 2=treated with drainage 3=required graft removal or bypass
Lymphatic disruption Lymphedema Lymphocoele Lymph fistula	1=no treatment required 2=aspiration, drainage 3=exploration with closure of lymphatics
Ureteric injury Complete obstruction Partial obstruction Urinoma (closed leak) Urinary fistula	1=resolved spontaneously 2=required drainage, diversion 3=surgical correction or nephrectomy required
Endovascular repair: deployment related complications	
Failed deployment with or without conversion	1=no complications from attempted endovascular procedure, hospital stay not prolonged after endovascular repair 2=conversion to open repair, no permanent disability 3=significant permanent disability that impairs employment, function or ability to live or death
Operative bleeding	1=autotransfusion <2 units, no homologous transfusion 2=>2units autologous, < 3 units homologous, limited incision for control 3=>3units homologous, laparotomy, thoracotomy, or necessitated exposure in addition to initial vessel cutdown to control bleeding
Aortic dissection (within 30 days of repair)	1=incidentally noted, asymptomatic 2=resolved with endovascular repair 3=open repair or fatal
Arterial perforation or rupture	1=spontaneous closure 2=stent graft or limited retroperitoneal iliac repair at primary procedure 3=laparotomy
Access artery dissection or thrombosis	1=incidentally noted, non-flow limiting dissection, local repair/prophylactic patch closure of access artery 2=stent, limited retroperitoneal bypass, or necessitated return to operating room for thrombosis 3=conversion to open AAA repair
Peripheral microembolization	1=resolution without tissue loss 2=minor tissue loss, including toe or ray amputation 3=major amputation or significant tissue loss
Peripheral macroembolization	1=resolution with intraoperative embolectomy at primary procedure 2=embolectomy or other minor secondary operation, minor tissue loss 3=arterial bypass or major arterial repair, major amputation
Access site haematoma	1=spontaneous resolution 2=surgical evacuation 3=nerve compression or associated arterial repair
Access site false aneurysm	1=resolved spontaneously, with compression or thrombin therapy 2=surgical repair 3=ruptured

Access site lymphocoele, lymphorrhea, lymphedema	1=resolution with or without aspiration, minor edema easily controlled with elastic support 2=open drainage or repair 3=Permanent debilitating oedema
Access site infection	1=resolved with oral antibiotics 2=operative drainage, IV antibiotics 3=major debridement, artery repair
Fever of unknown origin	1=prolonged hospital stay
Endovascular repair: implant-related complications Intraoperative endograft limb obstruction	1=resolved at primary procedure 2=limited retroperitoneal repair or thrombectomy 3=bypass or conversion
Postoperative endograft limb obstruction	2=resolved with endovascular repair, minor tissue loss, including toe or ray amputation 3=lytic therapy or open operative repair, major amputation
Buttock, leg claudication/ischaemia	1=transient 2=persistent but not disabling (controlled with exercise or pharmacotherapy) 3=sufficiently disabling to necessitate intervention
Death Procedure-related *or* device –related Verified (by autopsy, direct surgical observation, or definitive Imaging of endograft) *or* probable (consistent clinical picture) *or* indeterminate	
No complications	

Figure 2. The Acute Physiology and Chronic Health Evaluation (APACHE) II severity of disease classification system, adapted from Knaus *et al.*[412]

bpm, beats per minute; A-a DO_2 , alveolar-arterial oxygen gradient; PaO_2, partial pressure of oxygen in arterial blood; FiO_2, inspired oxygen concentration; HCO_3, bicarbonate; ABG, arterial blood gas.

Total APACHE II Score is the sum of Acute Physiology Score points (**A**) + Age points (**B**) + Chronic Health Points (**C**).

†Calculated using the following formula:

A-a $DO_2 = (713 \times FiO_2) - (1.25 \times PaCO_2) - PaO_2$

‡Points assigned only if the patient has a history of severe organ system insufficiency or immuno-compromise. These states must have been evident *prior* to this hospital admission and conform to the following criteria:

Liver: Biopsy proven cirrhosis and documented portal hypertension; episodes of past upper gastrointestinal bleeding attributed to portal hypertension; or prior episodes of hepatic failure/encephalopathy/coma.

Cardiovascular: New York Heart Association Class IV

Respiratory: Chronic restrictive, obstructive, or vascular disease resulting in severe exercise restriction, that is, inability to climb stairs or perform household duties; or documented chronic hypoxia, hypercapnia, secondary polycythaemia, severe pulmonary hypertension (>40mmHg), or respirator dependency.

Renal: Receiving chronic dialysis.

Immuno-compromised: The patient has received therapy that suppresses resistance to infection, for example, immuno-suppression, chemotherapy, radiation, long term or recent high dose steroids; or has a disease that is sufficiently advanced to suppress resistance to infection, for example, leukaemia, lymphoma, acquired immuno-deficiency syndrome (AIDS).

APACHE II: Acute Physiology Score Component (A)									
Physiologic Variable	**High Abnormal Range**						**Low abnormal Range**		
	+4	**+3**	**+2**	**+1**	**0**	**+1**	**+2**	**+3**	**+4**
Temperature – core (°C)	○ ≥41	○ 39-40.9		○ 38.5-38.9	○ 36-38.4	○ 34-35.9	○ 32-33.9	○ 30-31.9	○ ≤29.9
Mean arterial pressure (mmHg)	○ ≥160	○ 130-159	○ 110-129		○ 70-109		○ 50-69		○ ≤49
Heart rate - ventricular response (bpm)	○ ≥180	○ 140-179	○ 110-139		○ 70-109		○ 55-69	○ 40-54	○ ≤39
Respiratory rate – non-ventilated or ventilated (breaths/min)	○ ≥50	○ 35-49		○ 25-34	○ 12-24	○ 10-11	○ 6-9		○ ≤5
Oxygenation: A-aDO_2 or PaO_2 (mmHg) a. $FiO_2 \geq 0.5$ record A-aDO_2[†]	○ ≥500	○ 350-499	○ 200-349		○ <200				
b. $FiO_2 < 0.5$ record only PaO_2					○ >70	○ 61-70		○ 55-60	○ <55
Arterial PH	○ ≥7.7	○ 7.6-7.69		○ 7.5-7.59	○ 7.33-7.49		○ 7.25-7.32	○ 7.15-7.24	○ <7.15
Serum HCO_3 (venous mmol/L) [Not preferred, use only if no ABGs available]	○ ≥52	○ 41-51.9		○ 32-40.9	○ 22-31.9		○ 18-21.9	○ 15-17.9	○ <15
Serum sodium (mmol/L)	○ ≥180	○ 160-179	○ 155-159	○ 150-154	○ 130-149		○ 120-129	○ 111-119	○ ≤110
Serum potassium (mmol/L)	○ ≥7	○ 6-6.9		○ 5.5-5.9	○ 3.5-5.4	○ 3-3.4	○ 2.5-2.9		○ <2.5
Serum creatinine (mmol/L) Double point score for acute renal failure	○ ≥.310	○ .180-.300	○ .130-.170		○ .050-.120		○ <.050		
Packed cell volume (%)	○ ≥60		○ 50-59.9	○ 46-49.9	○ 30-45.9		○ 20-29.9		○ <20
White blood count (x 10^9/L)	○ ≥40		○ 20-39.9	○ 15-19.9	○ 3-14.9		○ 1-2.9		○ <20
Glasgow Coma Score (GCS) points: Point score = 15 minus actual GCS									
A. Total Acute Physiology Score: Sum of the 12 individual variable points									

APACHE II: Age Score (B) and Chronic Health Score (C) Components					
	Points				
	0	**2**	**3**	**5**	**6**
B. Age Score	○ ≤44	○ 45-54	○ 55-64	○ 65-74	○ ≥75
C. Chronic Health Score‡		○ Elective non-operative patients		○ Non-operative or emergency post-operative patients	

Figure 3. Medical Outcomes Study 36-Item Short-Form (SF-36) Health Survey (Standard English – Australia/New Zealand Version 1.0), for measurement of health-related quality of life (HRQoL).[413]

SF-36 Health Survey

Instructions: This questionnaire asks for your views about your health, how you feel and how well you are able to do your usual activities.
Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is:

(circle one)

Excellent 1
Very good 2
Good 3
Fair 4
Poor 5

2. *Compared to one year ago*, how would you rate your health *now*?

(circle one)

Much better than one year ago 1
Somewhat better now than one year ago 2
About the same as one year ago 3
Somewhat worse now than one year ago 4
Much worse now than one year ago 5

3. 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?

(circle one number on each line)

Activities	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. *Vigorous activities*, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
b. *Moderate activities*, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
c. Lifting or carrying groceries	1	2	3
d. Climbing *several* flights of stairs	1	2	3
e. Climbing *one* flight of stairs	1	2	3
f. Bending, kneeling or stooping	1	2	3
g. Walking *more than one kilometre*	1	2	3
h. Walking *half a kilometre*	1	2	3
i. Walking *100 metres*	1	2	3
j. Bathing or dressing yourself	1	2	3

4. During the *past 4 weeks*, have you had any of the following problems with your work or other regular daily activities *as a result of your physical health*?

(circle one number on each line)

	Yes	No
a. Cut down on the *amount of time* you spent on work or other activities	1	2
b. *Accomplished less* than you would like	1	2
c. Were limited in the *kind* of work or other activities	1	2
d. Had difficulty performing the work or other activities (for example, it took extra effort)	1	2

5. During the *past 4 weeks*, 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 depressed or anxious)?

(circle one number on each line)

	Yes	No
a. Cut down on the *amount of time* you spent on work or other activities	1	2
b. *Accomplished less* than you would like	1	2
c. Didn't do work or other activities as carefully as usual	1	2

6. During the *past 4 weeks*, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?

(circle one)

Not at all 1
Slightly 2
Moderately 3
Quite a bit 4
Extremely 5

7. How much bodily pain have you had during the *past 4 weeks*?

(circle one)

No bodily pain 1
Very mild 2
Mild 3
Moderate 4
Severe 5
Very severe 6

8. During the *past 4 weeks* how much did *pain* interfere with your normal work (including both work outside the home and housework)?

(circle one)

Not at all 1
A little bit 2
Moderately 3
Quite a bit 4
Extremely 5

9. These questions are about how you feel and how things have been with you *during the past 4 weeks*. For each question, please give the answer that comes closest to the way you have been feeling. How much of the time during the *past 4 weeks*:

(circle one number on each line)

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?	1	2	3	4	5	6
b. Have you been a very nervous person?	1	2	3	4	5	6
c. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
d. Have you felt calm and peaceful?	1	2	3	4	5	6
e. Did you have a lot of energy?	1	2	3	4	5	6
f. Have you felt down?	1	2	3	4	5	6
g. Did you feel worn out?	1	2	3	4	5	6
h. Have you been a happy person?	1	2	3	4	5	6
i. Did you feel tired?	1	2	3	4	5	6

10. During the *past 4 weeks*, how much of the time has your *physical health or emotional problems* interfered with your social activities (like visiting with friends, relatives, etc.)?

(circle one)

All of the time 1
Most of the time 2
Some of the time 3
A little of the time 4
None of the time 5

11. How TRUE or FALSE is *each* of the following statements for you?

(circle one number on each line)

	Definitely true	Mostly true	Don't know	Mostly fale	Definitely false
a. I seem to get sick a little easier than other people	1	2	3	4	5
b. I am as healthy as anybody I know	1	2	3	4	5
c. I expect my health to get worse	1	2	3	4	5
d. My health is excellent	1	2	3	4	5

Appendix 5: Abbreviations

A	adenine
AAA	abdominal aortic aneurysm
A-aDO_2	alveolar-arterial oxygen gradient
ABG	arterial blood gas
ACCP	American College of Chest Physicians
ACTH	adrenocorticotrophic hormone
ADCC	antibody dependent cell mediated cytotoxicity
AIDS	acquired immunodeficiency syndrome
ALL	acute lymphocytic leukaemia
AML	acute myelogenous leukaemia
ANOVA	analysis of variance
APACHE II	Acute Physiology and Chronic Health Evaluation II
APC	antigen presenting cell
APO	apolipoprotein
aPTT	activated partial thromboplastin time
ARDS	adult respiratory distress syndrome
ARF	acute renal failure
ASA	American Society of Anesthesiologists (physical status classification)
AUC	area under the curve
av.	average
AVP	arginine vasopressin
B	B lymphocyte

BDI-II	Beck Depression Inventory-II
BMC	bone mineral content
BMI	body mass index
bpm	beats per minute
C	cytosine
°C	degrees celcius
CABG	coronary artery bypass graft
CARS	compensatory anti-inflammatory response syndrome
CBA	cytometric bead array
CCF	congestive cardiac failure
CD	cluster of differentiation antigen
CES-D	Center for Epidemiological Studies-Depression Scale
CFA	common femoral artery
CIA	common iliac artery
CLL	chronic lymphocytic leukaemia
CML	chronic myelogenous leukaemia
conc.	concentration
COX-2	cyclooxygenase type 2
CPB	cardiopulmonary bypass
CRF	corticotrophin-releasing factor
CRP	C-reactive protein
CT	computed tomography
CVA	cerebrovascular accident

CXR	chest x-ray
DEXA	dual energy x-ray absorptiometry
DIC	disseminated intravascular coagulopathy
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphates
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders-IV
ECG	electrocardiograph
ELISA	enzyme-linked immunosorbent assay
ELR	glutamic acid-leucine-arginine
EVAR	endovascular aneurysm repair
FcγR	receptor for constant fragment (Fc) of immunoglobulin G
FEV_1	forced expiratory volume in one second
FFM	fat free mass
FFMI	fat free mass index
FFST	fat free soft tissue mass
FiO_2	inspired oxygen concentration
FITC	fluoroscein isothiocyanate isomer-1
FM	fat mass
FMI	fat mass index
FMLP	formyl-methionyl-leucyl-phenylalanine
g	acceleration due to gravity (unit of force)
G	guanine
GA	general anaesthetic

GABA	gamma-amino butyrate
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GPI	glycosyl-phosphatidylinositol
H	histidine
HCO_3	bicarbonate
HDU	high dependency unit
HGH	human growth hormone
HLA	human leukocyte antigen
HPA	hypothalamo-pituitary-adrenocortical
HPLC	high performance liquid chromatography
HRQoL	health-related quality of life
hrly.	hourly
5-HETE	5-hydroxyeicosatetranoic acid
ICAM-1	intercellular adhesion molecule-1
ICE	interleukin-1β converting enzyme
ICU	intensive care unit
IFN-γ	interferon-gamma
Ig	immunoglobulin
IL	interleukin
IL-1ra	interleukin-1 receptor antagonist
IMA	inferior mesenteric artery
IMVS	Institute of Medical and Veterinary Science
INR	international normalised ratio

IRI	ischaemia-reperfusion injury
ITAM (+)	immunoreceptor tyrosine-based activation motif
ITIM (–)	immunoreceptor tyrosine-based inhibition motif
IV	intravenous
kDa	kilodalton
K-EDTA	K-ethylenediamine tetra-acetic acid
kg	kilogram
LDL	low density lipoprotein
LOS	length of stay
LPS	lipopolysaccharide
M	mole
MAP	mean arterial pressure
MCP-1	macrophage chemotactic protein-1
MCS	mental component summary score
MFI	mean fluorescence intensity
mg	milligram
mg/ml	milligrams per millilitre
MHC	major histocompatibility complex
MI	myocardial infarction
mins	minutes
MIP-1α	macrophage inflammatory protein-1 alpha
ml	millilitre
MNA	Mini Nutritional Assessment
Mo	monocyte
Mϕ	macrophage

MODS	multiple organ dysfunction syndrome
MOF	multiple organ failure
mRNA	messenger ribonucleic acid
MRSA	methicillin resistant *Staphylococcus aureus*
NA	neutrophil antigen
N/A	not applicable
NADPH	dihydronicotinamide adenine dinucleotide
ND	not determined
NHL	non-Hodgkin's lymphoma
NK	natural killer (cell)
nmol/24 hours	nanomol per twenty-four hours
NS	not significant
O_2	oxygen
PAF	platelet-activating factor
$PaCO_2$	partial pressure of carbon dioxide in arterial blood
PaO_2	partial pressure of oxygen in arterial blood
PCV	packed cell volume
PBS	phosphate buffered saline
PBSG	phosphate buffered saline containing glucose
pck/day	packet per day
PCR	polymerase chain reaction
PCR-SSP	sequence specific polymerase chain reaction
PCS	physical component summary score
PEM	protein energy malnutrition

PFA	profunda femoral artery
PFT	pulmonary function test
PGE_2	prostaglandin E_2
pg/ml	picograms per millilitre
Plt	platelet
PMA	phorbol myristate acetate
PMN	polymorphonuclear leukocyte
PND	paroxysmal nocturnal dyspnoea
POD	post-operative day
POEE	post-operative edema and effusion
POMC	pro-opiomelanocortin
PTFE	polytetrafluoroethylene
R	arginine
RPE	R. phycoerythrin
SBP	systolic blood pressure
SCCM	Society of Critical Care Medicine
SCCM/ESICM/ACCP/ATS/SIS	Society of Critical Care Medicine/The European Society of Intensive Care Medicine/The American College of Chest Physicians/American Thoracic Society/Surgical Infection Society
SD	standard deviation
SEM	standard error of the mean
SFA	superficial femoral artery
sFcγR	soluble receptor for constant fragment (Fc) of immunoglobulin G

SF-36	Medical Outcomes Study 36-Item Short-Form Health Survey
sIL-2R	soluble interleukin-2 receptor
sIL-6R	soluble interleukin-6 receptor
SMA	superior mesenteric artery
SMM	estimated skeletal muscle mass
SNP	single nucleotide polymorphism
SNS	sympathetic nervous system
SPSS	Statistical Package for Social Sciences
STAI	Spielberger State-Trait Anxiety Inventory
STAI Form Y-1	Spielberger State-Anxiety Scale
STAI Form Y-2	Spielberger Trait-Anxiety Scale
SvO_2	mixed venous oxygen saturation
SVS/ISCVS	Joint Council of The Society for Vascular Surgery and the North American Chapter of the International Society for Cardiovascular Surgery
T	T lymphocyte
TAAA	thoracoabdominal aortic aneurysm
TAE	tris-acetate
TGF-β	transforming growth factor-beta
$T_{H(1)}$	T helper cell (subset 1)
TIA	transient ischaemic attack
TIVA	total intravenous anaesthesia
TNF-α	tumour necrosis factor-alpha
TNF-β	tumour necrosis factor-beta (lymphotxin α)

UFC	urinary free cortisol
UK	United Kingdom
µl	microlitre
UV	ultra-violet
VC	vital capacity
VCAM-1	vascular cell adhesion molecule-1
vs.	versus
WBC	white blood cell
%	percent

REFERENCES

1. Auerbach O, Garfinkel L. Atherosclerosis and aneurysm of aorta in relation to smoking habits and age. *Chest.* 1980; **78**: 805-9.

2. Wilmink AB, Quick CR. Epidemiology and potential for prevention of abdominal aortic aneurysm. *Br J Surg.* 1998; **85**: 155-62.

3. Castleden WM, Mercer JC. Abdominal aortic aneurysms in Western Australia: descriptive epidemiology and patterns of rupture. *Br J Surg.* 1985; **72**: 109-12.

4. Bengtsson H, Bergqvist D, Sternby NH. Increasing prevalence of abdominal aortic aneurysms. A necropsy study. *Eur J Surg.* 1992; **158**: 19-23.

5. Lilienfeld DE, Gunderson PD, Sprafka JM, Vargas C. Epidemiology of aortic aneurysms: I. Mortality trends in the United States, 1951 to 1981. *Arteriosclerosis.* 1987; **7**: 637-43.

6. Cheng SW, Ting AC, Lau H, Wong J. Epidemiology of atherosclerotic peripheral arterial occlusive disease in Hong Kong. *World J Surg.* 1999; **23**: 202-6.

7. Fowkes FG, Macintyre CC, Ruckley CV. Increasing incidence of aortic aneurysms in England and Wales. *Bmj.* 1989; **298**: 33-5.

8. Nasim A, Sayers RD, Thompson MM, Healey PA, Bell PR. Trends in abdominal aortic aneurysms: a 13 year review. *Eur J Vasc Endovasc Surg.* 1995; **9**: 239-43.

9. Cronenwett JL. Abdominal Aortic Aneurysms: Predicting the Natural History. In: Yao JST, Pearce WH, editors. *Progress In Vascular Surgery.* Connecticut: Appleton & Lange; 1997. p. 127-38.

10. Lederle FA, Johnson GR, Wilson SE, *et al.* Rupture rate of large abdominal aortic aneurysms in patients refusing or unfit for elective repair. *JAMA.* 2002; **287**: 2968-72.

11. Bown MJ, Sutton AJ, Bell PR, Sayers RD. A meta-analysis of 50 years of ruptured abdominal aortic aneurysm repair. *Br J Surg.* 2002; **89**: 714-30.

12. Ernst CB. Abdominal aortic aneurysm. *N Engl J Med.* 1993; **328**: 1167-72.

13. Zarins CK, Harris EJ, Jr. Operative repair for aortic aneurysms: the gold standard. *J Endovasc Surg.* 1997; **4**: 232-41.

14. Menard MT, Chew DK, Chan RK, *et al.* Outcome in patients at high risk after open surgical repair of abdominal aortic aneurysm. *J Vasc Surg.* 2003; **37**: 285-92.

15. Biancari F, Leo E, Ylonen K, Vaarala MH, Rainio P, Juvonen T. Value of the Glasgow Aneurysm Score in predicting the immediate and long-term outcome after elective open repair of infrarenal abdominal aortic aneurysm. *Br J Surg.* 2003; **90**: 838-44.

16. Blankensteijn JD, Lindenburg FP, Van der Graaf Y, Eikelboom BC. Influence of study design on reported mortality and morbidity rates after abdominal aortic aneurysm repair. *Br J Surg.* 1998; **85**: 1624-30.

17. Zeebregts CJ, Geelkerken RH, van der Palen J, Huisman AB, de Smit P, van Det RJ. Outcome of abdominal aortic aneurysm repair in the era of endovascular treatment. *Br J Surg.* 2004; **91**: 563-8.

18. Zarins CK, White RA, Moll FL, *et al.* The AneuRx stent graft: four-year results and worldwide experience 2000. *J Vasc Surg.* 2001; **33**: S135-45.

19. Makaroun MS, Chaikof E, Naslund T, Matsumura JS. Efficacy of a bifurcated endograft versus open repair of abdominal aortic aneurysms: a reappraisal. *J Vasc Surg.* 2002; **35**: 203-10.

20. Alric P, Hinchliffe RJ, MacSweeney ST, Wenham PW, Whitaker SC, Hopkinson BR. The Zenith aortic stent-graft: a 5-year single-center experience. *J Endovasc Ther.* 2002; **9**: 719-28.

21. Garcia-Madrid C, Josa M, Riambau V, Mestres CA, Muntana J, Mulet J. Endovascular versus open surgical repair of abdominal aortic aneurysm: a comparison of early and intermediate results in patients suitable for both techniques. *Eur J Vasc Endovasc Surg.* 2004; **28**: 365-72.

22. Brewster DC, Geller SC, Kaufman JA, *et al.* Initial experience with endovascular aneurysm repair: comparison of early results with outcome of conventional open repair. *J Vasc Surg.* 1998; **27**: 992-1003; discussion 4-5.

23. May J, White GH, Yu W, *et al.* Concurrent comparison of endoluminal versus open repair in the treatment of abdominal aortic aneurysms: analysis of 303 patients by life table method. *J Vasc Surg.* 1998; **27**: 213-20; discussion 20-1.

24. Zarins CK, White RA, Schwarten D, *et al.* AneuRx stent graft versus open surgical repair of abdominal aortic aneurysms: multicenter prospective clinical trial. *J Vasc Surg.* 1999; **29**: 292-305; discussion 6-8.

25. Amundsen S, Skjaerven R, Trippestad A, Soreide O. Abdominal aortic aneurysms--a study of factors influencing postoperative mortality. Norwegian Aortic Aneurysm Trial. *Eur J Vasc Surg.* 1989; **3**: 405-9.

26. Diehl JT, Cali RF, Hertzer NR, Beven EG. Complications of abdominal aortic reconstruction. An analysis of perioperative risk factors in 557 patients. *Ann Surg.* 1983; **197**: 49-56.

27. Katz DJ, Stanley JC, Zelenock GB. Operative mortality rates for intact and ruptured abdominal aortic aneurysms in Michigan: an eleven-year statewide experience. *J Vasc Surg.* 1994; **19**: 804-15; discussion 16-7.

28. Johnston KW, Scobie TK. Multicenter prospective study of nonruptured abdominal aortic aneurysms. I. Population and operative management. *J Vasc Surg.* 1988; **7**: 69-81.

29. Brady AR, Fowkes FG, Greenhalgh RM, Powell JT, Ruckley CV, Thompson SG. Risk factors for postoperative death following elective surgical repair of abdominal aortic aneurysm: results from the UK Small Aneurysm Trial. On behalf of the UK Small Aneurysm Trial participants. *Br J Surg.* 2000; **87**: 742-9.

30. Steyerberg EW, Kievit J, de Mol Van Otterloo JC, van Bockel JH, Eijkemans MJ, Habbema JD. Perioperative mortality of elective abdominal aortic aneurysm surgery. A clinical prediction rule based on literature and individual patient data. *Arch Intern Med.* 1995; **155**: 1998-2004.

31. McCabe CJ, Coleman WS, Brewster DC. The advantage of early operation for abdominal aortic aneurysm. *Arch Surg.* 1981; **116**: 1025-9.

32. Hannan EL, Kilburn H, Jr., O'Donnell JF, *et al.* A longitudinal analysis of the relationship between in-hospital mortality in New York State and the volume of abdominal aortic aneurysm surgeries performed. *Health Serv Res.* 1992; **27**: 517-42.

33. Samy AK, Murray G, MacBain G. Glasgow aneurysm score. *Cardiovasc Surg.* 1994; **2**: 41-4.

34. Dimick JB, Stanley JC, Axelrod DA, *et al.* Variation in death rate after abdominal aortic aneurysmectomy in the United States: impact of hospital volume, gender, and age. *Ann Surg.* 2002; **235**: 579-85.

35. Kantonen I, Lepantalo M, Salenius JP, Matzke S, Luther M, Ylonen K. Mortality in abdominal aortic aneurysm surgery-the effect of hospital volume, patient mix and surgeon's case load. *Eur J Vasc Endovasc Surg.* 1997; **14**: 375-9.

36. Detsky AS, Abrams HB, Forbath N, Scott JG, Hilliard JR. Cardiac assessment for patients undergoing noncardiac surgery. A multifactorial clinical risk index. *Arch Intern Med.* 1986; **146**: 2131-4.

37. Johnston KW. Multicenter prospective study of nonruptured abdominal aortic aneurysm. Part II. Variables predicting morbidity and mortality. *J Vasc Surg.* 1989; **9**: 437-47.

38. Amundsen S, Trippestad A, Viste A, Soreide O. Abdominal aortic aneurysms-a national multicentre study. *Eur J Vasc Surg.* 1987; **1**: 239-43.

39. Gouny P, Bertrand M, Coriat P, Kieffer E. Perioperative cardiac complications of surgical repair of infrarenal aortic aneurysms. *Ann Vasc Surg.* 1989; **3**: 328-34.

40. Biancari F, Heikkinen M, Lepantalo M, Salenius JP. Glasgow Aneurysm Score in patients undergoing elective open repair of abdominal aortic aneurysm: a Finnvasc study. *Eur J Vasc Endovasc Surg.* 2003; **26**: 612-7.

41. Cuypers PW, Gardien M, Buth J, *et al.* Cardiac response and complications during endovascular repair of abdominal aortic aneurysms: a concurrent comparison with open surgery. *J Vasc Surg.* 2001; **33**: 353-60.

42. Nesi F, Leo E, Biancari F, *et al.* Preoperative risk stratification in patients undergoing elective infrarenal aortic aneurysm surgery: evaluation of five risk scoring methods. *Eur J Vasc Endovasc Surg.* 2004; **28**: 52-8.

43. Lette J, Waters D, Lassonde J, *et al.* Multivariate clinical models and quantitative dipyridamole-thallium imaging to predict cardiac morbidity and death after vascular reconstruction. *J Vasc Surg.* 1991; **14**: 160-9.

44. Rutherford RB, Baker JD, Ernst C, *et al.* Recommended standards for reports dealing with lower extremity ischemia: revised version. *J Vasc Surg.* 1997; **26**: 517-38.

45. Eagle KA, Coley CM, Newell JB, *et al.* Combining clinical and thallium data optimizes preoperative assessment of cardiac risk before major vascular surgery. *Ann Intern Med.* 1989; **110**: 859-66.

46. Kertai MD, Steyerberg EW, Boersma E, *et al.* Validation of two risk models for perioperative mortality in patients undergoing elective abdominal aortic aneurysm surgery. *Vasc Endovascular Surg.* 2003; **37**: 13-21.

47. Vanzetto G, Machecourt J, Blendea D, *et al.* Additive value of thallium single-photon emission computed tomography myocardial imaging for prediction of perioperative events in clinically selected high cardiac risk patients having abdominal aortic surgery. *Am J Cardiol.* 1996; **77**: 143-8.

48. Krupski WC, Nehler MR. How to avoid cardiac ischemic events associated with aortic surgery. *Semin Vasc Surg.* 2001; **14**: 235-44.

49. Poldermans D, Boersma E, Bax JJ, *et al.* Bisoprolol reduces cardiac death and myocardial infarction in high-risk patients as long as 2 years after successful major vascular surgery. *Eur Heart J.* 2001; **22**: 1353-8.

50. Poldermans D, Boersma E, Bax JJ, *et al.* The effect of bisoprolol on perioperative mortality and myocardial infarction in high-risk patients undergoing vascular surgery. Dutch Echocardiographic Cardiac Risk Evaluation Applying Stress Echocardiography Study Group. *N Engl J Med.* 1999; **341**: 1789-94.

51. Warltier DC. Beta-adrenergic-blocking drugs: incredibly useful, incredibly underutilized. *Anesthesiology.* 1998; **88**: 2-5.

52. Leng GC, Fowkes FG. Epidemiology and risk factors for peripheral arterial disease. In: Beard JD, Gaines PA, editors. *Vascular and Endovascular Surgery.* 2nd ed. London: Harcourt Publishers Limited; 2001. p. 1-26.

53. Fowkes FG, Housley E, Cawood EH, Macintyre CC, Ruckley CV, Prescott RJ. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *Int J Epidemiol.* 1991; **20**: 384-92.

54. Dewhurst G, Wood DA, Walker F, *et al.* A population survey of cardiovascular disease in elderly people: design, methods and prevalence results. *Age Ageing.* 1991; **20**: 353-60.

55. European Working Group on Critical Limb Ischaemia. Second European Consensus Document on chronic critical leg ischemia. *Circulation.* 1991; **84**: IV1-26.

56. Rutherford RB. Vascular surgery-comparing outcomes. *J Vasc Surg.* 1996; **23**: 5-17.

57. Rose G, McCartney P, Reid DD. Self-administration of a questionnaire on chest pain and intermittent claudication. *Br J Prev Soc Med.* 1977; **31**: 42-8.

58. Kannel WB, Shurtleff D. The natural history of arteriosclerosis obliterans. *Cardiovasc Clin.* 1971; **3**: 37-52.

59. Bengt F. Investigation and general management. In: Dormandy J, Stock G, editors. *Critical leg ischaemia.* Berlin: Springer; 1990. p. 41-8.

60. Brothers TE, Robison JG, Sutherland SE, Elliott BM. Racial differences in operation for peripheral vascular disease: results of a population-based study. *Cardiovasc Surg.* 1997; **5**: 26-31.

61. Pomposelli FB, Jr., Arora S, Gibbons GW, *et al.* Lower extremity arterial reconstruction in the very elderly: successful outcome preserves not only the limb but also residential status and ambulatory function. *J Vasc Surg.* 1998; **28**: 215-25.

62. Veith FJ, Gupta SK, Wengerter KR, *et al.* Changing arteriosclerotic disease patterns and management strategies in lower-limb-threatening ischemia. *Ann Surg.* 1990; **212**: 402-14.

63. Australian Government Department of Health and Ageing. *National Health Morbidity (Casemix) Database: Australian Hospital Morbidity Data, AR-DRG v5.0, 2000-2001.* Available from: http://www.health.gov.au/internet/wcms/publishing.nsf/Content/health-casemix-report-hospmo26.htm

64. Baumgartner I, Schainfeld R, Graziani L. Management of peripheral vascular disease. *Annu Rev Med.* 2005; **56**: 249-72.

65. Virkkunen J, Heikkinen M, Lepantalo M, Metsanoja R, Salenius JP. Diabetes as an independent risk factor for early postoperative complications in critical limb ischemia. *J Vasc Surg.* 2004; **40**: 761-7.

66. Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP. The natural history of the systemic inflammatory response syndrome (SIRS). A prospective study. *JAMA.* 1995; **273**: 117-23.

67. Davies MG, Hagen PO. Systemic inflammatory response syndrome. *Br J Surg.* 1997; **84**: 920-35.

68. Vincent JL. Dear SIRS, I'm sorry to say that I don't like you. *Crit Care Med.* 1997; **25**: 372-4.

69. Bone RC. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). *Ann Intern Med.* 1996; **125**: 680-7.

70. Vilcek J. The cytokines: an overview. In: Thomson AW, Lotze MT, editors. *The Cytokine Handbook.* 4th ed. London: Academic Press; 2003. p. 3-18.

71. Slotman GJ, Burchard KW, Williams JJ, D'Arezzo A, Yellin SA. Interaction of prostaglandins, activated complement, and granulocytes in clinical sepsis and hypotension. *Surgery.* 1986; **99**: 744-51.

72. Lamy M, Deby-Dupont G. Is sepsis a mediator-inhibitor mismatch? *Intensive Care Med.* 1995; **21 Suppl 2**: S250-7.

73. Abraham E, Wunderink R, Silverman H, *et al.* Efficacy and safety of monoclonal antibody to human tumor necrosis factor alpha in patients with sepsis syndrome. A randomized, controlled, double-blind, multicenter clinical trial. TNF-alpha MAb Sepsis Study Group. *JAMA.* 1995; **273**: 934-41.

74. Fisher CJ, Jr., Dhainaut JF, Opal SM, *et al.* Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *JAMA.* 1994; **271**: 1836-43.

75. Dinarello CA, Gelfand JA, Wolff SM. Anticytokine strategies in the treatment of the systemic inflammatory response syndrome. *JAMA.* 1993; **269**: 1829-35.

76. Platzer C, Meisel C, Vogt K, Platzer M, Volk HD. Up-regulation of monocytic IL-10 by tumor necrosis factor-alpha and cAMP elevating drugs. *Int Immunol.* 1995; **7**: 517-23.

77. Bone RC, Grodzin CJ, Balk RA. Sepsis: a new hypothesis for pathogenesis of the disease process. *Chest.* 1997; **112**: 235-43.

78. Fukushima R, Alexander JW, Gianotti L, Ogle CK. Isolated pulmonary infection acts as a source of systemic tumor necrosis factor. *Crit Care Med.* 1994; **22**: 114-20.

79. Bone RC. The pathogenesis of sepsis. *Ann Intern Med.* 1991; **115**: 457-69.

80. Ibbotson GC, Wallace JL. Beneficial effects of prostaglandin E2 in endotoxic shock are unrelated to effects on PAF-acether synthesis. *Prostaglandins.* 1989; **37**: 237-50.

81. Petrak RA, Balk RA, Bone RC. Prostaglandins, cyclo-oxygenase inhibitors, and thromboxane synthetase inhibitors in the pathogenesis of multiple systems organ failure. *Crit Care Clin.* 1989; **5**: 303-14.

82. Stephens KE, Ishizaka A, Larrick JW, Raffin TA. Tumor necrosis factor causes increased pulmonary permeability and edema. Comparison to septic acute lung injury. *Am Rev Respir Dis.* 1988; **137**: 1364-70.

83. Tracey KJ, Lowry SF, Cerami A. Cachetin/TNF-alpha in septic shock and septic adult respiratory distress syndrome. *Am Rev Respir Dis.* 1988; **138**: 1377-9.

84. Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N Engl J Med.* 1990; **323**: 645-55.

85. Gomez-Jimenez J, Salgado A, Mourelle M, *et al.* L-arginine: nitric oxide pathway in endotoxemia and human septic shock. *Crit Care Med.* 1995; **23**: 253-8.

86. Miyauchi T, Tomobe Y, Shiba R, *et al.* Involvement of endothelin in the regulation of human vascular tonus. Potent vasoconstrictor effect and existence in endothelial cells. *Circulation.* 1990; **81**: 1874-80.

87. Eiseman B, Beart R, Norton L. Multiple organ failure. *Surg Gynecol Obstet.* 1977; **144**: 323-6.

88. McCutchan JA, Wolf JL, Ziegler EJ, Braude AI. Ineffectiveness of single-dose human antiserum to core glycolipid (E. coli J5) for prophylaxis of bacteremic, gram-negative infections in patients with prolonged neutropenia. *Schweiz Med Wochenschr Suppl.* 1983; **14**: 40-5.

89. Cotran RS, Kumar V, Robbins SL, editors. *Robbins Pathologic Basis of Disease*. 5th ed. Philadelphia: W.B. Saunders Company; 1994.

90. Hinshaw LB. Sepsis/septic shock: participation of the microcirculation: an abbreviated review. *Crit Care Med.* 1996; **24**: 1072-8.

91. Sigurdsson GH, Christenson JT, el-Rakshy MB, Sadek S. Intestinal platelet trapping after traumatic and septic shock. An early sign of sepsis and multiorgan failure in critically ill patients? *Crit Care Med.* 1992; **20**: 458-67.

92. Eisenberg PR. Endothelial cell mediators of thrombosis and fibrinolysis: Review in depth. *Coronary Artery Diseases.* 1991; **2**: 129-66.

93. Fourrier F, Chopin C, Goudemand J, *et al.* Septic shock, multiple organ failure, and disseminated intravascular coagulation. Compared patterns of antithrombin III, protein C, and protein S deficiencies. *Chest.* 1992; **101**: 816-23.

94. Gando S, Kameue T, Nanzaki S, Nakanishi Y. Cytokines, soluble thrombomodulin and disseminated intravascular coagulation in patients with systemic inflammatory response syndrome. *Thromb Res.* 1995; **80**: 519-26.

95. Levi M, ten Cate H, van der Poll T, van Deventer SJ. Pathogenesis of disseminated intravascular coagulation in sepsis. *JAMA.* 1993; **270**: 975-9.

96. van der Pol W, van de Winkel JG. IgG receptor polymorphisms: risk factors for disease. *Immunogenetics.* 1998; **48**: 222-32.

97. Cipolle MD, Pasquale MD, Cerra FB. Secondary organ dysfunction. From clinical perspectives to molecular mediators. *Crit Care Clin.* 1993; **9**: 261-98.

98. Gando S, Kameue T, Nanzaki S, Nakanishi Y. Disseminated intravascular coagulation is a frequent complication of systemic inflammatory response syndrome. *Thromb Haemost.* 1996; **75**: 224-8.

99. Levy MM, Fink MP, Marshall JC, *et al.* 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med.* 2003; **31**: 1250-6.

100. Bone RC, Balk RA, Cerra FB, *et al.* Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest.* 1992; **101**: 1644-55.

101. Randow F, Syrbe U, Meisel C, *et al.* Mechanism of endotoxin desensitization: involvement of interleukin 10 and transforming growth factor beta. *J Exp Med.* 1995; **181**: 1887-92.

102. Syrbe U, Meinecke A, Platzer C, Makki A, Asadullah K, Klug C. Improvement of monocyte function - a new therapeutic approach? In: Reinhart K, Eyrich K, Sprung CL, editors. *Sepsis: Current Perspectives in Pathophysiology and Therapy.* Berlin: Springer-Verlag; 1994. p. 473-500.

103. Bone RC. Why sepsis trials fail. *JAMA.* 1996; **276**: 565-6.

104. Munoz C, Carlet J, Fitting C, Misset B, Bleriot JP, Cavaillon JM. Dysregulation of in vitro cytokine production by monocytes during sepsis. *J Clin Invest.* 1991; **88**: 1747-54.

105. Faist E, Storck M, Hultner L, *et al.* Functional analysis of monocyte activity through synthesis patterns of proinflammatory cytokines and neopterin in patients in surgical intensive care. *Surgery.* 1992; **112**: 562-72.

106. Cheadle WG, Hershman MJ, Wellhausen SR, Polk HC. Role of monocytic HLA-DR expression following trauma in predicting clinical outcome. In: Faist E, Ninneman J, Green D, editors. *Immune Consequences of Trauma, Shock and Sepsis.* Berlin: Springer-Verlag; 1989. p. 119-22.

107. Browder W, Williams D, Pretus H, *et al.* Beneficial effect of enhanced macrophage function in the trauma patient. *Ann Surg.* 1990; **211**: 605-12; discussion 12-3.

108. Livingston DH, Appel SH, Wellhausen SR, Sonnenfeld G, Polk HC, Jr. Depressed interferon gamma production and monocyte HLA-DR expression after severe injury. *Arch Surg.* 1988; **123**: 1309-12.

109. Gibbons RA, Martinez OM, Lim RC, Horn JK, Garovoy MR. Reduction in HLA-DR, HLA-DQ and HLA-DP expression by Leu-M3+ cells from the peripheral blood of patients with thermal injury. *Clin Exp Immunol.* 1989; **75**: 371-5.

110. Wakefield CH, Carey PD, Foulds S, Monson JR, Guillou PJ. Changes in major histocompatibility complex class II expression in monocytes and T cells of patients developing infection after surgery. *Br J Surg.* 1993; **80**: 205-9.

111. Hamilton G, Hofbauer S, Hamilton B. Endotoxin, TNF-alpha, interleukin-6 and parameters of the cellular immune system in patients with intraabdominal sepsis. *Scand J Infect Dis.* 1992; **24**: 361-8.

112. Fisher CJ, Jr., Opal SM, Dhainaut JF, *et al.* Influence of an anti-tumor necrosis factor monoclonal antibody on cytokine levels in patients with sepsis. The CB0006 Sepsis Syndrome Study Group. *Crit Care Med.* 1993; **21**: 318-27.

113. Dofferhoff AS, Bom VJ, de Vries-Hospers HG, *et al.* Patterns of cytokines, plasma endotoxin, plasminogen activator inhibitor, and acute-phase proteins during the treatment of severe sepsis in humans. *Crit Care Med.* 1992; **20**: 185-92.

114. Leser HG, Gross V, Scheibenbogen C, *et al.* Elevation of serum interleukin-6 concentration precedes acute-phase response and reflects severity in acute pancreatitis. *Gastroenterology.* 1991; **101**: 782-5.

115. Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E. Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. *Chest.* 1993; **103**: 565-75.

116. Edwards SW. Cell signalling by integrins and immunoglobulin receptors in primed neutrophils. *Trends Biochem Sci.* 1995; **20**: 362-7.

117. Friese RS, Rehring TF, Wollmering M, *et al.* Trauma primes cells. *Shock.* 1994; **1**: 388-94.

118. Watson F, Robinson JJ, Edwards SW. Neutrophil function in whole blood and after purification: changes in receptor expression, oxidase activity and responsiveness to cytokines. *Biosci Rep.* 1992; **12**: 123-33.

119. Watson F, Lowe GM, Robinson JJ, Galvani DW, Edwards SW. Phospholipase D-dependent and -independent activation of the neutrophil NADPH oxidase. *Biosci Rep.* 1994; **14**: 91-102.

120. Botha AJ, Moore FA, Moore EE, Kim FJ, Banerjee A, Peterson VM. Postinjury neutrophil priming and activation: an early vulnerable window. *Surgery.* 1995; **118**: 358-64; discussion 64-5.

121. Botha AJ, Moore FA, Moore EE, Sauaia A, Banerjee A, Peterson VM. Early neutrophil sequestration after injury: a pathogenic mechanism for multiple organ failure. *J Trauma.* 1995; **39**: 411-7.

122. Anderson BO, Brown JM, Harken AH. Mechanisms of neutrophil-mediated tissue injury. *J Surg Res.* 1991; **51**: 170-9.

123. Koike K, Moore FA, Moore EE, Poggetti RS, Tuder RM, Banerjee A. Endotoxin after gut ischemia/reperfusion causes irreversible lung injury. *J Surg Res.* 1992; **52**: 656-62.

124. Waydhas C, Nast-Kolb D, Trupka A, *et al.* Posttraumatic inflammatory response, secondary operations, and late multiple organ failure. *J Trauma.* 1996; **40**: 624-30; discussion 30-1.

125. Moore FA, Moore EE. Evolving concepts in the pathogenesis of postinjury multiple organ failure. *Surg Clin North Am.* 1995; **75**: 257-77.

126. Pape HC, Auf'm'Kolk M, Paffrath T, Regel G, Sturm JA, Tscherne H. Primary intramedullary femur fixation in multiple trauma patients with associated lung contusion-a cause of posttraumatic ARDS? *J Trauma.* 1993; **34**: 540-7; discussion 7-8.

127. Bone RC. Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. *Crit Care Med.* 1996; **24**: 163-72.

128. Sweeney KJ, Evoy D, Sultan S, *et al.* Endovascular approach to abdominal aortic aneurysms limits the postoperative systemic immune response. *Eur J Vasc Endovasc Surg.* 2002; **23**: 303-8.

129. Norwood MG, Bown MJ, Lloyd G, Bell PR, Sayers RD. The clinical value of the systemic inflammatory response syndrome (SIRS) in abdominal aortic aneurysm repair. *Eur J Vasc Endovasc Surg.* 2004; **27**: 292-8.

130. Bown MJ, Nicholson ML, Bell PR, Sayers RD. The systemic inflammatory response syndrome, organ failure, and mortality after abdominal aortic aneurysm repair. *J Vasc Surg.* 2003; **37**: 600-6.

131. Cohen JD, Singer P, Grunberg G, Grozovski E, Sulkes J, Zelikovski A. Outcome after elective infrarenal aortic aneurysm surgery. *World J Surg.* 1998; **22**: 278-82.

132. Ramdev P, Rayan SS, Sheahan M, *et al.* A decade experience with infrainguinal revascularization in a dialysis-dependent patient population. *J Vasc Surg.* 2002; **36**: 969-74.

133. Mertens RA, O'Hara PJ, Hertzer NR, Krajewski LP, Beven EG. Surgical management of infrainguinal arterial prosthetic graft infections: review of a thirty-five-year experience. *J Vasc Surg.* 1995; **21**: 782-90; discussion 90-1.

134. Chalmers RT, Wolfe JH, Cheshire NJ, *et al.* Improved management of infrainguinal bypass graft infection with methicillin-resistant Staphylococcus aureus. *Br J Surg.* 1999; **86**: 1433-6.

135. Arnaout MA. Structure and function of the leukocyte adhesion molecules CD11/CD18. *Blood.* 1990; **75**: 1037-50.

136. Lofgren R, Serrander L, Forsberg M, Wilsson A, Wasteson A, Stendahl O. CR3, FcγRIIA and FcγRIIIB induce activation of the respiratory burst in human neutrophils: the role of intracellular Ca^{2+}, phospholipase D and tyrosine phosphorylation. *Biochim Biophys Acta.* 1999; **1452**: 46-59.

137. Roitt IM. *Roitt's Essential Immunology*, 9th ed. Oxford: Blackwell Science; 1997.

138. Aosasa S, Ono S, Mochizuki H, *et al.* Activation of monocytes and endothelial cells depends on the severity of surgical stress. *World J Surg.* 2000; **24**: 10-6.

139. Arnaout MA, Spits H, Terhorst C, Pitt J, Todd RF, 3rd. Deficiency of a leukocyte surface glycoprotein (LFA-1) in two patients with Mo1 deficiency. Effects of cell activation on Mo1/LFA-1 surface expression in normal and deficient leukocytes. *J Clin Invest.* 1984; **74**: 1291-300.

140. Wakefield CH, Carey PD, Foulds S, Monson JR, Guillou PJ. Polymorphonuclear leukocyte activation. An early marker of the postsurgical sepsis response. *Arch Surg.* 1993; **128**: 390-5.

141. Chatila TA, Geha RS, Arnaout MA. Constitutive and stimulus-induced phosphorylation of CD11/CD18 leukocyte adhesion molecules. *J Cell Biol.* 1989; **109**: 3435-44.

142. Hughes BJ, Hollers JC, Crockett-Torabi E, Smith CW. Recruitment of CD11b/CD18 to the neutrophil surface and adherence-dependent cell locomotion. *J Clin Invest.* 1992; **90**: 1687-96.

143. Smith CW, Rothlein R, Hughes BJ, *et al.* Recognition of an endothelial determinant for CD18 - dependent human neutrophil adherence and transendothelial migration. *J Clin Invest.* 1988; **82**: 1746-56.

144. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J Clin Invest.* 1989; **83**: 2008-17.

145. Diamond MS, Staunton DE, de Fougerolles AR, *et al.* ICAM-1 (CD54): a counter-receptor for Mac-1 (CD11b/CD18). *J Cell Biol.* 1990; **111**: 3129-39.

146. Capron M, Kazatchkine MD, Fischer E, *et al.* Functional role of the alpha-chain of complement receptor type 3 in human eosinophil-dependent antibody-mediated cytotoxicity against schistosomes. *J Immunol.* 1987; **139**: 2059-65.

147. Nathan CF. Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. *J Clin Invest.* 1987; **80**: 1550-60.

148. Shappell SB, Toman C, Anderson DC, Taylor AA, Entman ML, Smith CW. Mac-1 (CD11b/CD18) mediates adherence-dependent hydrogen peroxide production by human and canine neutrophils. *J Immunol.* 1990; **144**: 2702-11.

149. van Sorge NM, van der Pol WL, van de Winkel JG. FcγR polymorphisms: Implications for function, disease susceptibility and immunotherapy. *Tissue Antigens.* 2003; **61**: 189-202.

150. Tarnok A, Bocsi J, Pipek M, *et al.* Preoperative prediction of postoperative edema and effusion in pediatric cardiac surgery by altered antigen expression patterns on granulocytes and monocytes. *Cytometry.* 2001; **46**: 247-53.

151. Shih HC, Su CH, Lee CH. Alternations of surface antigens on leukocytes after severe injury: correlation with infectious complications. *Intensive Care Med.* 1998; **24**: 152-6.

152. Foulds S, Cheshire NJ, Schachter M, Wolfe JH, Mansfield AO. Endotoxin related early neutrophil activation is associated with outcome after thoracoabdominal aortic aneurysm repair. *Br J Surg.* 1997; **84**: 172-7.

153. Foulds S, Mireskandari M, Kalu P, *et al.* Visceral ischemia and neutrophil activation in sepsis and organ dysfunction. *J Surg Res.* 1998; **75**: 170-6.

154. Norwood MG, Horsburgh T, Bown MJ, Sayers RD. Neutrophil activation occurs in the lower-limbs of patients undergoing elective repair of abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg.* 2005; **29**: 390-4.

155. Barry MC, Kelly C, Burke P, Sheehan S, Redmond HP, Bouchier-Hayes D. Immunological and physiological responses to aortic surgery: effect of reperfusion on neutrophil and monocyte activation and pulmonary function. *Br J Surg.* 1997; **84**: 513-9.

156. Swartbol P, Norgren L, Parsson H, Truedsson L. Endovascular abdominal aortic aneurysm repair induces significant alterations in surface adhesion molecule expression on donor white blood cells exposed to patient plasma. *Eur J Vasc Endovasc Surg.* 1997; **14**: 48-59.

157. Sanders LA, Feldman RG, Voorhorst-Ogink MM, *et al.* Human immunoglobulin G (IgG) Fc receptor IIA (CD32) polymorphism and IgG2-mediated bacterial phagocytosis by neutrophils. *Infect Immun.* 1995; **63**: 73-81.

158. Salmon JE, Edberg JC, Kimberly RP. Fcγ receptor III on human neutrophils. Allelic variants have functionally distinct capacities. *J Clin Invest.* 1990; **85**: 1287-95.

159. van de Winkel JG, Capel PJ. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunol Today.* 1993; **14**: 215-21.

160. Ernst LK, van de Winkel JG, Chiu IM, Anderson CL. Three genes for the human high affinity Fc receptor for IgG (FcγRI) encode four distinct transcription products. *J Biol Chem.* 1992; **267**: 15692-700.

161. van Vugt MJ, Reefman E, Zeelenberg I, Boonen G, Leusen JH, van de Winkel JG. The alternatively spliced CD64 transcript FcγRIb2 does not specify a surface-expressed isoform. *Eur J Immunol.* 1999; **29**: 143-9.

162. Yuan FF, Wong M, Pererva N, *et al.* FcγRIIA polymorphisms in Streptococcus pneumoniae infection. *Immunol Cell Biol.* 2003; **81**: 192-5.

163. Lin S, Cicala C, Scharenberg AM, Kinet JP. The FcεRIβ subunit functions as an amplifier of FcεRIγ-mediated cell activation signals. *Cell.* 1996; **85**: 985-95.

164. Dombrowicz D, Lin S, Flamand V, Brini AT, Koller BH, Kinet JP. Allergy-associated FcRβ is a molecular amplifier of IgE- and IgG-mediated in vivo responses. *Immunity.* 1998; **8**: 517-29.

165. Kerst JM, de Haas M, van der Schoot CE, *et al.* Recombinant granulocyte colony-stimulating factor administration to healthy volunteers: induction of immunophenotypically and functionally altered neutrophils via an effect on myeloid progenitor cells. *Blood.* 1993; **82**: 3265-72.

166. Buckle AM, Hogg N. The effect of IFN-γ and colony-stimulating factors on the expression of neutrophil cell membrane receptors. *J Immunol.* 1989; **143**: 2295-301.

167. Huizinga TW, van der Schoot CE, Jost C, *et al.* The PI-linked receptor FcRIII is released on stimulation of neutrophils. *Nature.* 1988; **333**: 667-9.

168. Wakefield CH, Carey PD, Foulds S, Monson JR, Guillou PJ. Surgery and the release of a neutrophil Fc gamma receptor. *Am J Surg.* 1995; **170**: 277-84.

169. Middelhoven PJ, Van Buul JD, Hordijk PL, Roos D. Different proteolytic mechanisms involved in FcγRIIIb shedding from human neutrophils. *Clin Exp Immunol.* 2001; **125**: 169-75.

170. Middelhoven PJ, van Buul JD, Kleijer M, Roos D, Hordijk PL. Actin polymerization induces shedding of FcγRIIIb (CD16) from human neutrophils. *Biochem Biophys Res Commun.* 1999; **255**: 568-74.

171. Moldovan I, Galon J, Maridonneau-Parini I, *et al.* Regulation of production of soluble Fcγ receptors type III in normal and pathological conditions. *Immunol Lett.* 1999; **68**: 125-34.

172. Fossati G, Moots RJ, Bucknall RC, Edwards SW. Differential role of neutrophil Fcγ receptor IIIB (CD16) in phagocytosis, bacterial killing, and responses to immune complexes. *Arthritis Rheum.* 2002; **46**: 1351-61.

173. Shen L, Guyre PM, Fanger MW. Polymorphonuclear leukocyte function triggered through the high affinity Fc receptor for monomeric IgG. *J Immunol.* 1987; **139**: 534-8.

174. Ruiz P, Gomez F, Lopez R, Chien P, Rossman MD, Schreiber AD. Granulocyte Fcγ receptor recognition of cell bound and aggregated IgG: effect of γ-interferon. *Am J Hematol.* 1992; **39**: 257-63.

175. Guyre PM, Graziano RF, Vance BA, Morganelli PM, Fanger MW. Monoclonal antibodies that bind to distinct epitopes on FcγRI are able to trigger receptor function. *J Immunol.* 1989; **143**: 1650-5.

176. Fanger NA, Voigtlaender D, Liu C, *et al.* Characterization of expression, cytokine regulation, and effector function of the high affinity IgG receptor FcγRI (CD64) expressed on human blood dendritic cells. *J Immunol.* 1997; **158**: 3090-8.

177. Fanger NA, Wardwell K, Shen L, Tedder TF, Guyre PM. Type I (CD64) and type II (CD32) Fcγ receptor-mediated phagocytosis by human blood dendritic cells. *J Immunol.* 1996; **157**: 541-8.

178. Williams TE, Nagarajan S, Selvaraj P, Zhu C. Concurrent and independent binding of Fcγ receptors IIa and IIIb to surface-bound IgG. *Biophys J.* 2000; **79**: 1867-75.

179. Allen E, Bakke AC, Purtzer MZ, Deodhar A. Neutrophil CD64 expression: distinguishing acute inflammatory autoimmune disease from systemic infections. *Ann Rheum Dis.* 2002; **61**: 522-5.

180. Qureshi SS, Lewis SM, Gant VA, Treacher D, Davis BH, Brown KA. Increased distribution and expression of CD64 on blood polymorphonuclear cells from patients with the systemic inflammatory response syndrome (SIRS). *Clin Exp Immunol.* 2001; **125**: 258-65.

181. Fischer G, Schneider EM, LL LM, *et al.* CD64 surface expression on neutrophils is transiently upregulated in patients with septic shock. *Intensive Care Med.* 2001; **27**: 1848-52.

182. Layseca-Espinosa E, Perez-Gonzalez LF, Torres-Montes A, *et al.* Expression of CD64 as a potential marker of neonatal sepsis. *Pediatr Allergy Immunol.* 2002; **13**: 319-27.

183. Shehab El-Din SA, Aref SE, Salama OS. Assessment of certain neutrophil receptors, opsonophagocytosis and soluble intercellular adhesion molecule 1 (ICAM 1) following thermal injury. *Annals of Burns and Fire Disasters.* 1998; **11**: 27-33.

184. Wagner C, Deppisch R, Denefleh B, Hug F, Andrassy K, Hansch GM. Expression patterns of the lipopolysaccharide receptor CD14, and the FCγ receptors CD16 and CD64 on polymorphonuclear neutrophils: data from patients with severe bacterial infections and lipopolysaccharide-exposed cells. *Shock.* 2003; **19**: 5-12.

185. Spark JI, Scott DJ. Role of the neutrophil in the development of systemic inflammatory response syndrome and sepsis following abdominal aortic surgery. *Br J Surg.* 2001; **88**: 1583-9.

186. Warmerdam PA, van de Winkel JG, Vlug A, Westerdaal NA, Capel PJ. A single amino acid in the second Ig-like domain of the human Fcγ receptor II is critical for human IgG2 binding. *J Immunol.* 1991; **147**: 1338-43.

187. Clark MR, Clarkson SB, Ory PA, Stollman N, Goldstein IM. Molecular basis for a polymorphism involving Fc receptor II on human monocytes. *J Immunol.* 1989; **143**: 1731-4.

188. Clark MR, Stuart SG, Kimberly RP, Ory PA, Goldstein IM. A single amino acid distinguishes the high-responder from the low-responder form of Fc receptor II on human monocytes. *Eur J Immunol.* 1991; **21**: 1911-6.

189. Parren PW, Warmerdam PA, Boeije LC, *et al.* On the interaction of IgG subclasses with the low affinity FcγRIIa (CD32) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG2. *J Clin Invest.* 1992; **90**: 1537-46.

190. Salmon JE, Edberg JC, Brogle NL, Kimberly RP. Allelic polymorphisms of human Fcγ receptor IIA and Fcγ receptor IIIB. Independent mechanisms for differences in human phagocyte function. *J Clin Invest.* 1992; **89**: 1274-81.

191. Bredius RG, de Vries CE, Troelstra A, *et al.* Phagocytosis of Staphylococcus aureus and Haemophilus influenzae type B opsonized with polyclonal human IgG1 and IgG2 antibodies. Functional hFcγRIIa polymorphism to IgG2. *J Immunol.* 1993; **151**: 1463-72.

192. Bredius RG, Fijen CA, De Haas M, *et al.* Role of neutrophil FcγRIIa (CD32) and FcγRIIIb (CD16) polymorphic forms in phagocytosis of human IgG1- and IgG3-opsonized bacteria and erythrocytes. *Immunology.* 1994; **83**: 624-30.

193. Ory PA, Clark MR, Kwoh EE, Clarkson SB, Goldstein IM. Sequences of complementary DNAs that encode the NA1 and NA2 forms of Fc receptor III on human neutrophils. *J Clin Invest.* 1989; **84**: 1688-91.

194. Ory PA, Goldstein IM, Kwoh EE, Clarkson SB. Characterization of polymorphic forms of Fc receptor III on human neutrophils. *J Clin Invest.* 1989; **83**: 1676-81.

195. Sanders LA, van de Winkel JG, Rijkers GT, *et al.* Fcγ receptor IIa (CD32) heterogeneity in patients with recurrent bacterial respiratory tract infections. *J Infect Dis.* 1994; **170**: 854-61.

196. Yee AM, Phan HM, Zuniga R, Salmon JE, Musher DM. Association between FcγRIIa-R131 allotype and bacteremic pneumococcal pneumonia. *Clin Infect Dis.* 2000; **30**: 25-8.

197. Platonov AE, Shipulin GA, Vershinina IV, Dankert J, van de Winkel JG, Kuijper EJ. Association of human FcγRIIa (CD32) polymorphism with susceptibility to and severity of meningococcal disease. *Clin Infect Dis.* 1998; **27**: 746-50.

198. Bredius RG, Derkx BH, Fijen CA, *et al.* Fcγ receptor IIa (CD32) polymorphism in fulminant meningococcal septic shock in children. *J Infect Dis.* 1994; **170**: 848-53.

199. Domingo P, Muniz-Diaz E, Baraldes MA, *et al.* Associations between Fc gamma receptor IIA polymorphisms and the risk and prognosis of meningococcal disease. *Am J Med.* 2002; **112**: 19-25.

200. Fijen CA, Bredius RG, Kuijper EJ. Polymorphism of IgG Fc receptors in meningococcal disease. *Ann Intern Med.* 1993; **119**: 636.

201. van der Pol WL, Huizinga TW, Vidarsson G, *et al.* Relevance of Fcγ receptor and interleukin-10 polymorphisms for meningococcal disease. *J Infect Dis.* 2001; **184**: 1548-55.

202. Kobayashi T, Westerdaal NA, Miyazaki A, *et al.* Relevance of immunoglobulin G Fc receptor polymorphism to recurrence of adult periodontitis in Japanese patients. *Infect Immun.* 1997; **65**: 3556-60.

203. Kobayashi T, Sugita N, van der Pol WL, *et al.* The Fcγ receptor genotype as a risk factor for generalized early-onset periodontitis in Japanese patients. *J Periodontol.* 2000; **71**: 1425-32.

204. Kobayashi T, Yamamoto K, Sugita N, *et al.* The Fcγ receptor genotype as a severity factor for chronic periodontitis in Japanese patients. *J Periodontol.* 2001; **72**: 1324-31.

205. Sugita N, Kobayashi T, Ando Y, *et al.* Increased frequency of FcγRIIIb-NA1 allele in periodontitis-resistant subjects in an elderly Japanese population. *J Dent Res.* 2001; **80**: 914-8.

206. Loos BG, Leppers-Van de Straat FG, Van de Winkel JG, Van der Velden U. Fcγ receptor polymorphisms in relation to periodontitis. *J Clin Periodontol.* 2003; **30**: 595-602.

207. Ziegeler S, Tsusaki BE, Collard CD. Influence of genotype on perioperative risk and outcome. *Anesthesiology.* 2003; **99**: 212-9.

208. Shastri KA, Logue GL, Stern MP, Rehman S, Raza S. Complement activation by heparin-protamine complexes during cardiopulmonary bypass: effect of C4A null allele. *J Thorac Cardiovasc Surg.* 1997; **114**: 482-8.

209. Brull DJ, Montgomery HE, Sanders J, *et al.* Interleukin-6 gene -174g→c and -572g→c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. *Arterioscler Thromb Vasc Biol.* 2001; **21**: 1458-63.

210. Grocott HP, Newman MF, El-Moalem H, Bainbridge D, Butler A, Laskowitz DT. Apolipoprotein E genotype differentially influences the proinflammatory and anti-inflammatory response to cardiopulmonary bypass. *J Thorac Cardiovasc Surg.* 2001; **122**: 622-3.

211. Yende S, Quasney M, Zhang Q, Frederick K, Kessler L, Wunderink RG. Impact of cytokine gene polymorphisms on outcomes of coronary artery bypass graft surgery. *Chest.* 2002; **121**: 86S.

212. Stuber F, Petersen M, Bokelmann F, Schade U. A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-α concentrations and outcome of patients with severe sepsis. *Crit Care Med.* 1996; **24**: 381-4.

213. Roth-Isigkeit A, Hasselbach L, Ocklitz E, *et al.* Inter-individual differences in cytokine release in patients undergoing cardiac surgery with cardiopulmonary bypass. *Clin Exp Immunol.* 2001; **125**: 80-8.

214. Majetschak M, Flohe S, Obertacke U, *et al.* Relation of a TNF gene polymorphism to severe sepsis in trauma patients. *Ann Surg.* 1999; **230**: 207-14.

215. Carter MJ, Di Giovine FS, Cox A, *et al.* The interleukin 1 receptor antagonist gene allele 2 as a predictor of pouchitis following colectomy and IPAA in ulcerative colitis. *Gastroenterology.* 2001; **121**: 805-11.

216. Bown MJ, Horsburgh T, Nicholson ML, Bell PR, Sayers RD. Cytokines, their genetic polymorphisms, and outcome after abdominal aortic aneurysm repair. *Eur J Vasc Endovasc Surg.* 2004; **28**: 274-80.

217. Holmes CL, Russell JA, Walley KR. Genetic polymorphisms in sepsis and septic shock: role in prognosis and potential for therapy. *Chest.* 2003; **124**: 1103-15.

218. Tang GJ, Huang SL, Yien HW, *et al.* Tumor necrosis factor gene polymorphism and septic shock in surgical infection. *Crit Care Med.* 2000; **28**: 2733-6.

219. Mira JP, Cariou A, Grall F, *et al.* Association of TNF2, a TNF-α promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. *JAMA.* 1999; **282**: 561-8.

220. Stuber F, Udalova IA, Book M, *et al.* -308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. *J Inflamm.* 1995; **46**: 42-50.

221. Schluter B, Raufhake C, Erren M, *et al.* Effect of the interleukin-6 promoter polymorphism (-174 G/C) on the incidence and outcome of sepsis. *Crit Care Med.* 2002; **30**: 32-7.

222. Raeburn CD, Sheppard F, Barsness KA, Arya J, Harken AH. Cytokines for surgeons. *Am J Surg.* 2002; **183**: 268-73.

223. Bown MJ, Nicholson ML, Bell PR, Sayers RD. Cytokines and inflammatory pathways in the pathogenesis of multiple organ failure following abdominal aortic aneurysm repair. *Eur J Vasc Endovasc Surg.* 2001; **22**: 485-95.

224. Swartbol P, Truedsson L, Norgren L. The inflammatory response and its consequence for the clinical outcome following aortic aneurysm repair. *Eur J Vasc Endovasc Surg.* 2001; **21**: 393-400.

225. Roumen RM, Hendriks T, van der Ven-Jongekrijg J, *et al.* Cytokine patterns in patients after major vascular surgery, hemorrhagic shock, and severe blunt trauma. Relation with subsequent adult respiratory distress syndrome and multiple organ failure. *Ann Surg.* 1993; **218**: 769-76.

226. Wang H, Czura CJ, Tracey KJ. Tumor necrosis factor. In: Thomson AW, Lotze MT, editors. *The Cytokine Handbook.* 4th ed. London: Academic Press; 2003. p. 838-60.

227. Kriegler M, Perez C, DeFay K, Albert I, Lu SD. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell.* 1988; **53**: 45-53.

228. Smith RA, Baglioni C. The active form of tumor necrosis factor is a trimer. *J Biol Chem.* 1987; **262**: 6951-4.

229. Beutler B, Greenwald D, Hulmes JD, *et al.* Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature.* 1985; **316**: 552-4.

230. Dinarello CA. Interleukin-1 Family [IL-1F1, F2]. In: Thomson AW, Lotze MT, editors. *The Cytokine Handbook.* 4th ed. London: Academic Press; 2003. p. 644-68.

231. Dinarello CA, Cannon JG, Wolff SM, *et al.* Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J Exp Med.* 1986; **163**: 1433-50.

232. Schindler R, Clark BD, Dinarello CA. Dissociation between interleukin-1β mRNA and protein synthesis in human peripheral blood mononuclear cells. *J Biol Chem.* 1990; **265**: 10232-7.

233. Kishimoto T. Interleukin-6 (IL-6). In: Thomson AW, Lotze MT, editors. *The Cytokine Handbook.* 4th ed. London: Academic Press; 2003. p. 281-304.

234. May LT, Ghrayeb J, Santhanam U, *et al.* Synthesis and secretion of multiple forms of β2-interferon/B-cell differentiation factor 2/hepatocyte-stimulating factor by human fibroblasts and monocytes. *J Biol Chem.* 1988; **263**: 7760-6.

235. Santhanam U, Ghrayeb J, Sehgal PB, May LT. Post-translational modifications of human interleukin-6. *Arch Biochem Biophys.* 1989; **274**: 161-70.

236. Van Snick J. Interleukin-6: an overview. *Annu Rev Immunol.* 1990; **8**: 253-78.

237. Houssiau F, Van Snick J. IL6 and the T-cell response. *Res Immunol.* 1992; **143**: 740-3.

238. Hoffbrand AV, Pettit JE. *Essential Haematology*, 3rd ed. Oxford: Blackwell Scientific Publications; 1993.

239. Ganong WF. *Review of Medical Physiology*, 19th ed. New York: McGraw-Hill; 1999.

240. Ding Y, Fu S, Zamarin D, Bromberg J. Interleukin-10. In: Thomson AW, Lotze MT, editors. *The Cytokine Handbook.* 4th ed. London: Academic Press; 2003. p. 603-25.

241. Groux H, Bigler M, de Vries JE, Roncarolo MG. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. *J Exp Med.* 1996; **184**: 19-29.

242. Groux H, O'Garra A, Bigler M, *et al.* A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature.* 1997; **389**: 737-42.

243. Daftarian PM, Kumar A, Kryworuchko M, Diaz-Mitoma F. IL-10 production is enhanced in human T cells by IL-12 and IL-6 and in monocytes by tumor necrosis factor-alpha. *J Immunol.* 1996; **157**: 12-20.

244. Jeannin P, Delneste Y, Seveso M, Life P, Bonnefoy JY. IL-12 synergizes with IL-2 and other stimuli in inducing IL-10 production by human T cells. *J Immunol.* 1996; **156**: 3159-65.

245. Tilg H, Atkins MB, Dinarello CA, Mier JW. Induction of circulating interleukin 10 by interleukin 1 and interleukin 2, but not interleukin 6 immunotherapy. *Cytokine.* 1995; **7**: 734-9.

246. Marietta EV, Chen Y, Weis JH. Modulation of expression of the anti-inflammatory cytokines interleukin-13 and interleukin-10 by interleukin-3. *Eur J Immunol.* 1996; **26**: 49-56.

247. Foey AD, Parry SL, Williams LM, Feldmann M, Foxwell BM, Brennan FM. Regulation of monocyte IL-10 synthesis by endogenous IL-1 and TNF-alpha: role of the p38 and p42/44 mitogen-activated protein kinases. *J Immunol.* 1998; **160**: 920-8.

248. Cohen SB, Parry SL, Feldmann M, Foxwell B. Autocrine and paracrine regulation of human T cell IL-10 production. *J Immunol.* 1997; **158**: 5596-602.

249. Aman MJ, Tretter T, Eisenbeis I, *et al.* Interferon-alpha stimulates production of interleukin-10 in activated CD4+ T cells and monocytes. *Blood.* 1996; **87**: 4731-6.

250. Fouqueray B, Boutard V, Philippe C, *et al.* Mesangial cell-derived interleukin-10 modulates mesangial cell response to lipopolysaccharide. *Am J Pathol.* 1995; **147**: 176-82.

251. Ishizaka S, Saito S, Yoshikawa M, Kimoto M, Nishiyama T. IL-10 production in mouse hepatocytes augmented by TGF-beta. *Cytokine.* 1996; **8**: 837-43.

252. Maeda H, Kuwahara H, Ichimura Y, Ohtsuki M, Kurakata S, Shiraishi A. TGF-beta enhances macrophage ability to produce IL-10 in normal and tumor-bearing mice. *J Immunol.* 1995; **155**: 4926-32.

253. Rep MH, Hintzen RQ, Polman CH, van Lier RA. Recombinant interferon-beta blocks proliferation but enhances interleukin-10 secretion by activated human T-cells. *J Neuroimmunol.* 1996; **67**: 111-8.

254. Wanidworanun C, Strober W. Predominant role of tumor necrosis factor-alpha in human monocyte IL-10 synthesis. *J Immunol.* 1993; **151**: 6853-61.

255. Chomarat P, Rissoan MC, Banchereau J, Miossec P. Interferon gamma inhibits interleukin 10 production by monocytes. *J Exp Med.* 1993; **177**: 523-7.

256. de Waal Malefyt R, Figdor CG, Huijbens R, *et al.* Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN-gamma or IL-10. *J Immunol.* 1993; **151**: 6370-81.

257. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med.* 1991; **174**: 1209-20.

258. Kalinski P, Storkus WJ, Thomson AW, Lotze MT. Interleukin-12 Family [IL-12, 23, 12RA and 27]. In: Thomson AW, Lotze MT, editors. *The Cytokine Handbook.* 4th ed. London: Academic Press; 2003. p. 383-408.

259. Okamura H, Lotze MT, Tsutsui H, *et al.* Interleukin-18 [IL-1F4]. In: Thomson AW, Lotze MT, editors. *The Cytokine Handbook.* 4th ed. London: Academic Press; 2003. p. 709-33.

260. Luster AD. Chemokines-chemotactic cytokines that mediate inflammation. *N Engl J Med.* 1998; **338**: 436-45.

261. Mukaida N, Ketlinsky SA, Matsushima K. Interleukin-8 and other CXC chemokines. In: Thomson AW, Lotze MT, editors. *The Cytokine Handbook.* 4th ed. London: Academic Press; 2003. p. 1049-81.

262. Rollins BJ. Chemokines. *Blood.* 1997; **90**: 909-28.

263. Puneet P, Moochhala S, Bhatia M. Chemokines in acute respiratory distress syndrome. *Am J Physiol Lung Cell Mol Physiol.* 2005; **288**: L3-15.

264. Murphy PM, Baggiolini M, Charo IF, *et al.* International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev.* 2000; **52**: 145-76.

265. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity.* 2000; **12**: 121-7.

266. Mukaida N, Harada A, Yasumoto K, Matsushima K. Properties of pro-inflammatory cell type-specific leukocyte chemotactic cytokines, interleukin 8 (IL-8) and monocyte chemotactic and activating factor (MCAF). *Microbiol Immunol.* 1992; **36**: 773-89.

267. Oppenheim JJ, Zachariae CO, Mukaida N, Matsushima K. Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu Rev Immunol.* 1991; **9**: 617-48.

268. Matsushima K, Morishita K, Yoshimura T, *et al.* Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. *J Exp Med.* 1988; **167**: 1883-93.

269. DiMango E, Zar HJ, Bryan R, Prince A. Diverse Pseudomonas aeruginosa gene products stimulate respiratory epithelial cells to produce interleukin-8. *J Clin Invest.* 1995; **96**: 2204-10.

270. Van Damme J, Van Beeumen J, Conings R, Decock B, Billiau A. Purification of granulocyte chemotactic peptide/interleukin-8 reveals N-terminal sequence heterogeneity similar to that of beta-thromboglobulin. *Eur J Biochem.* 1989; **181**: 337-44.

271. Yoshimura T, Robinson EA, Appella E, *et al.* Three forms of monocyte-derived neutrophil chemotactic factor (MDNCF) distinguished by different lengths of the amino-terminal sequence. *Mol Immunol.* 1989; **26**: 87-93.

272. Mukaida N. Interleukin-8: an expanding universe beyond neutrophil chemotaxis and activation. *Int J Hematol.* 2000; **72**: 391-8.

273. Schroder JM. The monocyte-derived neutrophil activating peptide (NAP/interleukin 8) stimulates human neutrophil arachidonate-5-lipoxygenase, but not the release of cellular arachidonate. *J Exp Med.* 1989; **170**: 847-63.

274. Bussolino F, Sironi M, Bocchietto E, Mantovani A. Synthesis of platelet-activating factor by polymorphonuclear neutrophils stimulated with interleukin-8. *J Biol Chem.* 1992; **267**: 14598-603.

275. Carveth HJ, Bohnsack JF, McIntyre TM, Baggiolini M, Prescott SM, Zimmerman GA. Neutrophil activating factor (NAF) induces polymorphonuclear leukocyte adherence to endothelial cells and to subendothelial matrix proteins. *Biochem Biophys Res Commun.* 1989; **162**: 387-93.

276. Detmers PA, Lo SK, Olsen-Egbert E, Walz A, Baggiolini M, Cohn ZA. Neutrophil-activating protein 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. *J Exp Med.* 1990; **171**: 1155-62.

277. Huber AR, Kunkel SL, Todd RF, 3rd, Weiss SJ. Regulation of transendothelial neutrophil migration by endogenous interleukin-8. *Science.* 1991; **254**: 99-102.

278. Walz A, Meloni F, Clark-Lewis I, von Tscharner V, Baggiolini M. [Ca2+]i changes and respiratory burst in human neutrophils and monocytes induced by NAP-1/interleukin-8, NAP-2, and gro/MGSA. *J Leukoc Biol.* 1991; **50**: 279-86.

279. Geiser T, Dewald B, Ehrengruber MU, Clark-Lewis I, Baggiolini M. The interleukin-8-related chemotactic cytokines GRO alpha, GRO beta, and GRO gamma activate human neutrophil and basophil leukocytes. *J Biol Chem.* 1993; **268**: 15419-24.

280. Bacon KB, Flores-Romo L, Aubry JP, Wells TN, Power CA. Interleukin-8 and RANTES induce the adhesion of the human basophilic cell line KU-812 to human endothelial cell monolayers. *Immunology.* 1994; **82**: 473-81.

281. Dahinden CA, Kurimoto Y, De Weck AL, Lindley I, Dewald B, Baggiolini M. The neutrophil-activating peptide NAF/NAP-1 induces histamine and leukotriene release by interleukin 3-primed basophils. *J Exp Med.* 1989; **170**: 1787-92.

282. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science.* 1989; **243**: 1464-6.

283. Jinquan T, Moller B, Storgaard M, *et al.* Chemotaxis and IL-8 receptor expression in B cells from normal and HIV-infected subjects. *J Immunol.* 1997; **158**: 475-84.

284. Massberg S, Messmer K. The nature of ischemia/reperfusion injury. *Transplant Proc.* 1998; **30**: 4217-23.

285. Williams FM. Neutrophils and myocardial reperfusion injury. *Pharmacol Ther.* 1996; **72**: 1-12.

286. Norwood MG, Bown MJ, Sutton AJ, Nicholson ML, Sayers RD. Interleukin 6 production during abdominal aortic aneurysm repair arises from the gastrointestinal tract and not the legs. *Br J Surg.* 2004; **91**: 1153-6.

287. Germann G, Drucke D, Steinau HU. Adhesion receptors and cytokine profiles in controlled tourniquet ischaemia in the upper extremity. *J Hand Surg [Br].* 1997; **22**: 778-82.

288. Seekamp A, Jochum M, Ziegler M, van Griensven M, Martin M, Regel G. Cytokines and adhesion molecules in elective and accidental trauma-related ischemia/reperfusion. *J Trauma.* 1998; **44**: 874-82.

289. Holmberg A, Bergqvist D, Westman B, Siegbahn A. Cytokine and fibrinogen response in patients undergoing open abdominal aortic aneurysm surgery. *Eur J Vasc Endovasc Surg.* 1999; **17**: 294-300.

290. Galle C, De Maertelaer V, Motte S, *et al.* Early inflammatory response after elective abdominal aortic aneurysm repair: a comparison between endovascular procedure and conventional surgery. *J Vasc Surg.* 2000; **32**: 234-46.

291. Soong CV, Halliday MI, Barclay GR, Hood JM, Rowlands BJ, Barros D'Sa AA. Intramucosal acidosis and systemic host responses in abdominal aortic aneurysm surgery. *Crit Care Med.* 1997; **25**: 1472-9.

292. Hamano K, Gohra H, Noda H, *et al.* Increased serum interleukin-8: correlation with poor prognosis in patients with postoperative multiple organ failure. *World J Surg.* 1998; **22**: 1077-81.

293. Froon AH, Greve JW, Van der Linden CJ, Buurman WA. Increased concentrations of cytokines and adhesion molecules in patients after repair of abdominal aortic aneurysm. *Eur J Surg.* 1996; **162**: 287-96.

294. Baigrie RJ, Lamont PM, Kwiatkowski D, Dallman MJ, Morris PJ. Systemic cytokine response after major surgery. *Br J Surg.* 1992; **79**: 757-60.

295. Jedynak M, Siemiatkowski A, Gacko M, Mroczko B, Borkowski J, Szmitkowski M. [Serum concentration of interleukin-12 (IL-12) in patients undergoing abdominal aortic aneurysm repair-preliminary report]. *Pol Arch Med Wewn.* 2004; **112**: 1173-9.

296. Ben-Abraham R, Weinbroum AA, Lotan D, *et al.* Interleukin-8 secretion following cardiopulmonary bypass in children as a marker of early postoperative morbidity. *Paediatr Anaesth.* 2002; **12**: 156-61.

297. Lotan D, Zilberman D, Dagan O, *et al.* β-chemokine secretion patterns in relation to clinical course and outcome in children after cardiopulmonary bypass: continuing the search to abrogate systemic inflammatory response. *Ann Thorac Surg.* 2001; **71**: 233-7.

298. Sablotzki A, Friedrich I, Muhling J, *et al.* The systemic inflammatory response syndrome following cardiac surgery: different expression of proinflammatory cytokines and procalcitonin in patients with and without multiorgan dysfunctions. *Perfusion.* 2002; **17**: 103-9.

299. Hensler T, Heidecke CD, Hecker H, *et al.* Increased susceptibility to postoperative sepsis in patients with impaired monocyte IL-12 production. *J Immunol.* 1998; **161**: 2655-9.

300. Weighardt H, Heidecke CD, Emmanuilidis K, *et al.* Sepsis after major visceral surgery is associated with sustained and interferon-gamma-resistant defects of monocyte cytokine production. *Surgery.* 2000; **127**: 309-15.

301. Daniels L. Good nutrition for good surgery: clinical and quality of life outcomes. *Aust Prescr.* 2003; **26**: 136-40.

302. Mora RJ. Malnutrition: organic and functional consequences. *World J Surg.* 1999; **23**: 530-5.

303. Kyle UG, Morabia A, Slosman DO, Mensi N, Unger P, Pichard C. Contribution of body composition to nutritional assessment at hospital admission in 995 patients: a controlled population study. *Br J Nutr.* 2001; **86**: 725-31.

304. Corish CA. Pre-operative nutritional assessment. *Proc Nutr Soc.* 1999; **58**: 821-9.

305. Warnold I, Lundholm K. Clinical significance of preoperative nutritional status in 215 noncancer patients. *Ann Surg.* 1984; **199**: 299-305.

306. Spark JI, Robinson JM, Gallavin L, *et al.* Patients with chronic critical limb ischaemia have reduced total antioxidant capacity and impaired nutritional status. *Eur J Vasc Endovasc Surg.* 2002; **24**: 535-9.

307. Persson MD, Brismar KE, Katzarski KS, Nordenstrom J, Cederholm TE. Nutritional status using mini nutritional assessment and subjective global assessment predict mortality in geriatric patients. *J Am Geriatr Soc.* 2002; **50**: 1996-2002.

308. Beattie AH, Prach AT, Baxter JP, Pennington CR. A randomised controlled trial evaluating the use of enteral nutritional supplements postoperatively in malnourished surgical patients. *Gut.* 2000; **46**: 813-8.

309. Studley HO. Percentage weight loss: a basic indicator of surgical risk in patients with chronic peptic ulcer. *JAMA.* 1936; **106**: 458-60.

310. Dempsey DT, Mullen JL, Buzby GP. The link between nutritional status and clinical outcome: can nutritional intervention modify it? *Am J Clin Nutr.* 1988; **47**: 352-6.

311. Malone DL, Genuit T, Tracy JK, Gannon C, Napolitano LM. Surgical site infections: reanalysis of risk factors. *J Surg Res.* 2002; **103**: 89-95.

312. Jose DG, Good RA. Absence of enhancing antibody in cell mediated immunity to tumour heterografts in protein deficient rats. *Nature.* 1971; **231**: 323-5.

313. Chandra RK, Kumari S. Nutrition and immunity: an overview. *J Nutr.* 1994; **124**: 1433S-5S.

314. Jose DG, Cooper WC, Good RA. How protein deficiency enhances cellular immunity. *JAMA.* 1971; **218**: 1428-9.

315. Kramer TR, Good RA. Increased in vitro cell-mediated immunity in protein-malnourished guinea pigs. *Clin Immunol Immunopathol.* 1978; **11**: 212-28.

316. Good RA, Lorenz E. Nutrition and cellular immunity. *Int J Immunopharmacol.* 1992; **14**: 361-6.

317. Hatada T, Miki C. Nutritional status and postoperative cytokine response in colorectal cancer patients. *Cytokine.* 2000; **12**: 1331-6.

318. Miki C, Iriyama K, Mayer AD, *et al.* Energy storage and cytokine response in patients undergoing liver transplantation. *Cytokine.* 1999; **11**: 244-8.

319. O'Flaherty L, Bouchier-Hayes DJ. Immunonutrition and surgical practice. *Proc Nutr Soc.* 1999; **58**: 831-7.

320. Senkal M, Kemen M, Homann HH, Eickhoff U, Baier J, Zumtobel V. Modulation of postoperative immune response by enteral nutrition with a diet enriched with arginine, RNA, and omega-3 fatty acids in patients with upper gastrointestinal cancer. *Eur J Surg.* 1995; **161**: 115-22.

321. Weimann A, Bastian L, Bischoff WE, *et al.* Influence of arginine, omega-3 fatty acids and nucleotide-supplemented enteral support on systemic inflammatory response syndrome and multiple organ failure in patients after severe trauma. *Nutrition.* 1998; **14**: 165-72.

322. Weissman C. The metabolic response to stress: an overview and update. *Anesthesiology.* 1990; **73**: 308-27.

323. Breslow MJ, Parker SD, Frank SM, *et al.* Determinants of catecholamine and cortisol responses to lower extremity revascularization. The PIRAT Study Group. *Anesthesiology.* 1993; **79**: 1202-9.

324. Hall GM. The anaesthetic modification of the endocrine and metabolic response to surgery. *Ann R Coll Surg Engl.* 1985; **67**: 25-9.

325. Chambrier C, Chassard D, Bienvenu J, *et al.* Cytokine and hormonal changes after cholecystectomy. Effect of ibuprofen pretreatment. *Ann Surg.* 1996; **224**: 178-82.

326. Kiecolt-Glaser JK, Page GG, Marucha PT, MacCallum RC, Glaser R. Psychological influences on surgical recovery. Perspectives from psychoneuroimmunology. *Am Psychol.* 1998; **53**: 1209-18.

327. Manyande A, Chayen S, Priyakumar P, *et al.* Anxiety and endocrine responses to surgery: paradoxical effects of preoperative relaxation training. *Psychosom Med.* 1992; **54**: 275-87.

328. Jessop DS. Stimulatory and inhibitory regulators of the hypothalamo-pituitary-adrenocortical axis. *Baillieres Best Pract Res Clin Endocrinol Metab.* 1999; **13**: 491-501.

329. Sternberg EM. Neuroendocrine regulation of autoimmune/inflammatory disease. *J Endocrinol.* 2001; **169**: 429-35.

330. Mulla A, Buckingham JC. Regulation of the hypothalamo-pituitary-adrenal axis by cytokines. *Baillieres Best Pract Res Clin Endocrinol Metab.* 1999; **13**: 503-21.

331. Naito Y, Tamai S, Shingu K, *et al.* Responses of plasma adrenocorticotropic hormone, cortisol, and cytokines during and after upper abdominal surgery. *Anesthesiology.* 1992; **77**: 426-31.

332. Udelsman R, Holbrook NJ. Endocrine and molecular responses to surgical stress. *Curr Probl Surg.* 1994; **31**: 653-720.

333. Deitch EA, Bridges RM. Stress hormones modulate neutrophil and lymphocyte activity in vitro. *J Trauma.* 1987; **27**: 1146-54.

334. Elenkov IJ, Chrousos GP. Stress, cytokine patterns and susceptibility to disease. *Baillieres Best Pract Res Clin Endocrinol Metab.* 1999; **13**: 583-95.

335. Parker SD, Breslow MJ, Frank SM, *et al.* Catecholamine and cortisol responses to lower extremity revascularization: correlation with outcome variables. Perioperative Ischemia Randomized Anesthesia Trial Study Group. *Crit Care Med.* 1995; **23**: 1954-61.

336. Kraaij V, de Wilde EJ. Negative life events and depressive symptoms in the elderly: a life span perspective. *Aging Ment Health.* 2001; **5**: 84-91.

337. Cheok F, Schrader G, Banham D, Marker J, Hordacre AL. Identification, course, and treatment of depression after admission for a cardiac condition: rationale and patient characteristics for the Identifying Depression As a Comorbid Condition (IDACC) project. *Am Heart J.* 2003; **146**: 978-84.

338. Baker RA, Andrew MJ, Schrader G, Knight JL. Preoperative depression and mortality in coronary artery bypass surgery: preliminary findings. *ANZ J Surg.* 2001; **71**: 139-42.

339. Burg MM, Benedetto MC, Rosenberg R, Soufer R. Presurgical depression predicts medical morbidity 6 months after coronary artery bypass graft surgery. *Psychosom Med.* 2003; **65**: 111-8.

340. Langeluddecke P, Fulcher G, Baird D, Hughes C, Tennant C. A prospective evaluation of the psychosocial effects of coronary artery bypass surgery. *J Psychosom Res.* 1989; **33**: 37-45.

341. McKhann GM, Borowicz LM, Goldsborough MA, Enger C, Selnes OA. Depression and cognitive decline after coronary artery bypass grafting. *Lancet.* 1997; **349**: 1282-4.

342. Barefoot JC, Helms MJ, Mark DB, *et al.* Depression and long-term mortality risk in patients with coronary artery disease. *Am J Cardiol.* 1996; **78**: 613-7.

343. Frasure-Smith N. In-hospital symptoms of psychological stress as predictors of long-term outcome after acute myocardial infarction in men. *Am J Cardiol.* 1991; **67**: 121-7.

344. Frasure-Smith N, Lesperance F, Talajic M. Depression following myocardial infarction. Impact on 6-month survival. *Jama.* 1993; **270**: 1819-25.

345. Ford DE, Mead LA, Chang PP, Cooper-Patrick L, Wang NY, Klag MJ. Depression is a risk factor for coronary artery disease in men: the precursors study. *Arch Intern Med.* 1998; **158**: 1422-6.

346. Coons SJ, Rao S, Keininger DL, Hays RD. A comparative review of generic quality-of-life instruments. *Pharmacoeconomics.* 2000; **17**: 13-35.

347. Klevsgard R, Risberg BO, Thomsen MB, Hallberg IR. A 1-year follow-up quality of life study after hemodynamically successful or unsuccessful surgical revascularization of lower limb ischemia. *J Vasc Surg.* 2001; **33**: 114-22.

348. Tretinyak AS, Lee ES, Kuskowski MM, Caldwell MP, Santilli SM. Revascularization and quality of life for patients with limb-threatening ischemia. *Ann Vasc Surg.* 2001; **15**: 84-8.

349. Wilson IB, Cleary PD. Linking clinical variables with health-related quality of life. A conceptual model of patient outcomes. *JAMA.* 1995; **273**: 59-65.

350. Goyal TM, Idler EL, Krause TJ, Contrada RJ. Quality of life following cardiac surgery: impact of the severity and course of depressive symptoms. *Psychosom Med.* 2005; **67**: 759-65.

351. Mayou RA, Gill D, Thompson DR, *et al.* Depression and anxiety as predictors of outcome after myocardial infarction. *Psychosom Med.* 2000; **62**: 212-9.

352. Perski A, Feleke E, Anderson G, *et al.* Emotional distress before coronary bypass grafting limits the benefits of surgery. *Am Heart J.* 1998; **136**: 510-7.

353. Leonard B. Stress, depression and the activation of the immune system. *World J Biol Psychiatry.* 2000; **1**: 17-25.

354. Connor TJ, Leonard BE. Depression, stress and immunological activation: the role of cytokines in depressive disorders. *Life Sci.* 1998; **62**: 583-606.

355. Kronfol Z. Immune dysregulation in major depression: a critical review of existing evidence. *Int J Neuropsychopharmacol.* 2002; **5**: 333-43.

356. Anisman H, Ravindran AV, Griffiths J, Merali Z. Endocrine and cytokine correlates of major depression and dysthymia with typical or atypical features. *Mol Psychiatry.* 1999; **4**: 182-8.

357. van West D, Maes M. Activation of the inflammatory response system: A new look at the etiopathogenesis of major depression. *Neuroendocrinol Lett.* 1999; **20**: 11-7.

358. Spielberger CD, Gorsuch RJ, Lushene R, Vagg PR, Jacobs GA. *The Stait-Trait Anxiety Inventory for Adults: Sampler Set Manual, Test, Scoring Key.* Palo Alto, CA: Consulting Psychologists Press; 1983.

359. Salmon P, Pearce S, Smith CC, *et al.* Anxiety, type A personality and endocrine responses to surgery. *Br J Clin Psychol.* 1989; **28 (Pt 3)**: 279-80.

360. Salmon P. Surgery as a psychological stressor: Paradoxical effects of preoperative emotional state on endocrine responses. In: 3rd ISIS Conference; 1991 1992; Padova, Italy: John Wiley & Sons; 1991. p. 193-8.

361. Volodos NL, Shekhanin VE, Karpovich IP, Troian VI, Gur'ev Iu A. [A self-fixing synthetic blood vessel endoprosthesis]. *Vestn Khir Im I I Grek.* 1986; **137**: 123-5.

362. Parodi JC, Palmaz JC, Barone HD. Transfemoral intraluminal graft implantation for abdominal aortic aneurysms. *Ann Vasc Surg.* 1991; **5**: 491-9.

363. Greenhalgh RM, Brown LC, Kwong GP, Powell JT, Thompson SG. Comparison of endovascular aneurysm repair with open repair in patients with abdominal aortic aneurysm (EVAR trial 1), 30-day operative mortality results: randomised controlled trial. *Lancet.* 2004; **364**: 843-8.

364. Gorham TJ, Taylor J, Raptis S. Endovascular treatment of abdominal aortic aneurysm. *Br J Surg.* 2004; **91**: 815-27.

365. Maher MM, McNamara AM, MacEneaney PM, Sheehan SJ, Malone DE. Abdominal aortic aneurysms: elective endovascular repair versus conventional surgery-evaluation with evidence-based medicine techniques. *Radiology.* 2003; **228**: 647-58.

366. Boyle JR, Goodall S, Thompson JP, Bell PR, Thompson MM. Endovascular AAA repair attenuates the inflammatory and renal responses associated with conventional surgery. *J Endovasc Ther.* 2000; **7**: 359-71.

367. Elmarasy NM, Soong CV, Walker SR, *et al.* Sigmoid ischemia and the inflammatory response following endovascular abdominal aortic aneurysm repair. *J Endovasc Ther.* 2000; **7**: 21-30.

368. Rowlands TE, Homer-Vanniasinkam S. Pro- and anti-inflammatory cytokine release in open versus endovascular repair of abdominal aortic aneurysm. *Br J Surg.* 2001; **88**: 1335-40.

369. Odegard A, Lundbom J, Myhre HO, *et al.* The inflammatory response following treatment of abdominal aortic aneurysms: a comparison between open surgery and endovascular repair. *Eur J Vasc Endovasc Surg.* 2000; **19**: 536-44.

370. Swartbol P, Norgren L, Albrechtsson U, *et al.* Biological responses differ considerably between endovascular and conventional aortic aneurysm surgery. *Eur J Vasc Endovasc Surg.* 1996; **12**: 18-25.

371. Bolke E, Jehle PM, Storck M, *et al.* Endovascular stent-graft placement versus conventional open surgery in infrarenal aortic aneurysm: a prospective study on acute phase response and clinical outcome. *Clin Chim Acta.* 2001; **314**: 203-7.

372. Morikage N, Esato K, Zenpo N, Fujioka K, Takenaka H. Is endovascular treatment of abdominal aortic aneurysms less invasive regarding the biological responses? *Surg Today.* 2000; **30**: 142-6.

373. Pearson S, Hassen T, Spark JI, Cabot J, Cowled P, Fitridge R. Endovascular repair of abdominal aortic aneurysm reduces intraoperative cortisol and perioperative morbidity. *J Vasc Surg.* 2005; **41**: 919-25.

374. Thompson JP, Boyle JR, Thompson MM, Strupish J, Bell PR, Smith G. Cardiovascular and catecholamine responses during endovascular and conventional abdominal aortic aneurysm repair. *Eur J Vasc Endovasc Surg.* 1999; **17**: 326-33.

375. Salartash K, Sternbergh WC, 3rd, York JW, Money SR. Comparison of open transabdominal AAA repair with endovascular AAA repair in reduction of postoperative stress response. *Ann Vasc Surg.* 2001; **15**: 53-9.

376. American Society of Anesthesiologists. New classification of physical status. *Anesthesiology.* 1963; **24**: 111.

377. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis.* 1987; **40**: 373-83.

378. de Groot V, Beckerman H, Lankhorst GJ, Bouter LM. How to measure comorbidity. a critical review of available methods. *J Clin Epidemiol.* 2003; **56**: 221-9.

379. Johnston KW, Rutherford RB, Tilson MD, Shah DM, Hollier L, Stanley JC. Suggested standards for reporting on arterial aneurysms. Subcommittee on Reporting Standards for Arterial Aneurysms, Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery and North American Chapter, International Society for Cardiovascular Surgery. *J Vasc Surg.* 1991; **13**: 452-8.

380. Serotec Ltd. *Serotec Worksheet 1: Direct Immunofluoresence Staining for Flow Cytometry*. [PDF on Internet]. [Updated 2002]. Available from: http://serotec.oxi.net/html/ws01.htm

381. Rodriguez JA, Bodman-Smith KB, Raynes JG. Neutrophil responses to CRP are not dependent on polymorphism of human FcgammaRIIA (R131H). *Clin Exp Immunol.* 2004; **138**: 271-7.

382. Bux J, Stein EL, Santoso S, Mueller-Eckhardt C. NA gene frequencies in the German population, determined by polymerase chain reaction with sequence-specific primers. *Transfusion.* 1995; **35**: 54-7.

383. Bayer HealthCare LLC. ACS:180: Cortisol (COR) + E product insert. New York: Bayer Corporation; 2000.

384. Smeets HJ, Kievit J, Dulfer FT, van Kleef JW. Endocrine-metabolic response to abdominal aortic surgery: a randomized trial of general anesthesia versus general plus epidural anesthesia. *World J Surg.* 1993; **17**: 601-6; discussion 6-7.

385. Traynor C, Paterson JL, Ward ID, Morgan M, Hall GM. Effects of extradural analgesia and vagal blockade on the metabolic and endocrine response to upper abdominal surgery. *Br J Anaesth.* 1982; **54**: 319-23.

386. Miller J, Crapo L. The biochemical diagnosis of hypercortisolism. *Endocrinologist.* 1994; **4**: 7-16.

387. Omran ML, Morley JE. Assessment of protein energy malnutrition in older persons, part I: History, examination, body composition, and screening tools. *Nutrition.* 2000; **16**: 50-63.

388. Vellas B, Guigoz Y, Garry PJ, *et al.* The Mini Nutritional Assessment (MNA) and its use in grading the nutritional state of elderly patients. *Nutrition.* 1999; **15**: 116-22.

389. Guigoz Y, Vellas B, Garry PJ. Assessing the nutritional status of the elderly: The Mini Nutritional Assessment as part of the geriatric evaluation. *Nutr Rev.* 1996; **54**: S59-65.

390. Guigoz Y, Vellas B, Garry PJ. Mini Nutritional Assessment: a practical assessment tool for grading the nutritional status of elderly patients. *Facts Res Gerontol.* 1994; **4 (suppl 2)**: 15-59.

391. Gazzotti C, Pepinster A, Petermans J, Albert A. Interobserver agreement on MNA nutritional scale of hospitalized elderly patients. *J Nutr Health Aging.* 1997; **1**: 23.

392. Heymsfield SB, Nunez C, Testolin C, Gallagher D. Anthropometry and methods of body composition measurement for research and field application in the elderly. *Eur J Clin Nutr.* 2000; **54 Suppl 3**: S26-32.

393. Jebb SA, Elia M. Techniques for the measurement of body composition: a practical guide. *Int J Obes Relat Metab Disord.* 1993; **17**: 611-21.

394. Ellis KJ. Human body composition: in vivo methods. *Physiol Rev.* 2000; **80**: 649-80.

395. Heymsfield SB, Waki M, Kehayias J, *et al.* Chemical and elemental analysis of humans in vivo using improved body composition models. *Am J Physiol.* 1991; **261**: E190-8.

396. Barac-Nieto M, Spurr GB, Lotero H, Maksud MG. Body composition in chronic undernutrition. *Am J Clin Nutr.* 1978; **31**: 23-40.

397. Hansen RD, Raja C, Aslani A, Smith RC, Allen BJ. Determination of skeletal muscle and fat-free mass by nuclear and dual-energy x-ray absorptiometry methods in men and women aged 51-84 y [(1-3)]. *Am J Clin Nutr.* 1999; **70**: 228-33.

398. VanItallie TB, Yang MU, Heymsfield SB, Funk RC, Boileau RA. Height-normalized indices of the body's fat-free mass and fat mass: potentially useful indicators of nutritional status. *Am J Clin Nutr.* 1990; **52**: 953-9.

399. Proctor DN, O'Brien PC, Atkinson EJ, Nair KS. Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. *Am J Physiol.* 1999; **277**: E489-95.

400. Heymsfield SB, McManus C, Stevens V, Smith J. Muscle mass: reliable indicator of protein-energy malnutrition severity and outcome. *Am J Clin Nutr.* 1982; **35**: 1192-9.

401. Heymsfield SB, Smith R, Aulet M, *et al.* Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry. *Am J Clin Nutr.* 1990; **52**: 214-8.

402. Beck AT, Steer RA, Brown GK. *Beck Depression Inventory-Second Edition Manual.* San Antonio, TX: The Psychological Corporation; 1996.

403. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. *Appl Psychol Meas.* 1977; **1**: 385-401.

404. Smarr KL. Measures of Depression and Depressive Symptoms. *Arthritis Rheum.* 2003; **49**: S134-S46.

405. Zich JM, Attkisson CC, Greenfield TK. Screening for depression in primary care clinics: the CES-D and the BDI. *Int J Psychiatry Med.* 1990; **20**: 259-77.

406. Penninx BW, Beekman AT, Honig A, *et al.* Depression and cardiac mortality: results from a community-based longitudinal study. *Arch Gen Psychiatry.* 2001; **58**: 221-7.

407. Beekman AT, Deeg DJ, Van Limbeek J, Braam AW, De Vries MZ, Van Tilburg W. Criterion validity of the Center for Epidemiologic Studies Depression scale (CES-D): results from a community-based sample of older subjects in The Netherlands. *Psychol Med.* 1997; **27**: 231-5.

408. Ray JJ. Measuring trait anxiety in general population samples. *J Soc Psychol.* 1984; **123**: 189-93.

409. Shindo S, Ogata K, Kubota K, *et al.* Vascular prosthetic implantation is associated with prolonged inflammation following aortic aneurysm surgery. *J Artif Organs.* 2003; **6**: 173-8.

410. Talmor M, Hydo L, Barie PS. Relationship of systemic inflammatory response syndrome to organ dysfunction, length of stay, and mortality in critical surgical illness: effect of intensive care unit resuscitation. *Arch Surg.* 1999; **134**: 81-7.

411. Chaikof EL, Blankensteijn JD, Harris PL, *et al.* Reporting standards for endovascular aortic aneurysm repair. *J Vasc Surg.* 2002; **35**: 1048-60.

412. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med.* 1985; **13**: 818-29.

413. Ware JE, Snow KK, Kosinski M, Gandek B. *SF-36 Health Survey manual and interpretation guide*. Boston, MA: The Health Institute, New England Medical Center; 1993.

414. Prinssen M, Buskens E, Blankensteijn JD. Quality of life endovascular and open AAA repair. Results of a randomised trial. *Eur J Vasc Endovasc Surg.* 2004; **27**: 121-7.

415. Hernandez-Osma E, Cairols MA, Marti X, Barjau E, Riera S. Impact of treatment on the quality of life in patients with critical limb ischaemia. *Eur J Vasc Endovasc Surg.* 2002; **23**: 491-4.

416. Ballard JL, Abou-Zamzam AM, Teruya TH, Bianchi C, Petersen FF. Quality of life before and after endovascular and retroperitoneal abdominal aortic aneurysm repair. *J Vasc Surg.* 2004; **39**: 797-803.

417. Hallin A, Bergqvist D, Fugl-Meyer K, Holmberg L. Areas of concern, quality of life and life satisfaction in patients with peripheral vascular disease. *Eur J Vasc Endovasc Surg.* 2002; **24**: 255-63.

418. Hays RD, Prince-Embury S, Chen H. *RAND-36 Health Status Inventory: manual.* San Antonio, TX: The Psychological Corporation; 1998.

419. Hays RD, Sherbourne CD, Mazel RM. The RAND 36-Item Health Survey 1.0. *Health Econ.* 1993; **2**: 217-27.

420. Ware JE, Jr., Kosinski M, Bayliss MS, McHorney CA, Rogers WH, Raczek A. Comparison of methods for the scoring and statistical analysis of SF-36 health profile and summary measures: summary of results from the Medical Outcomes Study. *Med Care.* 1995; **33**: AS264-79.

421. Ware JE, Kosinski M. Interpreting SF-36 summary health measures: a response. *Qual Life Res.* 2001; **10**: 405-13; discussion 15-20.

422. Dawson-Saunders B, Trapp RG. *Basic & Clinical Biostatistics*, 2nd ed. Connecticut: Appleton & Lange; 1994.

423. Hadden JW. Immunodeficiency and cancer: prospects for correction. *Int Immunopharmacol.* 2003; **3**: 1061-71.

424. Opal SM. The uncertain value of the definition for SIRS. Systemic inflammatory response syndrome. *Chest.* 1998; **113**: 1442-3.

425. Syk I, Brunkwall J, Ivancev K, *et al.* Postoperative fever, bowel ischaemia and cytokine response to abdominal aortic aneurysm repair-a comparison between endovascular and open surgery. *Eur J Vasc Endovasc Surg.* 1998; **15**: 398-405.

426. Jedynak M, Siemiatkowski A, Gacko M, Mroczko B, Borkowski J. Serum concentrations of MCP-1 and RANTES in patients during aortic surgery: the relationship with ischemia-reperfusion. *Arch Immunol Ther Exp (Warsz).* 2004; **52**: 201-7.

427. Rosenbloom AJ, Pinsky MR, Bryant JL, Shin A, Tran T, Whiteside T. Leukocyte activation in the peripheral blood of patients with cirrhosis of the liver and SIRS. Correlation with serum interleukin-6 levels and organ dysfunction. *JAMA.* 1995; **274**: 58-65.

428. van der Pol WL, Jansen MD, Sluiter WJ, *et al.* Evidence for non-random distribution of Fcγ receptor genotype combinations. *Immunogenetics.* 2003; **55**: 240-6.

429. van Sorge NM, van der Pol WL, Jansen MD, *et al.* Severity of Guillain-Barre syndrome is associated with Fcγ Receptor III polymorphisms. *J Neuroimmunol.* 2005; **162**: 157-64.

430. Carcao MD, Blanchette VS, Wakefield CD, *et al.* Fcγ receptor IIa and IIIa polymorphisms in childhood immune thrombocytopenic purpura. *Br J Haematol.* 2003; **120**: 135-41.

431. van Schie RC, Wilson ME. Evaluation of human FcγRIIA (CD32) and FcγRIIIB (CD16) polymorphisms in Caucasians and African-Americans using salivary DNA. *Clin Diagn Lab Immunol.* 2000; **7**: 676-81.

432. Fromont P, Bettaieb A, Skouri H, *et al.* Frequency of the polymorphonuclear neutrophil Fc gamma receptor III deficiency in the French population and its involvement in the development of neonatal alloimmune neutropenia. *Blood.* 1992; **79**: 2131-4.

433. Torio A, Marin L, Muro M, Alvarez-Lopez MR, Garcia-Alonso AM. Determination of NA gene frequencies in the Spanish population by polymerase chain reaction with sequence-specific primers. *Eur J Immunogenet.* 1998; **25**: 393-4.

434. Hessner MJ, Curtis BR, Endean DJ, Aster RH. Determination of neutrophil antigen gene frequencies in five ethnic groups by polymerase chain reaction with sequence-specific primers. *Transfusion.* 1996; **36**: 895-9.

435. Hakanson E, Rutberg H, Jorfeldt L, Wiklund L. Endocrine and metabolic responses after standardized moderate surgical trauma: influence of age and sex. *Clin Physiol.* 1984; **4**: 461-73.

436. Chernow B, Alexander HR, Smallridge RC, *et al.* Hormonal responses to graded surgical stress. *Arch Intern Med.* 1987; **147**: 1273-8.

437. Nistrup-Madsen S, Fog-Moller F, Christiansen C, Vester-Andersen T, Engquist A. Cyclic AMP, adrenaline and noradrenaline in plasma during surgery. *Br J Surg.* 1978; **65**: 191-3.

438. Hughes JW, Watkins L, Blumenthal JA, Kuhn C, Sherwood A. Depression and anxiety symptoms are related to increased 24-hour urinary norepinephrine excretion among healthy middle-aged women. *J Psychosom Res.* 2004; **57**: 353-8.

439. Salmon P, Pearce S, Smith CC, *et al.* The relationship of preoperative distress to endocrine and subjective responses to surgery: support for Janis' theory. *J Behav Med.* 1988; **11**: 599-613.

440. Butcher SK, Chahal H, Nayak L, *et al.* Senescence in innate immune responses: reduced neutrophil phagocytic capacity and CD16 expression in elderly humans. *J Leukoc Biol.* 2001; **70**: 881-6.

441. Rink L, Cakman I, Kirchner H. Altered cytokine production in the elderly. *Mech Ageing Dev.* 1998; **102**: 199-209.

442. Bauer ME. Stress, glucocorticoids and ageing of the immune system. *Stress.* 2005; **8**: 69-83.

443. Boscaro M, Paoletta A, Scarpa E, *et al.* Age-related changes in glucocorticoid fast feedback inhibition of adrenocorticotropin in man. *J Clin Endocrinol Metab.* 1998; **83**: 1380-3.

444. Norman JG, Fink GW. The effects of epidural anesthesia on the neuroendocrine response to major surgical stress: a randomized prospective trial. *Am Surg.* 1997; **63**: 75-80.

AMENDMENTS

CHAPTER 1: INTRODUCTION, LITERATURE REVIEW AND AIMS OF CURRENT STUDY

1.2.1 Pathophysiology of SIRS

Page 13, line 19

Erratum: Bone *et al.*

Should read: Bone

Bone, R.C. is the sole author of the article (reference 69) to which reference is made.

CHAPTER 4: DISCUSSION

4.6 Immunological Parameters

Addendum

As described in Chapter 2, Section 2.4, the pre-operative peripheral venous blood sample drawn from all subjects for purposes including quantitation of pre-operative PMN integrin and immunoglobulin receptor expression and plasma cytokine assays was obtained within seven days, and in most cases within 24 hours, of their proposed surgery. Whilst the application of a more strictly standardised protocol governing the timing of pre-operative blood sampling is likely to have further minimised the introduction of potential confounding effects, thereby increasing the reliability of findings relating to pre-operative immunological data, this was prevented by constraints imposed by availability and access to subjects in the current study. As discussed in Chapter 4, Section 4.6.1, the issue of variability in pre-operative sampling times is of

greatest relevance to the observed relationship between pre-operative CD11b expression and post-operative SIRS severity.

Whilst variability in sites of blood sampling at the intra- and post-operative time points occurred in the current study as a consequence of available vascular access, as described in detail in Chapter 2, Section 2.4, published evidence argues against any significant influence of this variability on the measured immunological parameters. In their study of CD16b expression amongst subjects undergoing elective open AAA repair, Spark *et al.*[185] demonstrated no significant difference in levels of expression of this PMN immunoglobulin receptor between paired arterial and venous blood samples drawn at pre-, intra- and post-operative time points. Similarly, the finding by Norwood *et al.* [286] that no significant differences in IL-6 levels existed between paired radial artery and femoral vein blood samples drawn at pre-, intra- and post-operative time points amongst a cohort of patients undergoing elective open AAA repair, tends to negate any suggestion that sampling site variability may have confounded findings relating to cytokine data in the current study.

As discussed in Chapter 2, Sections 2.5.1 and 2.5.2, the methodology employed for determination of PMN CD11b, CD64 and CD16b expression was established prior to its application in the current study and included the demonstration of intra- and inter-assay reproducibility.

It is similarly contended that the method employed for plasma cytokine assays in the current study, using the BD® Cytometric Bead Array (CBA) Human Inflammation Kit

(BD Biosciences, San Diego, California, USA) in accordance with the manufacturer's instructions, as reported in Chapter 2, Section 2.7, was characterised by a high degree of precision with data demonstrating intra- and inter-assay reproducibility provided by the manufacturer. Evidence of inter-assay reproducibility, in particular, suggests that whilst the use of several kits was necessitated by the fact that the number of samples to be assayed exceeded that which can be performed using a single kit, this is unlikely to have been associated with significant data variability.

4.8.3.3 Measures of General Post-operative Morbidity

Page 229, line 12

Erratum: were experience

Should read: were experienced

4.9.4 Depression and Post-operative SIRS and Sepsis

Page 235, line 2

Erratum: depression my influence

Should read: depression may influence

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

5.6.1 The Immuno-inflammatory Response

Page 255, line 2

Erratum: inflammatory events that EVAR

Should read: inflammatory events than EVAR