INFLAMMATORY MARKER COMPARISON BETWEEN PATIENTS WITH ACUTE CORONARY SYNDROME UNDERGOING ON-PUMP VERSUS OFF-PUMP CORONARY ARTERY BYPASS GRAFT SURGERY

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DECLARATION OF INDEPENDANT WORK

I, Helena D Potgieter, do hereby declare that this research project submitted to the Central University of Technology, Free State for the degree MAGISTER TECHNOLOGIAE BIOMEDICAL TECHNOLOGY is my own independent work that has not been submitted to any institution by me or any other person in fulfilment of the requirements for the attainment of any qualification.

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SIGNATURE OF STUDENT

……..

DATE

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"The whole pupose of education is to turn mirrors into windows." ≈ S. Harris

ABBREVIATIONS, ACRONYMS & SYMBOLS

ABBREVIATION	TERMINOLOGY
%	Percentage
&	And
°C	Degree celsius
ACS	Acute coronary syndrome
AKS	Akute koronêre sindroom
AMI	Acute myocardial infarction
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CAM-1	Cell adhesion molecule-1
CAMs	Cell adhesion molecules
cCAMs	Circulating CAMs
CHD	Coronary heart disease
Cm	Centimetre
CABG	Coronary artery bypass graft
CRP	C-reactive protein
CUT	Central University of Technology, Free State
CV	Correlation coefficient
e.g.	For example
EASIA	Enzyme amplified sensitivity immunoassay
EC	Extracorporeal circulation
et al.	And others
etc.	Etcetera
FBC	Full blood count
GCP	Good clinical practice
GI	Gastrointestinal
HDL	High-density lipoprotein
HMWK	High-molecular-weight kininogen
ICAM-1	Intracellular adhesion molecule-1
ICU	Intensive care unit
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-13	Interleukin-13
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IκB protein	Inhibitory κB protein
iNOS	Inducible nitric oxide synthases
kDa	kDalton
LDL	Low-Density lipoprotein
Ln	Normal logarithm
MCP-1	Monocyte chemo attractant protein-1
mg/l	Milligrams per litre

ABBREVIATION	TERMINOLOGY
MI	Myocardial infarction
Min	Minutes
mmHg	Millimetre mercury
mmol/l	Milimoles per litre
MMPs	Matrix metalloproteinases
MODS	Multi-organ dysfunction syndrome
N/L ratio	Neutrophil/lymphocyte ratio
ng/l	Nanograms per litre
NHLS	National Health Laboratory Services
Nm	Nanometer
NO	Nitric oxide
NSTEMI	Non-ST-Elevation myocardial infarction
NF-kB	Nuclear factor κappa Beta
On-pump CABG	On-pump coronary artery bypass graft
Off-pump CABG	Off-pump coronary artery bypass graft
oxLDL	Oxidized low-density lipoprotein
***p*-value**	Probability
PAF	Platelet-activating factor
PCT	Procalcitonin
pg/ml	Picogram per millilitres
Rpm	Revolution per minutes
ROS	Reactive oxygen species
S	Seconds
SIRS	Systemic inflammatory response syndrome
SMC	Smooth muscle cell
STEMI	ST-Segment elevation myocardial infarction
TF	Tissue factor
TMB	Tetramethylbenzydine
TNF-α	Tumor necrosis factor-alpha
TRACE technology	Time-resolved amplified cryptate emission technology
UA	Unstable angina
UFS	University Free State
VCAM-1	Vascular Cell Adhesion Molecule-1
WCC	White Cell Count
yyyy/mm/dd	Year/Month/Day
B	Beta
µl	Micro litres

IMPORTANT DEFINITIONS

TERM	DEFINITION
Acute coronary syndrome	May be defined as the underlining process of platelet-rich thrombus formation on the surface of a ruptured or an eroded plaque formation within the coronary artery (Undas, Szułdrzynski, Stepien, Zalewski, Godlewski, Tracz, Pasowicz, Zmudka, 2008).
Atherosclerosis	Diffuse systemic process that affects the function of coronaries as well as peripheral vessels (Thanyasiri, Celermajer, Adams, 2005).
Body mass index (BMI)	This index reflects a person's weight in terms of his/her height. This is defined as the weight in kilogram divided by height in meters squared (kg/m^2).
Beta values/logistic regression	It is a type of predictive model that can be used when the target variable is a categorical variable with two categories - for example live/die. It does not involve decision trees and is more akin to nonlinear regression such as fitting a polynomial to a set of data values (Weisstein, 1999 - 2010)
Coronary artery disease (CAD)	Caused by atherosclerosis or atherosclerotic plaque growth (Apple, 2001).
C-reactive protein (CRP)	Acute phase protein that has been identified in the past as a useful inflammatory marker as well as a predictor of mortality (Gortney & Sanders, 2007).
Endothelium	Single-cell lining covering the internal surface of blood vessels, cardiac valves, and numerous body cavities (Schächinger, Britten, Elsner, Walter, Scharrer, Zeiher, 1999; Galley & Webster, 2004).
Full blood count (FBC)	The standard FBC measures hematocrit, haemoglobin, leukocyte count, Neutrophil/lymphocyte (N/L) ratio, White cell count (WCC), and platelet count (Anderson, Ronnow, Horne, Carlquist, May, Tami, Bair, Jensen, Muhlestein, 2007; Gibson, Croal, Cuthbertson, Small, Ifezulike, Gibson, Jeffrey, Buchan, El-Shafei, Hillis, 2007).
Gamma distribution	The gamma distribution is a two-parameter family of continuous probability distributions and is related to the beta distribution and arises naturally in processes for which the waiting times between Poisson distributed events are relevant. It has two free parameters labelled and α (The challenge of developing statistical literacy, reasoning and thinking (Weisstein, 1999 - 2010).
Generalised extreme value (GEV) distribution	The GEV distribution is one of the probability distributions used to model extreme events (Coles, 2001).
Human Interleukin-6 (IL-6)	A 184 A.A. prototypic pleiotrophic cytokine that belongs to a family of 20 kDa polypeptide cytokines having a four-long-chain α-helix bundle structure, with potential O and N-glycosylation sites, and a significant homology with G-CSF (BioSource, 1998).
Lognormal distribution	A continous distribution in which the logarithm of a variable has a normal distribution (Weisstein, 1999 - 2010).
Non-st-segment-elevation myocardial infarction (NSTEMI)	Also known as a "heart attack". The patient presents with chest pain, dyspnoea, jaw or arm pain, as well as weakness (Pollack & Braunwald, 2008).
Normal distribution	The normal distribution or Gaussian distribution is a continuous probability distribution that often gives a good description of data that cluster around the mean. The graph of the associated probability density function is bell-shaped with a peak at the mean, and is known as the Gaussian function or bell-curve (Weisstein, 1999 - 2010)
Morbidity	Morbidity refers to people who have a disease or condition but have not yet died of this.
Obese	A person is obese when his/her BMI is 30 kg/m^2 or higher.
Overweight	A person is overweight when his/her BMI is between 25 kg/m^2 and below 30 kg/m^2.
On-pump coronary artery bypass graft	A stable operative site and a bloodless field while ensuring end-organ perfusion and cooling of the patient during cardio-respiratory arrest (Morganstern & Kanchuger, 2008).
Prevalence	Prevalence refers to the percentage of people in the population who have a condition at any point in time.

TERM	DEFINITION
Procalcitonin (PCT)	PCT is a 116 amino acid protein (Meisner, Tschaikowsky, Schmidt, Schuttler, 1996), produced in the C-cells of the thyroid gland, and acts as the precursor of calcitonin.
ST-segment-elevation myocardial infarction (STEMI)	The "ST-elevation" in ST-segment-elevation myocardial infarction (STEMI) refers to a certain pattern or section of the ECG also known as the Q-wave (Mathis, Meswani, Spinler, 2001).
Student's t-test distribution	Is a continuous probability distribution that arises in the problem of estimating the mean of a normally distributed population when the sample size is small (Weisstein, 1999 - 2010).
Systemic inflammatory response syndrome (SIRS)	The body's response to an infectious or non-infectious insult. This ''inflammatory'' response consists of pro- and anti-inflammatory components (Larmann & Theilmeier, 2004).
Tumor necrosis factor-alpha (TNF-α)	TNF-α is a polypeptide pro-inflammatory cytokine produced mainly by endothelial, smooth muscle cells, macrophages, monocytes and adipose cells. It is also produced by B-cells and T-cells and by fibroblasts. It acts as a potent pyrogen, and activates neutrophils and vascular endothelial cells (Biglioli, Cannata, Alamanni, Naliato, Porqueddu, Zanobini, Tremoli, Parolari, 2003).
Unstable angina (UA)	A syndrome that is intermediate between stable angina and MI (Fogoros, 2006).
Zero-inflated Lognormal distribution	Zero-inflated distributions are used to model count data that have many zero counts. For example, the zero-inflated Poisson distribution might be used to model count data for which the proportion of zero counts is greater than expected on the basis of the mean of the non-zero counts (Ridout, Hinde, Demetrio, 2001)

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SUMMARY

The World Health Organization (WHO) has warned in 2005 that: "Coronary heart disease is now one of the leading causes of death worldwide. It is on the rise and has become a true pandemic that respects no borders" (WHO, 2005). The Heart and Stroke Foundation of South Africa more specifically estimates that approximately 33 (thirty-three) people per day will die of a heart attack in South Africa. Despite the already high death toll resulting from AIDS in South Africa, death from a chronic disease, also including heart disease, will increase from 565 deaths per day in the year 2000, to 666 deaths per day by 2010 (Steyn, 2007).

Acute coronary syndrome (ACS) is an 'umbrella term' describing a heterogeneous spectrum of clinical symptoms compatible with acute myocardial ischaemia (Monaco, Mathur, Martin, 2005; ACC/AHA, 2007) and an ongoing inflammatory process resulting from atherosclerosis. ACS can either be treated medically (pharmacological treatment), by percutaneous coronary intervention (PCI), or by performing coronary artery bypass graft (CABG) surgery either through on-pump or off-pump CABG surgery. By treating the ACS patient by means of CABG surgery, an inflammatory response is further triggered on top of the already existing inflammation resulting from atherosclerosis. This leads to a systemic inflammatory response (SIR), which may eventually lead to systemic inflammatory response syndrome (SIRS). This study focuses on the inflammatory response initiated by the CABG technique applied during the revascularisation of the ACS patient. Many past studies compared on-pump and off-pump CABG surgery, arguing not only the advantages and disadvantages of these surgeries, but also the outcomes regarding SIRS. Both types of surgery are associated with an inflammatory response resulting from tissue trauma and the use of the extracorporeal circulation (EC) in CABG surgery (Quaniers, Leruth, Albert, Limet, Defraigne, 2006).

This non-randomised, observational study primarily aimed to assess and compare the pre- and the post-operative inflammatory markers between (n=60) patients with ACS undergoing either on-pump CABG (n=30) or off-pump CABG surgery (n=30). A secondary objective was to ascertain whether a correlation exists between the pre-operative risk factors, the surgical procedure and the pre- and post-operative inflammatory markers. Three inflammatory markers - full blood count (FBC), procalcitonin (PCT) and C-reactive protein (CRP) - were analysed employing normal routine laboratory analysis. Interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) were analysed using an enzyme amplified sensitivity immunoassay (EASI) method. The inflammatory markers were

analysed pre-operatively (baseline) and post-operatively and at different time intervals (24, 48, 72, 96 and 120 hours post-operatively).

Pre-operatively, all the leucocytes were already elevated in both CABG groups, as could be expected in patients with ACS resulting from the already existing atherosclerotic process and the consequent pre-operative existing inflammatory response. A significant pre-operative difference was moreover detected in respect of the lymphocytes between the two CABG groups (p=0.03024). A significant post-operative difference was also detected between the two CABG groups. The following significantly elevated levels were detected in the on-pump CABG surgical group: for WCC at 24 hours (p=0.00761), 48 hours (p=0.01520) and 72 hours (p=0.00004); for neutrophils at 24 hours (p=0.17422), 96 hours (p=0.18611) and 120 hours (p=0.12872); for lymphocytes at 48 hours (p=0.04829) and at 96 hours (p=0.01982); and, for PCT at 24 hours (p=0.00811), 48 hours (p=0.00966) and 72 hours (p=0.01823) . However, measurable values of IL-6 levels were found to be higher in the off-pump CABG surgical group, with significant differences manifesting between the two CABG groups at 96 hours (p=0.05352) and 120 hours (p=0.09729). No differences between the two groups could be demonstrated for eosinophils, basophils, monocytes, CRP and TNF-α.

In conclusion: despite the demonstrable inflammatory responses in both CABG groups, no difference in clinical outcomes was observed. The inflammatory responses evoked by on-pump and off-pump CABG procedures will, for some time to come, remain an area of interest for future research, but they are certainly not the only factors to have a bearing on surgical outcomes. The impact of intra-operative events needs to be elucidated further - and in more detail - in order to attempt to determine the relationship of these events on the extent of inflammatory responses and clinical outcomes, irrespective of whether the procedure is performed with or without cardiopulmonary bypass.

OPSOMMING

Die Wêreldgesondheidsorganisasie (WGO) het reeds in 2005 gewaaarsku dat: "Koronêre hartsiekte een van die vernaamste oorsake van dood is ter wêreld. Dit is aan die toeneem en het pandemiese afmetings aangeneem wat alle grense oorskrei" (WHO, 2005). Volgens die Hart-en-beroertestigting van Suid-Afrika sal ongeveer 33 (drie-en-dertig) mense per dag in Suid-Afrika weens hartaanvalle sterf. Ongeag die reeds hoë mortaliteit as gevolg van VIGS in Suid-Afrika, sal die mortaliteit as gevolg van 'n chroniese siekte, soos byvoorbeeld hartsiektes, vanaf 565 sterftes per dag in 2000 tot 666 sterftes per dag teen 2010 toeneem (Steyn, 2007).

Akute koronêre sindroom (AKS) is 'n oorkoepelende term beskrywend van 'n heterogene spektrum kliniese simptome - geassosieër met akute miokardiale ischemie - (Monaco, *et al.*, 2005; ACC/AHA, 2007) en 'n voortdurende inflammatoriese proses vanweë aterosklerose. AKS kan óf medies behandel word (farmakologiese behandeling), óf deur middel van 'n stent intervensie, óf deur middel van 'n koronêre vatomleiding - met of sonder kardiopulmonêre omleiding chirurgie. Deur die AKS-pasiënt te onderwerp aan kardiopulmonêre omleiding chirurgie, word 'n inflammatoriese respons ontlok - bo en behalwe die reeds bestaande inflammatoriese proses vanweë aterosklerose. Dit gee aanleiding tot 'n sistemiese-inflammatoriese respons (SIR), wat uiteindelik kan lei tot sistemiese-inflammatoriese respons sindroom (SIRS). Hierdie studie fokus op die inflammatoriese respons wat deur beide koronêre vatomleiding-chirurgie tegnieke gedurende die hervaskularisering van die AKS-pasiënt teweeggebring word. In die verlede het verskeie studies die voor- en nadele van koronêre vatomleiding-chirurgie met of sonder kardiopulmonêre omleiding vergelyk, sowel as die gevolge van beide soorte chirurgië en SIRS beredeneer. Beide tipe chirurgiese prosedures hou verband met 'n inflammatoriese respons vanweë weefseltrauma en die impak as gevolg van koronêre vatomleiding-chirurgie (Quaniers, Leruth, Albert, Limet, Defraigne, 2006).

Die hoofdoel van hierdie nie-ewekansige, waarnemingstudie was 'n beoordeling en 'n vergelyking van die pre- en post-operatiewe inflammatoriese merkers onder pasiënte met AKS (n=60) wat met kardiopulmonêre omleiding (n=30) of sonder kardiopulmonêre omleiding (n=30) koronêre vatomleiding-chirurgie ondergaan het. Die sekondêre oogmerk was om te bepaal of daar 'n korrelasie is tussen die pre-operatiewe risikofaktore, die chirurgiese prosedure en die pre- en post-operatiewe inflammatoriese merkers. Drie inflammatoriese merkers - volbloedtelling (VBT), procalcitonin (PCT) en C-reaktiewe proteïen (CRP) - is by wyse van normale roetine

laboratoriumanalise ontleed. Interleukin-6 (IL-6) en tumor-nekrose faktor alpha (TNF-α) is ontleed deur middel van ‘n ensiemversterkte sensitiwiteits immuno bepalings metode. Die inflammatoriese merkers is pre-operatief ontleed (basaal) en post-operatief op verskillende tydsintervalle (24, 48, 72, 96 en 120 uur post-operatief).

In die geval van beide koronêre vatomleiding-chirurgie groepe was al die leukosiete - soos te wagte in die geval van pasiënte met AKS - reeds pre-operatief verhoog as gevolg van ‘n reeds teenwoordige aterosklerotiese proses en die gevolglike inflammatoriese respons. ‘n Beduidende, pre-operatiewe verskil is by die twee koronêre vatomleiding-chirurgie groepe ten opsigte van die limfosiete bevind (p=0.03024). ‘n Beduidende post-operatiewe verskil is ook by die twee koronêre vatomleiding-chirurgie groep bespeur. Die volgende beduidend verhoogde vlakke het by die groep voorgekom wat met kardiopulmonêre omleiding geopereer is: in die geval van witseltellings op 24 uur (p=0.00761), 48 uur (p=0.01520) en 72 uur (p=0.00004); vir neutrofiele op 24 uur (p=0.17422), 96 uur (p=0.18611) en 120 uur (p=0.12872), vir limfosiete op 48 uur (p=0.04829) en 96 uur (p=0.01982); en vir PCT op 24 uur (p=0.00811), 48 uur (p=0.00966) en 72 uur (p=0.01823). Meetbare waardes van IL-6-vlakke was egter hoër by die groep wat sonder kardiopulmonêre omleiding chirurgie geopereer is, met beduidende verskille op 96 uur (p=0.05352) en 120 uur (p=0.09729). Geen verskille het tussen die twee groepe voorgekom ten opsigte van eosinofiele, basofiele, monosiete, CRP en TNF-α nie.

Ten slotte: ongeag die beduidende inflammatoriese response by beide koronêre vatomleiding-chirurgie groepe, is daar geen verskil in die kliniese resultate waargeneem nie. Die inflammatoriese response wat teweeggebring word deur beide koronêre vatomleiding-chirurgie prosedures, sal nog vir ‘n geruime tyd die fokus van toekomstige navorsing bly. Dit is egter nie die enigste faktore wat chirurgiese uitkomste bepaal nie. Die impak van intra-operatiewe prosedures moet verder en in meer detail toegelig word ten einde te probeer bepaal wat die verhouding van hierdie gebeure op die omvang van die inflammatoriese response en die kliniese resultate is, ongeag of die prosedure met of sonder kardiopulmonêre omleiding uitgevoer word.

CHAPTER 1: INTRODUCTION

1.1 Introduction

The World Health Organization (WHO) has warned in 2005 that: "Coronary heart disease is now one of the leading causes of death worldwide. It is on the rise and has become a true pandemic that respects no borders" (WHO, 2005).

In South Africa, the number of deaths from coronary heart disease (CHD) in 2002 was estimated at 27,013 in a population size of 44,759 million (WHO, 2005). Cardiovascular disease and its major component coronary heart disease (CHD) exists in epidemic proportions in Westernized developed countries, but is also an increasing problem in the developing world, contributing to significant morbidity and premature mortality in vulnerable populations (Yusuf, Reddy, Ounupuu, Anand, 2001).

The burden of cardiovascular disease overall is predicted to rise by approximately 150% in the developing world by the year 2016. Therefore it is essential to understand the determinants of CHD by measuring the incidence, outcomes and variance over time across different populations. This in turn is crucial to define the treatment, risk factor reduction and therapeutic improvements, necessary for the treatment of patients with acute coronary syndrome (ACS) (Roger, 2007).

Atherosclerosis is an ongoing inflammatory process that will eventually lead to the development of endothelial dysfunction which may lead to a heart attack and death. The inflammatory response involves various mechanisms such as complement activation, endotoxin release, leukocyte activation, the expression of adhesion molecules, and the release of endogenous substances including oxygen-free radicals, macrophages, neutrophils, as well as cytokines (Ascione, Lloyd, Underwood, Lotto, Pitsis, Angelini, 2000; Wan, Izzat, Lee, Wan, Tang, Yim, 1999).

Considering the risk factors of the patient the cardiologist will decide on the manner of treatment. Accordingly, the patient will either undergo on-pump coronary artery bypass graft (CABG) surgery and off-pump coronary artery bypass graft (CABG) surgery, which on its own causes another detrimental effect, placing an inflammatory response on top of the already existing inflammatory process.

Both types of CABG surgery, on-pump and off-pump CABG are associated with an inflammatory response due to tissue trauma. It is postulated that the bypass circuit used in on-pump CABG surgery induces an independent additional inflammatory response. Despite numerous well designed published studies, it is still difficult to define the relative role played by the bypass circuit compared to the inevitable surgical insult in the induction of the inflammatory reaction (Quaniers *et al.,* 2006).

1.2 Purpose and aim of the study

To compare the inflammatory response in patients with acute coronary syndrome undergoing on-pump and off-pump CABG surgery by measuring pre-determined inflammatory markers in the peri-operative period.

1.3 Objective

- This is a prospective longitudinal observational pilot study, where the primary objective of this study will be to assess and compare the pre- and post-operative inflammatory markers between 60 patients with ACS undergoing either on-pump or off-pump CABG surgery.
- The secondary objective is to assess if there is a correlation between the pre-operative risk factors, the surgical procedure and the pre- and post-operative inflammatory markers.

CHAPTER 2: LITERATURE REVIEW

2.1 Background

Acute coronary syndrome is an "umbrella term" describing a heterogeneous spectrum of clinical symptoms compatible with acute myocardial ischaemia (Monaco, *et al.*, 2005; ACC/AHA, 2007) and an ongoing inflammatory process resulting from atherosclerosis. ACS can either be treated medically (pharmacological treatment), by percutaneous coronary intervention (PCI), or by performing CABG surgery either through on-pump or off-pump CABG surgery.

By treating the ACS patient with surgery, an inflammatory response is further triggered on top of the already existing inflammation resulting from atherosclerosis. This leads to a SIR, which may eventually lead to SIRS. This study will focus on the inflammatory response initiated by the CABG technique applied during the revascularisation of the ACS patient. Many studies have in the past compared on-pump and off-pump CABG surgery, arguing both the advantages and disadvantages of these surgeries, and also the outcomes with regard to SIRS. Both types of CABG surgery are associated with an inflammatory response resulting from tissue trauma, and the use of the extracorporeal circulation (EC) during on-pump CABG surgery (Quaniers *et al.,* 2006).

Because of the inflammatory response that is initiated, inflammatory markers such as C-reactive protein (CRP), procalcitonin (PCT) (Gram, 2002), proteases and cytokine mediators like interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) are released. Thus the direct injury to the vessel wall causes endothelial and smooth muscle cells of the large arteries to become transcriptionally active when exposed to certain pathogenic pro-inflammatory stimuli (Libby, 1995). Although it is postulated in certain studies that the inflammatory response is more pertinent in on-pump CABG surgery as a result of the use of the EC, it is still difficult to determine whether the EC or the surgery itself plays a major role in the stimulation of the inflammatory reaction.

This chapter constitutes the literature review of this study and the following aspects will be discussed:

- Atherosclerosis
- Coronary artery bypass graft surgery
- SIRS and CABG surgery

- Inflammatory markers useful in the detection of an inflammatory response and CABG

2.2 Atherosclerosis

According to the American Heart Association (Sävykoski, 2003), atherosclerosis is a disease of large and medium-sized arteries characterised by thickening and hardening of the vascular wall. Atherosclerosis can be described as one of the most frequent pathological entities that form part of a broad spectrum of cardiovascular diseases. Yet, what exactly causes it is still unknown. Atherosclerosis can further be described as a diffuse systemic process of coronary arteries as well as peripheral vessels, and it involves the development of so-called plaques in the inner lining of the arteries. Over time, the build-up in an artery grows large enough to narrow the artery significantly enough to decrease the blood flow through it. Atherosclerosis causes an inflammatory response resulting from damage to the arterial endothelium, thereby causing a chronic inflammation at every stage - from initiation to progression - and, eventually, it will lead to plaque rupture (Libby, Ridker, Maseri, 2002).

2.2.1 Risk factors

The AHA (2002) has identified several risk factors for CHD and of atherosclerosis. Both the increasing number and the increasing severity of risk factors increase the risk of developing CHD. Additional risk factors, such as increased age, heredity, race, gender, diabetes mellitus (DM), and increased plasma homocysteine (Thanyasiri, Celermajer, Adams, 2005; Apple, 2001), are considered major risk factors for the development of CHD. There are many contributing factors in the development of this disease, e.g. hypertension, smoking, and hyperlipidemia, which are considered to be the major classical risk factors.

2.2.2 Atherosclerosis - an inflammatory disease

Atherosclerosis can further be defined as a “chronic inflammatory disorder”. It is thought to begin with damage to the innermost layer of the artery called the endothelium. The initial lesion will form at a small part of the injured intima, which is caused by increased turbulence of blood. This turbulence is characteristic at certain sites of the arterial bed, such as the orifices of branches, bifurcations and curvatures that cause changes in blood flow. At this injured site, a non-specific

inflammatory response is provoked and the existing lesions now contain inflammatory cells, mostly macrophages and other inflammatory cells (Khan, 2006).

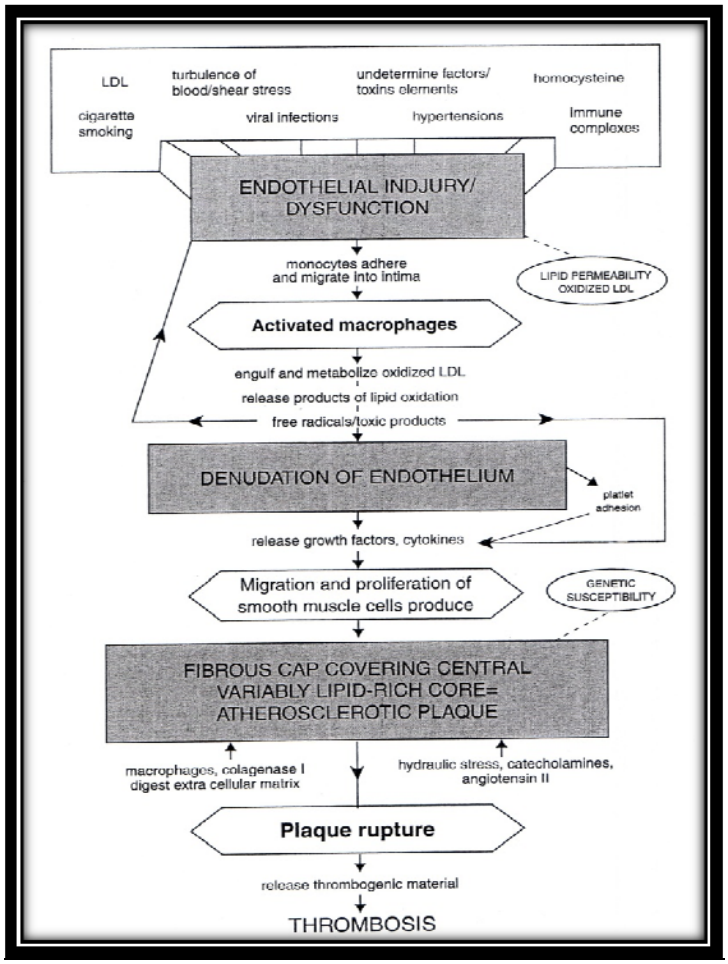


Figure 2.1 Pathogenesis of atherosclerosis (atheroma) plaque (Adapted from Khan, 2006)

In figure 2.1 Khan (2006) explains the pathogenesis of atherosclerosis. The formation of an atheroma is the result of hemodynamic forces that causes arterial injury, activating an inflammatory response with the accumulation of lipid substances from the circulating blood. The damaged area is then walled off by "nature's band-aid" called a protective fibrous cap and the smooth muscle cells assist in this healing process.

Activated inflammatory cells stimulate the release of large amounts of inflammatory factors and cytokines, which promote more inflammation and associated tissue damage. Factors that contribute to endothelial dysfunction include IL-6, IL-1β and TNF-α. These cytokines are found to be elevated in patients with a variety of inflammatory diseases. They are also found in atherosclerotic tissue. Elevated levels of CRP are also present, which causes a reduction in the production of nitric oxide (NO) and consequently diminishes its bioactivity. NO is a toxic gas that is harmful to the

endothelium and it also possibly plays a role in the manifestations of sepsis and septic shock (Szmitko, Chao-Hung, Weisel, de Almeida, Anderson, Verma, 2003).

The cytokines, IL-1 and TNF-α, and also lipopolysaccharide (LPS), ultraviolet irradiation, growth factors, oxygen free radicals, oxidative stress and micro-organisms play an essential role in the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Papparella, Yau, Young, 2002). NFκB is a protein complex that is involved in the regulation of transcription of DNA and many pro-inflammatory genes. Normally, NF-κB is bound to the inhibitory κB (IκB) protein in the cytoplasm of different cells, such as leukocytes and endothelial cells. When stimulated, the NF-κB-IκB complex is phosphorylated and the IκB protein is dissociated and inactivated. Phosphorylation of the NF-κB-IκB complex is accomplished by two specific kinases (IKKα/IKK1 and IKKβ/IKK2). NF-κB translocates to the nucleus, where, binding to DNA, it is able to induce the expression of several inflammatory mediators including pro-inflammatory cytokines, inducible nitric oxide synthases (iNOS) and adhesion molecules (figure 2.2) (Papparella, Yau, Young, 2002).

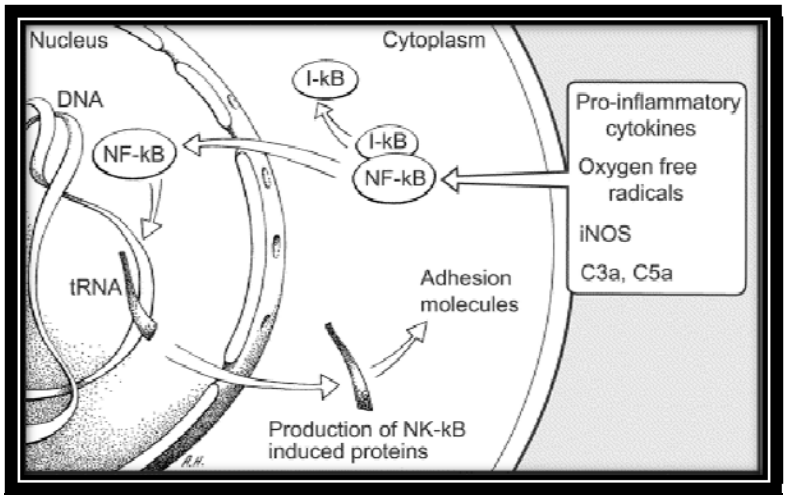


Figure 2.2 **Pathways leading to the activation of NF-kB and the production of adhesion molecules (Adapted from Papparella, Yau, Young, 2002)**

Various genes are induced in the atherosclerotic lesion regulated by NF-κB proteins, including the genes encoding TNF-α, IL-1β, macrophage colony-stimulating factor (M-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), monocyte chemotactic protein-1 (MCP-1), tissue factor (TF), VCAM-1, intercellular adhesion molecule-1 (ICAM-1). Ischaemia quickly reduces IκB cytoplasmic levels, causing the translocation of NF-κB. The cytokine IL-10 stops NF-κB activity through the inhibition of the IκB phosphorylation and NF-κB-DNA binding (Papparella, Yau, Young, 2002).

2.2.3 Endothelial activation and inflammatory response

Nitric oxide (NO) is an endothelium-derived relaxing factor, and is formed by the conversion of the amino acid L-arginine to NO and L-citrulline by the enzyme NO synthase, and plays a pivotal role in the regulation of vascular tone and vasomotor function (Szmitko *et al.,* 2003). A reduced production or availability of NO and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors, such as endothelin-1 (ET-1), angiotensin, and oxidants (Szmitko *et al.,* 2003) are present during endothelial dysfunction.

NO has various functions, such as protection against vascular injury and inflammation. It further inhibits leukocyte endothelium adhesion, and it keeps the vascular smooth muscles in a non-proliferative state, while it also limits platelet aggregation. However, traditional cardiovascular risk factors such as hypertension, obesity, diabetes, smoking and hypercholesterolaemia contribute to the destruction of the vascular endothelium (Berliner, Navab, Fogelman, Frank, Demer, Edwards, Watson, Lusis, 1995).

When the endothelium fails to elicit NO-mediated vasodilation, it will result in reduced bioavailability of endothelium-derived NO resulting from decreased formation or accelerated degradation. Thus, vasomotor dysfunction may be indicative of the amount of endothelial dysfunction that can be present even before the development of atherosclerosis and can serve as an independent predictor of future cardiovascular events (Behrendt & Ganz, 2002).

Angiotensin II is a vasoconstrictor associated with hypertension, inhibition of NO action and the production of reactive oxygen species (ROS). Angiotensin II stimulates the production of pro-inflammatory cytokines such as interleukin (IL)-6, and also MCP-1 and VCAM-1 on the endothelial cells (Griendling, Ushio-Fukai, Lassègue, Alexander, 1997). There are other risk factors that can further contribute to endothelial dysfunction, such as elevated CRP levels and reduced NO production, which will lead to reduced bio-activity. Consequently, an inflammatory response will be triggered within the wall of the vessel, which sets the stage for the initiation and progression of an atherosclerotic lesion (Szmitko *et al.,* 2003).

Levels of P-selectin are found to be increased intracellularly by mildly oxidised LDL (ox-LDL) and can be released by a variety of substances including high-density lipoprotein (HDL). Mildly oxidised LDL-

induced endothelial cells can produce the potent monocyte activators, MCP-1 and monocyte colony stimulating factor (M-CSF) (Gawaz, Neuman, Dickfeld, Koch, Laugwitz, Adelsberger, Langenbrink, Page, Deumeier, Schömig, Brand, 1998).

Under normal circumstances, the leukocytes will attach to the endothelium; however, when hypercholesterolaemia (figure 2.3) is present, the leukocytes are resistant to firm leukocyte adhesion. Low-density lipoprotein (LDL) is rapidly transported across an intact endothelium and becomes trapped in the three-dimensional cage works of fibres and fibrils secreted by arterial wall cells. The cells of the arterial wall secrete oxidative products from multiple pathways that initiate lipid oxidation (Berliner *et al.,* 1995).

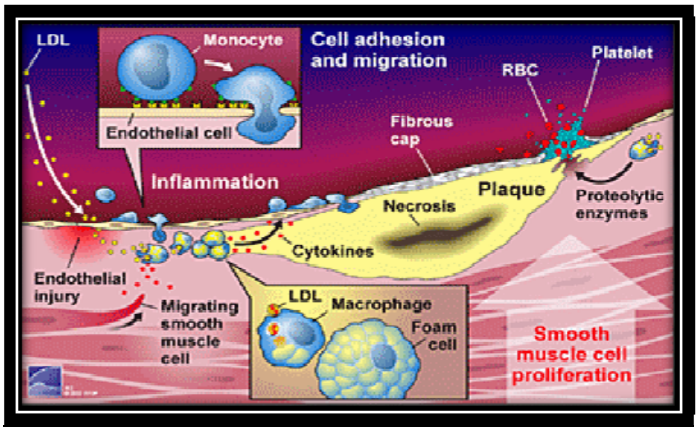


Figure 2.3 **Endothelial dysfunction: Setting the stage for inflammation (Adapted from: National Diabetes Education Initiative, 2002)**

The endothelium becomes activated by oxidised-LDL (oxLDL) and changes its biological characteristics in part by reducing the intracellular concentration of NO (Szmitko *et al.*, 2003). This oxidative modification of the trapped LDL occurs in two stages: the first stage occurs before monocytes are recruited and results in the oxidisation of lipids in LDL with little change in apolipoprotein-B; the second stage begins when monocytes are recruited to the lesion in the vessel wall converting into lipid-filled macrophages that contributes further to the existing inflammatory process with their enormous oxidative capacity. During the second stage LDL is further oxidised but the protein part of the LDL is also modified. This leads to a loss of recognition in the LDL receptor of the scavenger receptors and/or the oxidised LDL receptor (Paoletti, Gotto, Hajjar, 2004).

Activated monocytes will transmigrate into the tunica intima, i.e. the innermost layer of the arterial wall, passing between the endothelial cells. These monocytes are then directed along a

concentration gradient of MCP-1, via interaction with the monocyte receptor chemokine (C-C motif) receptor 2 (CCR2). These monocytes will now, within the arterial intima, develop into macrophages expressing scavenger receptors (SR), such as SR-A, CD36, and LOX-1, which internalise modified lipoproteins. These lipoprotein particles give rise to lipid-laden macrophages - also known as foam cells - that characterise early atherosclerotic lesions, e.g. fatty streak. Within this developing atheroma, the foam cells will start secreting pro-inflammatory cytokines (e.g. IL-1, IL-6, TNF-α), which will, in turn, lead to constant stimulation of adherent leukocytes, enhanced expression of scavenger receptors, and macrophage replication (Szmitko *et al.,* 2003).

T cells, (binding to adhesion molecules, such as VCAM-1), dendritic cells, and mast cells are also recruited into the intima, thereby further contributing to the inflammatory response (Hansson, Libby, Schönbeck, Yan, 2002). Inside the intima, the T cells may become activated through encountering antigens, such as ox-LDL, and will subsequently secrete cytokines that can influence macrophage activity. The engagement between CD40/CD40L, activated T cells and macrophages may lead to the formation of TF, matrix metalloproteinases (MMPs), and pro-inflammatory cytokines that will further contribute to the inflammatory response. With mast-cell degranulation, TNF-α, heparin and serine proteases are released. T cells, all of the said risk factors that contribute to endothelial dysfunction and the subsequent inflammation response remain. The formed atheroma will progress from a fatty streak to a more complex lesion (Szmitko *et al.,* 2003).

2.2.4 The development of inflammation and atherosclerosis

When the fatty streak develops into a complex lesion, it is characterised by the proliferation of smooth muscle cells (SMCs), their migration toward the intima, and their production of collagen (Szmitko *et al.,* 2003). The continuous release of cytokines, such as MCP-1, by the activated endothelial cells, T cells, and foam cells (figure 2.3) will not only perpetuate the inflammatory response and lipid accumulation within the atheroma, but it also has an effect on the smooth muscle cell activity (Schonbeck & Libby, 2001). Growth factors and cytokines are released by the foam cells that promote the migration of smooth muscle cells, neointimal proliferation stimulation and the continuous accumulation of lipids, which further support endothelial cell dysfunction. Thus, the fatty streak is formed by the T cells, the foam cells and the smooth muscle cells. This also includes platelet adherence and aggregation (Khan, 2006).

The lesions will continue to grow out towards the adventitia until a critical point is reached. When the lesion reaches a point where it can no longer expand outwards, it will start to intrude on the lumen. The lesion now grows larger and larger as a result of the movement of new nuclear cells entering at the shoulder regions of the lesion, increased production of monocytes, macrophages and smooth muscle cells, and, the accumulation of extracellular lipid in a necrotic core (Berliner *et al.*, 1995).

New collagen production is required for the preservation of the fibrous cap, but this production is limited by pro-inflammatory cytokines, such as interferon (IFN)-γ that are secreted by the activated T cells (Libby *et al.,* 2002). The accumulated oxLDL has toxic effects, this leading to necrosis and apoptosis of the core and also to increased proteolytic activity and lipid accumulation. Together with oxLDL toxicity, the enzyme lipoprotein-associated phospholipase-A_2 reduces macrophage death when inhibited (Paoletti *et al.,* 2004).

Lipid droplets are released and phagocytized upon the death of macrophage foam cells. This further contributes to the inflammatory response, and SMC death contributes to the reduction of collagen synthesis and to a subsequent thinning of the fibrous cap. The over-expression of MMPs within the plaque (mediated by IL-1ß, TNF-α, oxLDL, and within CD40L), the interstitial collagenases, and within the gelatinases enhances the thinning of the fibrous cap, owing to the degradation of the supportive collagen. Once the fibrous cap is weakened, the plaque is vulnerable to rupture, which will give rise to acute thrombotic complications and the occurrence of ACS such as unstable angina pectoris and myocardial infarction (MI) (Paoletti *et al.,* 2004).

A complicated atherosclerotic lesion will cause thrombosis of the coronary arteries and this may lead to an acute myocardial infarction (AMI). Most coronary thrombi are associated with small tears in the vessel wall, which increases the risk of plaque rupture and the exposure of large pools of extracellular lipids. Foam cells, originating from macrophages, will infiltrate the fibrous caps of ruptured plaques and then interact with the T-lymphocytes and smooth the muscle cells via growth factors and cytokines (Van der Wal, Becker, van der Loos, Das, 1994).

The mechanisms of ACS encompass elements of thrombosis and vasoconstriction that are superimposed on atherosclerotic lesions (Libby *et al.*, 2002). ACS can therefore be defined as the formation of an acute thrombus in an atherosclerotic coronary artery. As mentioned before, such a thrombus can either completely occlude the coronary artery, thereby depriving the heart muscles of

blood flow and consequently causing ischaemia and necrosis, or partially occlude the coronary artery, which reduces the blood flow across the blockage, thereby causing angina (Gibler, Cannon, Blomkalns, Char, Drew, Hollander, Jaffe, Jesse, Newby, Ohman, 2005).

Thus, in ACS patients, there is an already existing inflammatory process resulting from atherosclerosis. These patients can be treated through CABG surgery, which by itself also provokes an inflammatory response that aggravates the already existing inflammatory process.

2.3 Mechanisms involved in the inflammatory response during coronary artery bypass graft (CABG) surgery

2.3.1 Inflammatory response provoked during on-pump CABG surgery

When an inflammatory process is present in the body, this constitutes the body's first line of defence against the injuring agent (Warren, Smith, Alexiou, Rogers, Jaward, Vincent, Darzi, Athanasiou, 2009). When a patient undergoes on-pump CABG surgery with prolonged anaesthesia, an inflammatory process is activated. This process involves the activation of various multiple, interdependent and redundant inflammatory systems that further aggravates the inflammatory process at humoral and cellular levels (figure 2.4) (Chong, Hampton, Shimamoto, Pohlman, Verrier, 2003). Eventually, this inflammatory response can become so exaggerated that, in the end, it tends to damage the very host it is trying to protect (Warren *et al.*, 2009).

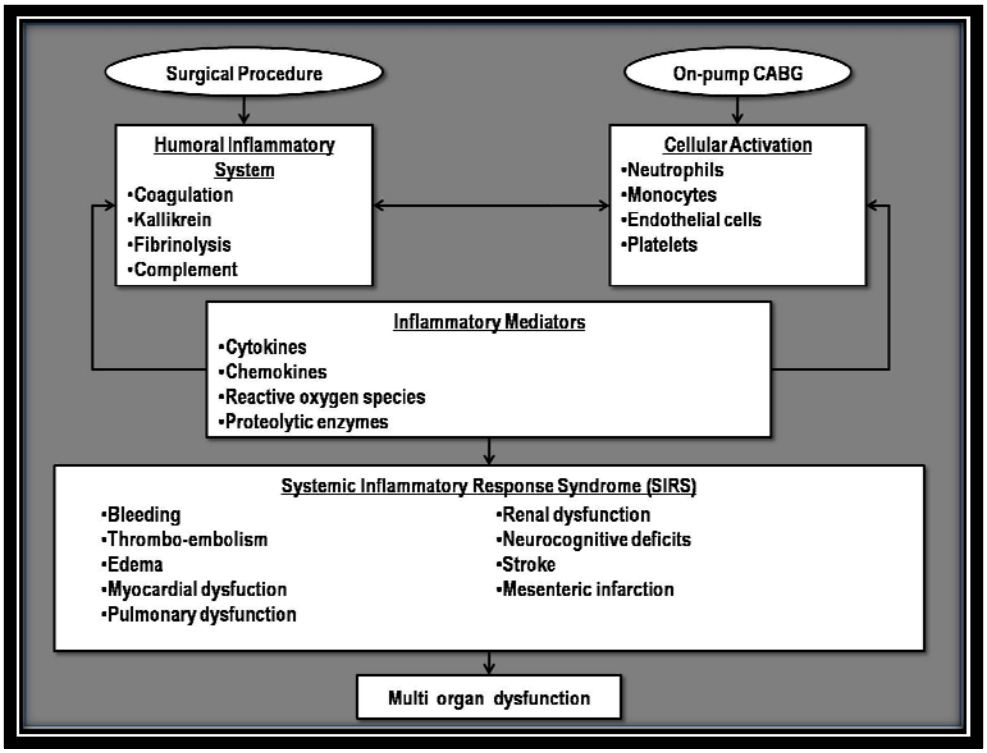


Figure 2.4 **Activation of humoral inflammatory cascades and multiple cell types leads to elaboration of inflammatory mediators, resulting in SIRS. This schematic highlights the interdependence and redundancy of these systems, providing opportunity for amplification at multiple steps (Adapted from Chong *et al.*, 2003)**

Cardiac surgery initiates a potent stress response that causes the stimulation of stress hormones such as cortisol, growth hormones and nor-epinephrine. These stimulated stress hormones has a major effect on the body such as muscle catabolism, hyperglycaemia, and changes in the immune system. The induced cytokine response can further contribute to multi organ dysfunction. All these stress response components activate the inflammatory response and the release of stress hormones, including adrenaline, noradrenalin, adrenocortical hormone and cortisol. Other non-specific activators such as surgical trauma, blood loss/transfusion and hypothermia agitate the stress response further (Kapoor & Ramachandran, 2004).

On-pump CABG surgery activates the immune response by three different mechanisms: (1) direct activation of the immune response resulting from blood surface/component interaction, (2) ischaemia/reperfusion injury to organs as a result of aortic cross-clamping, and, (3) directly through systemic endotoxemia. Therefore, the inflammatory response initiated in the body during on-pump CABG surgery may lead to widespread activation of and injury to the endothelium, which may eventually lead to organ injury and dysfunction (Kapoor & Ramachandran, 2004).

2.3.2 Components of the inflammatory response to on-pump CABG surgery

During on-pump CABG surgery the inflammatory response can be divided into an "early" and "late" phase. During the early phase blood components comes into contact with the non-endothelial surfaces of the bypass circuit i.e. contact activation. The late phase is characterized by ischaemia/reperfusion injury (I/R injury) and endotoxemia (figure 2.5) (Warren *et al.*, 2009).

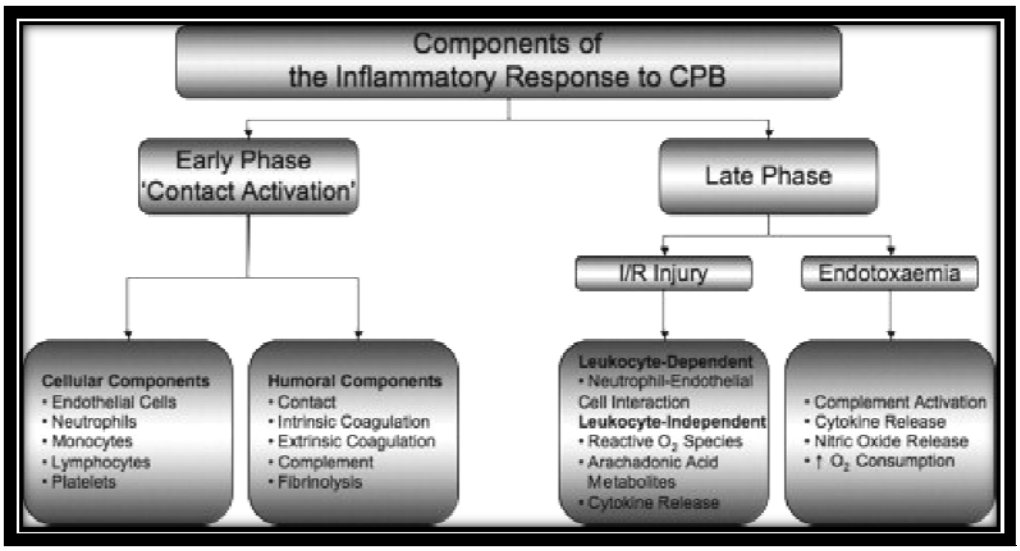


Figure 2.5 **A summary of the inflammatory response to on-pump CABG surgery (Adapted from Warren *et al.*, 2009). (I/R injury = ischaemia/reperfusion injury)**

2.3.2.1 Early phase: contact activation

2.3.2.1.1 Humoral inflammatory system

During on-pump CABG surgery, the inflammatory cascades are activated by surgical trauma, the components of the bypass circuit, such as membrane oxygenators and roller pumps, bio-incompatible artificial surfaces and the oxidative stress caused by ischaemia and reperfusion. Subsequently, together with the initiated coagulation cascade, the humoral inflammatory system (figure 2.5) (coagulation, kallikrein, fibrinolysis and complement system) is activated, and the products of the humoral inflammatory cascades will activate almost every cell type in the body. These activated cells will release pro-inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α), thereby further aggravating the inflammatory cascades and creating a vicious circle of inflammatory events that can eventually lead to multi-organ failure (Chong *et al.*, 2003).

2.3.2.1.2 Kallikrein sytem (Contact system)

The contact system consists of four primary plasma proteins: (1) factors XII (Hageman factor), (2) Factor XI, (3) pre-kallikrein, and (4) high-molecular-weight kininogen (HMWK). During on-pump CABG surgery, the coagulation and kallikrein systems (both associated with the plasma protein Factor XII) are activated when they come into contact with the surfaces of the bypass circuit and the exposed sub-endothelial extracellular matrix leading to the production of Factor XIIa from Factor XII (Chong *et al.,* 2003; Warren *et al.,* 2009). Factor XIIa initiates two inflammatory systems: (a) the intrinsic coagulation pathway via Factor XIa activation, and (b) via kallikrein cascades (figure 2.6) (Warren *et al.*, 2009).

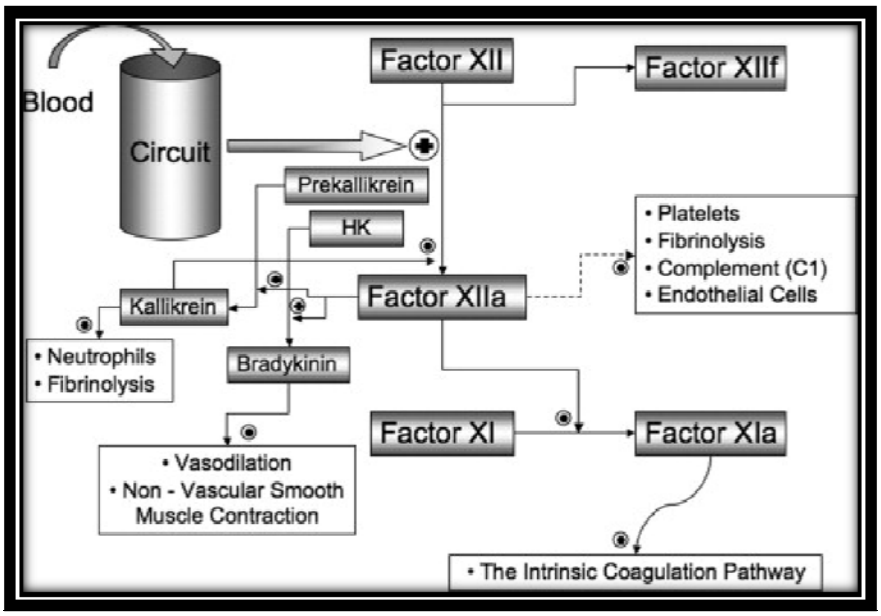


Figure 2.6 **Activation of the contact system by on-pump CABG surgery (Adapted from Warren *et al.*, 2009)**

Kallikrien is converted from pre-kallikrein in the presence of HMWK and Factor XIIa pre-kallikrein (a protease with serine at its active site) (figure 2.6). Kallikrein, together with Factor XIIa, activate more Factor XII in a positive feedback loop. Kallikrein can also activate several inflammatory pathways through the stimulation of neutrophils releasing superoxide and hydrogen peroxide as well as proteolytic enzymes such as elastase and cathepsin. Furthermore, kallikrein supports the release of bradykinin from surface bound HMWK, which is a powerful inducer of smooth muscle contraction and increased capillary permeability. This contributes to the already present injury by causing edema and fascilitating the movement of polymorphonuclear cells into the tissues leading to the intensification of destructive proteolytic enzymes (Warren *et al.*, 2009).

Kallikrein and bradykinin are also directly responsible for the activation of the fibrinolytic system (figure 2.6) by activating pro-urokinase. Bradykinin also stimulates the release of tissue plasminogen activator (t-PA) from the endothelial cells. Lastly, kallikrein enhances the activation of the complement system by cleaving C5 to produce C5a, a potent anaphylatoxin containing numerous inflammatory properties. Thus, the activation of the Factor XII/kallikrein sytem is present during cardiac surgical procedures, and the activation of the kallikrein-kinin system is related to the development of possible post-operative organ dysfunction (Warren *et al.,* 2009).

2.3.2.1.3 Coagulation system

The classic coagulation system consists of an extrinsic pathway which is activated by tissue factor, an intrinsic pathway which is activated by the contact system and a common pathway. The classic coagulation model is valuable in the interpretation of laboratory findings and our understanding of physiologic and abnormal hemostatic conditions. Because cellular components are not incorporated in this model it contributed to several limitations of the model. A novel cell-based concept of hemostases was developed and replaced the classic coagulation cascade model (Hartmann, Sucker, Boehm, Koch, Loer, Zacharowski, 2006).

The new cell-based model occurs in three phases: (1) initiation, (2) amplification and (3) propagation (figure 2.7). The new model takes into consideration the effect of tissue factor-expressing cells and platelets in the hemostatic process. These cells provide a surface for the initiation phase of coagulation which involves the activation of Factor VII to Factor VIIa by tissue factor. The result of vessel injury is thus the formation of a TF-FVIIa complex responsible for the initiation of coagulation. Surgical trauma and inflammation have been shown *in vitro* as well as clinical studies to be the main causes of intravascular tissue factor expression. Additional to increased tissue factor expression on monocyte surfaces, further sources of Factor VII activators have been reported in cardiac surgery. During the amplification phase small amounts of thrombin is formed. This thrombin is responsible for the activation of platelets and the activation of Factor V, Factor VIII and Factor IX on platelet surfaces. In the propagation phase large amounts of thrombin are produced that contribute to the formation of a stable fibrin clot. Thrombin also has the function of stimulating sub-endothelial smooth muscle cells to contract, consequently stopping blood loss from an injured vessel (Warren *et al.*, 2009).

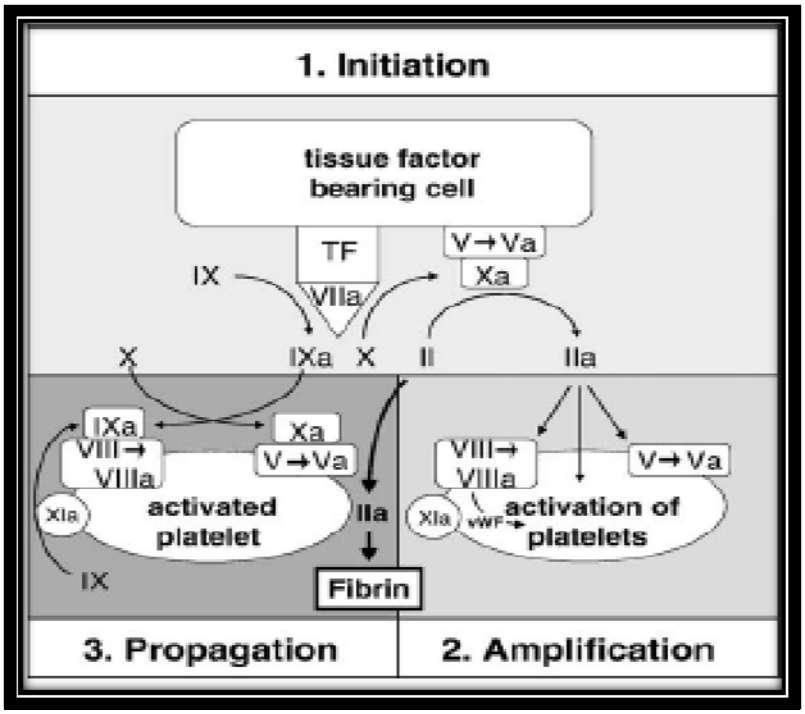


Figure 2.7 **Cell-based coagulation models. In the initiation phase, Factors VIII and IX are activated by Factor VII bound to tissue factor. Small amounts of thrombin generated in this phase activate platelets in the amplification phase. In the propagation phase, huge amounts of thrombin lead to fibrin formation. Abbreviation: vWF=von Willebrand factor; TF=tissue factor. (Adapted from Hartmann *et al.*, 2006)**

Thrombin will, during the use of the extra corporeal circuit (EC), split fibrinogen to fibrin, activating Factor XIII to crosslink fibrin and activate platelets via the thrombin-specific receptors. This thrombin mediated platelet activation releases von Willebrand factor (vWF) from platelet α-granules. Together with the release of vWF from activated endothelial cells vWF assists in platelet aggregation, and promotes the chemotaxis of monocytes and neutrophils (figure 2.7) (Chong *et al.,* 2003). If there is an injury present thrombin will aim to stimulate the production of a variety of growth factors, inducing the production of chemoattractants and vasoactive substances. This will promote neutrophil adhesion, attraction of macrophages, and increase the vascular permeability (Edmunds & Colman, 2006).

Looking at the classic coagulation system, when blood is exposed to the artificial surfaces of the bypass circuit the intrinsic coagulation pathway is activated - particularly Factor XII. In the presence of HMWK, Factor XIIa activates Factor XI to Factor XIa which is the first step of the intrinsic coagulation pathway (figure 2.6) (Warren *et al.,* 2009). Results of Bosclair and colleagues suggest

that the extrinsic coagulation system might also be activated in the setting of EC by means of intravascular tissue factor expression (Boisclair, Lane, Philippou, Esnouf, Sheikh, Hunt, Smith, 1993).

During on-pump CABG surgery the coagulation cascade is de-activated by systemic heparinization (activation of antithrombin III) to prevent clot formation within the bypass circuit. However, systemic heparinization has certain side effects such as platelet activation that will cause heparin-induced thrombocytopenia. This again will lead to elevated levels of thrombin-antithrombin throughout the bypass circuit. Due to this progressive thrombin production, (mainly within the pericardial wound) a coagulopathy can form that is responsible for many of the thromboembolic and non-surgical bleeding complications related to cardiac surgery (Warren *et al.*, 2009).

In the presence of HMWK pre-kallikrein is converted to kallikrein (a protease with serine at its active site) (figure 2.6), by Factor XIIa. Kallikrein, together with Factor XIIa, activate Factor XII. Kallikrein can activate several inflammatory pathways through the stimulation of neutrophils releasing superoxide and hydrogen peroxide as well as proteolytic enzymes such as elastase and cathepsin. Furthermore, kallikrein supports the release of bradykinin from surface bound HMWK, which is a powerful inducer of smooth muscle contraction and increased capillary permeability. This contributes further to the already present injury by causing edema and fascilitating the movement of polymorphonuclear cells into the tissues leading to the intensification of destructive proteolytic enzymes (Warren *et al.,* 2009).

Kallikrein and bradykinin are also directly responsible for the activation of the fibrinolytic system (figure 2.7) by activating pro-urokinase. Bradykinin also stimulates the release of tissue plasminogen activator (t-PA) from the endothelial cells. Lastly, kallikrein enhances the activation of the complement system by cleaving C5 to produce C5a, a potent anaphylatoxin containing numerous inflammatory properties. Thus, the activation of the Factor XII/kallikrein sytem is present during cardiac surgical procedures, and the activation of the kallikrein-kinin system is related to the development of possible post-operative organ dysfunction (Warren *et al.,* 2009).

2.3.2.1.4 Fibrinolytic system

During on-pump CABG surgery fibrinolysis is activated and hyper-fibrinolysis is thought to occur secondary to the coagulation factor activation. Thrombin and the activation of Factor XIIa induce the release of tissue-type plasminogen activating factor from the endothelial cells. Plasminogen is

converted to plasmin which is responsible for the breakdown the fibrin clot and the formation of several degradation products such as D-dimer (Waren *et al.,* 2009).

Fibrinolysis is indirectly influenced by kallikrein through the activation of pro-urokinase and directly through the cleaving of plasminogen to plasmin. Plasmin activates Factor XII causing another positive feedback cycle. Thus, fibrinolysis disrupts hemostasis, creating fibrin split products as well as worsening of the inflammatory process. Fibrin stimulates the production of TNF-α and IL-1β through monocytes and chemokine secretion by the endothelial cells, as well as fibroblasts and neutrophils. Fibrin-degradation products are associated in platelet dysfunction, endothelial injury and weakened fibrin production (Chong *et al.,* 2003). Therefore, for the duration of the on-pump CABG surgery, the fibrinolysis process will carry on, particularly in the pericardial wound (Warren *et al.,* 2009).

2.3.2.1.5 Complement system

The complement system plays an important role in the body's humoral defence mechanisms and it is also a potent initiator and mediator of inflammation.

The complement system can be activated by one or more of the following: (1) blood components being in direct contact with the artificial surfaces of the bypass circuit, (2) the surgical procedure itself, (3) I/R injury, (4) endotoxin, (5) products of the fibrinolytic and the kallikrein-kinin cascades, and (6) administration of drugs such as heparin and protamine (Chong *et al.,* 2003).

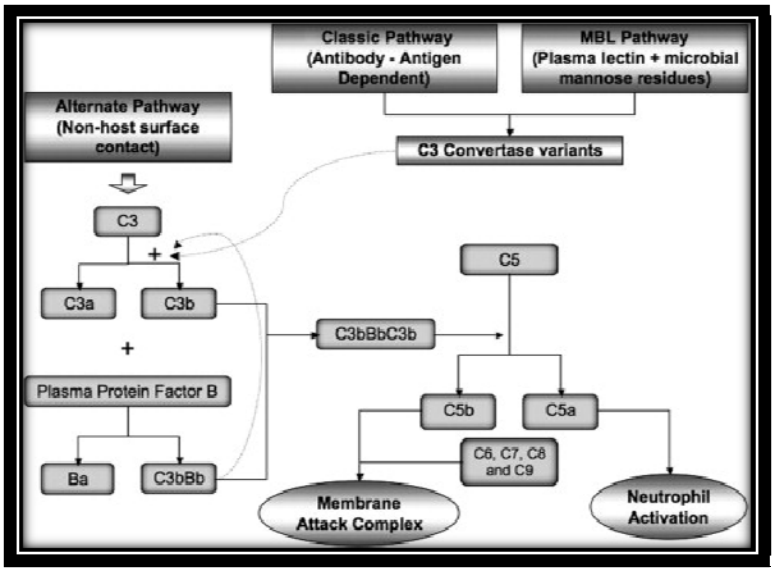


Figure 2.8 Activation of the complement system (Adapted from Warren *et al.*, 2009)

Complement activation can be divided into three different pathways (figure 2.8): (1) the classical pathway (immune complex-dependent), (2) the alternative pathway (antibody-independent pathway) that can be activated by numerous triggers, such as foreign surfaces, damaged tissue, shear forces, hypoxia-induced oxygen free radicals, and tissue plasminogen activator, and (3) the third pathway is initiated by the binding of mannose-binding lectin (MBL) to carbohydrate moieties on the surfaces of bacteria, yeast, parasites and viruses (Warren *et al.,* 2009; Chong *et al.,* 2003). All these pathways come together to form C3, and the subsequent activation products C3a and C5a are powerful inflammatory mediators, which are more elevated in on-pump CABG surgery than in off-pump CABG surgery (Vedin, 2006).

2.3.3 Inflammatory mediators

2.3.3.1 Cytokines

The release of cytokines such as TNF-α, interleukins and interferons are stimulated by the conversion to laminar flow, blood contact with the artificial bypass surface, cold cardiac ischaemia, oxidative stress, and endotoxin. These cytokines play an important role in the immune system as mediators of inflammatory effects. During cardiac surgery, cytokines are responsible for affecting the haemodynamic regulation, and contribute to myocardial stunning, lung injury, and renal dysfunction (Larmann & Theilmeier, 2004). Cytokine production is activated by stimuli, such as infection, trauma, foreign material and haemorrhage. During the inflammatory process, the monocytes, macrophages and endothelial cells secrete cytokines. All of these play a role in the development of SIRS and multi-organ failure (Vedin, 2006).

2.3.3.2 Chemokines

Chemokines are a cytokine subfamily with chemotactic functions. Their function is to recognise, recruit, remove, and repair inflammatory tissue. The chemokines, MCP-1 and macrophage inflammatory protein-1 (MIP-1a) and the cytokine IL-1β play a pivotal role in *in vivo* macrophage recruitment and activation. These macrophages have the ability to arrange acute and chronic inflammatory responses mainly through T-cell chemotaxis and regulation of transendothelial migration of monocytes, dendritic cells, and natural killer cells (Castelheim, Hoel, Videm, Fosse, Pharo, Svennevig, Fiane, Mollnes, 2008).

2.3.3.3 Oxidative stress and reactive oxygen species (ROS)

Oxidative stress describes the imbalance between local anti-oxidant defences and the increased bioactivity of reactive oxygen species (ROS), which can contribute to endothelial and myocardial injury (Biglioli, Cannata, Alamanni, Naliato, Porqueddu, Zanobini, Tremoli, Parolari, 2003). Cell membranes and DNA are damaged by ROS, causing proteins to become dysfunctional and bring about changes in transcriptional programmes. During on-pump CABG surgery and aorta cross-clamping, the myocardium undergoes ischaemia, which causes hypoxic cellular damage. Reperfusion (re-opening of the aorta) follows after ischaemia and this leads to the production of oxidative stress, the stimulation of various inflammatory pathways and the recruitment of neutrophils into the post-ischaemic tissue. All of this further contributes to the already existing ischaemic tissue damage. Thus, this oxidative burst of neutrophils can be seen as one of the cytotoxic designs of the leukocyte population (Larmann & Theilmeier, 2004).

2.3.3.4 Proteolytic enzymes

Neutrophil elastase (NE) is an aserine proteinase consisting of 267 amino acids and variable glycosylation, migrating with a molecular mass of 28 to 31 kDaltons (kDa). NE is synthesised in the promyelocytic stage of myeloid development and stored in large quantities, in its active form, in neutrophil azurophil granules. NE is associated with pulmonary emphysema, a main component of chronic onstructive pulmonary disease (COPD) (Shapiro, Goldstein, Houghton, Kobayashi, Kelley, Belaaouaj, 2003). In the absence of normal repair, proteolysis leads to tissue destruction and airspace enlargement within the lungs. NE also has catalytic properties against various extracellular matrix substrates, such as the highly resistant elastin that forms part of the structural stability of the lung (Shapiro *et al.,* 2003). Once up-regulated, NE disturbs the function of the lung permeability barrier and induces the release of pro-inflammatory cytokine such as IL-6 (Ng, Wan, Yim, Arifi, 2002). It is also known to be used post-operatively as a marker of pulmonary injury subsequent to on-pump CABG surgery (Ng *et al.,* 2002).

2.3.4 Cellular components

2.3.4.1 On-pump CABG surgery activates endothelial cells

During on-pump CABG surgery, the endothelial cells are activated mainly by thombin, C5a, IL-1β and TNF-α contributing to the inflammatory response. IL-1β and TNF-α activate cell adhesion molecules

P-selectin and later E-selectin, which are involved in the early stages of neutrophil and monocyte adhesion. These cytokines are also involved in the activation of ICAM-1 and VCAM-1, which binds neutrophils and monocytes firmly to the endothelium, thus starting leukocyte trafficking to the extracellular space (Day & Taylor, 2005). During on-pump CABG, the endothelial cells produce different anti-coagulants and homeostatic agents. The TF inhibitor inhibits Factor Xa reversibly and inhibits indirectly the Factor VIIa-TF complex that drives the extrinsic coagulation pathway (Warren *et al.,* 2009).

2.3.4.2 On-pump CABG surgery activates leukocytes

On-pump CABG surgery is associated with leukocyte activation together with elevated levels of NE, pro-inflammatory cytokines and the formation of platelet-leukocyte bindings. This leukocyte activation happens as a result of elevated levels of thrombin, kallikrein and C5a. Shortly after on-pump CABG is started, C5a is produced and this stimulates neutophil chemotaxis, degranulation and superoxide generation. Other mediators responsible for leukocyte activation during on-pump CABG surgery are IL-1β, TNF-α, IL-8, C5b-9, Factor XIIa, heparin and histamine (Day & Taylor, 2005).

2.3.4.3 On-pump CABG surgery activates neutrophils

With the activation of the inflammatory response, neutrophils will migrate through the vessel walls into the surrounding tissues. Through the actions of IL-8 the adhesion grip between neutrophils and endothelial cells are reduced and will subsequently set up the way of migration (Warren *et al.*, 2009). Activated neutrophils release a number of cytotoxic enzymes (NE, lysozymes and myeloperoxidase), from intracellular granules as well as oxygen free radicals and hydrogen peroxide. During on-pump CABG surgery, the activated circulating neutrophils will increase and infiltrate the myocardium, subsequently increasing the perivascular oedema and leukocyte transmigration into the extracellular matrix (Day & Taylor, 2005) and contributing to reperfusion injury (Larmann & Theilmeier, 2004).

2.3.4.4 On-pump CABG surgery activates monocytes

Compared with other systems, such as complement activation and neutrophils, the activation of monocytes is slower during an on-pump CABG surgical process. Monocytes are activated by potent agonists, such as C5a, thrombin, platelet Factor IV and bradykinin, because of the contact of the

blood with the non-endothelial cell surfaces of the bypass circuit. Monocyte activation will peak shortly after the start of on-pump CABG surgery. Subsequently, inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8 and MCP-1), reactive oxygen, nitrogen intermediates and prostaglandins (Menasche & Edmunds, 2003) are produced and released. Monocytes activated by pro-inflammatory cytokines, will produce and present tissue factor on its surface, both in the pericardial wound and in the bypass circuit (Warren *et al.,* 2009).

2.3.4.5 On-pump CABG surgery activates platelets

During on-pump CABG surgery, platelets are activated by thrombin in both the pericardial wound and within the bypass circuit. As the on-pump CABG surgical procedure continues, activated complement (C5b-9), leukotrines, plasmin platelet-activating factor, surface contact and collagenases will further activate platelets (Menasche & Edmunds, 2003). Platelets can also be activated by mediators such as surgical wound heparin dosing, hyperthermia and direct contact with the EC. Activated platelets may further bind to fibrinogen, vWF, and fibrinonectin on the circuit surfaces. Furthermore, activated platelets will bind to each other through fibrinogen bridges, express P-selectin and the formation of monocytes and neutrophil bindings by binding P-selectin glycoprotein 1. Circulating and bound platelets may release their granules containing chemo attractants, coagulation-proteins and vasoactive substances, which can contribute to the formation of micro-emboli. The circuit-priming fluids of the bypass circuit will decrease platelet production, causing a decrease in the post-operative coagulopathy, but this is not sufficient enough to prevent thrombocytopenia (Warren *et al.,* 2009).

2.3.5 Late phase

During the late phase, the activated humoral and cellular components diminish. This may result from the binding sites on the biomaterial surfaces of the EC, which make the surface much more biocompatible (Warren *et al.,* 2009).

2.3.6 Ischaemia/reperfusion injury (I/R injury)

I/R injury is when the blood flow to the heart and the lungs is cut off through aorta cross-clamping during on-pump CABG causing the heart and the lungs to become ischaemic. On releasing the cross-clamp both will become fully perfused. Reperfusion after cross-clamping produces high levels of

reactive oxygen species (ROS) (Warren *et al.,* 2009). Therefore, this ischaemic process contributes further to the already existing inflammatory process, causing an increase in capilliary permeability, interstitial fluid accumulation, leukocytosis, coagulopathy and end-organ dysfunction. I/R injury cause undesirable effects that may range from post-ischaemic organ dysfunction to permanent tissue damage including myocellular necrosis. I/R injury is associated with an inflammatory response that is mediated by cytokines, chemokines, and adhesion molecules, and also the recruitment of neutrophils, monocytes and other inflammatory cells that contribute to the damage of the ischaemic myocardium (Levy & Tanaka, 2003).

2.3.7 Endotoxin

Endotoxin (lipopolysaccharide (LPS)) is released from the cell wall of Gram-negative bacteria during their breakdown, and plays an intrinsic role in the development of SIRS. LPS are responsible for the activation and release of inflammatory cytokines or complements. According to a study done by Larmann & Theilmeier (2004), on-pump CABG surgery is associated with increased systemic endotoxin levels and usually gut translocation is perceived to be the primary source. Enteric mucosal ischaemia is the result of splanchnic vasoconstriction that occurs during on-pump CABG surgery. This may cause changes in microbial viability and intestinal permeability.

However, it remains difficult to establish/demonstrate a clear connection between variables such as the duration of the surgery, levels of intestinal permeability, and the endotoxin levels after on-pump CABG surgery. Elevated levels of endotoxin will activate complement via a different pathway, which will stimulate the production of pro-inflammatory cytokines, NO, and will increase levels of post-operative oxygen use (Warren *et al.,* 2009).

Thus, on-pump CABG surgery triggers an inflammatory response that is intense and complex and may lead to morbidity and mortality. The question now arises whether off-pump CABG surgery also initiates the same intense inflammatory responses as does on-pump CABG.

2.3.8 Inflammatory response in off-pump CABG surgery

According to Vallely and colleagues, all the humoral and cellular responses (figure 2.2 - 2.6), and also the inflammatory mediators - as discussed in on-pump CABG surgery - are the same, but only to a lesser degree. They also maintain that off-pump CABG surgery has certain advantages over on-

pump CABG whereby inflammatory insults, such as blood contact with the use of the EC and the ischaemic/reperfusion injury associated with cardioplegic arrest and non-physiological flow, are excluded. The inflammatory response activated during off-pump CABG surgery is due to the surgical trauma itself, manipulation of the heart, pericardial suction, heparin and anaesthesia (Vallely, Bannon, Kritharides, 2001).

According to a study done by Menasche & Edmunds (2003), off-pump CABG surgery is associated with decreased levels of neutrophil elastase, reactive oxidants and leukocyte counts. During off-pump CABG surgery, the complement system is activated and can be seen as an early response to an inflammatory stimulus that triggers and amplifies the acute inflammatory response (Vallely *et al.,* 2001). A marked decrease is noted in C3a, C5b-9 (Menasche & Edmunds, 2003). During off-pump CABG surgery, pro-inflammatory cytokines, such as TNF-α, IL-6 and particularly IL-8 are released and may cause a decrease in leukocyte/endothelial cell activation. The neutrophil and monocyte counts, neutrophil elastase, and E-selectin concentrations are also decreased with off-pump CABG surgery (Menasche & Edmunds, 2003).

In addition, off-pump CABG surgery also attenuates platelet β-thromboglobulin and PCT levels, and effects a reduction in ROS produced injury (Raja & Dreyfus, 2005). Factor XIIa may play an important role in the thrombophilic state post-operativley in off-pump CABG surgery, because the coagulation and fibrinolytic systems are linked to the inflammatory cascade (Valley *et al.*, 2001). Thus, although off-pump CABG surgery has the same activated processes earlier discussed regarding on-pump CABG, they here seem to be less pronounced. The reason for this might be the one perceived advantage that, during off-pump CABG surgery, many of the inflammatory insults associated with on-pump CABG are eliminated, such as for instance use of the EC and the I/R injury.

2.4 Coronary artery bypass graft (CABG) surgery

The question arises as to how the surgeon, or the referring cardiologist, or especially the patient (who needs CABG surgery) decides whether and when to choose off-pump CABG surgery versus conventional on-pump CABG surgery. Many factors will influence this decision. Some anti-inflammatory strategies have shown certain successes; however on-pump CABG surgery increases the risk factor by tipping the balance between exaggerated and expected inflammatory responses to the development of SIRS and other inflammatory response complications. Many patients can handle the on-pump CABG surgical process, even though the EC induces an inflammatory response and

oxidative and coagulatory stress. On the other hand, off-pump CABG surgery has over the years shown many advantages in comparison with on-pump CABG. Off-pump CABG surgery has certain outcome variables like a shorter hospital stay and a shorter surgical intubation time, which make this type of procedure more favourable, especially for high-risk, multimorbid and older patients (Larmann & Theilmeier, 2004).

2.4.1 On-pump CABG surgery

In the early 20th century, gifted visionary investigators looked beyond the mere technical problems of the perfusion of isolated organs. These inventors then integrated mechanical concepts, while simultaneously appreciating physiological issues - to address the clinical needs of open-heart surgery (Galletti, 1993). A breakthrough in routine cardiac surgery on the non-beating heart was made on 6 May 1953, when John H. Gibbon performed the first successful on-pump CABG surgery in Philadelphia, USA (Larmann & Theilmeier, 2004). This form of surgery can be defined as a stable operative site and a bloodless field while ensuring end-organ perfusion and cooling of the patient during cardio-respiratory arrest (Morganstern & Kanchuger, 2008).

Over the past 50 years, on-pump CABG surgery has developed in such a way that it has become a standard procedure in cardiac surgery, one that is performed on more than half a million patients worldwide per year (Warren *et al.,* 2009). However, on-pump CABG surgery is now being challenged (Murphy, Ascione, Angelini, 2004) by the renaissance of off-pump CABG surgery. Despite improvements over the years in surgical techniques and cardioplegic agents, along with the introduction of membrane oxygenators and in-line filtration, there is nevertheless a persistent presence of a major systemic inflammatory response after on-pump CABG surgery (Sisillo, Marino, Juliano, Beverini, Salvi, Alamanni, 2007).

2.4.2 Advantages of on-pump CABG surgery

One of the advantages of on-pump CABG surgery is that it allows the cardiothoracic surgeon to perform delicate work in a bloodless and motionless field. Cardiac arrest is achieved by infusing a cold solution (cardioplegia) through the coronary artery circulation, and this solution is used for the duration of the surgery to keep the heart motionless and cool (Raja & Dreyfus, 2004). This surgical procedure is also most beneficial especially in diabetic patients where revascularisation is complex and difficult to accomplish. On-pump CABG is also contemplated in patients with diffuse disease, or

in patients with extensive internal thoracic artery grafting. For low-risk patients, on-pump CABG surgery may be beneficial and these patients may therefore, in effect, receive more effective revascularisation (Lytle, 2007).

One of the major disadvantages provoked by on-pump CABG surgery is the inflammatory response. Several modifications both to the bypass circuit and the surgical procedure were however introduced over the years to address this disadvantages (Menasche & Edmunds, 2003):

- heparin-coated bypass circuit
- enhances biocompatibility;
- reduces contact activation;
- decreases cardiovascular, respiratory, hemostatic and neurologic dysfunction post-operatively.
- modified ultra-filtration
- leukocyte filtration - development of leukocyte-depleting filters for the bypass circuit
- complement inhibitors

Pharmacological strategies, according to Raja & Dreyfus (2005), include the following:

- Aprotinin
- Pentoxifylline (improves blood flow through blood vessels and therefore helps with blood circulation in the arms and legs)
- High doses of Vitamin C and D
- N-Acetylcysteine,
- Allopurinol
- Phosphodiesterase inhibitors
- Mannitol
- Corticosteroids

2.4.3 Disadvantages and complications of on-pump CABG surgery

Very early on it already became evident that cold myocardial ischaemia and laminar flow during on-pump CABG had certain disadvantages. These disadvantages included complement activation, coagulation, activation of platelets and leukocytes (an initial neutropenia followed by leukocytosis) (Benetti, Rizzardi, Concetti, Bergese, Zappetti, 1999), and also a complete alteration of the lymphocytes (Menasche & Edmunds, 2003).

With on-pump CABG, the following multiple external contributory factors can trigger or provoke a systemic inflammatory response intra-operatively:

- Contact activation of plasma protein systems is initiated when the blood is exposed to artificial surfaces in the bypass circuit (Larmann & Theilmeier, 2004; Takenaka, Ogawa, Wada, Hirata, Terblanche, Brett, 2006).
- Generation of shear forces from the roller pumps driving the blood through the bypass circuit (Takenaka *et al.,* 2006).
- Pulsatile flow is converted to laminar flow (Takenaka *et al.,* 2006).
- The heart is exposed to global cold I/R injury with cardioplegic protection (Takenaka *et al.,* 2006).
- Endotoxaemia and operative trauma (Paparella, Yau, Young, 2002).
- Hypothermia is triggered, as blood is expelled through the extracorporeal circuit (Takenaka *et al.,* 2006).

On-pump CABG surgery therefore contributes to the inflammatory response through the activation of endothelial cells, leukocytes, platelets (to a lesser extent) (Menasche & Edmunds, 2003), the complement system and the coagulation cascade (Takenaka *et al.,* 2006). Monocyte activation plays a major role in thrombin generation via expression of tissue factor and release of pro-inflammatory cytokine mediators such, as TNF-α and IL-6 (Day & Taylor, 2005). Unlike neutrophils, monocytes have a unique biology that involves the pathophysiology of both inflammation and coagulation. Monocytes are one of the primary sources of pro- and anti-inflammatory cytokines after activation. Monocyte and platelet activation can both increase during and after on-pump CABG surgery (Greilich, Brouse, Rinder, Jessen, Rinder, Eberhart, Whitten, Smith, 2008).

The coagulation cascade is directly influenced by an increased tissue factor expression and Factor X (ten) activity. Monocyte-platelet conjugates are formed when activated platelets bind to monocytes. Consequently the tissue factor-dependent thrombotic potential is amplified (Parratt & Hunt, 1998). With on-pump CABG surgery there is an increase in the inflammatory marker cytokines IL-6 and interleukin-10 (IL-10) levels and adhesion molecules such as ICAM-1 and P-selectin (Czerny, Baumer, Kilo, Lassnigg, Hamwi, Vukovich, Wolner, Grimm, 2000).

On-pump CABG surgery is also associated with an acute phase reaction of protease cascades, leukocyte, and neutrophil activation. An elevation in the neutrophil/lymphocyte (N/L) ratio and

platelet activation may result in tissue injury, which post-operatively may reflect on a chronic inflammatory process (Gibson, Croal, Cuthbertson, Small, Ifezulike, Gibson, Jeffrey, Buchan, El-Shafei, Hillis, 2007). Prolonged mechanical ventilation may result in a further retention of leukocytes in the pulmonary bed (Tomic, Russwurm, Möller, Claus, Blaess, Brunkhorst, Bruegel, Bode, Bloos, Wippermann, Wahlers, Deigner, Thiery, Reinhart, Bauer, 2005) and the total white cell count (WCC) and the numbers of circulating neutrophils will increace. Activated platelets are being deposited onto the endothelial cells, which in turn attach to the circulating leukocytes via P-selectin. Consequently, leukocyte-platelet conjugates form, increasing during aortic cross-clamping and on-pump CABG surgery (Larmann & Theilmeier, 2004).

The major disadvantage associated with on-pump CABG surgery is the inflammatory response provoked by the procedure itself, and is thus associated with:

- low blood pressure,
- increased heart rate,
- increased body temperature,
- leucocytosis,
- tissue oedema (Czerny *et al.*, 2000).

On-pump CABG surgery *per se*, and the disadvantages associated with the surgery have been identified as contributory factors to SIRS post-operatively (Johansson-Synnergren, Nilsson, Begtsson, Jeppsson, Wiklund, 2004). These factors may further contribute to a prolonged hospital stay after surgery (Czerny *et al.,* 2000). This may further cause renal, pulmonary and neurological complications and bleeding dysfunction (Biglioli *et al.,* 2003), which may often lead to multiple organ dysfunction and death (Diegeler, Doll, Rauch, Haberer, Walther, Falk, Gummert, Autschbach, Mohr, 2000).

The following vascular complications during on-pump CABG surgery can contribute to mortality and morbidity:

- Direct injury to the vessel wall secondary to the cannula placement
- Embolisation
- Placement of intravascular cannula can result in a retroperitoneal hematoma
- Ischaemia
- Malperfusion of the lower extremities (Lawton, 2006)

The following risk factors are associated with cardiovascular complications post-operatively:

- Peripheral vascular disease
- Emergency surgery
- Presence of unstable angina (UA)
- Duration of the surgical procedure
- Poor pre-operative ventricular function (Lawton, 2006)

Lung injury, ranging from minor sub-clinical functional changes to respiratory distress syndrome, is a very common complication with on-pump CABG surgery. The lungs are in a deflated state and the diaphragm becomes passively displaced. Because the lungs receive blood from the bronchial arteries, pulmonary artery blood flow becomes minimal, thereby contributing to pulmonary ischaemia during on-pump CABG surgery. The sequestration of neutrophils in the lung microvasculature results in the release of oxygen free radicals and proteolytic enzymes with resultant oedema, with increased capillary permeability (Ng, Wan, Yim, Arifi, 2002). Heparin-protamine complexes, mechanical shear stress and contact with the non-endothelial surfaces of the EC lead to the activation of plasma enzyme systems. This will consequently lead to an inflammatory response, the magnitude of which will adversely influence the outcome post-operatively (Holmes, Connolly, Paull, Hill, Guyton, Ziegler, Hall, 2002).

Gatro intestinal (GI) complications such as injury to the small and the large bowel, gallbladder, liver and the pancreas are post-operative complications that may be the result of on-pump CABG surgery. Other GI complications include reduced GI blood flow, together with systemic anti-coagulation, causing GI bleeding, gastritis, cholecystitis, ischaemic colitis, hepatic damage and pancreatitis. Mortality is greatly increased when severe GI complications occur post-operatively, which is likely the reflection of a generalised low cardiac output state. However, the incidence of GI complications to occur post-operatively is about 1% (Halm, 1996).

Therefore, an early diagnosis and treatment of possible SIRS after on-pump CABG surgery might improve the outcome of post-operative organ dysfunction and coagulation disorders (Strüber, Cremer, Gohrbandt, Hagl, Jankowski, Völker, Rückoldt, Martin, Haverich, 1999).

2.5 Off-pump CABG surgery

The fisrt off-pump CABG surgery done on a beating heart was carried out in 1910 by Carrel. Since the 1980's, this form of surgery has become more prominent with improved techniques for surgery on the beating heart (Benetti *et al.,* 1999). Though off-pump CABG has a reduced inflammatory response it cannot be totally prevented due to the surgical procedure itself (Larmann & Theilmeier, 2004). Other contributing factors that evoke an inflammatory response during surgery is the manipulation of the heart, pericardial suction, heparin, protamine, a variety of drugs that causes an increase in the activation of acute inflammatory markers, C3a, C5b-9, pro-inflammatory cytokines (TNF-α, IL-6, Interleukin-8 (IL-8), NE, and reactive oxidants (Menasche & Edmunds, 2003).

2.5.1 Advantages of off-pump CABG surgery

Many advantages of off-pump CABG surgery have been highlighted over the past few years such as the avoidance of blood contact activation and global ischaemia and reperfusion injury. This permits minimal invasive surgery and accordingly, sets the stage for a milder inflammatory response (Larmann & Theilmeier, 2004). Gu and colleagues reported a dampened inflammatory response (C3a, leukocyte elastase, platelet activation) with increased concentrations of IL-8 and TNF-α which are avoided when off-pump CABG surgery was performed (Gu, Mariani, van Oeveren, Grandjean, Boonstra, 1998). Peri-operative cytokine release and oxidative stress are, however, also reduced with sternotomy, possibly because of reduced ROS production in off-pump CABG surgery. The peri-operative release of inflammatory markers, such as interleukin-2 (IL-2) receptor, IL-6 and TNF-α is also considerably lower in off-pump CABG surgery (Kapoor & Ramachandran, 2004).

It seems that certain subpopulations of patients, such as elderly patients, obese patients (Murphy *et al.,* 2004), and also patients with a high risk of inflammation-related complications may benefit more from circumventing the pro-inflammatory stress that is linked to on-pump CABG surgery. Off-pump CABG surgery is thus the technique of choice for these particular patients (Larmann & Theilmeier, 2004). These patients also have a particularly high risk of neurological complications subsequent to on-pump CABG cardiac surgery, but off-pump CABG surgery has demonstrated a neuroprotective effect in these groups. Furthermore, the off-pump CABG procedure reduces myocardial injury, renal dysfunction, and the possible development of SIRS (Murphy *et al.,* 2004).

Off-pump CABG surgery has the advantage of simplicity and it avoids the inflammatory response (Cooley, 2000), utilizes a cardiac stabilizer and occludes the coronary artery proximal and/or distal of the stenosis with temporary ligations. Only the dependent myocardial tissue is exposed to brief ischaemia (Larmann & Theilmeier, 2004). During off-pump CABG surgery there is an unrestricted full course of intravenous heparin and hemodilution. This leads to reduced platelet consumption as well as less activation of the fibrinolytic system. This contributes to frequently reported reduction of bleeding complications, mediastinal blood losses and blood transfusions, but also to early thrombotic complications (Larmann & Theilmeier, 2004; Biglioli *et al.,* 2003).

If compared to on-pump CABG surgery, off-pump CABG surgery can achieve equivalent multi-vessel revascularization and post-operatively these patients had shorter ventilation time, earlier extubation, and were discharged earlier from hospital (Ascione *et al.,* 2000), with a reduced stroke rate (Dempsey & Arrowsmith, 2005). Off-pump CABG surgery has a reduction in cost and significantly better short-term outcome, particularly in high risk groups. Reduced morbidity and peri-operative costs, in turn, increase the quantity and quality of care that can be delivered by healthcare systems (Murphy *et al.,* 2004).

A marked reduction in monocyte activation and adhesion is detected during and after off-pump CABG surgery and this may be the reason for a reduction in post-cardiac surgical complications (Holmes *et al.,* 2002). Greilich and colleagues were the first to demonstrate that off-pump CABG surgery suppresses the increase in monocyte-platelet conjugates associated with on-pump CABG surgical procedures (Greilich *et al.,* 2008). Several monocyte-secreted cytokines, including IL-8, promote organ dysfunction. Lower cytokine levels, associated with off-pump CABG surgery, may lead to a reduced systemic inflammatory cascade, which result in fewer related complications (Laffey, Boylan, Cheng, 2002; Cheng, Bainbridge, Martin, Novick; Evidence-based peri-operative clinical outcomes research group, 2005).

Overall, it seems that post-operatively off-pump CABG surgery has lower concentrations of cytokines, inflammatory markers, neutrophils, and the patient has lesser chance to develop SIRS (Castellheim, Hoel, Videm, Fosse, Pharo, Svennevig, Fiane, Mollnes, 2008).

2.5.2 Disadvantages and complications of off-pump CABG surgery

Off-pump CABG surgery makes huge demands on the surgeon and it requires a high level of vigilance on the part of both the anaesthetist and the whole surgical team. An adequate exposure of the anastomosis site must be obtained with restrained cardiac motion and the myocardium has to be protected from ischaemia during coronary artery flow interruption. The heart must be displaced to compress the ventricular wall, and, if possible, the surgeon must use a technique that allows coronary perfusion while the anastomosis is performed. The anaesthetist must consequently be prepared to handle severe haemodynamic alterations, transient deterioration of cardiac pump function, and acute intra-operative myocardial ischaemia. The team must at all times be prepared for conversion to on-pump CABG in the case of either sustained ventricular fibrillation or cardiovascular collapse (Chassot, van der Linden, Zaugg, Mueller, Spahn, 2004).

Furthermore, off-pump CABG surgery causes tissue trauma through cardiac manipulation, pericardial suction, and physiological stress responses, and leads to elevated pro-inflammatory markers. But, altogether, the magnitude of the inflammatory response is significantly less with off-pump than with on-pump CABG surgery, because it induces not only lower activation of the inflammatory mediators but also reduced morbidity (Ascione *et al.,* 2000). There is no difference in risk-adjusted mortality, but a higher rate of repeat revascularisation in patients undergoing off-pump CABG surgery is evident. However, patients with severe left ventricular dysfunction requiring multiple grafts, and also patients with certain patterns of coronary artery disease (CAD) (e.g. diffuse disease, calcification, intramyocardial and small targets) may not be considered for off-pump CABG surgery (Lytle, 2007).

Another disadvantage that can result in significant morbidity, post-operatively, is hyper-coagulability. Injured tissue will release significant amounts of tissue factor into the circulation and during sternotomy there is an increase in the release of thrombin-antithrombin complexes and tissue plasmin activator. Furthermore, bleeding from the sternal bone will initiate pro-coagulant factors (Dewey & Edgerton, 2003).

2.5.3 Comparison between on-pump and off-pump CABG surgery

Pre-operatively the cardiologist now has to decide according to the history and current situation of the patient, which of the two surgical interventions would be more beneficial to the patient. Off-

pump CABG surgery offers a prognostic advantage over on-pump CABG surgery in high-risk patients with severe surgical risk from complicated CAD, and these patients have better outcomes. The pre-operative optimisation of high-risk patients plays a crucial role in determining the clinical outcome for both methods of myocardial revascularisation (Ngaage, 2003).

On-pump CABG surgery causes myocardial damaging effects and does not prevent post-operative cardiac dysfunction (Ngaage, 2003). Elderly patients and multi-morbid patients (Larmann & Theilmeier, 2004) are considered high-risk surgical patients because of their reduced functional capacity. Lytle (2007) has reached similar conclusions to those indicated by the bulk of previously published data. The short-term risks appear to be slightly less for patients undergoing off-pump CABG surgery, while the long-term results may be somewhat better for those undergoing on-pump CABG surgery. Lytle states that off-pump and on-pump CABG surgery are complementary techniques for the achievement of myocardial revascularisation, and each technique has its own advantages and disadvantages in patients with specific characteristics (Lytle, 2007).

2.6 Systemic inflammatory response syndrome (SIRS) and CABG surgery

SIRS is more often present post-operatively in some patients (for example in patients with metabolic syndrome and patients with pre-operative elevated TNF-α and PCT levels), and SIRS is moreover believed to contribute to morbidity and mortality (Parolari, Camera, Alamanni, Naliato, Polvani, Agrifoglio, Brambilla, Biancardi, Mussoni, Biglioli, Tremoli, 2007). SIRS may be initiated during CABG surgery and the inflammatory response is mediated by a complex interaction between the nervous, endocrine, immune, and haematopoietic systems (the system in the body which is responsible for the production of blood cells). A post-operative stress response can be related to the impact of surgical wounds and surgical conditions, e.g. (1) the type of surgery, (2) duration of the operation, (3) volume of blood loss, and (4) complications. The inflammatory response is further mediated by other contributory factors, such as an increase in (1) metabolic rate, (2) stress hormone levels, and (3) CRP levels. During and after CABG, various inflammatory cytokines are released. The cytokine known as IL-6 especially appears to be a major mediator (Takenaka *et al.,* 2006).

2.6.1 On-pump CABG surgery and SIRS

On-pump CABG surgery induces an inflammatory response and this results from the fact that the blood is exposed to large artificial surfaces of the bypass circuit and to the shear forces of the rollers

(Parolari *et al.,* 2007). In addition, multiple external factors (biomaterial-dependent and biomaterial-independent) are associated with the use of on-pump CABG surgery (table 2.1). These include: (1) the generation of shear forces from roller pumps driving blood through the bypass circuit; (2) hypothermia, as blood is passed through the extracorporeal circuit; and (3) contact activation of plasma protein systems, as circulating blood is exposed to artificial surfaces that contribute to the generation of possible peri-operative SIRS (Takenaka *et al.,* 2006; Weigand, Hörner, Bardenheuer, Bouchon, 2004).

Table 2.1 Factors influencing the systemic inflammatory response during on-pump CABG surgery (Adapted from Raja & Dreyfuss, 2005)

Factors influencing systemic inflammatory response	
Biomaterial-dependent factors*	Type of extracorporeal circuit Type of oxygenator and pump
Biomaterial-independent factors	Extracorporeal perfusion factors Composition of the priming solution Cardioplegia Pulsatile or non-pulsatile perfusion Temperature during on-pump CABG
Pre-operative factors	Morbid conditions
Peri-operative haemodynamic factors	Low cardiac output Splanchnic hypo-perfusion
Anaesthetic techniques	Thoracic epidural anaesthesia Anaesthetic agents and drugs Lung management during on-pump CABG surgery
Surgical factors	Incision and approach Duration Cardiotomy blood management
Shear stress	
Transfusion	
Post-operative factors	Continuous renal replacement therapies Mechanical ventilation

*Related to the composition of the synthetic surface of the circuit, such as poor left ventricular function or diabetes.

Post-operatively, there are three very distinct mechanisms present in activating the inflammatory response:

- Activation of the immune system, following the exposure of the blood components with the circuit
- I/R injury to the end-organs as a result of aortic cross-clamping
- Endotoxaemia may indirectly activate the inflammatory cascade (Raja & Dreyfuss, 2005).

It would thus seem that the post-operative inflammatory response associated with on-pump CABG surgery is activated by the surgical trauma itself, by blood loss or transfusion, and by hypothermia (Takenaka *et al.,* 2006; Weigand *et al.*, 2004).

SIRS is often present in critically ill patients, especially those patients in an intensive care unit (ICU) (Castelli, Condemi, Munari, Savi, Carro, Vanelli**,** 2001). Such patients frequently present with clinical characteristics of infection, organ dysfunction, and various severities of SIRS (Table 2.2). Differences in body temperature, heart rate, WCC, and respiratory rate can also be detected (Robertson & Coopersmith, 2006).

Table 2.2 Consensus definition of a spectrum of clinical entities that result in organ failure (Adapted from Robertson & Coopersmith, 2006)

Sepsis	**SIRS caused by infection**
Severe sepsis	**Sepsis with at least one organ dysfunction or hypo-perfusion**
Septic shock	**Severe sepsis associated with hypotension that is resistant to adequate fluid resuscitation**
Bacteraemia	**The presence of viable bacteria in the blood stream**
MODS	**Impairment of two or more organ systems in an acutely ill patient where homeostasis cannot be maintained without therapeutic intervention**

SIRS is deemed to be present if two or more of the following four criteria are present:

- temperature >38°C or <36°C,
- heart rate >90 beats/minute,
- respiratory rate >20 breaths/minute or $PaCO_2$ <4.3 kPa, or
- WCC <4 x 10^9/l or >12 x 10^9/l or >10% immature forms (Takenaka *et al.,* 2006).

2.6.2 Off-pump CABG surgery and SIRS

Off-pump CABG surgery has been argued to reduce the many inflammatory responses associated with the use of the EC and the ischaemia/reperfusion injury associated with cardioplegic arrest and non-physiological flow (Vallely *et al.*, 2001). It is associated with reduced post-operative rise in markers of SIRS, such as C3a, C5a, TNF-α, or IL-6 and IL-8. However, there is still uncertainty regarding the significance of these data, because IL-8 and C3a are linked to the amount of surgical tissue injury, IL-10 has anti-inflammatory properties and heparin inhibits complement activation (Chassot *et al.,* 2004). Although, off-pump CABG surgery post-operatively presents with a lower inflammatory response (Vallely *et al.*, 2001) it is not necessarily associated with marked benefits such as decreased morbidity or post-operative mortality (Quaniers *et al.,* 2006).

2.6.3 Inflammatory markers associated with SIRS

Patients with a heavy burden of CAD would display definitely higher inflammatory markers than those without. Therefore, inflammatory markers can be seen as a very important determinant of a patient's present and future outcomes (Tziakas, Chalikias, 2007; Cusack, Marber, 2002; Richter, Meisner, Tassani, Barankay, Dietrich, Braun, 2000). VCAM-1, ICAM-1 and endothelial leukocyte adhesion molecule-1 (E-selectin) are known to modulate cell-endothelium interactions. These cell-adhesion molecules (CAMs) play an intrinsic role in adhesion and transendothelial migration of monocytes, which together result in the formation of fatty streaks, and the subsequent release of soluble forms of adhesion molecules of circulating CAMs (cCAMs). Elevated levels of cCAMs are present in individuals with Type 2 diabetes mellitus, hypertension, and in individuals who smoke. The surface expression of CAMs has a common response to a variety of stimuli, such as, for example, cytokines. In elderly patients the leukocytes produce elevated IL-1, IL-6 and TNF-α after stimulation with lipopolysaccharide. Thus the adhesion of monocytes to endothelial cells induces the expression and release of IL-1β by monocytes, which, in turn, stimulate IL-6 by endothelial cells and the up-regulation of VCAM-1 on the endothelial surface (Richter *et al.,* 2000). The cytokines often associated with release during and after on-pump CABG surgery include TNF-α, IL-1, IL-2, IL-6, IL-8, and IL-l0 (Hall, Smith, Rocker, 1997).

2.6.4 Cytokines and SIRS

2.6.4.1 Inflammatory cytokines

Inflammatory cytokines like TNF-α stimulate the secretion of other pro-inflammatory cytokines, including, IL-1, IL-6, IL-8, IL-10 and IL-12. IL-6, specifically, is a multifunctional pro-inflammatory cytokine that plays an important role in I/R injury (Ishida, Kimura, Imamaki, Ishida, Shimura, Kohno, Sakurai, Miyazaki, 2006). These cytokines play a pivotal role in the inflammatory cascade and are released from macrophages within the vessel wall during an inflammatory response. Distant inflammatory effects are mediated, including activation of hepatic genes encoding the acute phase reactants fibrinogen and CRP. As part of the acute-phase reaction, IL-6, IL-8, and TNF-α consequently release neutrophils from the bone marrow (Diegeler *et al.,* 2000).

2.6.4.2 Anti-inflammatory cytokines

Anti-inflammatory cytokines, such as IL-4, IL-10, IL-11 and IL-13, will inhibit the inflammatory processes by reducing the pro-inflammatory cytokines. Cytokines may also influence the production of other cytokines and further also induce the expression of cytokine receptors and enzymes, like inducible NO synthase and cyclooxygenase-2. These enzymes, in turn, contribute to the inflammatory response (Castellheim *et al.,* 2008). Inflammatory markers, such as Procalcitonin (PCT), CRP and full blood count (FBC), have been found to be good markers for the detection of SIRS in a patient (Diegeler *et al.,* 2000). CRP induces synthesis of cytokines, CAMs, and tissue factor in monocytes and endothelial cells. The TF activates the extrinsic cascade, providing a link between inflammation and thrombosis (Gonzalez, Selwyn, 2003; Koh, Han, Quon, 2005). Therefore, SIRS can be present more often in some post-intervention patients, e.g. in patients with metabolic syndrome. Pre-operative elevated TNF-α, micro-albuminuria and PCT are believed to contribute to post-operative morbidity and mortality (Parolari *et al.,* 2007).

2.6.5 C-reactive protein and SIRS

According to Biancari and fellow researchers, a patient with a high pre-operative CRP level of >10 mg/l may be associated with a significantly increased risk of post-operative infection, low cardiac output syndrome and overall post-operative death. Furthermore, an elevated CRP level, pre-operatively, can result from an inflammatory process or from active atherosclerotic lesions (Biancari, Lahtinen, Lepojärvi, Rainio, Salmela, Pokela, Lepojärvi, Satta, Juvonen, 2003).

SIRS accelerates the atherosclerotic process and causes an increase in plasma CRP levels. CRP can therefore be seen as a useful marker for risk stratification to identify patients at risk of atherosclerotic complications, for the prediction of future coronary events (Tardiff, Heinonen, Orloff, Libby, 2006; Blake & Ridker, 2002), and, further, to confirm the presence of acute organic diseases, such as MI, deep-vein thrombosis and infection or chronic conditions (Whicher, 1998). A strong and consistent relationship between inflammatory markers and the risk of future coronary vascular events can thus be implicated (Tardiff *et al.,* 2006; Blake & Ridker, 2002).

Furthermore, CRP is also useful in the diagnosis and monitoring of post-operative infectious complications within the setting of an intensive care unit (ICU) when microbiological testing is either too slow or impossible (Whicher, 1998). Increased plasma CRP concentrations, post-operatively, are

indicators of the following: (1) an acute or chronic inflammation, which may be accompanied by bacterial infections (the most potent stimulus to CRP production); (2) autoimmune or immune complex disease; and (3) tissue necrosis (Whicher, 1998).

2.6.6 Procalcitonin and SIRS

Early differentiation between SIRS after cardiac surgery, and the development of peri-operative infection are crucial towards enabling appropriate antibiotic therapy initiation and prevention of subsequent complications. PCT is therefore helpful in differentiating between infectious and non-infectious causes of complications in patients, and in differentiating between SIRS, sepsis, severe sepsis, and septic shock. PCT levels can further increase with the increasing severity of the inflammatory response to the infection, and are therefore helpful in assessing the severity of the infection, the prognosis of the disease, and the response to therapeutic measures (Reinhart & Karzai, 2000).

Irrespective of the surgical technique used, an increase in serum PCT concentration after cardiac surgery appears to be related to post-operative SIRS. PCT is thus related to the cytokine cascade and to endotoxin release as induced by the surgical procedure itself (Aouifi, Piriou, Blanc, Bouvier, Bastien, Chiari, Rousson, Evans, Lehot, 1999) and not by the ischaemia. According to Macrina and co-researchers it is important to emphasise that there is not yet an established normal range of PCT concentrations - after cardiac surgery - with which to define the cut-off values that are useful for the identification of complications including infections (Macrina, Tritapepe, Pompei, Sciangula, Evangelista, Toscano, Criniti, Brancaccio, Puddu, 2005).

Some recommendations regarding PCT as an inflammatory marker:

- Cut-off values for clinical decision-making are essential and must be context specific.
- “Normal” values, in general are <0.5 µg/l (Whicher, Bienvenu, Monneret, 2001).

Following surgery and trauma:

- In the first four days after trauma or surgery, levels may rise to <10 µg/l.
- Levels higher than this suggest infection (Whicher, *et al.*, 2001).

2.6.7 Tumor necrosis factor-α and SIRS

Although TNF-α is currently a less firmly established test, it can be used as a marker for SIRS, in conjunction with sepsis, trauma and heart failure (BioSource, 1999; Rostami, 2002). An elevated TNF-α concentration is an indicator for an ongoing chronic inflammatory response, indicating an excessive and/or prolonged inflammatory reaction with systemic manifestation. In patients with heart failure, an elevated TNF-α level is characteristic of a subgroup of patients with pronounced cachexia and a significantly poorer prognosis (Ridker, Rifai, Pfeffer, Sacks, Lepage, Braunwald, 2000; Volk, Keysser, Burmester, 1998).

2.6.8 Summary of SIRS and CABG surgery

If the balance tips too far in the direction of a prolonged amplified inflammation, the inflammatory events will ultimately lead to SIRS, which is characterised by tachycardia, fever or hypothermia, leukocytosis or leukopaenia and tachypnoea (Larmann & Theilmeier, 2004). The inflammatory response to on-pump CABG surgery may also produce dysfunction of multiple organ systems, the collective clinical picture being termed “post-perfusion syndrome”. The most common clinical manifestation of this syndrome is seen in the respiratory system, possibly of ischaemic injury to the lungs’ endothelial cells, which may cause acute pulmonary failure - also known as acute respiratory distress syndrome (Larmann & Theilmeier, 2004). In acutely ill patients, an alteration of organ function can develop in such a way that homoeostasis cannot be maintained and this is known as multiple organ dysfunction syndrome (MODS). MODS affects physiologic systems not classically thought of as organs, including the haematologic system, the immune system or the endocrine system. Therefore, organ dysfunction after on-pump CABG surgery correlates with the activation of the humoral mediators of inflammation, with the subsequent involvement of neutrophils and endothelial cells resulting in tissue damage, elevated plasma C3a levels and elevated TNF-α (Marshall, 2001).

However, there are many anti-inflammatory strategies to reduce the inflammatory response and the incidence of SIRS resulting from on-pump CABG (Miller & Levy, 1997). In comparison with on-pump CABG surgery, off-pump CABG surgery permits minimal invasive surgery because of the avoidance of blood contact activation with the bypass circuit, and also ischaemia/reperfusion injury, thereby setting the stage for both a lesser inflammatory response and a reduced chance of developing SIRS (Larmann & Theilmeier, 2004).

2.7 Inflammatory markers useful in the detection of an inflammatory response during CABG surgery

An inflammatory response is expected after CABG surgery, and it can sometimes be difficult to distinguish between an infection and an inflammatory response. The immune system consists of two main parts: the inflammatory cells (granulocytes, lymphocytes and monocytes/macrophages) and the soluble mediators (cytokines e.g. IL-6, acute-phase proteins and complement factors) (Vedin, 2006). Cytokines, e.g. IL-6, are mediators of metabolic, immunological and endocrine responses to an inflammatory injury (Vallely *et al.*, 2001). The granulocytes can be subdivided into three different cell types: neutrophils, eosinophils and basophils (Vedin, 2006). Non-specific inflammatory markers, e.g. CRP (marker of the acute-phase response) have been used to compare the SIRS differences between on-pump CABG surgery and off-pump CABG surgery post-operatively (Vallely *et al.*, 2001).

2.7.1 C-reactive protein (CRP)

2.7.1.1 Mechanism and prognostic value of CRP

CRP is a sensitive, non-specific, prototypical acute-phase inflammatory protein (Takahashi, Anzai, Yoshikawa, Maekawa, Asakura, Satoh, Mitamura, Ogawa, 2003) that has been identified as a useful inflammatory marker, especially pre-operatively, in the context of atherosclerosis (Tardiff *et al.,* 2006). CRP has a half-life of 5 - 7 hours, lack of diurnal variation, and independency towards age and sex (Biasucci, Liuzzo, Grillo, Caligiuri, Rebuzzi, Buffon, Summaria, Ginnetti, Fadda, Maseri, 1999), and is produced in the liver through the stimulation of various cytokines, such as IL-1, TNF-α (Takahashi *et al.*, 2003), and also IL-6. IL-6 is specifically involved in both the stimulation of CRP and its release from the adipose tissue in response to the innate immune system (Gortney & Sanders, 2007).

There is a direct relation between elevated CRP levels and adipocyte secretion in obese individuals, especially in those individuals with pre-operative risk factors, such as metabolic syndrome, smoking and being Type 2 diabetics. These high-risk individuals have higher baseline values than have healthy individuals and have twice the risk of developing CHD (Blake & Ridker, 2002; Gortney & Sanders, 2007). According to the relevant literature, CRP levels can be seen as an independent predictor of short-term and long-term mortality (Gortney & Sanders, 2007). Elevated CRP levels, especially, may predict recurrent instability among patients admitted with ACS or undergoing invasive procedures, such as percutaneous coronary angioplasty, stenting or CABG surgery (Piccoli,

Cerquetani, Pastena, Posteraro, Amici, Romeo, La Carrubba, Salustri, 2008). Raised circulating levels of CRP may be useful to predict future coronary events in patients with stable angina or unstable angina (UA) (Whicher, 1998).

In an acute event, the plasma CRP concentrations elevate after six hours, reach a peak at 48 hours and decline by half over the next 48 hours. A single measurement is therefore only useful to indicate inflammation within about three days of an acute event. With the incidence of a MI, the CRP concentration elevates within a few hours after the onset of pain. It peaks on the third or fourth day (Whicher, 1998). CRP levels will fall towards normal over a period of 7-10 days, and if not, post-operative elevated CRP levels can be an indication of an infection or of thromboembolic complications (Biancari *et al.*, 2003; Kangasniemi, Biancari, Luukkonen, Vuorisalo, Satta, Pokela, Juvonen, 2006).

2.7.1.2 C-reactive protein and on-pump CABG surgery

Although an elevated CRP level, pre-operatively has been associated with an unfavourable outcome after CABG surgery (Piccoli *et al.*, 2008), it is still the most important predictor of post-operative complications (Fransen, Maessen, Elenbaas, 1999; Boeken, Feindt, Zimmermann, Kalweit, Petzold, Gams, 1998; Aouifi *et al.,* 1999), particularly where the inflammatory response is due to I/R injury, endotoxaemia, hypothermia, contact of the blood with the artificial surface of the bypass circuit and the surgical trauma itself (Piccoli *et al.*, 2008). Although, the interpretation of CRP values in the on-pump CABG surgery setting remains difficult to interpret (Piccoli *et al.,* 2008), patients with elevated CRP levels pre-operatively should not be considered for on-pump CABG surgery unless there is an urgent indication of uncomplicated post-operative recovery (Boeken *et al.,* 1998).

Elevated CRP levels - without an infection - may also be induced by post-operative pain resulting from the surgical procedure itself (Celebi, Koner, Menda, Balci, Hatemi, Korkut, Esen, 2006; Vanek, Brucek, Straka, Klepetar, Maly, 2002).

2.7.1.3 C-reactive protein and off-pump CABG surgery

One of the major reasons for performing off-pump CABG surgery in preference to on-pump CABG surgery is to reduce the systemic inflammatory response induced by the EC, and to improve post-operative organ function and patient outcome, especially in high-risk patients (Raja & Dreyfus,

2005). Several studies have revealed the benefit of off-pump CABG surgery regarding the lower release of pro-inflammatory cytokines and, based on these findings CRP is released mainly because of both the surgical trauma itself and also the anaesthesiological management, and its release is only moderately increased with the use of off-pump surgery (Serrano, Souza, Lopes, Fernandes, Nicolau, Blotta, Ramires, Hueb, 2009).

2.7.2 Procalcitonin (PCT)

2.7.2.1 Mechanism and prognostic value of PCT

PCT is a 116-amino-acid protein (Meisner, Tschaikowsky, Schmidt, Schuttler, 1996), produced in the C-cells of the thyroid gland, and acts as the precursor of calcitonin. PCT has a longer *in vivo* half-life of between 24-32 hours post-operatively (Jebali, Hausfater, Abbes, Aouni, Riou, Ferjani, 2007) in blood than does calcitonin, which has a short half-life of 10 minutes (Meisner, 2000). In comparison with tests - such as CRP, IL-6 or FBC - PCT is used to monitor patients at risk of sepsis. PCT has been reported to rise earlier than CRP after the onset of sepsis. During controlled sepsis, PCT decreases earlier than CRP (Jebali *et al.*, 2007). A specific protease cleaves all PCT to calcitonin, katacalcin, and an N-terminal residue. Under normal circumstances PCT levels are therefore undetectable (<0.1 ng/ml) in healthy humans. PCT concentrations >0.5 ng/ml indicate an acute infection (Meisner, 2000). A PCT value of >1.5 ng/ml on the second post-operative day, strongly indicates an infectious complication. However, the origin of the infection is not revealed and must be found as quickly as possible (Jebali *et al.*, 2007).

During severe infections presenting with systemic manifestations, PCT levels may increase until they exceed 100 mg/l. Remarkably, large amounts of PCT produced during infections do not cause an increase in either plasma calcitonin levels or activity (Reinhart & Karzai, 2000). Elevated PCT levels can be seen in severe cases of bacterial, non-bacterial, sepsis and also in multi-organ dysfunction syndrome (MODS) (Jebali *et al.*, 2007; Boeken, Feindt, Micek, Petzold, Schulte, Gams, 2000). Therefore, PCT is used to follow up patients with severe bacterial infections, sepsis or septic shock (Meisner, Rauschmayer, Schmidt, Feyrer, Cesnjevar, Bredle, Tschaikowsky, 2002). PCT levels may elevate after exposure to conditions of haemorrhagic shock, and also after CABG surgery as a result of translocation of bacteria or of bacterial products caused by poor gut perfusion (Reinhart & Karzai, 2000). A weak stimulation of PCT can be detected in patients with cardiac failure. PCT may also have prognostic value in cardiac-assist devices by indicating those patients with septic hypotension and poor prognosis (Meisner *et al.,* 1996).

2.7.2.2 Procalcitonin and on-pump CABG surgery

On-pump CABG surgery *per se* induces an acute inflammatory response that may lead to SIRS, and elevated PCT levels may be expected. Because of this inflammatory response, conventional clinical and biological signs may be misleading in the diagnosis of post-operative complications, particularly infection. Patients may present post-operatively with increased PCT levels without any evidence of severe infections (Meisner *et al.,* 1996). In a study done by Aouifi and colleagues, a moderate to temporary increase was detected in serum PCT levels 24 hours post-operatively. This resulted from the inflammatory response triggered by the surgical procedure itself and was not related specifically to on-pump CABG surgery (Aouifi *et al.*, 1999).

2.7.2.3 Procalcitonin and off-pump CABG surgery

Elevated PCT levels after surgical procedures may be explained by normal PCT kinetics. Therefore, in both on-pump CBAG surgery and off-pump CABG surgery an acute phase response and cytokine production (especially IL-6) is present (Fransen, Maessen, Dentener, Senden, Geskes, Buurman, 1998) this leading to an increase in PCT levels after cardiac surgery, which is related to the cytokine cascade, rather than to the surgical trauma itself or anaesthetics. Although, both CABG surgical procedures induce an inflammatory response in patients post-operatively, lower PCT levels have been noted after off-pump CABG surgery (Bitkover, Hansson, Valen, Vaage, 2000). This might be as a result of minimised surgical trauma in comparison with on-pump CABG surgery.

2.7.3 Full blood count (FBC)

2.7.3.1 Mechanism and prognostic value of FBC

A full blood count (FBC) is an inexpensive, readily available, accessible circulating marker of an inflammatory state and has been correlated with CHD since the 1920s. But in general, it is underutilised for its risk predictive ability as an inflammatory marker (Anderson, Ronnow, Horne, Carlquist, May, Tami, Bair, Jensen, Muhlestein, 2007). The standard FBC measures haematocrit, haemoglobin, neutrophil/lymphocyte (N/L) ratio, WCC, platelet count and the leukocyte count, consisting of the following: (1) lymphocytes, (2) neutrophils, (3) monocytes, (4) basophils and (5) eosinophils (Anderson *et al.,* 2007; Gibson *et al.*, 2007).

2.7.3.2 Leukocytes

A FBC is an independent predictor of cardiovascular events in healthy subjects - even in those with stable and unstable coronary syndromes. Leukocytes play a major role in such inflammatory processes and form an important part of the immune defences (Anderson *et al.,* 2007). There seems to be a strong link in the prognosis of patients who have stable CHD after a previous MI, and between leukocyte counts and the risk of AMI. High leukocyte counts are associated with an increased risk of re-infarction or death, even after adjusting for confounding risk factors. In general, the risk of developing CHD is two times higher in patients with WCC of 8 to 10 x 10^9/l, compared with patients with counts of 4 - 6 x 10^9/l (Shankar, Mitchell, Rochtchina, Wang, 2007).

An elevated leukocyte count also correlates positively with cigarette smoking, serum total cholesterol, serum triglycerides, clotting factors, haematocrit, fasting glucose levels, diastolic blood pressure, serum LDL cholesterol, forced expiratory volume, forced vital capacity, and height. Thus, elevated leukocyte levels have been shown to be independent predictors of CHD, and prognostic indicators of future cardiovascular outcomes (Shankar *et al.,* 2007).

2.7.3.3 Leukocytes and CABG surgery

Irrespective of the technique used, leukocytes are activated during CABG surgery. Comparison of on-pump and off-pump CABG surgery clearly reveals that both groups have elevated leukocyte levels post-operatively, even if higher levels are admittedly present in the on-pump CABG group (Lim, Anderson, Leong, Pepe, Salamonsen, Rosenfeldt, 2007). Prolonged mechanical ventilation during on-pump CABG surgery may consequently result in a further retention of leukocytes in the pulmonary bed (Tomic *et al.*, 2005). Leukocyte filters during on-pump CABG surgery have the potential to remove leukocytes, thereby reducing contact of activated leukocytes with the endothelium of target organs (Lim *et al.,* 2007).

2.7.3.4 Haematocrit and white cell count (WCC)

The haematocrit and WCC have individually been proposed as risk markers for cardiovascular disease (Anderson *et al.,* 2007). An elevated WCC can be seen as a positive predictor of mortality post-operatively (Madjid, Awan, Willerson, Casscells, 2004; Dacey, DeSimone, Braxton, Leavitt, Lahey, Klemperer, Westbrook, Olmstead, O'Connor, 2003). Patients with metabolic syndrome

(insulin-resistant group) have more than a three-fold risk of cardiovascular mortality. These patients present with a combination of both high WCC (6.8×10^9 cells/l and above) and triglyceride levels (1.98 mmol/l and above) (Shankar *et al.,* 2007). These patients are at increased risk of diabetes and also future cardiovascular events, this resulting both in a general decline in quality of life and an increase of the burden on all health care systems (Lohsoonthorn, Dhanamun, Williams, 2006).

2.7.3.5 White cell count in CABG surgery

During both on-pump and off-pump CABG surgery, the total WCC and the numbers of circulating neutrophils increase as a result of the inflammatory response being triggered by the surgery itself. However, a marked increase in the WCC is noted during on-pump CABG, peaking especially after 36 to 60 hours, post-operatively, and this possibly results from the use of the bypass circuit. Activated platelets are being deposited onto the endothelial cells, which again attach to the circulating leukocytes via P-selectin (Abdelhadi, Gurm, Van Wagoner, Chung, 2004). Consequently, leukocyte-platelet conjugates form and increase during aortic cross-clamping and on-pump CABG surgery (Larmann & Theilmeier, 2004).

2.7.3.6 Platelets

Platelets play a critical role in atherothrombotic disease, and extreme platelet counts are well recognised as risk factors for haemorrhage (with very low counts) or for thrombosis (with very high counts). Platelet indexes have specifically been linked to cardiovascular risk. Although individual parameters might provide modest predictive ability, a complete count has more substantial advantages (Anderson *et al.,* 2007).

2.7.3.7 Platelets in CABG surgery

During CABG surgery, platelets are probably initially activated by thrombin, which is the most potent platelet agonist. During on-pump CABG surgery the artificial surface of the bypass circuit and the membrane oxygenator are important sources for both platelet and leukocyte activation (Serrano *et al.,* 2009). Furthermore, platelets possess several protease-activated receptors to most of these agonists and to collagen that plays an important role in adhesion and thrombus formation. Elevated platelet to platelet aggregation, and also aggregation platelet to monocytes, and neutrophils to monocytes can be seen especially in on-pump CABG surgery (Menasche & Edmunds, 2003). One of

the advantages of off-pump CABG surgery is the reduction of bleeding and less need for transfusion. In on-pump CABG surgery the EC impairs the coagulation system by damaging the plasma factors and platelets. In off-pump CABG surgery the coagulation system remains undamaged and therefore the same numbers of pro-coagulative inductors (tissue factor) are exposed in systemic and coronary circulation (Suwalski, Suwalski, Filipiak, Postuła, Majstrak, Opolski, 2008).

2.7.3.8 Lymphocytes in CABG surgery

Activated lymphocytes play an important role in the inflammatory response and in the immune response triggered by CABG procedures. In the immune system, the lymphocytes are specific for each antigen and present a slow response, while the innate immune system is non-specific and presents a rapid response. Lymphocytes can be divided into T-lymphocytes and B-lymphocytes. The main purpose of T-lymphocytes is to fight infected or mutated cells, while B-lymphocytes are responsible for the humoral response, producing antibodies that act on extra-cellular elements, such as toxins and bacteria (Blacher, Neumann, Jung, Lucchese, Ribeiro, 2005).

Activated lymphocytes during on-pump CABG surgery are associated with an immune response. This immune response is due to the I/R injury that occur in the heart and in the lungs during the surgery. During off-pump CABG surgery, there is a similar degree of lymphocyte activation because of the surgical trauma itself and not because of the use of cardiopulmonary bypass *per se* (Kotani, Hashimoto, Sessler, Muraoka, Wang, O'Connor, Matsuki, 2000 & Blacher *et al.,* 2005).

2.7.3.9 Monocytes in CABG surgery

Monocytes/macrophages and T-lymphocytes are prevalent and pathogenic within unstable coronary artery plaques (Anderson *et al.,* 2007). Monocytes and macrophages (tissue monocytes) are relatively large, long-lived cells that are involved in both acute and chronic inflammation. Monocytes respond to chemical signals, are mobile, phagocytise micro-organisms and cell fragments, produce and secrete chemical mediators, participate in the immune response, and generate cytotoxins (Biglioli *et al.*, 2003).

Monocytes, being activated during on-pump and off-pump CABG surgery, play a major role in thrombin formation. Continuous oozing and bleeding from the cut edges and particularly from the sterna spongiosa into the surgical field, even after meticulous haemostasis, may further activate

monocytes after contact with the pericardium (Biglioli *et al.*, 2003). Monocytes also produce and release many inflammatory mediators during acute inflammation. These mediators include pro-inflammatory cytokines (principally TNF-α, IL-1ß, IL-6, IL-8, and MCP-1), reactive oxygen and nitrogen intermediates, and prostaglandins. Monocytes also possess CRP receptors, which, when activated, strongly upgrade pro-inflammatory cytokine production. Monocytes are the major source of the early-response cytokines IL-1ß and TNF-α, which play an important role in directing both neutrophils and monocytes to local sites of inflammation. Monocytes are also the major producer of IL-8, which, too, is produced by the neutrophils and induces neutrophil chemotaxis (Menasche & Edmunds, 2003).

By using cell savers (Greilich *et al.,* 2008) during off-pump CABG surgery, the cellular inflammatory response is further attenuated, consequently decreasing the neutrophil and monocytes counts, neutrophil elastase, and E-selectin concentrations (Raja & Dreyfus, 2005). With the avoidance of anti-fibrinolytic agents (with anti-inflammatory properties) during on-pump CABG, the monocytes levels are elevated and the monocytosis is more brisk in the on-pump CABG group than in the off-pump CABG surgery group (Greilich *et al.,* 2008).

2.7.3.10 Platelet-monocyte conjugate formations in CABG surgery

During on-pump CABG surgery, leukocyte-depleting filters cause monocytes and platelets to bind together, initiating a platelet-monocyte conjugate formation. This formation takes place through direct adhesion and the induction of thrombin and fibrin, thus avoiding vascular compromise, e.g. thrombosis (Greilich *et al.*, 2008; Biglioli *et al.*, 2003; Weerasinghe, Athanasiou, Philippidis, Day, Mandal, Warren, Anderson, Taylor, 2006). This platelet-monocyte conjugate formation is at its lowest at two hours post-operatively. Such suppression of monocyte activation and adhesion may lead to a reduction of post-operative complications. Especially in those high-risk patients with pre-operative risk factors, the depletion of this formation might play a role in post-operative bleeding (Tatoulis, Rice, Davis, Goldblatt Marasco, 2006). Off-pump CABG surgery, in comparison with on-pump CABG surgery, is associated with less post-operative bleeding; the contribution of monocytes to post-operative bleeding may therefore potentially play a role in this difference (Weerasinghe *et al.,* 2006).

2.7.3.11 Basophils in CABG surgery

Other cells, such as mast cells and basophils are also involved in the inflammatory response during cardiac surgery (Levy & Tanaka, 2003). Basophils are related to delayed hypersensitivity response and are triggered by IgE binding to antigen.

Basophils release inflammatory mediators such as:

- pre-formed mediators (e.g. histamine, bradykinin)
- new mediators (e.g. prostaglandins, leukotrienes)
- chemotactic factors (attracts neutrophils and eosinophils)

The numbers of the basophils increase during an inflammatory process, and will accumulate at the site of inflammation. At this site, the basophils will discharge the contents of their granules, releasing a variety of mediators, such as:

- histamine
- serotonin
- prostaglandins, and
- leukotrienes

The release of these mediators will increase the blood flow to the inflammatory area and contribute to the inflammatory process (Moses, 2008).

2.7.3.12 Neutrophils in CABG surgery

Neutrophils, as main inflammatory cells, are also present during sepsis (Levy & Tanaka, 2003) and arrive at the scene of inflammation to begin the process of phagocytosis and the release of cytotoxins (Menasche & Edmunds, 2003). Neutrophils may also be pathogenic, e.g. with leukocyte-platelet aggregation formation, and are therefore intimately involved with infarct healing resulting from reperfusion injury (Anderson *et al.,* 2007).

An elevated neutrophil count and a depressed lymphocyte count during CABG may be associated with a worsened outcome post-operatively, especially in the setting of on-pump CABG surgery (Gibson *et al.*, 2007; Murphy & Angelini, 2004). Furthermore, during on-pump CABG surgery the

activated neutrophils will infiltrate the myocardium and consequently contribute to the reperfusion injury, which occurs after the re-opening of the aorta (Serrano *et al.*, 2009).

During on-pump CABG surgery, leukocyte counts decrease in response to haemodilution and increase moderately after operation and only a few neutrophils attach to synthetic surfaces, to each other, or to platelets and monocytes. The organs and tissues experience periods of ischaemia followed by reperfusion (lung, heart and brain), and as a result, express adhesion receptors and reactive oxidants, and are sources of neutrophil chemo-attractant. Neutrophils also produce arachidonate metabolites, prostaglandins, leukotrienes, and platelet-activating factor (PAF). Furthermore, vasoactive and cytotoxic substances are produced and released into both the extracellular environment and the circulation (Menasche & Edmunds, 2003).

Neutrophils may also accumulate in the ischaemic and reperfused myocardium, participating directly in myocardial injury through the forming of aggregates that alter the flow properties of blood in the microvasculature. The released neutrophils will consequently activate substances, such as oxygen free radicals, proteases, and pro-inflammatory cytokines (Levy & Tanaka, 2003; Dacey *et al.*, 2003). The total WCC and neutrophil counts were found to be higher in the on-pump CABG group, probably owing to the activated neutrophils becoming “trapped” within the tissues during on-pump CABG surgery (Al-Ruzzeh, Hoare, Marczin, Asimakopoulos, George, Taylor, Amrani, 2003*).*

2.7.3.13 Neutrophil/lymphocyte (N/L) ratio in CABG surgery

The N/L ratio is a simple index of the systemic inflammatory response. On-pump and off-pump CABG surgery are both associated with an acute-phase reaction of protease cascades, leukocyte and neutrophil activation, and also an elevation in the N/L ratio and in platelet activation. During on-pump CABG surgery, the N/L ratio is elevated and this may further contribute to tissue injury, which may post-operatively reflect on a chronic inflammatory process (Gibson *et al*, 2007). Although the N/L ratio may assist with risk stratification in patients undergoing surgical revascularisation, it is as yet unclear whether the elevation of the N/L ratio directly contributes to the observed poorer outcome (Gibson *et al*, 2007).

2.7.3.14 Leukocytes and SIRS

The activation of leukocytes, especially the neutrophils, plays an intrinsic role in the initiation of SIRS. Endothelial cells and leukocytes are immediately activated during on-pump CABG surgery as a result of the blood that comes into contact with the artificial surfaces and rollers of the bypass circuit. This leads to the activation of the complement system, coagulation cascades and various other mediators. Leukocytes are further aggravated by myocardial ischaemia and endothelial cells during aortic cross-clamping. All of these mediators are major sources of an inflammatory response during surgery (Samankatiwat, Samartzis, Lersithichai, Stefanou, Punjabi, Taylor, Gourlay, 2003).

2.7.4 Tumor necrosis factor-α (TNF-α)

2.7.4.1 Mechanism and prognostic value of TNF-α

Human TNF-α, also named cachectin, is a 57 a.a unglycosylated polypeptide cytokine, associated with coronary atheroma (BioSource, 1999; Rostami, 2002). TNF-α, a potent pro-inflammatory cytokine, is mainly produced by monocytes/macrophages, endothelial cells, smooth muscle cells, macrophages, adipose cells, and also by B-cells and T-cells and by fibroblasts (Biglioli *et al.*, 2003). It acts as a potent pyrogen, and activates neutrophils and vascular endothelial cells (Biglioli *et al.*, 2003).

The various biological activities of TNF-α may be classified as:

- *Antitumoral and growth regulatory activities:* TNF-α displays a selective toxicity for tumour and virus-infected cells. Conversely, TNF-α is angiogenic and stimulates the growth of cultured fibroblasts (BioSource, 1999).
- *Immunomodulatory and pro-inflammatory activities*: TNF-α activates macrophages, neutrophils and eosinophils, and also endothelial cells (that display procoagulant activity). It regulates the production of antibodies by B-cells and stimulates cytotoxic T-cells. It induces the production of several other inflammatory mediators such as IL-1, IL-6, colony stimulating factors, prostaglandins, PAF, collagenases (BioSource, 1999).
- *Metabolic activities*: TNF-α strongly inhibits lipoprotein lipase and adipocyte gene expression (BioSource, 1999).
- *TNF-α has a major pathogenic role*: In cachexia it is associated with chronic infectious diseases, in septic shock, where the neutralisation of TNF-α protects against the associated acute lethality, in graft rejection and graft-versus-host disease, and in

parasitic infections where TNF-α may provide some protection but also favours more severe forms of the disease (e.g. the cerebral form of malaria). TNF-α in combination with other cytokines, has been involved in several auto-immune diseases and even in the pathogenesis of arteriosclerosis (BioSource, 1999).

Abnormally high levels of serum TNF-α have been described in septic shock, graft rejection, parasitic infections, cancer, post-haemofiltrations and during *in vivo* cytokine (IL-2) therapy. Besides potentially providing an insight into pathogenesis, these determinations might also aid diagnosis (e.g. in graft rejection) and have prognostic value, e.g. in systemic infections (BioSource, 1999).

Obese individuals may, for example, have one or more of the following complications: hypertension, insulin resistance and impaired glucose metabolism. These complications contribute to the pathophysiology of the heart (Rostami, 2002). Adipose tissue has been implicated to contribute to the elevation of circulating TNF-α level in obesity. Furthermore, monocyte recruitment is enhanced into developing atherosclerotic lesions linking obesity with atherosclerosis (Blake & Ridker, 2002).

2.7.4.2 Tumor necrosis factor-α and on-pump CABG surgery

Several studies have investigated the release of TNF-α during and after on-pump CABG surgery, and have found a limited release of TNF-α during aortic cross-clamping, but it becomes more prominent after aortic de-clamping (Biglioli *et al.,* 2003). TNF-α soluble receptors increase significantly soon after on-pump CABG surgery, which can persist up to 48 hours post-operatively (Diegeler *et al.*, 2000).

Persistent TNF-α plasma levels may be indicative of patients being at increased risk for recurrent coronary events. For only a brief period of time (<6 hours), TNF-α is secreted, after stimulation, by the monocytes/macrophages and mast cells. The detection of bioactive TNF-α therefore implies that it was produced within the last four to six hours or is still being produced, whereas monomers and degradation products are often still detectable after 24 hours and even longer (Ridker *et al.,* 2000; Volk *et al.*, 1998). The activation cycle play an important role in cardiac dysfunction following cardiac surgery and cardiac myocytes can produce elevated levels of TNF-α post-operatively (Meldrum, Partrick, Cleveland, Shenkar, Meldrum, Raiesdana, Ayala, Brown, Harken, 2003).

2.7.4.3 Tumor necrosis factor-α and off-pump CABG surgery

TNF-α levels are significantly lower in the off-pump CABG surgical group than in the on-pump CABG surgical group, while there is further also a marked decrease in systemic inflammatory response in off-pump CABG surgery patients. This is due to both shortened time of mechanical ventilation and also shortened stay in the ICU and in-hospital stay times. Patients undergoing off-pump CABG surgery are generally also discharged a day earlier than those patients undergoing on-pump CABG surgery (Orhan, Sargin, Senay, Yuksel, Kurc, Tasdemir, Ozay, Aykut Aka, 2007). Yet, in a study done by Diegeler and colleagues, the opposite was found: there was an early significant post-operative increase in the circulating levels of TNF-α soluble receptors and no change was detected in the minimally invasive direct coronary artery bypass (MIDCAB) group (Diegeler *et al.*, 2000). Gomes and colleagues have since found TNF-α levels to be induced by the surgical procedure and that these will therefore be elevated post-operatively in those patients undergoing off-pump CABG surgery. Leukocytes are activated by the surgical stress; especially monocytes lead to the production of TNF-α and correlate with the serum interleukin-6 levels, which are also related to the degree of surgical stress (Gomes, Erlichman, Batista-Filho, Knobel, Almeida, Carvalho, Catani, Buffolo, 2003).

2.7.5 Interleukins

There are various pro- and anti-inflammatory cytokines. Anti-inflammatory cytokines are a series of immune-regulatory molecules that control the pro-inflammatory cytokine response. Together, with specific cytokine inhibitors and soluble cytokine receptors, certain cytokines regulate the human immune response. Their physiologic role in inflammation and their pathologic role in systemic inflammatory states are increasingly recognised (Opal & DePalo, 2000). Major anti-inflammatory cytokines include the following: IL-1 receptor antagonist (IL-1Ra) a natural inhibitor of interleukin-1β, has been shown to improve β-cell function and glycaemic control in patients with Type 2 diabetes (Herder, Brunner, Rathmann, Strassburger, Tabák, Schloot, Witte, 2009). Other anti-inflammatory markers are interleukin-4 (IL-4), IL-6, IL-10, and interleukin-13 (IL-13) (Opal & DePalo, 2000).

2.7.5.1 Interleukin-1 (IL-1)

IL-1 is a primary pro-inflammatory marker that potentiates the expression of adhesion molecules on vascular endothelial cells. This leads to the recruitment of leucocytes to the arterial wall in the early stages of atheromatous lesion development and to the activation of chemokines that promote migration of monocytes into the sub-endothelial space. Mononuclear cells release growth factors

that stimulate the proliferation of smooth muscle cells and lead to plaque progression (Bassuk, Rifai, Ridker, 2004).

2.7.5.2 Interleukin-4 (IL-4) and interleukin-13 (IL-13)

Both of these interleukins are anti-inflammatory cytokines that have an inhibitory effect on the production of several inflammatory cytokines (TNF-α, IL-6, and IL-8) from human monocytes, macrophages, and endothelial cells (Nathan, Preux, Feiss, Denizot, 2000).

2.7.5.3 Interleukin-8 (IL-8)

Interleukin-8 (IL-8) is a chemo-attractant for neutrophils and plays a definite role in lung injury that is associated with pulmonary sequestration subsequent to on-pump CABG surgery. IL-8 activates neutrophils & T-lymphocytes, and complement activation promotes IL-8 release. There is a strong correlation between IL-8 and post-operative Troponin I levels in patients undergoing CABG surgery. A lesser production of IL-8 is observed with off-pump CABG surgery (Wan *et al.,* 1999).

2.7.5.4 Interleukin-10 (IL-10)

IL-10 is an anti-inflammatory cytokine that is produced in the liver. IL-10 production is proportional to IL-8 release during on-pump CABG surgery and is therefore lower in off-pump CABG surgery. In combination with lower IL-6 and IL-8, a lower production of IL-10 has been associated with the use of heparin-coated bypass circuits (Wan *et al.,* 1999). IL-10 inhibits the production of TNF-α and NO, consequently protecting the ischaemic and reperfused myocardium through the suppression of neutrophil recruitment (Anguera, Miranda-Guardiola, Bosch, Filella, Sitges, Matín, Betriu, Sanz, 2002).

2.7.6 Interleukin-6 (IL-6)

2.7.6.1 Mechanism and prognostic value of IL-6

Human IL-6 is a 184 a.a prototypic pleiotrophic cytokine that belongs to a family of 20 kDa polypeptide cytokines having a four-long-chain α-helix bundle structure, with potential O-glycosylation and N-glycosylation sites, and a significant homology with granulocyte colony-stimulating factor (G-CSF) (BioSource, 1998). IL-6 is an interesting cytokine because it is produced by

various cells, including T-cells and B-cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, astrocytes, bone marrow, stroma cells and several tumour cells (BioSource, 1998; Huber, Sakkinen, Conze, Hardin, Tracy, 1999; Koh *et al.,* 2005). IL-6 induces the final maturation of B-cells into antibody producing cells, and stimulates T-cell growth and cytotoxic T-cell differentiation. IL-6 regulates the growth and differentiation of different cell types with major activities on the immune system, haematopoiesis, and inflammation. Elevated levels of IL-6 are a major inducer of the acute phase reactions in response to inflammation or tissue injury/hypoxia and these acute phase proteins can be used as an indicator of increased risk of future MI (BioSource, 1998; Koh *et al.,* 2005).

IL-6 specifically has both pro- and anti-inflammatory properties, and acts as a mediator of metabolic, immunological and endocrine responses to inflammatory injury (Vallely *et al.*, 2001; Biglioli *et al.*, 2003). IL-6 is a principal pro-coagulant cytokine that increases plasma concentrations of fibrinogen, plasminogen activator inhibitor Type 1, and CRP. Elevated levels of IL-6 are associated with increased risk of future MI in especially healthy men (Koh *et al.*, 2005). The IL-6-type cytokines use the same receptor gp130 subunit for signal transduction resulting in activation of the Janus Kinase pathway (BioSource, 1998; Huber *et al.,* 1999).

IL-6 can also induce a pro-thrombotic state with increased expression of fibrinogen, tissue factor, Factor VIII and von Willebrand factor, and also through the activation of endothelial cells and consequently increasing the platelet production (Roldán, Marin, Blann, Garcia, Marco, Sogorb, Lip, 2003). Elevated IL-6 levels have been detected through the stimulation of thrombopoietin in patients with coronary atherosclerosis both pre-operatively and post-operatively (Hedman, Larsson, Alam, Wallen, Nordlander, Samad*,* 2007).

Parallel detection of high concentrations of IL-6 and TNF-α suggests an excessive activation of the monocytes/macrophages, e.g. as seen in SIRS. Monocytes/macrophages secrete IL-6 within a few hours (<6 hours) after coming into contact with bacteria and/ bacterial toxins. In contrast to TNF-α, this secretion occurs over a period of 24 - 48 hours (Volk *et al.*, 1998). Isolated high IL-6 concentrations in combination with minimally elevated or normal TNF-α concentration especially when persistent over the course of several days, can point to the activation of non-immune cells. This may be caused by a recent release (within the past few days) of TNF-α from monocytes/macrophages or it may be a direct result of the interaction between bacteria or bacterial products, e.g. endothelial cells or keratinocytes (Volk *et al.,* 1998).

Serum IL-6 has a prognostic use in surgical or traumatic tissue injuries, infectious diseases, auto-immune diseases including graft rejection, and heart failure. Thus, IL-6 has multiple actions that are integrated within a complex cytokine network in which, several cytokines can induce (e.g. IL-1 and TNF-α) or are induced by IL-6. The final effects result from both synergistic or antagonistic activities between IL-6 and other cytokines (Volk *et al.,* 1998; BioSource, 1998). The indication of IL-6 measurements is of great value in, especially cardiac patients admitted to ICU's. The relevance of determining the activity of chronic inflammation is less significant, since this test offers few advantages in comparison with inflammatory markers, such as CRP or WCC response (Volk *et al.,* 1998).

It has also been determined that IL-6 has a significant association with post-operative fever (Mitchell, Grocott, Phillips-But, Mathew, Newman, Bar-Yosef, 2007). Post-operatively, IL-6 will be secreted within a few hours (<6hours) after coming into contact with monocytes/macrophages, this promoting neutrophil activation and a consequent release of free oxygen radicals. This secretion will continue for a period of 24 - 48 hours. IL-6 concentrations of >1000 ng/l are generally associated with a high mortality rate (Volk *et al.,* 1998).

2.7.6.2 Interleukin-6 and on-pump CABG surgery

The release of IL-6 and the subsequent elevated IL-6 levels after cardiac surgery, are possibly more related to the on-pump CABG surgical trauma itself than to ischaemia (Vallely *et al.*, 2001; Mei, Qiang, Liu, Wang, Feng, Long, Cheng, Xing, Li, Hu, 2007). One of the major sources of IL-6 secretion is the exposed myocardium to cardioplegic arrest. Another reason for elevated IL-6 levels with on-pump CABG surgery is the exclusion of the lungs from circulation (Biglioli *et al.*, 2003). Therefore, the functional lifespan of the neutrophils are prolonged by the release of IL-6 after on-pump CABG surgery, while modulating apoptosis (also known as "programmed cell death"), further enhances the inflammatory response (Mitchell *et al.,* 2007). UA patients presenting with increased levels of CRP together with elevated IL-6 levels may have a poor prognostic outcome (Volk *et al.,* 1998; Huber *et al.,* 1999).

Elevated pre-operative levels of CRP and IL-6 predict early graft occlusion after CABG surgery. Furthermore, CRP causes monocytes to synthesise tissue factor, and IL-6 seems to have pro-coagulant effects. Raised pre-operative IL-6 levels are predictors both of early graft occlusion and

late cardiovascular events after on-pump CABG surgery (Hedman *et al.,* 2007). According to a study done by Larmann & Theilmeier, elevated concentrations of the pro-inflammatory cytokines IL-6 and IL-8 were detected after on-pump CABG surgery, and elevated levels of the anti-inflammatory cytokine IL-10 - which suppresses IL-6 and IL-8 - were also detected during and after on-pump CABG surgery (Larmann & Theilmeier, 2004). These levels can reach a peak at 3 - 24 hours post-operatively and could be due to multiple reasons, such as the surgical procedure itself or even to other external factors, such as anaesthesia, blood loss, mechanical ventilation, or even pain (Sander, 2006).

2.7.6.3 Interleukin-6 and off-pump CABG surgery

Several studies have compared the release of IL-6 after CABG surgery in the past. In studies done by Wan, Corbi and Fransen, IL-6 levels were compared post-operatively between patients undergoing on-pump CABG surgery and off-pump CABG surgery. Although the IL-6 levels peaked 4 - 8 hours post-operatively in the off-pump CABG group there were no significant differences found between the two CABG groups (Corbi, Rahmati, Delwail, Potreau, Menu, Wijdenes, Lecron, 2000; Wan *et al.,* 1999; Fransen *et al.,* 1998; Franke, Lante, Fackeldey, Becker, Kurig, Zöller, Weinhold, Markewitz, 2005; Wan, Arifi, Wan, Yip, Sihoe, Thung, Wong, Yim, 2004). This elevation in the off-pump CABG group may be a strong indicator of a pro-inflammatory response.

Tatoulis and colleagues have found off-pump CABG surgery to be associated with reduced post-operative IL-6 cytokine response (Tatoulis *et al.,* 2006) and Ishida and colleagues suggest from their study that there may be a positive correlation between intra-operative blood loss and the release of elevated IL-6 levels after the surgical trauma caused by off-pump CABG surgery (Ishida *et al.*, 2006). Fransen and colleagues, in their study, found that the release of IL-6 levels was significantly elevated in the on-pump CABG surgical group but that it was delayed in the off-pump CABG surgical group due to the possible attribution of various anaeshestic procedures used (Fransen *et al.,* 1998; Franke *et al.,* 2005).

It seems therefore that on-pump CABG surgery with the use of the bypass circuit play a secondary role in the stimulation and release of IL-6 after heart surgery. However, surgical trauma and tissue trauma seems to play a bigger role in the release of IL-6 and the degree of elevated IL-6 levels are dependant on the degree of the surgical trauma inflicted (Vallely *et al.,* 2001).

CHAPTER 3: METHODOLOGY

3.1 Study location

The research study was conducted at Universitas hospital involving the Department of Cardiology (Universitas hospital), the Department of Cardiothoracic Surgery (Universitas Hospital), the Department of Chemical Pathology (NHLS) and the Department Haematology (NHLS). Universitas is a state/private hospital located in Bloemfontein, the capital city of the Free State, South Africa.

3.2 Study design

The clinical study was a prospective longitudinal observational study.

3.3 Study population

3.3.1 The number of subjects

Patients (n=60) diagnosed with ACS were recruited for the study. These patients were recruited from the Cardiology clinic, and were assigned to participate in the study upon granting informed consent (Appendix A). Each patient also received an information sheet (Appendix B) that provided the necessary information regarding the study in lay terms.

3.3.2 Subject identification

For the duration of the study and for the purpose of this dissertation, the participating patients were identified by their hospital number (UM number), to ensure patient confidentiality as far as possible.

3.3.3 Inclusion and exclusion criteria

The criteria were set to ensure a homogenous subject population.

3.3.3.1 Inclusion criteria

- Patients with acute CAD.
- Patient must be able to sit up-right.
- Patients must be available for pre- and post-operative interviews.
- The patient must be able to give informed consent.

3.3.3.2 Exclusion criteria

- Patients with existing organ failures other than chronic cardiac failure.
- Patients older than 85 years were excluded.

3.4 Study layout

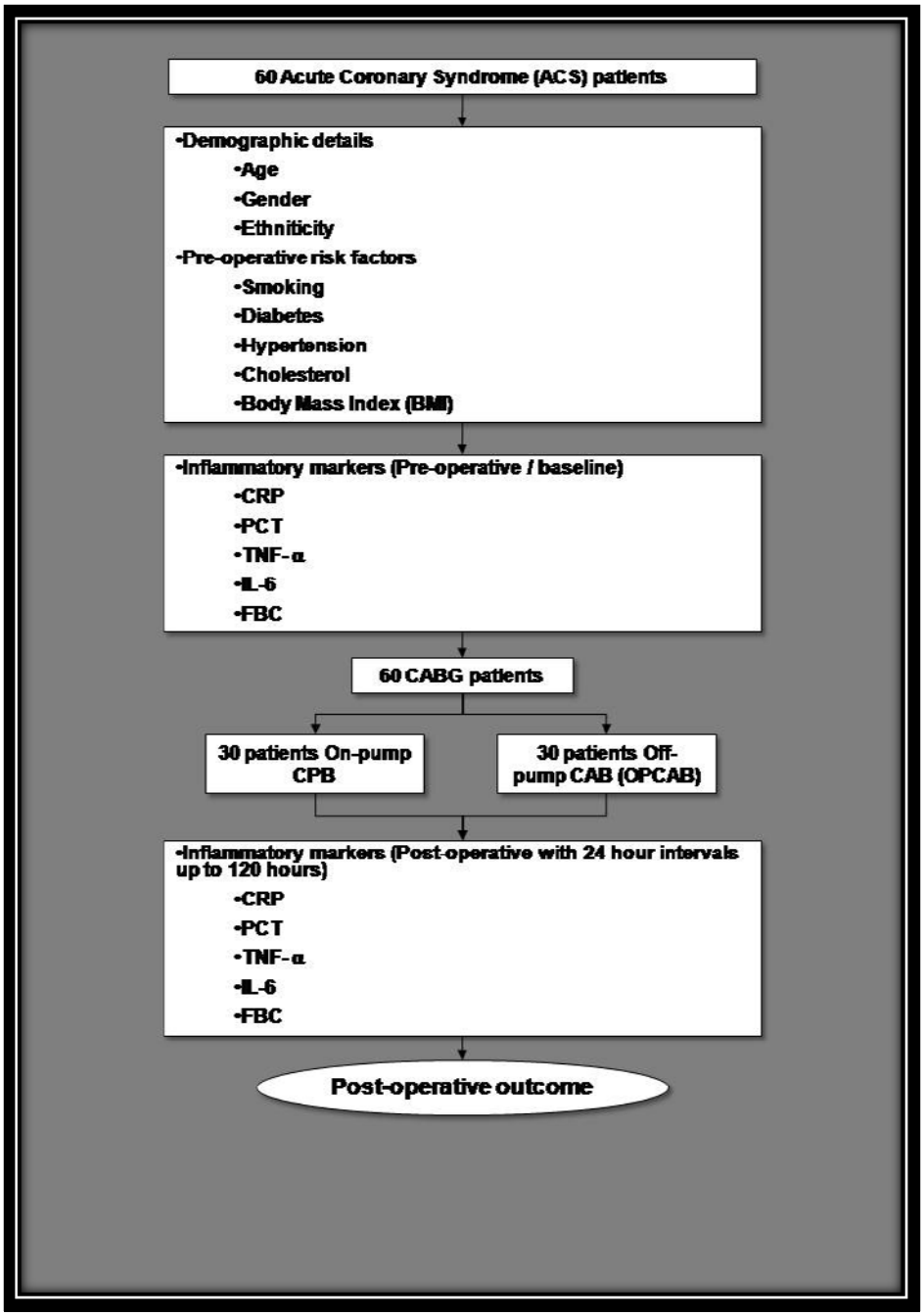


Figure 3.1 Study layout

This was a non-randomized, observational study where patients (n=60) with ACS, that met the inclusion criteria, were recruited for the study (figure 3.1). These sixty patients were divided into two groups - thirty patients that underwent on-pump CABG surgery and thirty patients that underwent off-pump CABG surgery. Pre-operatively the following "baseline" inflammatory markers were analyzed on both groups: CRP, PCT, FBC, IL-6 and TNF-α. Post-operatively, the

abovementioned inflammatory markers were again analyzed every twenty four hours for five days post-operatively. The various inflammatory markers were used in this particular study to determine which inflammatory markers are most useful in the detection of SIRS.

3.5 Special investigations

3.5.1 Sample collection

3.5.1.1 Pre-intervention (Baseline values)

Blood samples were drawn prior to intervention procedure and sent to the National Health Laboratory Services (NHLS, Universitas hospital) for analysis (table 3.1).

Table 3.1 Summary of sample collection for inflammatory markers

Test	Colour top tube	Pre-intervention & post-intervention	24 hours (day 1) after intervention	48 hours (day 2) after intervention	72 hours (day 3) after intervention	96 hours (day 4) after intervention	120 hours (day 5) after intervention
CRP, PCT, IL-6 & TNF-α	Two yellow top tubes (10ml)	✓	✓	✓	✓	✓	✓
FBC	**One purple top tube (5ml)**	✓	✓	✓	✓	✓	✓

One yellow top tube (10ml)

- Reference No: 367955
- Lot No: 9096805
- Becton Dickenson (BD) Vacutainer SST II Advance
- Expiry date: 2010-10

One purple top tube (5ml)

- Reference No: 368861
- Lot No: 9071288
- Becton Dickenson (BD) Vacutainer K2E-7.2mg Advance
- Expiry date: 2010-07

3.5.1.2 Post-Intervention

After the specified intervention procedure, blood samples were drawn at 24 hours intervals and sent to the National Health Laboratory Services (NHLS, Universitas hospital) for analysis (table 3.1).

One yellow top tube (10ml)

- Reference No: 367955
- Lot No: 9096805
- Becton Dickenson (BD) Vacutainer SST II Advance
- Expiry date: 2010-10

One purple top tube (5ml)

- Reference No: 368861
- Lot No: 9071288
- Becton Dickenson (BD) Vacutainer K2E-7.2mg Advance
- Expiry date: 2010-07

3.5.1.3 Laboratory analysis

The following inflammatory markers were analyzed pre- and post-operatively.

3.5.2 C-reactive protein

CRP was measured with the BeckmanCoulter Synchron LX20 (figure 3.2) from BeckmanCoulter, Johannesburg, South Africa (Serial No 1170) (BeckmanCoulter, 2000).

Figure 3.2 BeckmanCoulter Synchron LX20

3.5.2.1 Method of C-reactive protein

CRP is determined through a turbidimetric method. CRP combines with specific antibodies to form insoluble antigen-antibody complexes. The required amount of sample and CRP reagent volumes as specified by the manufacturer are automatically pipetted by the system into the cuvette. The Synchron LX system (figure 3.2) measures the change in absorbance at 340 nm (BeckmanCoulter, 2000).

3.5.2.2 Reference ranges for C-reactive protein

The degree of elevation for CRP (table 3.2) reflects the mass of activity of the inflamed tissue and this may be secondary to the underlying disease, as in MI.

Table 3.2 Reference ranges for C-reactive protein (NHLS, Universitas hospital, 2009)

Reference ranges	Interpretation
0.0 - 5.0 mg/l	Normal
>5.0 mg/ml	May be suggestive of an infection/ inflammatory process

3.5.2.3 Control precision of C-reactive protein

The CV for CRP Synchron Vigil control level 2 is 4% and the correlation coefficient (CV) for CRP Synchron Vigil control level 3 is 3%. In Appendix D the control precision results and control reference ranges for CRP is explained in detail.

3.5.3 Procalcitonin

PCT was measured with the Brahms Kryptor analyzer from Humor Diagnostica (figure 3.3), Pretoria, South Africa (Serial No F300) (Brahms, 2006).

Figure 3.3 Brahms Kryptor analyzer from Humor Diagnostica

3.5.3.1 Principle of time-resolved amplified cryptate emission (TRACE) technology

TRACE technology was used to measure PCT, whereby an emitted signal from an immunocomplex was measured with a time delay. TRACE technology is a non-radioactive energy transferred from a donor (a cage-like structure with a europium ion in the centre (Cryptate) to an acceptor, which is part of a chemically modified, light collecting algal protein (XL 665). The proximity of donor (Cryptate) and acceptor (XL 665) forms an immunocomplex. The fluorescent signal of the Cryptate was intensified by the spectral overlap between donor emission and acceptor absorption. The

acceptor signal has an extended lifespan allowing the measurement of temporary delayed fluorescence. After the sample was excited with a nitrogen laser at 337 nm, the donor (Cryptate) emits a long-life fluorescent signal in the milli-second range at 620 nm, while the acceptor (XL 665) generates a short-life signal in the range of nano-seconds at 665 nm. When both components bind in an immunocomplex, both the signal amplification and the prolonged life span of the acceptor signal occurr at 665 nm, which is measured in µ-seconds. This long-life signal was proportional to the concentration of the analyte. Non-specific signals are eliminated by the temporary delay of the fluorescence measurement, e.g. signals of the short-life and unbound acceptor XL 665 and the medium-specific interference signals conditional upon the natural fluorescence of the sample. Cryptate signals are generated at 620 nm and serve as an internal reference and are measured simultaneously with the long-life acceptor signal 665 nm which is the specific signal. Interfering influences, e.g. from turbid sera, are automatically corrected with the internally calculated ratio of the intensities at these wavelengths (Brahms, 2006).

3.5.3.2 Reference ranges for PCT

Table 3.3 indicates the reference ranges and interpretation of PCT levels for the diagnosis of systemic bacterial infection and sepsis.

Table 3.3 Reference ranges & interpretation of PCT levels (NHLS, Universitas hospital, 2009)

Reference ranges	Interpretation
Infections of lower respiratory tract:	
<0.10 ng/ml	**No signs of bacterial infection Therapy with antibiotics not recommended**
0.10 - <0.25 ng/ml	**Bacterial infection unlikely Antibiotic therapy not recommended**
0.25 - <0.50 ng/ml	**Bacterial infection possible Antimicrobial therapy recommended**
>0.50 ng/ml	**Suspected bacterial infection Antibiotic therapy strongly recommended**
Diagnosis of systemic infection:	
<0.5 ng/ml	**Local bacterial infection possible Systemic infection is unlikely**
≥0.5 - <2.0 ng/ml	**Systemic infection is possible**
≥2.0 - 10.0 ng/ml	**Systemic infection is likely unless other reasons are known**
≥10.0 ng/ml	**Pronounced systemic inflammatory reaction almost solely due to severe bacterial infection or septic shock**

3.5.3.3 Control Precision of proclacitonin

The CV for PCT KRYPTOR Control Level 1 is 3% and the CV for PCT Kryptor Control Level 2 is 2%. In Appendix D the control precision results and control reference ranges for PCT is explained in detail.

3.5.4 Full blood count

FBC were analysed on the Sysmex XE 2100 analyser from Roche, Johannesburg, South Africa (Serial No A3447) (figure 3.4). This instrument performed haematological analyses according to the RF/DC detection method, hydrodynamic focusing (DC Detection), flow cytometry method (using a semiconductor laser), and a SLS-haemoglobin method (Roche, 2004).

Figure 3.4 Sysmex XE 2100 analyser

3.5.4.1 Reference ranges for FBC

Table 3.4 indicates the FBC reference ranges for males and females.

Table 3.4 Full blood count reference ranges (NHLS, Universitas hospital, 2009)

	Female	Male
WCC 10^9/l	4.00 - 10.00	**4.00 - 10.00**
Platelets 10^9/l	178 - 400	**137 - 373**
Neutrophils 10^9/l	2.00 - 7.50	**2.00 - 7.50**
Lymphocytes 10^9/l	1.00 - 4.00	**1.00 - 4.00**
Monocytes 10^9/l	0.18 - 0.80	**0.18 - 0.80**
Eosinophils 10^9/l	0.00 - 0.45	**0.00 - 0.45**
Basophils 10^9/l	0.00 - 0.20	**0.00 - 0.20**

WCC = white cell count

3.5.4.2 Control precision for FBC

Table 3.5 indicates the control precision for FBC (Level 1, 2 and 3).

Table 3.5 Control precision for FBC

Control level 1:	CV for platelets 10^9/l was 6.3%, CV for WCC 10^9/l was 3.1%, CV for neutrophils 10^9/l was 4.6%, CV for lymphocytes 10^9/l was 4.1%, CV for monocytes 10^9/l was 9.3%, CV for eosinophils 10^9/l was 8.2%, CV for basophils 10^9/l was 3.8%.
Control level 2:	CV for platelets 10^9/l was 3.1%, CV for WCC 10^9/l was 2%, CV for neutrophils 10^9/l was 2.8%, CV for lymphocytes 10^9/l was 4.9%, CV for monocytes 10^9/l was 8.9%, CV for eosinophils 10^9/l was 8.5%, CV for basophils 10^9/l was 2.4%.
Control level 3:	CV for platelets 10^9/l was 2.1%, CV for WCC 10^9/l was 1.8%, CV for neutrophils 10^9/l was 2.5%, CV for lymphocytes 10^9/l was 3.8%, CV for monocytes 10^9/l was 6.1%, CV for eosinophils 10^9/l was 7.7%, CV for basophils 10^9/l was 1.8%.

CV = correlation coefficient

In Appendix D the control precision results and control reference ranges for level 1, 2 and 3 of FBC is explained in detail.

3.5.5 Tumor Necrosis Factor-α

TNF-α was determined through an EASIA (Enzyme Amplified Sensitivity Immunoassay) method form BioSource (figure 3.5), Belgium, Europe, provided by the company LabSpec, Johannesburg, South Africa (Lot no KAP1751: 96 tests) (BioSource, 2005).

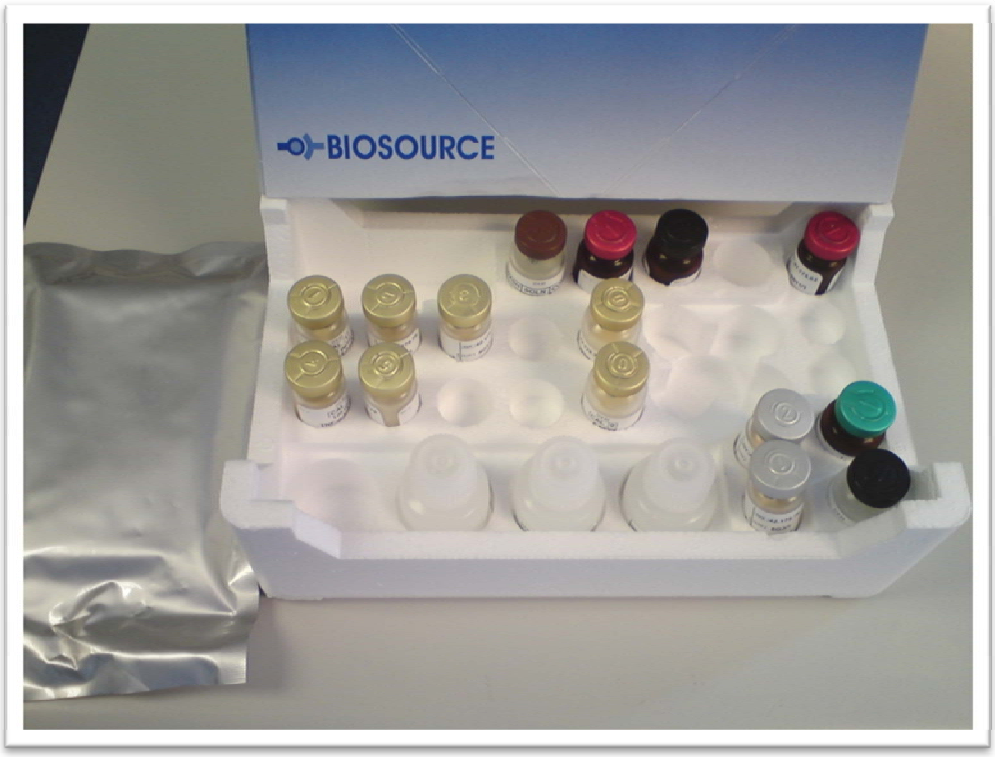


Figure 3.5 EASIA for TNF-α from BioSource

3.5.5.1 Reagents provided for TNF-α

Table 3.6 indicates the reagents provided for TNF-α analysis.

Table 3.6 Reagents provided for TNF-α (BioSource, 2005)

Reagents	96 test kit	Colour code	Reconstitution
Microtiterplate with 96 anti-TNF-α (monoclonal antibodies) coated wells	96 wells	Blue	Ready for use
Ab HRP	1 vial 0.75ml	Red	Add conjugate buffer Preparation of the conjugate solution: following the number of wells to be used, dilute the concentrated conjugate buffer in a clean glass vial e.g. 8 wells require 50µl concentrated conjugate and 500 µl conjugate buffer to form a working solution volume of 550 µl. Extemporaneous preparation is required. The diluted conjugate is stable for maximum one week at 2-8 ˚C
Cal N = 0 Value: 0 pg/ml OD units: 0.045	2 vials lyophil	Yellow	Add 6ml of distilled water in each vial
Cal N = 1 to 5 Value: 1: 6.8 pg/ml	5 vials lyophil	Yellow	Add 2ml distilled water in each vial

Reagents	96 test kit	Colour code	Reconstitution
2: 18 pg/ml 3: 52 pg/ml 4: 176 pg/ml 5: 518 pg/ml OD units: 1: 0.120 2: 0.259 3: 0.619 4: 1.435 5: 3.237			
Conjugate buffer: TRIS-Maleate buffer with bovine serum albumin, EDTA and thymol	1 vial 6ml	Red	Ready for use
Incubation buffer: TRIS-Maleate buffer with bovine serum albumin, EDTA and thymol	1 vial 6ml	Black	Ready for use
Medgenix Wash Solution Concentrate: Tris-HCL	1 vial 10ml	Brown	Dilute 200 x with distilled water (use a magnetic stirrer). 199 volumes of distilled water to 1 volume of Wash solution (200x) e.g. 398ml of distilled water and 2ml Wash Solution Concentrate. Discard unused Working Wash Solution at the end of the day
Control 1& 2 in human serum and thymol	2 vials lyophil	Silver	Add 2ml of distilled water
Chromogen TMB Concentration: (Tetramethylbenzydine) in Dimethylformamide The Chromogenic Solution should be colourless. The Chromogenic Solution must be dispensed within 15 min, following the last washing step of the microtiter plate	1 vial 1ml	Green	Revelation solution: pipette 0.2ml of the chromogen TMB into one of the vials of substrate buffer (H_2O_2 in acetate/citrate buffer). Extemporaneous preparation is recommended
Substrate Buffer: (H_2O_2 in acetate/citrate buffer)	3 vials 21ml	White	Ready for use
Stop Solution: H_2SO_4 1.8N	1 vial 6ml	Black	Ready for use

3.5.5.2 Handling notes for TNF-α

Samples and reagents were equilibrated to room temperature (18 - 25 ˚C) before use by gentle swirling. Clean disposable plastic pipettes were used for each reagent, standard, control and specimen in order to avoid cross-contamination. Pipetting delay was avoided from the first standard until the last sample - no longer than 30 minutes. Direct sunlight on the microtiter plate was avoided during incubation, by placing the microtiter plate on the horizontal shaker (Densey WE-MIXX-1, Serial no 90/235) in a cupboard. Incubation times were adhered to as described in the assay procedure. The unused strips were resealed in the silver bag provided with desiccant and stored at 2 - 8˚C (BioSource, 2005).

3.5.5.3 Assay procedure for TNF-α

The assay procedure as follows (BioSource, 2005):

- The assay strips were secured into the holding frame.
- Fifty microlitres of Incubation Buffer (table 3.6) were pipetted into all the wells.
- Thereafter 200µl of each standard, control or sample were pipetted into the appropriate wells according to the worklist.
- The microtiter plate was incubated at room temperature on a horizontal shaker at 700 rpm (± 100 rpm) for 2 hours in a cupboard.
- The liquid was poured out and gently shaken from the wells.
- The plate was washed according to the following procedure:
  - 0.4ml of Medgenix Wash Solution (table 3.6 for the preparation of the Medgenix Wash Solution) was dispensed into each well.
  - Afterwards the liquid was poured from the wells and gently shaken.
  - The microtiter plate was tapped (upside down) gently on a piece of paper towel.
  - This procedure was repeated two times.
- 100µl of standard 0 was pipetted into all the wells.
- This is followed by 50µl of anti-TNF-α conjugate being pipette into all the wells (table 3.6 for the preparation of the Conjugate Solution).
- The microtiter plate was incubated for 2 hours at room temperature on a horizontal shaker set at 700 rpm (± 100 rpm).
- The liquid was poured out and gently shaken from all the wells.
- The plate was washed according to the following procedure:
  - 0.4ml of Medgenix Wash Solution (table 3.6 for the preparation of the Medgenix Wash Solution) was dispensed into each well.
  - Afterwards the liquid was poured from the wells and gently shaken.
  - The microtiter plate was tapped gently on a piece of paper towel.
  - This procedure was repeated two times.
- 200µl of freshly prepared Chromogenic Solution was pipetted into each well within 15 minutes following the washing step (table 3.6 for the preparation of the Chromogenic Solution).
- The plate was incubated for 30 minutes at room temperature on an horizontal shaker set at 700 rpm (± 100 rpm) avoiding direct sunlight.

- 50µl of Stop Solution was pipette into each well (table 3.6 - Stop solution).
- The absorbances were read at 450 nm and 490 nm (reference filter: 630 or 650 nm) within 3 hours and the results were calculated.
- The micotiter plate was read using the Synergy ™ Microplate Reader, Biotek Instruments Incorporated, from Analytical Diagnostic Products, Johannesburg, South Africa (Serial No B6898).

3.5.5.4 Reference ranges for TNF-α

4.6 - 12.4 pg/ml (BioSource, 2005).

3.5.5.5 Control precision of TNF-α

The CV for the low level control for TNF-α was 9% and the CV for the high level control for TNF-α was 13%. In Appendix D the control precision results and control reference ranges for TNF-α is explained in detail.

3.5.6 Interleukin-6 (IL-6)

IL-6 was determined through an EASIA method form BioSource (figure 3.6), Belgium, Europe, provided by the company LabSpec, Johannesburg, South Africa (Catalogue no KAP1261: 96 tests) (BioSource, 2006).

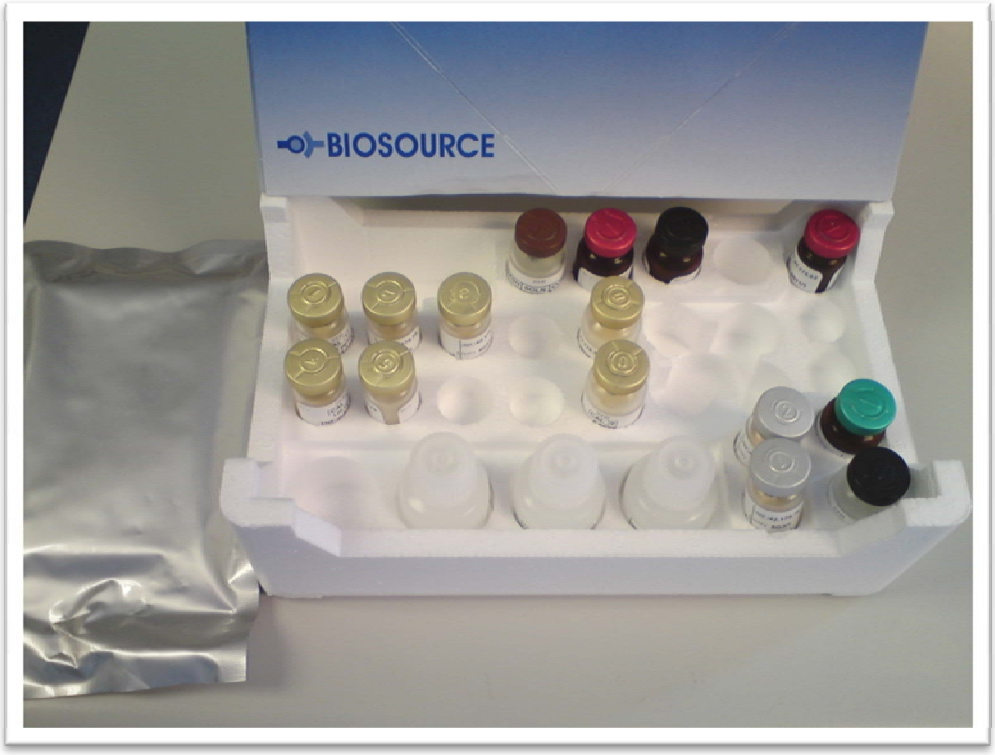


Figure 3.6 EASIA for IL-6 from BioSource

3.5.6.1 Reagents provided for IL-6

Table 3.7 indicates the reagents provided for IL-6 analysis.

Table 3.7 Reagents provided for IL-6 (BioSource, 2006)

Reagents	96 test kit	Colour code	Reconstitution
Microtiterplate with 96 anti-TNF-α (monoclonal antibodies) coated wells	96 wells	Blue	Ready for use
Conjugate: Ab HRP labelled anti-IL-6 (monoclonal antibodies) in Borate Buffer with bovine serum albumin and thymol	1 vial 11ml	Red	Ready for use
Cal N = 0 Value: 0 pg/ml OD units: 79	1 vial lyophil	Yellow	Add 1ml distilled water
Cal N = 1 to 5 Value: 1: 23.3 pg/ml 2: 68 pg/ml 3: 201 pg/ml 4: 633 pg/ml 5: 2560 pg/ml OD units: 1: 125 2: 193	5 vials lyophil	Yellow	Add 1ml distilled water in each vial

Reagents	96 test kit	Colour code	Reconstitution
3: 408 4: 1036 5: 3579			
Specimen Diluent: human serum with bovine serum albumin, benzamidin and thymol	3 vials lyophil	Black	Add 3ml distilled water in each vial
Incubation buffer: Borate buffer with bovine serum albumin, benzamidin and thymol	1 vial 11ml	Black	Ready for use
Medgenix Wash Solution Concentrate: Tris-HCL	1 vial 10ml	Brown	Dilute 200 x with distilled water (use a magnetic stirrer). 199 volumes of distilled water to 1 volume of Wash solution (200x) e.g. 398ml of distilled water and 2ml Wash Solution Concentrate. Discard unused Working Wash Solution at the end of the day
Control 1& 2 in human serum and thymol	2 vials lyophil	Silver	Add 1ml of distilled water
Chromogen TMB Solution: The Chromogenic Solution should be colourless. The Chromogenic Solution must be dispensed within 15 min, following the last washing step of the microtiter plate	1 vial 1ml	White	Ready for use
Stop Solution: 2N HCL	1 vial 25ml	White	Ready for use

3.5.6.2 Handling notes for IL-6

Samples and reagents were handled according to the methodology described in 3.5.2.4.2 (18 - 25˚C) before use by gentle swirling. Clean disposable plastic pipettes were used for each reagent, standard, control and specimen in order to avoid cross-contamination. Pipetting delay was avoided from the first standard until the last sample - no longer than 30 minutes. Direct sunlight on the microtiter plate was avoided during incubation, by placing the microtiter plate on the horizontal shaker (Densey WE-MIXX-1, Serial no 90/235) in a cupboard. Incubation times were adhered to as described in the assay procedure. The unused strips were resealed in the silver bag provided with desiccant and stored at 2 - 8˚C (BioSource, 2006).

3.5.6.3 Assay procedure for IL-6

The assay procedure is as follow (BioSource, 2006):

- The assay strips were secured into the holding frame.
- 50µl of Incubation Buffer (table 3.7) were pipetted into all the wells.

- Thereafter 100µl of each standard, control or sample were pipette into the appropriate wells according to the worklist.
- The microtiter plate was incubated at room temperature on a horizontal shaker at 700 rpm (± 100 rpm) for 1 hour.
- The liquid was poured out and gently shaken from all the wells.
- The plate was washed according to the following procedure:
  - 0.4ml of Medgenix Wash Solution (table 3.7 for the preparation of the Medgenix Wash Solution) was dispensed into each well.
  - Afterwards the liquid was poured from the wells and gently shaken.
  - The microtiter plate was tapped gently (upside down) on a piece of paper towel.
  - This procedure was repeated two times.
- Thereafter, 100µl of anti-IL-6-HRP conjugate (table 3.7) and 50µl specimen diluent (table 3.7) were pipetted into all the wells.
- The microtiter plate was incubated for 1 hour at room temperature on a horizontal shaker set at 700 rpm (± 100 rpm).
- The liquid was poured out and gently shaken from all the wells.
- The plate was washed according to the following procedure:
  - 0.4ml of Medgenix Wash Solution (table 3.7 for the preparation of the Medgenix Wash Solution) was dispensed into each well.
  - Afterwards the liquid was poured from the wells and gently shaken.
  - The microtiter plate was tapped gently on a piece of paper towel.
  - This procedure was repeated two times.
- 200µl of Chromogenic Solution (table 3.7 for the preparation of the Chromogenic solution) were pipetted into each well within 15 minutes following the washing step.
- The micotiter plate was incubated for 15 minutes at room temperature on a horizontal shaker set at 700 rpm (± 100 rpm) avoiding direct sunlight.
- 100µl of Stop Solution (table 3.7) was pipetted into each well.
- The absorbances were read at 450 nm and 490 nm (reference filter: 630 or 650 nm) within 3 hours and the results were calculated.
- The micotiter plate was read using the Synergy ^{TM} Microplate Reader, Biotek Instruments Incorporated, from Analytical Diagnostic Products, Johannesburg, South Africa (Serial No B6898).

3.5.6.4 Reference ranges for IL-6

0 - 50 pg/ml (BioSource, 2006).

3.5.6.5 Control precision of IL-6

The CV for the low level control for IL-6 was 7% and the CV for the high level control for IL-6 was 5%. In Appendix D the control precision results and control reference ranges for IL-6 is explained in detail.

3.6 Coronary artery bypass graft surgery

3.6.1 On-pump CABG surgical techniques

The patient was pre-operatively prepared for theatre:

- The following equipment were used:
  - Bypass circuit - Heart-Lung Machine, Stockert S5, Sorin Group, Italy (customized designed pump with five roller pump heads).
  - Sorin Biomedica Synthesis oxygenator, Sorin Group, Italy (Ref: 050239).
  - Medtronic Adult Membrane Pack, Medtronic Incorporated, Minneapolis, USA (Ref: M273102E).
  - Medtronic Myotherm 4:1, Medtronic Incorporated, Minneapolis, USA (Ref: M999214E).
  - Medex disposable dome for the Medex Transducer (for cardioplegia pressure measurement), Medex Medical LTD, Lancashire (Ref: MX960XYP1).
- The bypass circuit was de-aired with the following:
  - 1L Balsol Infusion, Fresenius Kabi for Bodene Limited trading as Intramed, PE, South Africa (Ref: FSB001000).
  - Intramed Mannitol 25%m/v, 12.5g, 50ml, Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: FSM250050).
  - 30mg of Heparin Sodium-Fresenius 1000 IU/ml, Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: J/8.2/405).
  - 1g Ranbaxy Ranzol Injection Cefazolin Sodium (Sterile) 1m/iv, Ranbaxy (SA) (Pty) LTD, North Centurion, South Africa (Ref: 30/20.1.1/0333, Code: MP/DRUGS/28/15/83).

- 500ml Gelofusion Solution for I.V Infusion I.V Plasma Substitute, B. Braun Medical (Pty) LTD, Randburg, South Africa (Ref: 31/8.4/0360).

- Preparation of the transducer for the measurement of the arterial cannula with the following:
  - Edwards Lifesciences Pressure Monitoring Set, Edwards Lifesciences, Irvine, United States of America (Ref: PX600FP).
  - CritiCare 0.9% Sodium Chloride Injection BP (200ml), Dismed CritiCare (Pty) LTD, Midrand, South Africa (Ref: 32/24/0128).
  - 30mg of Heparin Sodium-Fresenius 1000 IU/ml, Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: J/8.2/405).

Cardioplegia preparation for different surgeons with the following:

- Prof Smit's surgical technique:
  - Medsol Cardioplegic Induction Solution, Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: FSM01850I).
  - Medsol Cardioplegic Maintanance Solution, Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: FSM01850M).
  - Medsol Cardioplegic Reperfusion Solution, Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: FSM01850R).
  - Dextose-Fresenium 50% (20ml) (10ml inserted into each bag of cardioplegic solution), Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: V/24/222).

- Dr Long's surgical technique:
  - Intramed Ringer-Lactate Solution 1000ml Infusion, Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: FSR001000).
  - 50ml Albusol 4% Human Plasma Albumin, National Bioproducts Institute, Pinetown, South Africa (Ref: T/30.3/738).
  - 30ml Intramed Sodium Bicarbonate Injection 8.5% (50ml) m/v, Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: FSS850050).
  - 2 Ampoules Lignocaine HCl-Fresenius 2% (2ml Ampoules), Fresenius Kabi for Bodene Limited trading as Intramed, Port Elizabeth, South Africa (Ref: M/4/254).

- 4 Ampoules SABAX Magnesium Sulphate 50% Injection (1g/2ml) iv/im, Pharmacological Classification A.24 (Mineral Substitutes, Electrolytes), ADCOCK Ingram Critical Care (Pty) LTD, Johannesburg, South Africa (Ref: V/24/253).
- 1½ Ampoules SABAX Potassium Chloride 15% Injection, Pharmacological Classification A.24 (Mineral Substitutes, Electrolytes), ADCOCK Ingram Critical Care (Pty) LTD, Johannesburg, South Africa (Ref: V/24/218).

- The patient entered the theatre and thereafter the following procedures took place:
  - Soma-sensors were placed on the forehead and the back.
  - ECG sensors were placed on the back.
  - Anaesthetics were administered.
  - Median sternotomy was done by the surgeon and saphenous veins were harvested from the patient's legs by the assistant surgeon.
  - Internal Mammary arteries may also be mobilized by the surgeon.
  - 4mg/kg Heparin Sodium-Fresenius 1000 IU/ml are administered to the patient, Fresenius Kabi for Bodene Ltd. trading as Intramed, Port Elizabeth, South Africa (Ref: J/8.2/405).
- Arterial cannulation was done in the aorta and then de-aired via the pressure line to the transducer with the following:
  - Medtronic 24Fr Elongated One-Piece Arterial Cannula, Medtronic Inc. Minneapolis, USA (Ref: 77424).
  - Brittan Healthcare Pressure Monitoring Line Male/Female 183cm, Brittan Healthcare, Isando, South Africa (Ref: BG-VMML-MF-72).
- Venous cannulation of the right atrium with the following:
  - Edwards Lifesciences Dual Stage Drainage Cannula 32/40Fr x 40cm, Edwards Lifesciences LLC, Irvine USA (Ref: TR3240L,) or
  - Edwards Lifesciences Dual Stage Drainage Cannula 34/46Fr x 40cm, Edwards Lifesciences LLC, Irvine USA (Ref: TR3446L).
- Routine hypothermic CABG was started (28 - 30˚C).
- Cross-clamping of the ascending aorta.
- Infusion of cold blood cardioplegia, at ± 20 degrees, antegrade into the aortic root via the cardioplegia cannula.
- SARNS Coronary Perfusion Cannula 14Fr, Terumo Cardiovascular System Corporation, Michigan (Ref: L7722).

- Retrograde cardioplegia were administered in certain instances on demand by the surgeon.
- Edwards Lifesciences Retrograde Cardioplegia Catheter 14Fr x 27cm, Edwards Lifesciences LLC, Irvine, USA (Ref: RC014).
- Topical ice flush was applied onto the heart.
- Antegrade cardioplegia were repeated every ± 20minutes.
- Vessels were bypassed distally.
- At this stage the surgeon requested reperfusion cardioplegic solution to be administered.
- The ascending aorta was de-aired.
- The cross-clamp was removed.
- Blood flow into the bypassed vessels through the manifold.
- Proximal anastomoses were performed on the side-biting clamp.
- The patient was then weaned from the CABG.
- Protamine was administered to reverse the effects of the Heparin (¾ of heparin dosage = 3mg of protamine).
- Prosulf (Protamine Sulphate), CP Pharmaceuticals LTD, Wrexham (Ref: 4543/0234).
- Haemostasis was gained.
- Mediastinal and pericardial underwater drains were inserted.
- Tyco Healthcare Kendall Argyle Thoracic Catheter Straight, Tyco Healthcare United Kingdom, Gosport, United Kingdom (Ref: 8888-570549).
- The sternum were wired and closed by the surgeon.

3.6.2 Off-pump CABG surgical techniques

The patient entered the theatre and thereafter the following procedures took place:

- Soma-sensors were placed on forehead and the back.
- ECG sensors were placed on the back.
- Anaesthetics were administered.
- Median sternotomy was done by the surgeon and saphenous veins were harvested from the patient's legs by the assistant surgeon.
- Internal mammary arteries may also be mobilized by the surgeon.

- 1mg/kg Heparin Sodium-Fresenius 1000 IU/ml were administered to the patient, Fresenius Kabi for Bodene Ltd. trading as Intramed, Port Elizabeth, South Africa (Ref: J/8.2/405).
- Vessels were bypassed distally.
- The area of anastomosis was stabilized with the following:
- Medtronic Octopus 4, Medtronic Incorporated. Minneapolis, United States of America (Ref: 29400).
- Medtronic Starfish 2, Medtronic Incorporated, Minneapolis, United States of America (Ref: 29800).
- Genzyme stabilizer, Thebe Medical, South Africa (Ref: TX180010).
- The following coronary shunt was inserted according to the surgeon's choice.
- Medtronic Clearview 1.5mm, Medtronic Incorporated Minneapolis, United States of America (Ref: 31150).
- A water filled glove was placed posterior to the heart and steadily inflated in order to bring the heart more anterior if necessary or a posterior pericardial stitch and vaginal swab could be used for exposure of coronaries.
- Proximal anastomoses were performed by the side-biting clamp on the ascending aorta.
- Side-biting clamp was released.
- Protamine was administered to reverse Heparin (¾ of heparin dosage = protamine dosage).
- Prosulf (Protamine Sulphate), CP Pharmaceuticals LTD, Wrexham (Ref: 4543/0234).
- Haemostasis was gained.
- Mediastinal and Pericardial underwater drains were inserted.
- Tyco Healthcare Kendall Argyle Thoracic Catheter Straight, Tyco Healthcare United Kingdom, Gosport, United Kingdom (Ref: 8888-570549).
- Sternum was wired and closed by the surgeon (Department of Cardiothoracic Surgery, Universitas Hospital, 2009).

3.7 Statistical analysis

Data was analyzed using a Microsoft Excel spreadsheet. Lognormal, normal logarithm (*ln*), beta values, gamma distributions, histograms, Student's *t*-test and Satterthwaite adjusted *t*-test.

3.8 Ethical aspects and good clinical practice

3.8.1 Ethical clearance

The study was approved by the Ethics committee, University of the Free State (ETOVS no 51/07) (Appendix C) as well as the Clinical Head, Universitas Hospital.

3.8.2 Safety variables

3.8.2.1 Project safety

The research project was very safe. The CABG procedures were invasive and the collection of blood from the patient for laboratory analysis was non-invasive procedures.

3.8.2.2 Patient's safety

Patients participating in this study were well monitored and could at any time discontinue participation in the study.

3.8.3 Premature discontinuation of the study

The study could have been discontinued prematurely if the researcher or any of the study leaders felt that the patient's confidentiality might be breached or if any unethical procedures occurred.

3.8.4 Good clinical practice / quality assurance

The declaration of Helsinki's basic principle number 3 stated that research should be conducted only by scientifically qualified people and under the supervision of adequately qualified people (World Medical Association, 2002). Therefore, the whole research project was compiled by a registered cardiothoracic surgeon (Registered with the Health Professional Council of South Africa under the supervision of three study leaders).

3.9 Financial implications to the patient

The financial support for performing the tests stated in the protocol was the responsibility of the Department of Cardiothoracic Surgery and the Department Chemical Pathology (NHLS, Universitas hospital). No financial remuneration regarding the study's analysis was claimed from the patient.

3.10 Withdrawal criteria

Participation was completely voluntarily. Patients had the right to withdraw from this particular study at any time, irrespective the reason(s), without detriment to their medical care then, presently or in the future. The elimination of a patient from this particular study will not involve any penalty.

3.11 Subject information and informed consent

All the patients were informed about the purpose and necessity of the research project, the financial implications and the consequences as well as the adverse effects and their right to withdraw without any effects on them or their doctor-patient relationship. They signed an informed consent form (Appendix A) and received an information sheet (Appendix B) that they may have kept. The Ethical committee also approved the consent form (Appendix C). The information sheet was available in English and Afrikaans and the consent form was available in English, Afrikaans and in Sesotho.

3.12 Confidentiality

Personal details of every patient participating in this particular study were kept confidential, as far as possible. The confidentiality of this study was important. At no time during the research were any of the patients' identification made known to any other person as to whom the patient gave his/her consent.

CHAPTER 4: RESULTS

4.1 Introduction

All the numerical results for demographic data, risk factors and EuroSCORE (European System for Cardiac Operative Risk Evaluation) are presented in table format. The results of the ten inflammatory markers are represented in both tables and figures for both on-pump and off-pump CABG surgery. The results reflect pre-operative (baseline) and post-operative data at different time intervals (24, 48, 72, 96 and 120 hours post-operatively).

The process or approach that was followed in this chapter is the following:

- First, the data were summarised in terms of summary statistics and, graphically, in the form of histograms. This was done to gain a basic understanding of the data and their properties. The results of this investigation included important factors that needed to be taken into account when applying statistical methods, such as censoring and non-Normal distributions.
- The censoring was taken into account only in the sense that all statistics were calculated taking into account the decreasing sample size over time.
- The non-normal distributions are an important factor in the sense that most established statistical techniques assume that the Normal distribution is followed by the data. While these techniques are robust in respect of this assumption, it was endeavoured to transform the data to the Normal distribution whenever possible. In most cases, this was a simple process that yielded reliable results.
- Secondly, a preliminary comparison of the two groups was done that did not take into account any of the demographic data or pre-operative risk factors. This was first done visually, using the method of approximate confidence intervals where the means were plotted together with bands, which gave indications of how such means were likely to differ between samples. The idea was that roughly 95 out of 100 times where such a sample was taken; the mean would lie within those bands. Then, further expansion was done using an established statistical test widely applied in such cases.
- Thirdly, and most importantly, the ordinary least squares (OLS) regression technique was applied. This technique is useful because it allows the statistician to take both the demographic variables and the pre-operative risk factors into account. The results thus

obtained were the easiest to interpret because the effect of on-pump CABG can be quantified when that all the other parameters are constant. These results will be focused on in going forward, while the prior results should be interpreted merely as corroborating evidence.

4.1.1 Assumptions of normality for statistical tests

It was confirmed that the underlying assumptions, like a Normal distribution, hold true. This was to ensure the validity of standard statistical tests, such as the Student's *t*-test, in the analyses and conclusions. No tests were reported for normality during the performed exploratory phase, and a visual portrayal was presented of the data or lognormal data, which indicated that standard, robust tests had been applied. Here are the definitions of the statistical methods used.

4.1.2 Various statistical distributions used

- **Gamma distribution:** the gamma distribution is a two-parameter family of continuous probability distributions and is related to the beta distribution and arises naturally in processes for which the waiting times between Poisson-distributed events are relevant. It has two free parameters labelled θ and α (Weisstein, 1999-2010).
- **Beta values/logistic regression:** this is a type of predictive model that can be used when the target variable is a categorical variable with two categories - for example live/die. It does not involve decision trees and is similar to a non-linear regression in terms of fitting a polynomial to a set of data values (Weisstein, 1999-2010).
- **Normal distribution:** the normal distribution or Gaussian distribution is a continuous probability distribution that often gives a good description of data that cluster around the mean. The graph of the associated probability density function is bell-shaped with a peak at the mean, and is known as the Gaussian function or bell-curve (Weisstein, 1999-2010).
- **Lognormal distribution:** a continuous distribution in which the logarithm of a variable has a normal distribution (Weisstein, 1999-2010).
- **Zero-inflated lognormal distribution:** zero-inflated distributions are used to model count data that have many zero counts. For example, the zero-inflated Poisson distribution might be used to model count data for which the proportion of zero counts

is greater than expected on the basis of the mean of the non-zero counts (Ridout, Hinde, Demetrio, 2001).

- **Generalised extreme value (GEV) distribution:** the GEV distribution is one of the probability distributions used to model extreme events (Coles, 2001).
- **Student's *t*-test distribution:** is a continuous probability distribution that arises in the problem of estimating the mean of a normally distributed population when the sample size is small (Weisstein, 1999-2010).

4.2 Demographic data

Table 4.1 summarises the differences in patients' demographic data between the on-pump and off-pump CABG surgical groups.

Table 4.1 Demographic data

Demographic patient data	On-pump CABG (n=30)		Off-pump CABG(n=30)	
	Total of n-value	% of n-value	Total of n-value	% of n-value
Age (average):	59.13		61.2	
Gender:				
Male	27	90.00%	23	76.67%
Female	3	10.00%	7	23.33%
Ethnicity:				
White	24	80.00%	26	86.67%
Coloured	3	10.00%	3	10.00%
Black	3	10.00%	1	3.33%

4.3 Patient risk factors

Table 4.2 and 4.3 summarize the different pre-operative and post-operative risk factors between the on-pump and off-pump CABG surgical groups.

Table 4.2 Pre-operative risk factors

Risk factors	On-pump CABG (n=30)		Off-pump CABG(n=30)	
	Total of n-value	% of n-value	Total of n-value	% of n-value
Diabetes:				
• Not diabetic	24	80.00%	19	63.33%
• Diet	1	3.33%	5	16.67%
• Oral therapy	3	10.00%	4	13.33%
• Insulin	2	6.67%	2	6.67%

Risk factors	On-pump CABG (n=30)		Off-pump CABG(n=30)	
	Total of n-value	% of n-value	Total of n-value	% of n-value
Hypercholesterolemia:				
• No hypercholesterolemia	18	60.00%	14	46.67%
• Treated or >6.5 mmol/l	12	40.00%	7	23.33%
• Unknown	0	0.00%	9	30.00%
Cholesterol values (average):	4.12		4.49	
Hypertension:				
• No hypertension	11	36.67%	7	23.33%
• Treated or BP 140/90	19	63.33%	20	66.67%
• Unknown	0	0.00%	3	10.00%
Smoking:				
• Ex-smoker	6	20.00%	12	40.00%
• Never	6	20.00%	4	13.33%
• Current	18	60.00%	14	46.67%
Height in cm (average):	177.03		176.50	
Weight in kg (average):	87.13		91.47	
BMI (Average):	27.99		29.23	
Weight category:				
• Underweight [<18.5]	0	0.00%	0	0.00%
• Normal [18.5 - 25]	14	46.67%	10	33.33%
• Pre-obese [25 - 30] = overweight	7	23.33%	8	26.67%
• Obese [30 - 35]	6	20.00%	8	26.67%
• Severely obese [>35]	3	10.00%	8	26.67%

BP = blood pressure; BMI = body mass index; cm = centimetre; kg = kilogram; mmol/l = millimoles per litre

Table 4.3 Post-operative risk factors

Risk factors	On-pump CPB (n=30)		OPCAB (n=30)	
	Total of n-value	% of n-value	Total of n-value	% of n-value
Mortality:				
• No	30	100.00%	29	96.67%
• Yes <30 days	0	0.00%	1	3.33%
• Yes >30 days	0	0.00%	0	0.00%
Sternal wound infection:				
• No	29	96.67%	29	96.67%
• Yes	1	3.33%	1	3.33%
Septicaemia:				
• No	29	96.67%	29	96.67%
• Yes	1	3.33%	1	3.33%
SIRS during ICU stay:				
• No	10	33.33%	14	46.67%
• Yes	20	66.67%	16	53.33%

SIRS = systemic inflammatory response syndrome; ICU = intensive care unit

4.4 EuroSCORE: on-pump versus off-pump CABG surgery

EuroSCORE or the European System for Cardiac Operative Risk Evaluation is a method of calculating the predicted operative mortality for patients undergoing cardiac surgery (Available from http://www.euroscore.org/).

Table 4.4 summarises the EuroSCORE used to compare the on-pump versus off-pump CABG surgical groups.

Table 4.4 EuroSCORE: on-pump versus off-pump CABG surgery

EuroSCORE	On-pump CABG (n=30)		Off-pump CABG(n=30)	
	Total of n-value	% of n-value	Total of n-value	% of n-value
Chronic pulmonary disease:				
• No	25	83.33%	25	83.33%
• Yes	5	16.67%	5	16.67%
Extracardiac arteriopathy:				
• No	30	100.00%	28	93.33%
• Yes	0	0.00%	2	6.67%
Neurological dysfunction:				
• No	30	100.00%	30	100.00%
• Yes	0	0.00%	0	0.00%
Previous cardiac surgery:				
• No	29	96.67%	29	96.67%
• Yes	1	3.33%	1	3.33%
Serum creatinine >200 µmol/l				
• No	30	100.00%	30	100.00%
• Yes	0	0.00%	0	0.00%
Active endocarditis:				
• No	30	100.00%	30	100.00%
• Yes	0	0.00%	0	0.00%
Critical preoperative status:				
• No	30	100.00%	30	100.00%
• Yes	0	0.00%	0	0.00%
Unstable angina:				
• No	4	13.33%	6	20.00%
• Yes	26	86.67%	24	80.00%
LVEF moderate or 30-50%:				
• No	20	66.67%	21	70.00%
• Yes	10	33.33%	9	30.00%
LVEF poor or <30%:				

EuroSCORE	On-pump CABG (n=30)		Off-pump CABG(n=30)	
	Total of n-value	% of n-value	Total of n-value	% of n-value
• No	28	93.33%	29	96.67%
• Yes	2	6.67%	1	3.33%
Recent MI:				
• No	9	30.00%	15	50.00%
• Yes	21	70.00%	15	50.00%
Pulmonary hypertension:				
• No	30	100.00%	30	100.00%
• Yes	0	0.00%	0	0.00%
Emergency:				
• No	25	83.33%	29	96.67%
• Yes	5	16.67%	1	3.33%
Other than isolated CABG:				
• No	30	100.00%	30	100.00%
• Yes	0	0.00%	0	0.00%
Surgery on thoracic aorta:				
• No	30	100.00%	30	100.00%
• Yes	0	0.00%	0	0.00%
Post-infarct septal rupture:				
• No	30	100.00%	30	100.00%
• Yes	0	0.00%	0	0.00%
EuroSCORE (average %)		6.09%		5.49%
Median	30	4.03	30	3.33
25th percentile		2.64		1.83
75th percentile		7.12		6.02
p value (EuroSCORE (average %) median: comparison between on-pump and off-pump CABG surgery	0.3867			

LVEF = left ventricular ejection fraction; MI = myocardial infarction, CABG = coronary artery bypass graft surgery; % = percentage; n = total; µmol = micromoles

4.5 Inflammatory markers: on-pump and off-pump CABG surgery

Tables 4.5 to 4.24 reflect data for inflammatory markers (CRP, PCT, FBC, IL-6 and TNF-alpha) collected pre-operatively and post-operatively for on-pump and off-pump CABG surgery. The data are presented as pre-operative (baseline) and post-operative values at different time intervals (24, 48, 72, 96, and 120 hours respectively).

Table 4.5 C-reactive protein (on-pump CABG surgery)

C-reactive protein [mg/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	1.30	129.00	105.30	66.50	42.30	29.20
UM00444978	15.70	67.80	102.30	84.50	47.90	30.00
UM00444996	212.10	113.10	86.40	47.10	36.20	Discharged
UM00452998	37.50	98.60	198.10	179.70	131.40	160.90
UM00454075	30.10	139.80	134.00	43.00	52.90	35.20
UM00271364	82.30	100.90	76.70	31.00	23.20	66.30
UM00460094	47.10	116.30	179.00	156.20	68.90	47.20
UM00460160	22.90	81.20	96.90	79.40	63.50	50.50
UM00461761	86.70	187.10	247.50	189.30	107.80	87.80
UM00448673	6.00	92.40	132.50	70.80	33.00	Discharged
UM00462085	19.90	112.70	231.60	158.70	107.90	Discharged
UM00448963	6.70	108.70	122.20	58.30	50.50	Discharged
UM00449114	2.40	48.40	80.30	36.50	No sample	No sample
UM00464165	8.80	77.50	55.30	31.00	No sample	No sample
UM00466956	75.80	65.70	149.30	202.80	167.60	94.80
UM00054119	6.50	102.60	98.80	68.80	23.70	18.70
UM00089966	25.60	102.60	58.70	44.30	27.30	20.60
UM00472005	26.80	82.40	104.80	No sample	No sample	No sample
UM00430058	2.30	104.10	69.40	28.70	11.40	Discharged
UM00476086	20.20	119.20	107.00	55.60	35.70	Discharged
UM00469736	9.10	90.20	73.50	44.90	34.70	Discharged
UM00478175	80.90	99.20	115.10	129.40	No sample	Discharged
UM00478354	22.30	114.30	207.50	190.70	105.60	No sample
UM00479874	7.30	129.40	118.90	102.70	79.90	Discharged
UM00226204	15.30	115.80	127.60	53.90	26.50	Discharged
UM00480115	4.40	116.10	131.90	78.10	49.00	45.60
UM00397366	14.10	83.20	150.80	123.40	66.80	Discharged
UM00344738	16.70	82.20	58.70	52.50	50.60	59.70
UM00483018	0.00	144.30	230.50	149.60	80.10	Discharged
UM00494885	55.90	90.30	131.40	152.90	72.80	71.00
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	0.00	48.40	55.30	28.70	11.40	18.70
Maximum	212.10	187.10	247.50	202.80	167.60	160.90
Median	18.30	102.60	117.00	70.80	50.55	48.85
Average	32.09	103.84	126.07	93.46	61.43	58.39
SD	42.69	26.96	53.33	56.12	37.39	37.70
25th Percentile	6.85	84.95	89.03	47.10	34.95	31.30
75th Percentile	35.65	116.03	145.48	149.60	78.13	69.83

n = total; SD = standard deviation; mg/l = milligram per litre

Table 4.6 C-reactive protein (off-pump CABG surgery)

CRP [mg/l]						
UM Number	Pre operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	4.90	79.70	126.50	42.50	73.60	36.10
UM00129489	60.70	147.10	78.60	84.40	91.10	Discharged
UM00444354	15.80	33.30	64.50	51.00	Discharged	Discharged
UM00323069	0.08	112.00	55.10	28.70	17.70	Discharged
UM00259094	6.70	67.60	105.60	71.40	34.60	32.30
UM00352641	11.90	33.20	34.80	83.40	77.60	93.30
UM00447250	1.10	116.90	121.80	80.20	32.80	Discharged
UM00351965	38.20	103.40	73.90	38.10	89.00	176.30
UM00211678	10.60	11.50	41.40	64.10	Discharged	Discharged
UM00449979	2.10	80.50	114.80	117.40	117.40	32.90
UM00325501	12.00	77.20	84.50	102.80	74.80	Discharged
UM00459802	20.70	66.00	130.60	72.70	43.90	Discharged
UM00458992	2.20	147.70	204.80	97.20	57.90	57.60
UM00459940	54.00	100.30	124.50	108.00	58.90	50.10
UM00426535	1.80	109.90	82.80	37.30	33.40	Discharged
UM00343872	16.60	186.80	248.50	179.10	136.80	109.70
UM00463403	55.80	154.70	181.40	98.10	No sample	No sample
UM00465221	132.10	260.60	279.00	182.50	116.60	Discharged
UM00470093	5.90	114.40	84.50	41.50	70.70	67.40
UM00072833	0.00	151.50	126.10	91.70	70.90	40.60
UM00499583	2.10	48.70	21.30	12.60	13.30	8.30
UM00508146	84.40	90.40	83.90	42.30	Discharged	Discharged
UM00508999	8.40	63.00	150.80	140.50	Deceased	Deceased
UM00364680	1.00	65.20	148.20	173.30	156.80	101.50
UM00512436	24.00	21.00	45.60	41.60	41.60	25.20
UM00516293	4.60	24.10	6.30	6.30	No sample	No sample
UM00380508	16.30	142.50	203.80	190.90	120.30	93.40
UM00456515	15.70	133.30	236.10	216.10	210.60	144.70
UM00457930	0.13	120.20	110.70	83.50	44.90	Discharged
UM00336409	35.80	88.50	84.20	94.50	No sample	No sample
n	30.00	30.00	30.00	30.00	23.00	15.00
Minimum	0.00	11.50	6.30	6.30	13.30	8.30
Maximum	132.10	260.60	279.00	216.10	210.60	176.30
Median	11.25	95.35	108.15	83.45	70.90	57.60
Average	21.52	98.37	115.15	89.12	77.62	71.29
SD	29.82	54.19	68.00	55.06	47.96	47.58
25th Percentile	2.13	65.40	75.08	42.35	42.75	34.50
75th Percentile	23.18	130.03	143.80	106.70	103.85	97.45

n = total; SD = standard deviation; mg/l = milligram per litre

Table 4.7 Procalcitonin (on-pump CABG surgery)

Procalcitonin [µg/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	0.06	1.23	0.77	0.47	0.31	0.19
UM00444978	0.05	0.67	0.57	0.26	0.13	0.09
UM00444996	0.62	1.62	1.05	0.66	0.32	Discharged
UM00452998	0.17	4.94	2.63	1.38	0.99	1.00
UM00454075	0.13	5.20	5.00	2.55	2.19	1.46
UM00271364	0.11	11.03	6.73	3.45	1.71	0.89
UM00460094	0.06	1.46	0.95	0.80	0.50	0.27
UM00460160	0.57	2.32	1.38	0.78	0.43	0.21
UM00461761	0.13	2.83	1.39	0.86	0.64	0.43
UM00448673	0.07	0.17	0.16	0.14	0.11	Discharged
UM00462085	0.08	2.08	0.88	0.59	0.33	Discharged
UM00448963	0.09	1.06	0.80	0.45	0.30	Discharged
UM00449114	0.05	1.73	1.23	0.63	No sample	No sample
UM00464165	0.11	6.32	5.09	2.56	No sample	No sample
UM00466956	0.11	4.41	4.38	3.64	1.89	0.87
UM00054119	0.07	1.90	1.35	0.86	0.49	0.25
UM00089966	0.06	6.67	3.80	1.94	1.21	0.70
UM00472005	0.08	0.37	0.38	No sample	No sample	No sample
UM00430058	0.06	2.08	1.51	0.81	0.55	Discharged
UM00476086	0.00	1.21	0.72	0.37	0.38	Discharged
UM00469736	0.07	3.53	1.95	1.05	0.74	Discharged
UM00478175	0.17	0.56	0.32	0.28	No sample	Discharged
UM00478354	0.15	9.78	7.15	3.75	1.24	No sample
UM00479874	0.07	0.71	0.49	0.30	0.21	Discharged
UM00226204	0.00	4.86	3.27	1.89	1.03	Discharged
UM00480115	0.07	1.72	1.22	0.59	0.32	0.21
UM00397366	0.00	1.20	0.68	0.44	0.22	Discharged
UM00344738	0.08	1.81	1.42	0.73	0.46	0.58
UM00483018	0.05	1.68	0.93	0.57	0.38	Discharged
UM00494885	0.05	0.06	0.55	0.34	0.19	0.11
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	0.00	0.06	0.16	0.14	0.11	0.09
Maximum	0.62	11.03	7.15	3.75	2.19	1.46
Median	0.07	1.77	1.23	0.73	0.45	0.35
Average	0.11	2.84	1.96	1.14	0.66	0.52
SD	0.14	2.73	1.92	1.06	0.56	0.41
25th Percentile	0.06	1.20	0.73	0.45	0.31	0.21
75th Percentile	0.11	4.19	2.46	1.38	0.93	0.83

n = total; SD = standard deviation; µg/l = micrograms per litre

Table 4.8 Procalcitonin (off-pump CABG surgery)

PCT [µg/L]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	0.02	0.81	1.06	0.25	0.36	0.23
UM00129489	0.08	0.20	0.17	0.16	0.14	Discharged
UM00444354	0.05	0.07	0.06	0.05	Discharged	Discharged
UM00323069	0.00	0.63	0.47	0.30	0.20	Discharged
UM00259094	0.07	0.20	0.18	0.15	0.16	0.13
UM00352641	0.08	0.06	0.07	0.09	0.07	0.06
UM00447250	0.07	0.15	0.10	0.10	1.23	Discharged
UM00351965	0.57	9.14	6.60	2.96	2.09	1.12
UM00211678	0.37	0.15	0.12	0.13	Discharged	Discharged
UM00449979	0.05	0.19	0.17	0.14	0.14	0.10
UM00325501	0.08	0.41	0.29	0.21	0.15	Discharged
UM00459802	0.09	0.17	0.16	0.18	0.33	Discharged
UM00458992	0.07	0.70	0.51	0.40	0.36	0.29
UM00459940	0.09	1.73	1.26	0.71	0.36	0.23
UM00426535	0.02	2.65	2.03	1.23	0.86	Discharged
UM00343872	0.08	22.51	13.77	7.75	4.45	2.34
UM00463403	0.11	0.44	0.35	0.22	No sample	No sample
UM00465221	0.17	2.76	2.20	1.21	0.82	Discharged
UM00470093	0.05	2.32	1.48	0.83	0.68	0.34
UM00072833	0.08	0.50	0.34	0.17	0.16	0.10
UM00499583	0.06	0.17	0.11	0.08	0.08	0.07
UM00508146	0.00	0.19	0.16	0.14	Discharged	Discharged
UM00508999	0.07	0.09	0.09	0.08	Deceased	Deceased
UM00364680	0.05	8.75	7.48	3.25	1.65	0.76
UM00512436	0.09	0.08	0.28	0.17	0.17	0.08
UM00516293	0.08	4.65	2.14	2.14	No sample	No sample
UM00380508	0.08	6.23	5.37	3.70	2.34	1.08
UM00456515	0.12	1.07	1.12	1.89	3.31	8.55
UM00457930	0.13	0.69	0.48	0.45	0.38	Discharged
UM00336409	0.10	1.88	1.48	0.82	No sample	No sample
n	30.00	30.00	30.00	30.00	23.00	15.00
Minimum	0.00	0.06	0.06	0.05	0.07	0.06
Maximum	0.57	22.51	13.77	7.75	4.45	8.55
Median	0.08	0.57	0.41	0.24	0.36	0.23
Average	0.10	2.32	1.67	1.00	0.89	1.03
SD	0.11	4.53	2.98	1.63	1.15	2.17
25th Percentile	0.05	0.18	0.16	0.14	0.16	0.10
75th Percentile	0.09	2.21	1.48	1.12	1.05	0.92

n = total; SD = standard deviation; µg/l = microgram per litre

Table 4.9 White cell count (on-pump CABG surgery)

White cell count [10^9/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	5.38	12.56	13.94	12.95	8.45	7.58
UM00444978	6.63	18.77	17.77	14.04	11.95	12.39
UM00444996	15.90	25.70	20.63	20.45	13.41	Discharged
UM00452998	10.86	14.95	3.62	15.75	11.75	10.75
UM00454075	8.23	27.79	31.99	15.78	12.74	11.75
UM00271364	14.22	34.08	28.91	18.82	12.64	16.64
UM00460094	8.97	19.47	12.65	11.93	11.69	9.78
UM00460160	9.72	26.94	24.14	23.23	16.66	14.73
UM00461761	8.38	19.99	16.16	13.88	10.56	9.29
UM00448673	6.13	14.14	12.78	13.66	12.25	Discharged
UM00462085	6.58	17.12	18.80	13.99	11.02	Discharged
UM00448963	5.70	18.27	13.52	8.98	6.84	Discharged
UM00449114	9.81	15.27	19.55	18.57	No sample	No sample
UM00464165	11.14	22.91	24.21	20.24	No sample	No sample
UM00466956	7.75	7.75	19.99	19.55	10.59	11.93
UM00054119	10.78	26.78	19.97	12.58	10.92	11.72
UM00089966	11.20	14.32	10.06	12.08	10.38	10.38
UM00472005	2.03	12.88	10.88	No sample	No sample	No sample
UM00430058	8.58	21.34	18.21	13.63	10.95	Discharged
UM00476086	10.63	25.12	24.07	18.94	12.26	Discharged
UM00469736	11.60	35.44	34.69	23.94	20.45	Discharged
UM00478175	8.83	16.15	14.75	10.16	No sample	Discharged
UM00478354	11.95	29.72	26.05	19.07	14.57	No sample
UM00479874	8.76	16.69	17.09	14.84	13.84	Discharged
UM00226204	11.08	26.69	28.22	21.51	13.77	Discharged
UM00480115	10.68	23.27	28.80	26.79	20.62	17.18
UM00397366	9.51	11.85	15.63	12.81	11.20	Discharged
UM00344738	13.83	17.55	19.21	13.28	15.05	16.94
UM00483018	9.10	18.45	19.14	17.32	15.96	Discharged
UM00494885	9.58	18.53	17.56	15.43	15.38	13.02
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	2.03	7.75	3.62	8.98	6.84	7.58
Maximum	15.90	35.44	34.69	26.79	20.62	17.18
Median	9.55	18.65	18.97	15.43	12.26	11.84
Average	9.45	20.35	19.43	16.35	12.92	12.43
SD	2.82	6.68	6.91	4.32	3.15	2.97
25th Percentile	8.27	15.49	14.97	13.28	10.97	10.47
75th Percentile	11.03	25.56	24.12	19.07	14.39	14.30

n = total; SD = standard deviation

Table 4.10 White cell count (off-pump CABG surgery)

White cell count [10^9/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	8.14	6.26	8.18	3.88	10.15	9.34
UM00129489	14.01	15.75	14.31	11.95	11.92	Discharged
UM00444354	4.02	12.00	10.97	9.91	Discharged	Discharged
UM00323069	7.38	13.50	12.24	7.92	6.96	Discharged
UM00259094	5.47	13.37	10.25	9.10	9.06	10.36
UM00352641	7.44	16.26	13.42	11.18	11.04	9.56
UM00447250	10.47	16.00	13.48	9.80	9.32	Discharged
UM00351965	13.29	11.58	12.93	12.54	14.18	15.15
UM00211678	5.09	12.30	11.22	7.37	Discharged	Discharged
UM00449979	7.84	21.60	18.23	16.03	16.03	13.44
UM00325501	9.63	17.58	17.49	14.24	11.28	Discharged
UM00459802	9.58	20.78	15.27	12.93	16.73	Discharged
UM00458992	13.48	20.70	18.58	15.41	11.91	12.31
UM00459940	5.53	14.21	16.82	12.59	11.13	9.93
UM00426535	9.33	22.47	23.22	11.94	10.73	Discharged
UM00343872	5.98	16.16	14.89	12.87	10.29	9.32
UM00463403	9.94	12.20	11.80	9.34	No sample	No sample
UM00465221	12.49	16.19	14.09	10.83	10.56	Discharged
UM00470093	10.38	18.53	23.60	13.75	10.76	14.66
UM00072833	7.00	16.07	16.21	11.56	11.01	7.62
UM00499583	5.83	16.93	12.13	8.31	9.19	8.70
UM00508146	8.85	16.15	13.74	13.01	Discharged	Discharged
UM00508999	10.91	13.63	14.12	12.80	Deceased	Deceased
UM00364680	6.62	9.99	15.41	9.96	7.65	7.74
UM00512436	6.10	5.84	8.15	8.05	8.05	8.40
UM00516293	8.83	23.14	15.79	15.79	No sample	No sample
UM00380508	7.44	16.04	16.12	13.38	10.25	10.69
UM00456515	10.13	21.09	23.64	21.70	27.14	30.08
UM00457930	10.62	33.06	18.73	16.77	13.31	Discharged
UM00336409	5.12	7.30	7.17	6.10	No sample	No sample
n	30.00	30.00	30.00	30.00	23.00	15.00
Minimum	4.02	5.84	7.17	3.88	6.96	7.62
Maximum	14.01	33.06	23.64	21.70	27.14	30.08
Median	8.49	16.06	14.22	11.95	10.76	9.93
Average	8.56	15.89	14.74	11.70	11.68	11.82
SD	2.69	5.54	4.20	3.57	4.12	5.58
25th Percentile	6.23	12.57	12.16	9.46	9.74	9.01
75th Percentile	10.32	18.29	16.67	13.29	11.92	12.88

n = total; SD = standard deviation

Table 4.11 Neutrophils (on-pump CABG surgery)

Neutrophils [10^9/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	3.13	10.46	11.75	10.28	5.75	5.08
UM00444978	3.25	14.40	14.70	10.28	8.53	7.19
UM00444996	11.11	22.59	18.42	16.91	10.45	Discharged
UM00452998	7.47	11.21	1.52	13.45	9.05	8.40
UM00454075	5.14	24.12	29.78	14.60	10.85	9.14
UM00271364	8.66	23.17	25.06	13.74	8.91	9.15
UM00460094	5.22	15.91	9.65	8.88	7.52	5.75
UM00460160	4.88	23.33	19.46	18.89	10.83	9.52
UM00461761	5.16	15.99	13.67	10.78	6.85	6.73
UM00448673	1.33	9.37	9.00	7.61	5.75	Discharged
UM00462085	3.78	11.81	15.04	10.35	8.30	Discharged
UM00448963	3.06	13.89	11.19	6.62	3.86	Discharged
UM00449114	5.21	9.93	15.27	14.48	No sample	No sample
UM00464165	6.41	19.82	21.33	17.04	No sample	No sample
UM00466956	3.84	6.37	11.59	10.36	8.54	10.74
UM00054119	6.85	20.35	15.86	9.56	7.81	5.97
UM00089966	7.40	10.90	11.36	8.64	6.06	7.43
UM00472005	0.41	10.57	7.18	no sample	No sample	No sample
UM00430058	5.24	14.30	12.20	9.13	7.84	Discharged
UM00476086	6.86	10.80	14.44	14.60	8.72	Discharged
UM00469736	7.82	31.15	23.94	19.73	15.38	Discharged
UM00478175	5.77	11.47	11.92	8.93	No sample	Discharged
UM00478354	6.87	21.10	21.65	13.16	8.68	No sample
UM00479874	4.73	11.02	13.07	10.30	8.11	Discharged
UM00226204	7.20	16.39	25.06	16.43	9.71	Discharged
UM00480115	5.29	19.50	24.45	22.37	16.39	12.02
UM00397366	6.08	10.43	12.08	9.61	7.19	Discharged
UM00344738	9.90	14.76	12.10	8.50	11.11	13.64
UM00483018	3.62	12.55	14.97	9.18	11.06	Discharged
UM00494885	4.60	15.42	13.10	10.82	9.66	7.72
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	0.41	6.37	1.52	6.62	3.86	5.08
Maximum	11.11	31.15	29.78	22.37	16.39	13.64
Median	5.23	14.35	14.06	10.36	8.61	8.06
Average	5.54	15.44	15.36	12.25	8.96	8.46
SD	2.31	5.67	6.17	3.98	2.72	2.45
25th Percentile	4.03	10.93	11.79	9.18	7.59	6.85
75th Percentile	6.87	19.74	19.20	14.60	10.27	9.43

n = total; SD = standard deviation

Table 4.12 Neutrophils (off-pump CABG surgery)

UM Number	Neutrophils [10^9/l]					
	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	5.01	4.57	6.45	3.02	8.22	6.44
UM00129489	11.12	13.99	12.02	9.08	7.99	Discharged
UM00444354	2.39	10.15	8.93	6.58	Discharged	Discharged
UM00323069	3.53	11.95	9.29	5.08	4.87	Discharged
UM00259094	1.83	10.74	7.49	5.51	5.11	5.64
UM00352641	4.47	13.93	10.49	7.94	7.74	6.41
UM00447250	4.96	12.48	11.73	7.09	6.05	Discharged
UM00351965	9.73	9.95	11.43	9.51	10.49	10.45
UM00211678	2.61	10.22	8.29	4.64	Discharged	Discharged
UM00449979	4.39	18.64	15.22	13.72	13.72	3.02
UM00325501	6.50	12.31	13.82	11.55	7.03	Discharged
UM00459802	6.75	15.99	13.04	9.74	12.76	Discharged
UM00458992	7.67	155.00	13.75	10.66	6.90	6.83
UM00459940	3.64	12.60	14.53	10.20	8.84	7.21
UM00426535	4.49	19.23	19.71	6.78	5.62	Discharged
UM00343872	3.38	10.83	13.43	9.27	8.30	7.44
UM00463403	5.58	8.66	9.56	6.11	No sample	No sample
UM00465221	7.47	12.58	10.36	6.67	5.25	Discharged
UM00470093	6.06	16.27	17.23	9.28	7.21	8.85
UM00072833	4.77	9.64	13.03	9.17	7.38	5.07
UM00499583	3.42	15.37	9.36	5.14	5.79	5.09
UM00508146	4.96	9.37	10.29	7.51	Discharged	Discharged
UM00508999	3.70	6.82	11.42	9.20	Deceased	Deceased
UM00364680	4.06	7.74	11.59	7.57	5.40	5.02
UM00512436	3.38	5.03	5.69	5.49	5.49	4.48
UM00516293	5.45	20.15	11.65	11.65	No sample	No sample
UM00380508	4.56	4.17	5.59	9.57	6.41	6.84
UM00456515	6.79	14.97	19.86	17.36	21.71	20.46
UM00457930	6.16	24.13	14.80	10.57	8.25	Discharged
UM00336409	3.20	5.91	5.74	3.90	No sample	No sample
n	30.00	30.00	30.00	30.00	23.00	15.00
Minimum	1.83	4.17	5.59	3.02	4.87	3.02
Maximum	11.12	155.00	19.86	17.36	21.71	20.46
Median	4.67	12.13	11.51	8.51	7.21	6.44
Average	5.07	16.78	11.53	8.32	8.11	7.28
SD	2.08	26.54	3.72	3.05	3.75	4.07
25th Percentile	3.56	9.44	9.31	6.23	5.71	5.08
75th Percentile	6.14	15.27	13.67	9.70	8.28	7.33

n = total; SD = standard deviation

Table 4.13 Lymphocytes (on-pump CABG surgery)

UM Number	Lymphocytes [10^9/l]					
	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	1.84	1.26	1.55	1.89	2.06	1.89
UM00444978	2.13	0.88	1.33	2.20	2.29	3.44
UM00444996	2.78	1.18	0.91	2.23	1.94	Discharged
UM00452998	2.61	0.75	1.52	1.39	1.57	1.55
UM00454075	2.19	1.17	1.22	0.68	1.13	1.48
UM00271364	4.00	1.70	2.08	3.01	2.95	5.32
UM00460094	2.85	1.89	2.01	1.81	2.93	2.62
UM00460160	4.21	1.94	3.65	2.95	1.83	3.70
UM00461761	1.99	1.80	1.34	2.07	2.44	1.67
UM00448673	4.26	3.17	2.79	4.63	5.23	Discharged
UM00462085	2.02	1.54	1.88	0.98	1.99	Discharged
UM00448963	2.03	2.56	1.43	1.79	2.18	Discharged
UM00449114	6.54	2.14	2.64	1.30	No sample	No sample
UM00464165	2.81	0.82	1.84	1.74	No sample	No sample
UM00466956	2.74	0.62	2.60	3.91	1.60	0.89
UM00054119	2.94	2.14	2.80	2.43	2.34	4.08
UM00089966	2.42	1.30	1.93	2.62	3.59	1.91
UM00472005	1.47	1.03	0.76	No sample	No sample	No sample
UM00430058	2.37	1.07	1.82	2.32	2.40	Discharged
UM00476086	2.87	1.76	2.17	2.88	2.67	Discharged
UM00469736	2.71	1.35	3.82	2.59	3.29	Discharged
UM00478175	1.22	0.81	0.74	0.58	No sample	Discharged
UM00478354	3.51	2.38	2.84	2.86	3.93	No sample
UM00479874	3.11	0.83	2.77	3.15	4.48	Discharged
UM00226204	2.99	1.07	2.03	3.79	3.13	Discharged
UM00480115	4.69	1.70	2.53	2.97	3.24	4.12
UM00397366	2.83	1.30	2.52	2.36	3.02	Discharged
UM00344738	3.06	1.12	1.73	2.26	2.63	2.19
UM00483018	4.62	1.11	2.09	3.29	3.54	Discharged
UM00494885	4.29	2.19	3.02	3.53	4.15	3.89
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	1.22	0.62	0.74	0.58	1.13	0.89
Maximum	6.54	3.17	3.82	4.63	5.23	5.32
Median	2.82	1.30	2.02	2.36	2.65	2.41
Average	3.00	1.49	2.08	2.42	2.79	2.77
SD	1.12	0.61	0.78	0.95	0.97	1.31
25th Percentile	2.24	1.07	1.53	1.81	2.09	1.73
75th Percentile	3.41	1.87	2.63	2.97	3.28	3.84

n = total; SD = standard deviation

Table 4.14 Lymphocytes (off-pump CABG surgery)

Lymphocytes [10^9/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	2.36	0.63	1.07	0.49	1.01	1.59
UM00129489	1.65	0.88	1.29	2.03	2.74	Discharged
UM00444354	1.21	0.72	1.10	2.07	Discharged	Discharged
UM00323069	2.41	0.74	1.62	1.72	1.11	Discharged
UM00259094	2.58	1.44	1.58	2.02	2.27	2.95
UM00352641	2.38	1.37	1.99	2.37	2.29	1.97
UM00447250	4.48	0.80	0.94	1.99	2.25	Discharged
UM00351965	1.85	0.72	0.63	1.87	2.13	2.27
UM00211678	1.92	1.16	1.87	1.87	Discharged	Discharged
UM00449979	2.73	1.97	1.48	1.44	1.44	2.45
UM00325501	2.02	1.23	1.57	1.32	2.65	Discharged
UM00459802	2.36	0.62	1.13	2.33	2.84	Discharged
UM00458992	4.15	19.68	2.79	3.17	2.93	3.41
UM00459940	1.42	0.77	1.36	1.59	1.64	2.02
UM00426535	4.18	1.93	2.65	4.33	4.14	Discharged
UM00343872	1.87	1.57	0.85	1.03	1.39	0.99
UM00463403	3.16	0.85	0.91	1.94	No sample	No sample
UM00465221	4.10	2.25	2.54	2.89	3.76	Discharged
UM00470093	3.59	0.98	3.78	3.90	2.86	4.31
UM00072833	1.54	0.48	1.54	1.17	1.65	1.54
UM00499583	2.04	0.91	1.90	2.22	2.31	2.56
UM00508146	3.12	2.42	2.67	4.48	Discharged	Discharged
UM00508999	1.97	1.09	1.28	15.50	Deceased	Deceased
UM00364680	1.76	0.48	1.59	1.22	1.16	1.49
UM00512436	1.93	0.69	1.55	1.71	1.71	2.64
UM00516293	2.22	1.30	3.06	3.06	No sample	No sample
UM00380508	1.75	0.96	1.12	1.87	2.08	1.82
UM00456515	2.36	0.42	1.65	2.60	1.09	3.01
UM00457930	2.88	2.98	2.25	2.35	2.66	Discharged
UM00336409	1.35	0.76	0.90	0.73	No sample	No sample
n	30.00	30.00	30.00	30.00	23.00	15.00
Minimum	1.21	0.42	0.63	0.49	1.01	0.99
Maximum	4.48	19.68	3.78	15.50	4.14	4.31
Median	2.29	0.94	1.56	2.01	2.25	2.27
Average	2.44	1.76	1.69	2.58	2.18	2.33
SD	0.90	3.44	0.75	2.62	0.83	0.86
25th Percentile	1.86	0.73	1.12	1.62	1.54	1.71
75th Percentile	2.84	1.42	1.97	2.54	2.70	2.80

n = total; SD = standard deviation

Table 4.15 Monocytes (on-pump CABG surgery)

UM Number	Monocytes [10^9/l]					
	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	0.38	0.83	0.63	0.74	0.61	0.53
UM00444978	0.92	3.47	1.72	1.49	0.76	0.85
UM00444996	1.75	1.90	1.30	1.25	0.80	Discharged
UM00452998	0.70	0.75	0.65	0.83	0.89	0.68
UM00454075	0.67	2.50	0.99	0.50	0.71	0.85
UM00271364	1.29	0.34	1.76	0.94	0.71	1.16
UM00460094	0.62	1.67	0.97	1.18	1.01	1.02
UM00460160	0.48	1.67	0.94	1.23	0.67	1.19
UM00461761	1.14	0.80	1.13	0.97	1.16	0.81
UM00448673	0.30	1.58	0.98	1.31	1.00	Discharged
UM00462085	0.48	1.03	0.00	0.70	0.61	Discharged
UM00448963	0.48	0.18	0.88	0.49	0.55	Discharged
UM00449114	0.86	1.07	1.60	0.74	No sample	No sample
UM00464165	1.57	2.27	1.04	1.44	No sample	No sample
UM00466956	0.86	0.76	0.60	0.39	0.40	0.25
UM00054119	0.59	0.54	1.28	0.54	0.67	1.18
UM00089966	1.22	2.12	0.76	0.72	0.48	0.92
UM00472005	0.06	1.26	0.98	No sample	No sample	No sample
UM00430058	0.72	1.28	0.18	0.68	0.55	Discharged
UM00476086	0.75	2.01	1.20	1.29	0.66	Discharged
UM00469736	0.57	2.91	0.35	1.48	1.39	Discharged
UM00478175	1.20	0.48	0.44	0.40	No sample	Discharged
UM00478354	0.99	2.67	1.54	1.72	1.36	No sample
UM00479874	0.57	1.50	1.23	1.28	0.64	Discharged
UM00226204	0.66	2.38	1.13	1.23	0.78	Discharged
UM00480115	0.61	2.07	1.81	1.39	0.87	0.52
UM00397366	0.57	0.12	0.98	0.81	0.82	Discharged
UM00344738	0.72	1.67	1.34	0.66	1.26	1.08
UM00483018	0.76	2.40	2.05	0.35	1.24	Discharged
UM00494885	0.43	0.91	1.39	0.97	1.28	0.98
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	0.06	0.12	0.00	0.35	0.40	0.25
Maximum	1.75	3.47	2.05	1.72	1.39	1.19
Median	0.69	1.54	1.02	0.94	0.77	0.89
Average	0.76	1.50	1.06	0.96	0.84	0.86
SD	0.37	0.86	0.49	0.39	0.29	0.28
25th Percentile	0.57	0.81	0.79	0.68	0.65	0.71
75th Percentile	0.91	2.11	1.33	1.28	1.01	1.07

n = total; SD = standard deviation

Table 4.16 Monocytes (off-pump CABG surgery)

Monocytes [10^9/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	0.65	0.25	0.54	0.25	0.81	0.84
UM00129489	1.20	0.88	1.00	0.72	0.24	Discharged
UM00444354	0.29	1.09	0.92	1.12	Discharged	Discharged
UM00323069	1.21	0.78	1.21	0.81	0.70	Discharged
UM00259094	0.73	1.18	1.13	1.21	1.30	1.25
UM00352641	0.36	0.96	0.90	0.79	0.74	0.79
UM00447250	0.49	1.12	0.81	0.55	0.66	Discharged
UM00351965	1.65	0.91	0.87	1.13	0.43	0.76
UM00211678	0.45	0.92	1.03	0.79	Discharged	Discharged
UM00449979	0.37	0.99	1.46	0.74	0.74	1.16
UM00325501	0.83	2.81	2.10	1.28	1.22	Discharged
UM00459802	0.28	1.66	1.08	0.74	0.84	Discharged
UM00458992	1.29	1.50	1.30	1.37	1.45	1.34
UM00459940	0.37	0.82	0.91	0.76	0.55	0.56
UM00426535	0.50	1.30	0.86	0.72	0.78	Discharged
UM00343872	0.51	0.63	0.60	0.39	0.44	0.27
UM00463403	0.81	0.61	1.23	0.90	No sample	No sample
UM00465221	0.87	1.36	1.13	1.10	1.25	Discharged
UM00470093	0.48	1.28	0.47	0.49	0.49	1.03
UM00072833	0.51	0.96	1.59	1.18	0.99	0.73
UM00499583	0.31	0.64	0.82	0.70	0.67	0.64
UM00508146	0.51	0.48	0.76	0.74	Discharged	Discharged
UM00508999	1.03	0.68	1.38	1.40	Deceased	Deceased
UM00364680	0.56	1.76	2.20	1.09	0.84	0.92
UM00512436	0.40	0.08	0.74	0.64	0.64	0.81
UM00516293	0.65	1.39	0.99	0.99	No sample	No sample
UM00380508	0.90	2.41	2.07	1.77	1.49	1.18
UM00456515	0.70	0.21	0.71	1.52	1.09	0.60
UM00457930	1.39	2.31	0.94	1.34	0.53	Discharged
UM00336409	0.36	0.57	0.39	0.37	No sample	No sample
n	30.00	30.00	30.00	30.00	23.00	15.00
Minimum	0.28	0.08	0.39	0.25	0.24	0.27
Maximum	1.65	2.81	2.20	1.77	1.49	1.34
Median	0.54	0.96	0.97	0.80	0.74	0.81
Average	0.69	1.08	1.07	0.92	0.82	0.86
SD	0.36	0.63	0.45	0.36	0.34	0.29
25th Percentile	0.41	0.65	0.81	0.72	0.60	0.69
75th Percentile	0.86	1.35	1.23	1.17	1.04	1.10

n = total; SD = standard deviation

Table 4.17 Eosinophils (on-pump CABG surgery)

Eosinophils [10^9/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	0.02	0.00	0.00	0.01	0.02	0.08
UM00444978	0.30	0.00	0.00	0.06	0.30	0.83
UM00444996	0.17	0.00	0.00	0.04	0.20	Discharged
UM00452998	0.07	2.24	0.00	0.06	0.22	0.12
UM00454075	0.23	0.00	0.00	0.00	0.03	0.28
UM00271364	0.26	0.00	0.00	0.94	0.05	0.17
UM00460094	0.26	0.00	0.01	0.06	0.22	0.38
UM00460160	0.12	0.00	0.07	0.14	0.33	0.29
UM00461761	0.08	0.00	0.00	0.04	0.10	0.07
UM00448673	0.19	0.00	0.00	0.10	0.25	Discharged
UM00462085	0.27	0.00	0.19	0.14	0.08	Discharged
UM00448963	0.08	0.00	0.01	0.07	0.21	Discharged
UM00449114	0.17	0.00	0.02	0.00	No sample	No sample
UM00464165	0.35	0.00	0.00	0.02	No sample	No sample
UM00466956	0.29	0.00	4.60	0.00	0.04	0.04
UM00054119	0.38	0.00	0.02	0.04	0.10	0.47
UM00089966	0.13	0.00	0.01	0.08	0.20	0.10
UM00472005	0.07	0.01	0.00	No sample	No sample	No sample
UM00430058	0.23	0.00	0.00	0.00	0.13	Discharged
UM00476086	0.12	10.05	6.02	0.15	0.18	Discharged
UM00469736	0.45	0.04	0.00	0.14	0.37	Discharged
UM00478175	0.61	0.00	0.15	0.23	No sample	Discharged
UM00478354	0.54	0.00	0.00	0.00	0.57	No sample
UM00479874	0.31	0.00	0.02	0.10	0.54	Discharged
UM00226204	0.22	0.00	0.00	0.04	0.12	Discharged
UM00480115	0.05	0.00	0.00	0.03	0.04	0.52
UM00397366	0.02	0.00	0.03	0.03	0.15	Discharged
UM00344738	0.12	0.00	0.00	0.00	0.03	0.02
UM00483018	0.35	0.00	0.00	0.00	0.10	Discharged
UM00494885	0.23	0.00	0.04	0.06	0.22	0.36
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	0.02	0.00	0.00	0.00	0.02	0.02
Maximum	0.61	10.05	6.02	0.94	0.57	0.83
Median	0.23	0.00	0.00	0.04	0.17	0.23
Average	0.22	0.41	0.37	0.09	0.18	0.27
SD	0.15	1.87	1.36	0.17	0.15	0.23
25th Percentile	0.12	0.00	0.00	0.01	0.09	0.09
75th Percentile	0.30	0.00	0.02	0.10	0.22	0.38

n = total; SD = standard deviation

Table 4.18 Eosinophils (off-pump CABG surgery)

Eosinophils [10^9/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	0.08	0.81	0.08	0.11	0.10	0.37
UM00129489	0.01	0.00	0.00	0.12	0.24	Discharged
UM00444354	0.11	0.00	0.01	0.10	Discharged	Discharged
UM00323069	0.18	0.01	0.10	0.26	0.21	Discharged
UM00259094	0.29	0.00	0.04	0.32	0.34	0.45
UM00352641	0.19	0.00	0.01	0.04	0.24	0.34
UM00447250	0.48	0.00	0.00	0.16	0.34	Discharged
UM00351965	0.04	0.00	0.00	0.03	0.14	0.00
UM00211678	0.10	0.00	0.01	0.07	Discharged	Discharged
UM00449979	0.31	0.00	0.05	0.11	0.11	0.81
UM00325501	0.26	0.00	0.00	0.07	0.34	Discharged
UM00459802	0.18	0.00	0.00	0.10	0.25	Discharged
UM00458992	0.34	2.67	0.00	0.18	0.61	0.70
UM00459940	0.09	0.00	0.00	0.04	0.10	0.13
UM00426535	0.14	0.00	0.00	0.10	0.16	Discharged
UM00343872	0.19	0.00	0.00	0.00	0.14	0.18
UM00463403	0.38	0.00	0.09	0.39	No sample	No sample
UM00465221	0.02	0.00	0.06	0.15	0.27	Discharged
UM00470093	0.20	0.00	0.00	0.05	0.18	0.43
UM00072833	0.16	0.00	0.03	0.03	0.11	0.25
UM00499583	0.04	0.00	0.02	0.24	0.40	0.40
UM00508146	0.23	0.00	0.01	0.23	Discharged	Discharged
UM00508999	0.20	0.00	0.01	0.00	Deceased	Deceased
UM00364680	0.22	0.00	0.02	0.09	0.24	0.27
UM00512436	0.37	0.04	0.16	0.20	0.20	0.46
UM00516293	0.43	0.00	0.06	0.06	No sample	No sample
UM00380508	0.15	0.00	0.16	0.13	0.20	0.11
UM00456515	0.26	0.00	0.00	0.00	2.99	0.00
UM00457930	0.18	3.31	0.00	0.17	0.27	Discharged
UM00336409	0.19	0.05	0.13	0.12	No sample	No sample
n	30.00	30.00	30.00	30.00	23.00	15.00
Minimum	0.01	0.00	0.00	0.00	0.10	0.00
Maximum	0.48	3.31	0.16	0.39	2.99	0.81
Median	0.19	0.00	0.01	0.11	0.24	0.34
Average	0.20	0.23	0.04	0.12	0.36	0.33
SD	0.12	0.77	0.05	0.10	0.59	0.23
25th Percentile	0.12	0.00	0.00	0.05	0.15	0.16
75th Percentile	0.26	0.00	0.06	0.17	0.31	0.44

n = total; SD = standard deviation

Table 4.19 Basophils (on-pump CABG surgery)

UM Number	Basophils [10^9/l]					
	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	0.01	0.01	0.01	0.03	0.02	0.00
UM00444978	0.03	0.02	0.02	0.01	0.06	0.07
UM00444996	0.08	0.03	0.00	0.02	0.01	Discharged
UM00452998	0.02	0.00	0.02	0.02	0.01	0.01
UM00454075	0.01	0.00	0.00	0.00	0.01	0.00
UM00271364	0.01	0.00	0.00	0.19	0.03	0.83
UM00460094	0.02	0.00	0.00	0.00	0.01	0.01
UM00460160	0.04	0.00	0.02	0.02	0.00	0.03
UM00461761	0.01	0.00	0.02	0.01	0.01	0.01
UM00448673	0.05	0.01	0.00	0.01	0.02	Discharged
UM00462085	0.03	0.00	0.00	0.00	0.04	Discharged
UM00448963	0.05	0.00	0.00	0.01	0.04	Discharged
UM00449114	0.03	0.00	0.02	0.00	No sample	No sample
UM00464165	0.01	0.00	0.00	0.00	No sample	No sample
UM00466956	0.01	0.00	0.60	0.00	0.01	0.01
UM00054119	0.02	0.00	0.02	0.01	0.01	0.02
UM00089966	0.02	0.00	0.00	0.01	0.05	0.01
UM00472005	0.02	0.00	0.00	No sample	No sample	No sample
UM00430058	0.02	0.00	0.00	0.00	0.03	Discharged
UM00476086	0.03	0.50	0.24	0.02	0.02	Discharged
UM00469736	0.05	0.00	0.00	0.00	0.02	Discharged
UM00478175	0.03	0.00	1.47	0.02	No sample	Discharged
UM00478354	0.04	0.00	0.03	0.00	0.03	No sample
UM00479874	0.04	0.00	0.00	0.01	0.07	Discharged
UM00226204	0.00	0.00	0.00	0.02	0.03	Discharged
UM00480115	0.04	0.00	0.00	0.03	0.08	0.00
UM00397366	0.01	0.00	0.02	0.01	0.02	Discharged
UM00344738	0.03	0.00	0.00	0.00	0.02	0.02
UM00483018	0.05	0.00	0.04	0.00	0.02	Discharged
UM00494885	0.03	0.02	0.02	0.05	0.08	0.07
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	0.08	0.50	1.47	0.19	0.08	0.83
Median	0.03	0.00	0.00	0.01	0.02	0.01
Average	0.03	0.02	0.09	0.02	0.03	0.08
SD	0.02	0.09	0.29	0.04	0.02	0.22
25th Percentile	0.01	0.00	0.00	0.00	0.01	0.01
75th Percentile	0.04	0.00	0.02	0.02	0.04	0.03

n = total; SD = standard deviation

Table 4.20 Basophils (off-pump CABG surgery)

Basophils [10^9/l]						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	0.03	0.00	0.03	0.01	0.00	0.09
UM00129489	0.01	0.00	0.00	0.00	0.72	Discharged
UM00444354	0.02	0.01	0.01	0.04	Discharged	Discharged
UM00323069	0.05	0.01	0.02	0.05	0.07	Discharged
UM00259094	0.04	0.01	0.01	0.04	0.04	0.07
UM00352641	0.04	0.00	0.03	0.03	0.03	0.04
UM00447250	0.05	0.00	0.00	0.02	0.03	Discharged
UM00351965	0.03	0.00	0.00	0.01	0.99	0.00
UM00211678	0.01	0.00	0.01	0.01	Discharged	Discharged
UM00449979	0.04	0.00	0.02	0.02	0.02	0.01
UM00325501	0.02	0.00	0.00	0.01	0.05	Discharged
UM00459802	0.01	0.00	0.00	0.03	0.03	Discharged
UM00458992	0.03	0.00	0.00	0.02	0.02	0.02
UM00459940	0.01	0.01	0.02	0.01	0.01	0.02
UM00426535	0.02	0.00	0.00	0.01	0.02	Discharged
UM00343872	0.09	0.00	0.01	0.00	0.01	0.18
UM00463403	0.02	0.00	0.01	0.00	No sample	No sample
UM00465221	0.02	0.00	0.01	0.01	0.03	Discharged
UM00470093	0.05	0.00	0.00	0.01	0.01	0.04
UM00072833	0.02	0.00	0.02	0.01	0.00	0.03
UM00499583	0.02	0.00	0.01	0.01	0.02	0.01
UM00508146	0.03	0.00	0.01	0.05	Discharged	Discharged
UM00508999	0.04	0.00	0.01	0.03	Deceased	Deceased
UM00364680	0.02	0.01	0.02	0.00	0.01	0.04
UM00512436	0.02	0.01	0.01	0.01	0.01	0.02
UM00516293	0.08	0.00	0.02	0.02	No sample	No sample
UM00380508	0.08	0.00	7.18	0.04	0.07	0.00
UM00456515	0.02	0.00	0.00	0.00	0.27	0.00
UM00457930	0.01	0.33	0.00	0.00	0.00	Discharged
UM00336409	0.02	0.01	0.01	0.00	No sample	No sample
n	30.00	30.00	30.00	30.00	23.00	15.00
Minimum	0.01	0.00	0.00	0.00	0.00	0.00
Maximum	0.09	0.33	7.18	0.05	0.99	0.18
Median	0.02	0.00	0.01	0.01	0.02	0.02
Average	0.03	0.01	0.25	0.02	0.11	0.04
SD	0.02	0.06	1.31	0.02	0.25	0.05
25th Percentile	0.02	0.00	0.00	0.01	0.01	0.01
75th Percentile	0.04	0.01	0.02	0.03	0.05	0.04

n = total; SD = standard deviation

Table 4.21 Interleukin-6 (on-pump CABG surgery)

Interleukin-6 (pg/ml)						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	0.00	40.23	4.29	0.00	0.00	8.18
UM00444978	6.24	232.53	239.33	105.30	39.26	48.00
UM00444996	42.17	30.52	0.00	8.18	15.95	Discharged
UM00452998	2.34	226.88	135.61	101.83	134.69	82.67
UM00454075	0.00	9.64	0.00	4.17	0.00	0.00
UM00271364	17.43	47.93	0.00	3.13	2.18	65.09
UM00460094	60.32	115.60	94.63	65.09	32.68	14.57
UM00460160	0.00	115.60	60.32	14.57	0.89	0.00
UM00461761	51.00	51.00	488.09	87.20	58.43	47.29
UM00448673	0.00	105.76	13.89	0.00	0.00	Discharged
UM00462085	0.00	205.05	93.69	141.95	0.00	Discharged
UM00448963	0.00	115.96	25.02	63.07	0.00	Discharged
UM00449114	0.00	239.39	29.66	0.00	No sample	No sample
UM00464165	0.00	21.26	5.00	2.30	No sample	No sample
UM00466956	13.13	263.22	515.12	222.60	145.85	0.00
UM00054119	0.00	29.40	64.04	11.12	15.93	26.52
UM00089966	0.00	65.97	39.02	13.05	1.50	1.50
UM00472005	23.63	134.28	98.68	No sample	No sample	No sample
UM00430058	0.00	34.21	21.71	0.00	0.00	Discharged
UM00476086	0.00	105.41	46.72	15.93	6.31	Discharged
UM00469736	24.59	71.74	47.68	39.99	31.33	Discharged
UM00478175	22.66	67.17	50.91	38.07	No sample	Discharged
UM00478354	0.00	133.08	103.12	50.91	37.21	No sample
UM00479874	0.00	64.60	61.18	34.64	10.68	Discharged
UM00226204	0.00	32.08	17.52	0.00	2.97	Discharged
UM00480115	0.00	98.84	0.00	2.97	0.00	0.00
UM00397366	0.00	39.78	20.95	0.00	0.00	Discharged
UM00344738	14.02	29.81	33.24	46.90	34.91	118.17
UM00483018	6.54	172.91	106.92	62.91	32.21	Discharged
UM00494885	18.05	44.33	58.44	2460.00	24.57	19.90
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	0.00	9.64	0.00	0.00	0.00	0.00
Maximum	60.32	263.22	515.12	2460.00	145.85	118.17
Median	0.00	69.45	47.20	15.93	8.49	17.24
Average	10.07	98.14	82.49	124.00	24.14	30.85
SD	16.33	73.53	124.87	452.24	38.05	36.83
25th Percentile	0.00	39.89	18.38	2.97	0.00	0.38
75th Percentile	16.58	128.80	94.40	63.07	32.56	47.82

n = total; SD = standard deviation; pg/ml = picogram per millilitre

Table 4.22 Interleukin-6 (off-pump CABG surgery)

Interleukin-6 (pg/ml)						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	0.00	96.56	56.74	5.26	0.00	0.00
UM00129489	302.46	92.68	137.35	50.91	38.29	Discharged
UM00444354	0.00	101.42	35.37	5.26	Discharged	Discharged
UM00323069	0.00	23.99	33.07	22.98	4.82	Discharged
UM00259094	0.00	134.97	39.13	17.94	11.89	36.10
UM00352641	0.00	12.90	75.44	54.26	51.23	45.18
UM00447250	0.00	63.50	29.73	2.34	0.00	Discharged
UM00351965	204.58	22.98	14.91	28.03	96.63	22.98
UM00211678	0.00	31.55	112.79	48.89	Discharged	Discharged
UM00449979	0.00	37.03	161.16	0.00	0.00	0.00
UM00325501	0.00	65.09	160.40	89.87	21.24	Discharged
UM00459802	0.00	34.59	25.06	28.87	36.49	Discharged
UM00458992	3.68	403.64	89.05	100.19	143.80	156.80
UM00459940	60.32	128.95	79.38	28.87	53.65	20.29
UM00426535	0.00	21.26	4.10	7.71	18.55	Discharged
UM00343872	0.00	225.47	115.04	98.33	38.94	50.08
UM00463403	0.00	231.62	56.47	16.74	No sample	No sample
UM00465221	28.48	142.24	132.31	31.19	15.84	Discharged
UM00470093	0.00	95.79	14.97	34.21	27.48	26.52
UM00072833	7.40	118.30	102.20	50.90	75.30	23.20
UM00499583	3.30	15.84	24.81	No sample	No sample	No sample
UM00508146	228.99	33.15	0.00	0.51	Discharged	Discharged
UM00508999	111.31	67.51	40.02	10.82	Deceased	Deceased
UM00364680	76.90	>2688.00	2262.00	1885.94	647.04	350.40
UM00512436	35.00	135.80	153.99	35.81	78.99	238.80
UM00516293	0.00	0.00	0.00	0.00	No sample	No sample
UM00380508	0.00	350.10	33.50	34.50	0.00	0.00
UM00456515	0.00	181.06	369.48	191.85	225.21	320.52
UM00457930	3.13	107.98	80.34	43.17	5.04	Discharged
UM00336409	0.00	134.97	39.13	17.94	No sample	No sample
n	30.00	30.00	30.00	29.00	22.00	14.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	302.46	2688.00	2262.00	1885.94	647.04	350.40
Median	0.00	96.18	56.60	28.87	31.99	31.31
Average	35.52	193.30	149.26	101.49	72.29	92.20
SD	77.01	480.71	405.84	345.57	139.41	122.83
25th Percentile	0.00	33.51	30.56	10.82	6.75	20.96
75th Percentile	23.21	135.59	114.47	50.90	69.89	130.12

n = total; SD = standard deviation; pg/ml = picogram per millilitre

Table 4.23 TNF-alpha (on-pump CABG surgery)

TNF-alpha (pg/ml)						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440462	7.22	6.60	9.02	19.76	12.53	10.49
UM00444978	12.53	7.44	12.27	8.56	10.49	11.76
UM00444996	24.22	15.78	15.30	22.67	18.81	Discharged
UM00452998	12.51	7.79	12.01	19.58	29.35	27.86
UM00454075	7.28	6.80	9.71	45.34	22.55	23.45
UM00271364	7.97	4.25	5.74	3.67	6.31	12.27
UM00460094	6.65	4.94	5.34	6.05	6.65	8.70
UM00460160	6.47	5.51	8.22	7.49	6.80	12.35
UM00461761	4.60	5.67	9.57	7.32	11.03	9.37
UM00448673	5.84	3.34	4.94	8.54	9.64	Discharged
UM00462085	6.50	4.51	6.21	5.74	4.34	Discharged
UM00448963	8.42	3.10	4.85	5.65	3.84	Discharged
UM00449114	9.80	6.18	10.39	7.79	No sample	No sample
UM00464165	4.16	4.30	17.05	31.28	No sample	No sample
UM00466956	16.78	22.76	10.15	12.82	12.46	15.05
UM00054119	6.80	3.40	14.31	19.33	20.28	20.28
UM00089966	8.54	5.89	8.30	14.31	12.10	20.94
UM00472005	12.10	9.04	16.45	No sample	No sample	No sample
UM00430058	6.60	4.89	14.41	5.69	7.72	Discharged
UM00476086	6.60	4.59	6.41	9.66	19.27	Discharged
UM00469736	24.03	21.85	15.93	24.31	26.78	Discharged
UM00478175	15.76	18.82	25.89	29.48	No sample	Discharged
UM00478354	4.15	4.59	5.52	6.22	6.22	No sample
UM00479874	10.63	5.52	7.48	10.63	25.10	Discharged
UM00226204	26.78	22.19	29.11	26.55	30.45	Discharged
UM00480115	27.88	23.16	24.03	26.32	29.30	22.19
UM00397366	12.01	14.41	25.57	22.03	22.03	Discharged
UM00344738	23.10	2.50	7.80	13.80	11.30	37.90
UM00483018	6.50	7.80	3.80	16.30	6.50	Discharged
UM00494885	16.30	11.30	11.30	11.30	13.80	13.10
n	30.00	30.00	30.00	29.00	26.00	14.00
Minimum	4.15	2.50	3.80	3.67	3.84	8.70
Maximum	27.88	23.16	29.11	45.34	30.45	37.90
Median	8.48	6.03	9.93	12.82	12.28	14.07
Average	11.62	8.96	11.90	15.45	14.83	17.55
SD	7.08	6.57	6.82	9.95	8.51	8.35
25th Percentile	6.60	4.59	6.68	7.49	7.03	11.88
75th Percentile	14.95	10.74	15.08	22.03	21.59	21.88

n = total; SD = standard deviation; pg/ml = picogram per millilitre

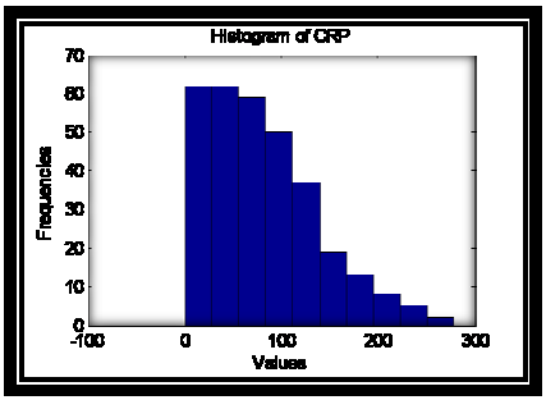
Table 4.24 TNF-alpha (off-pump CABG surgery)

TNF-alpha (pg/ml)						
UM Number	Pre-operative	Post-operative				
	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
UM00440157	8.79	6.80	9.26	10.74	6.00	6.60
UM00129489	5.41	5.41	8.10	12.53	13.04	Discharged
UM00444354	7.44	7.66	11.24	14.81	Discharged	Discharged
UM00323069	19.94	18.81	13.30	16.92	18.81	Discharged
UM00259094	16.70	19.00	25.54	25.68	21.69	15.06
UM00352641	6.96	4.15	6.65	11.76	9.71	9.09
UM00447250	13.56	9.09	11.51	14.91	19.15	Discharged
UM00351965	9.92	10.58	13.56	23.63	27.09	27.56
UM00211678	10.13	6.05	6.65	6.50	Discharged	Discharged
UM00449979	16.76	20.00	14.64	22.73	17.27	21.61
UM00325501	7.00	5.20	5.74	4.42	7.97	Discharged
UM00459802	6.97	2.73	5.20	7.49	10.18	Discharged
UM00458992	90.72	8.22	8.98	12.13	14.87	13.03
UM00459940	6.14	6.31	13.95	9.97	8.98	2.46
UM00426535	5.11	2.78	3.26	4.85	4.76	Discharged
UM00343872	9.42	7.29	12.04	11.62	11.00	15.85
UM00463403	20.26	14.80	24.04	23.61	No sample	No sample
UM00465221	18.00	16.05	19.25	14.38	20.42	Discharged
UM00470093	13.19	5.22	6.61	11.41	20.73	19.81
UM00072833	9.00	9.00	13.80	11.40	14.90	10.20
UM00499583	12.60	7.90	11.40	13.80	12.60	11.40
UM00508146	12.58	10.22	25.57	22.03	Discharged	Discharged
UM00508999	3.14	7.86	13.77	6.68	Deceased	Deceased
UM00364680	23.20	13.80	16.10	18.49	14.95	13.77
UM00512436	13.00	2.80	14.99	10.93	8.90	6.86
UM00516293	9.80	3.20	5.80	7.20	No sample	No sample
UM00380508	16.10	17.40	24.00	22.60	39.70	39.70
UM00456515	19.64	13.49	24.06	48.87	51.14	43.74
UM00457930	18.21	11.24	11.26	16.62	12.27	Discharged
UM00336409	16.45	19.00	25.54	25.68	No sample	No sample
n	30.00	30.00	30.00	30.00	23.00	15.00
Minimum	3.14	2.73	3.26	4.42	4.76	2.46
Maximum	90.72	20.00	25.57	48.87	51.14	43.74
Median	12.59	8.06	12.67	13.16	14.87	13.77
Average	14.87	9.74	13.53	15.48	16.79	17.11
SD	15.27	5.42	6.78	8.91	10.71	11.85
25th Percentile	7.77	5.57	8.32	10.78	9.94	9.65
75th Percentile	16.75	13.72	15.82	21.14	19.78	20.71

n = total; SD = standard deviation; pg/ml = picogram per millilitre

4.6 Statistical distribution histograms of inflammatory markers

Histograms (bar-charts) were used for the graphic representation of the frequency table. A Normal distribution histogram is 'bell'-shaped. Scrutiny of the histograms of figure 4.1 and figure 4.2 reveal that these histograms display a more pronounced skewness to the right or hardly any variation, and often a very high Katharsis (peakedness), i.e. much higher than normal.



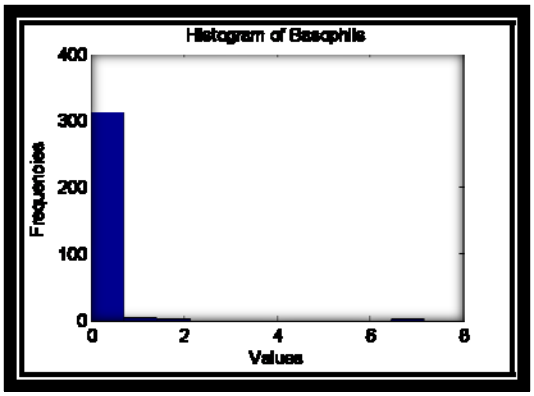
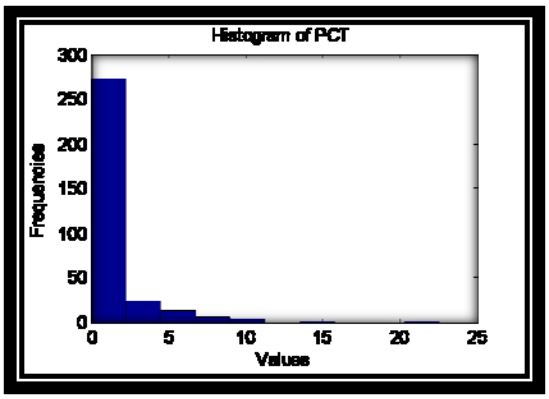


Figure 4.1 **Histogram of the actual C-reactive protein (CRP) values (left)**

Figure 4.2 **Histogram of the actual values for Basophils (right)**

After applying the natural logarithmic transformation on the raw data (i.e. base *e* ratio), it was observed that the resultant (transformed) data approached the lognormal distribution and subsequently the statistician proceeded with the hypothesis tests.

For the variables displayed in figures 4.3, 4.4, 4.5, 4.6, 4.7, 4.8 and figure 4.9, the lognormal very closely resembled the Gaussian (Normal) distribution. Fortunately, for all the variables tested in the raw or lognormally transformed state, the Student's *t*-test was applied, which is fairly robust with regard to deviations from normality. The power of these tests was assumed to be at acceptable levels.



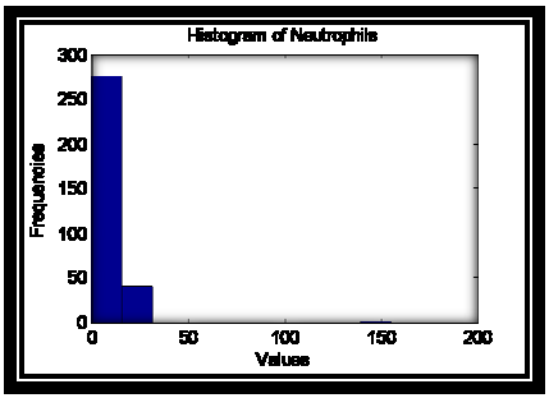
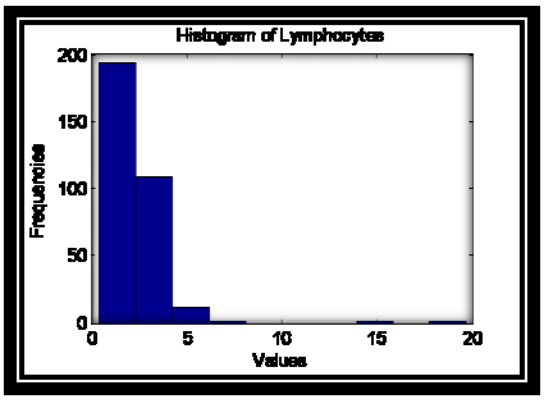


Figure 4.3 Histogram of the actual values of Procalcitonin (PCT) (left)

Figure 4.4 Histogram of the actual values for Neutrophils (right)



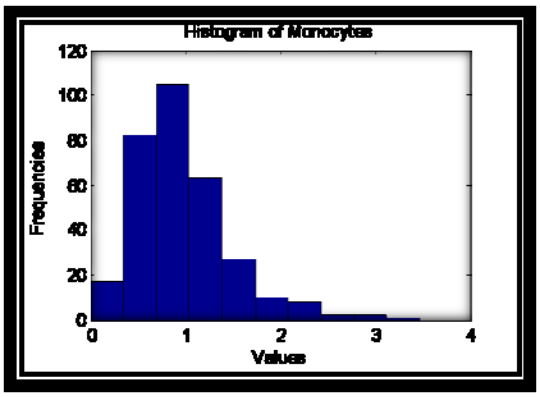
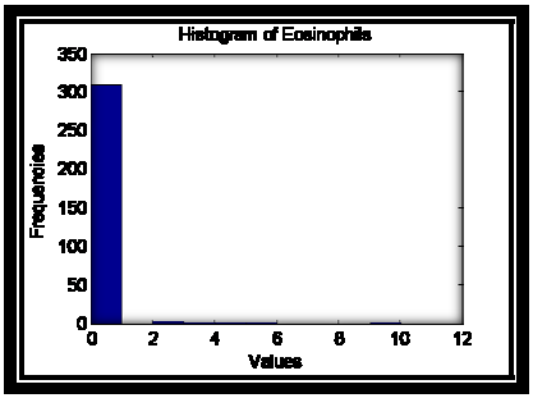


Figure 4.5 Histogram of the actual values for Lymphocytes (left)

Figure 4.6 Histogram of the actual values for Monocytes (right)



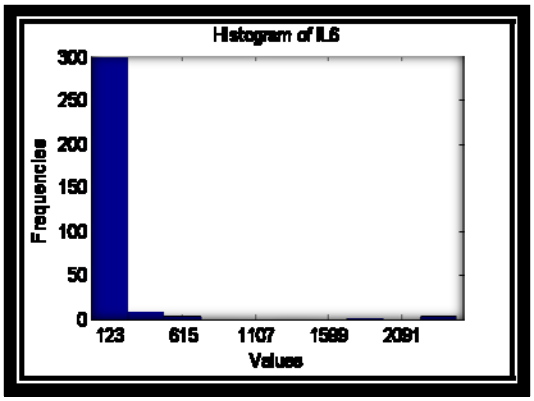


Figure 4.7 Histogram of the actual values for Eosinophils (left)

Figure 4.8 Histogram of the actual values for interleukin-6 (IL-6) (right)

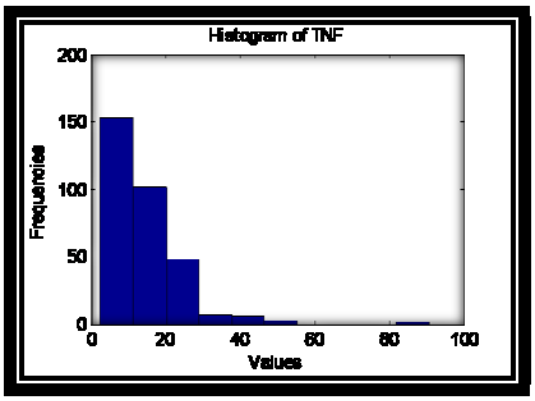
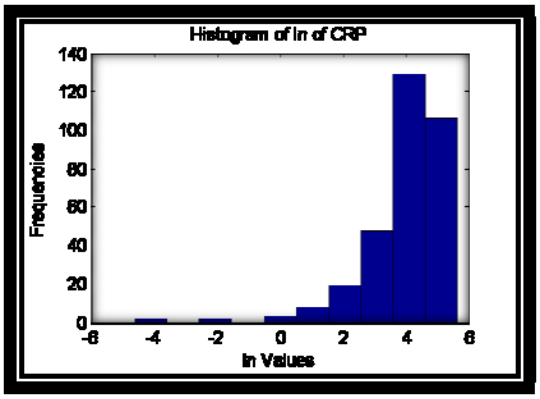


Figure 4.9 **Histogram of the actual values for tumour necrosis factor alpha (TNF-α)**

Figures 4.10, 4.11, 4.12, 4.13, 4.14, 4.15, 4.16 and figure 4.17 indicate that the variables (inflammatory markers) had approximately the following distributions:



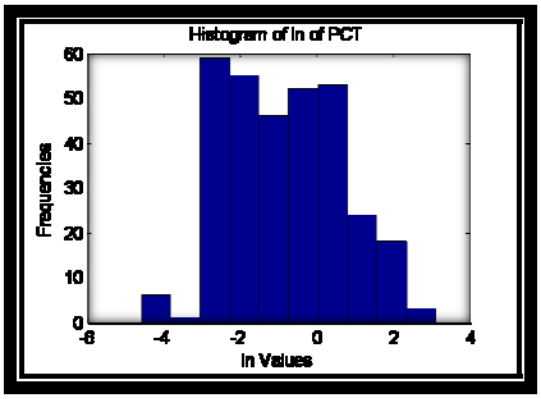
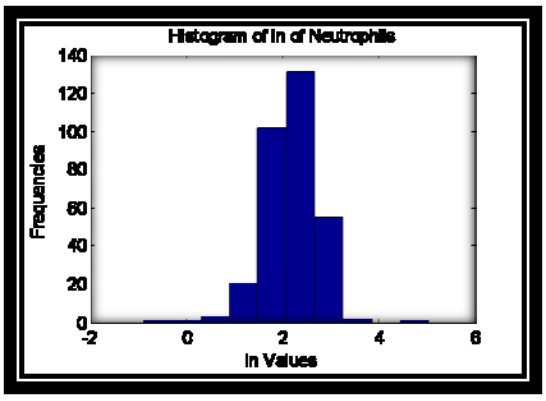


Figure 4.10 **Histogram of normal logarithm (ln) for C-reactive protein (CRP) (left)**

Figure 4.11 **Histogram of normal logarithm (ln) for procalcitonin (PCT) (right)**



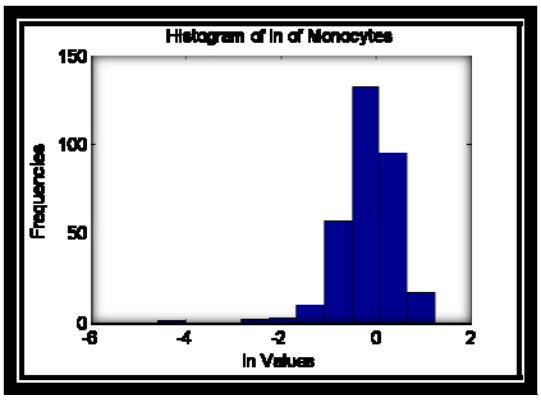
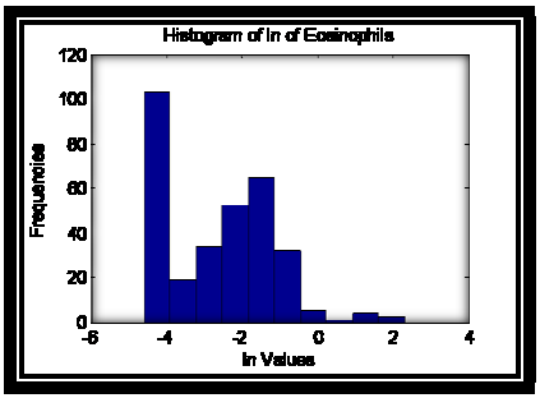


Figure 4.12 **Histogram of normal logarithm (ln) for Neutrophils (left)**

Figure 4.13 **Histogram of normal logarithm (ln) for Monocytes (right)**



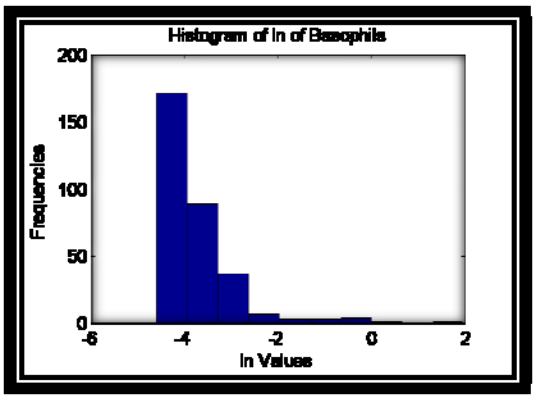
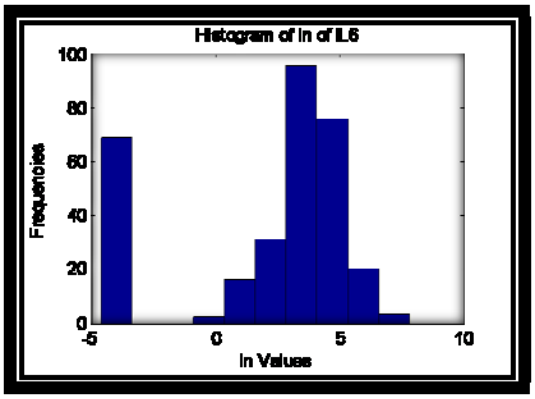


Figure 4.14 **Histogram of normal logarithm (ln) for Eosinophils (left)**

Figure 4.15 **Histogram of normal logarithm (ln) for Basophils (right)**



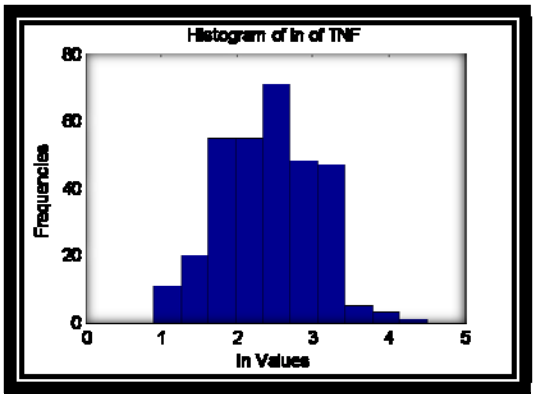


Figure 4.16 **Histogram of normal logarithm (ln) for interleukin-6 (IL-6) (left)**

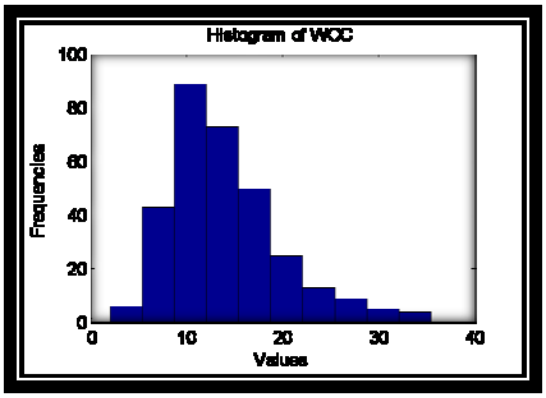
Figure 4.17 **Histogram of normal logarithm (ln) for tumour necrosis factor alpha (TNF-α) (right)**

Comparing the means was valid for the two variables that are approximately Gamma distributed, but not for any of the other variables. For the variables that are lognormally distributed, the logs were taken to obtain approximately Normal data, for which the comparisons were done in the usual ways (e.g. the means might be compared using Student's *t*-distribution). For the zero-inflated data it was best to separate the zero and the non-zero data and then to analyse those separately. Lastly, for the variables (basophils) it was suggested that only the proportion of zero values be investigated.

Table 4.25 Variables and approximate distributions

Variable	Approximate distribution
CRP	Gamma
PCT	Lognormal (some zeros)
WCC	Lognormal
Neutrophils	Lognormal
Lymphocytes	Lognormal
Monocytes	Gamma
Eosinophils	Zero-inflated lognormal
Basophils	Generalised extreme value
IL-6	Zero-inflated lognormal
TNF-α	Lognormal

As summarised in table 4.25, for a variable like the WCC (figure 4.18), the histogram of the raw data already resembled a Normal curve. The lognormal histogram (figure 4.19), however, was more symmetrical.



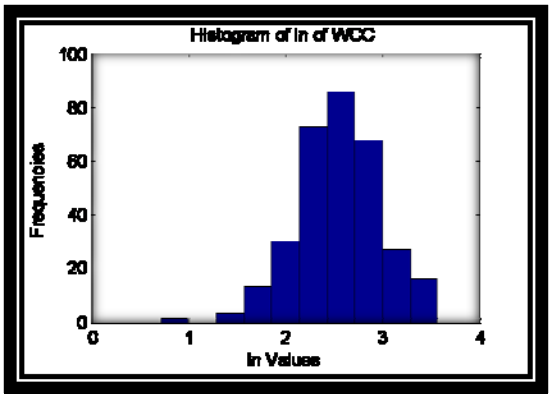
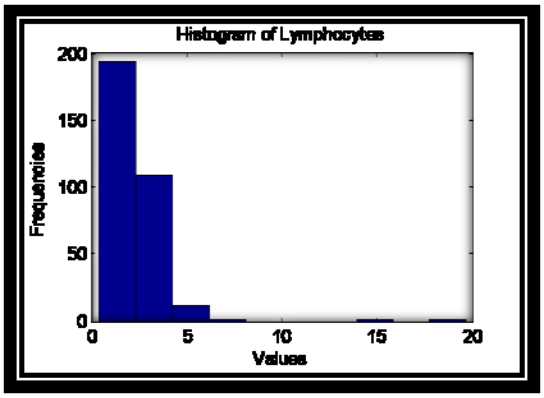


Figure 4.18 Histogram of the actual values for white cell count (WCC) (left)

Figure 4.19 Histogram of normal logarithm (ln) for white cell count (WCC) (right)

The histogram of the lymphocytes presented in figure 4.20, displays hardly any variance, but what little there is, tests to a positive skewness. The lognormal histogram of lymphocytes displayed in figure 4.21, is a classic and patently clear case of one where the logarithmic transformation led to what is undoubtedly perceived as a Normal distribution.



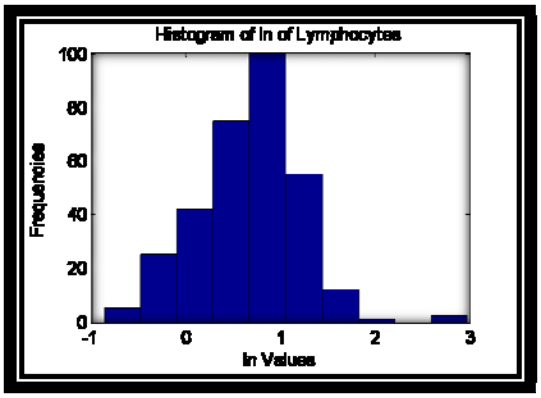


Figure 4.20 **Histogram of the actual values for lymphocytes (left)**

Figure 4.21 **Histogram of normal logarithm (ln) for lymphocytes (right)**

Given below are histograms of the data collected in respect of the inflammatory markers (hereafter referred to as dependent variables) for the two CABG surgical procedures done on (n=60) patients with ACS . In the case of the lognormal (*ln*) histograms the zeros were adjusted to 0.01, thus moving the spike in the histograms from $-\infty$ to -4.6.

4.7 Inflammatory markers: comparing on-pump and off-pump CABG surgical groups

Assuming that similar people were non-randomly assigned to the two groups, the results of the inflammatory markers for the two CABG groups were investigated. This was achieved by calculating the average values for each dependent variable (medical indicator) for each group and plotting these as they changed over time. Additionally, the approximate 95% confidence intervals for these averages were calculated according to the assumed distributions.

These confidence intervals are useful in that, where the intervals for the groups overlap, it indicates that the differences are not statistically significant; if, on the other hand, there is a separation of the intervals, then there is a statistically significant difference between the mean values of the groups at that specific point in time. In addition, if the lower limit of the on-pump CABG group is above the upper limit of the off-pump CABG surgical group, then it can be said with certainty that at least 95% (*p*-value <0.05), on average, of the on-pump CABG surgical group produce higher inflammatory marker values for the indictor in question (and *vice versa*).

4.7.1 C-reactive protein (CRP)

Figure 4.22 indicates the 95% confidence intervals using the mean values for CRP between on-pump and off-pump CABG surgical groups.

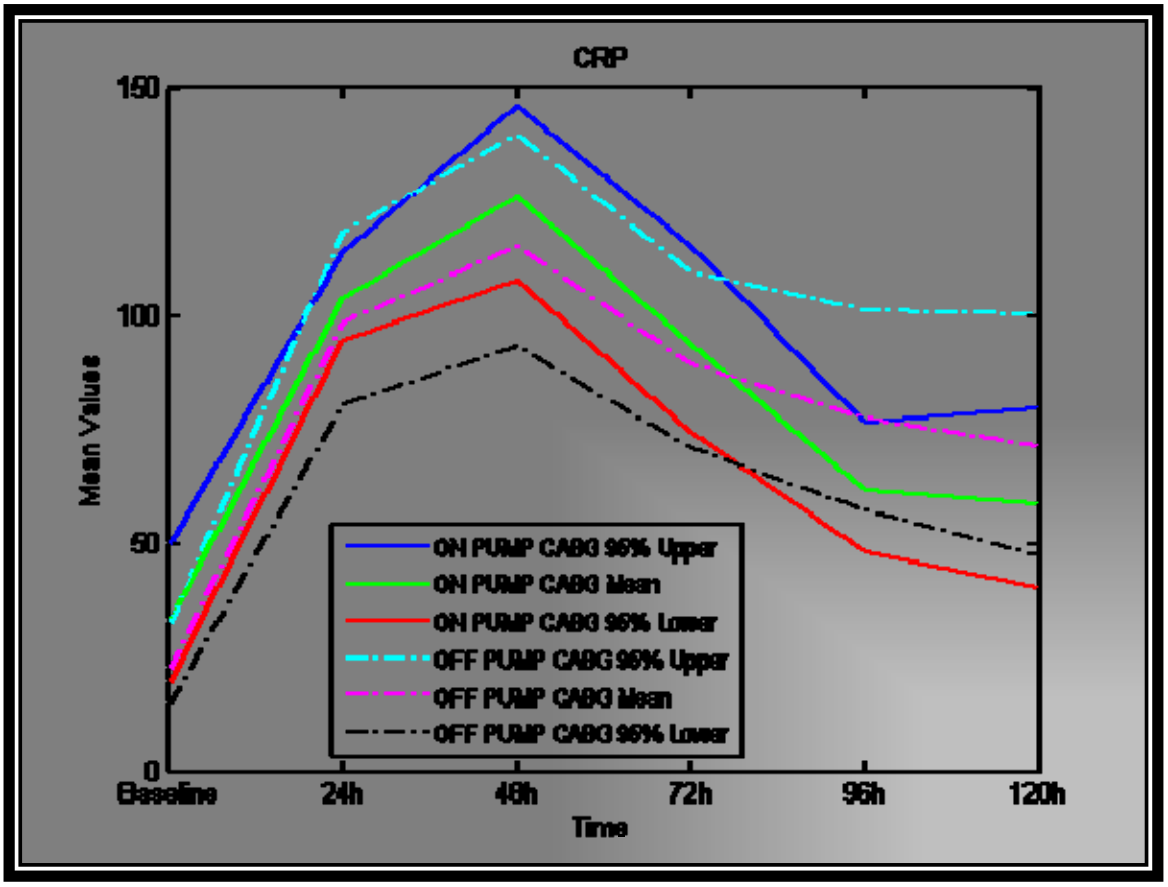


Figure 4.22 **The 95% confidence intervals using the mean values for CRP between on-pump and off-pump CABG surgical groups**

4.7.2 Procalcitonin (PCT)

Figure 4.23 indicates the 95% confidence intervals using the mean lognormal values for PCT between on-pump and off-pump CABG surgical groups.

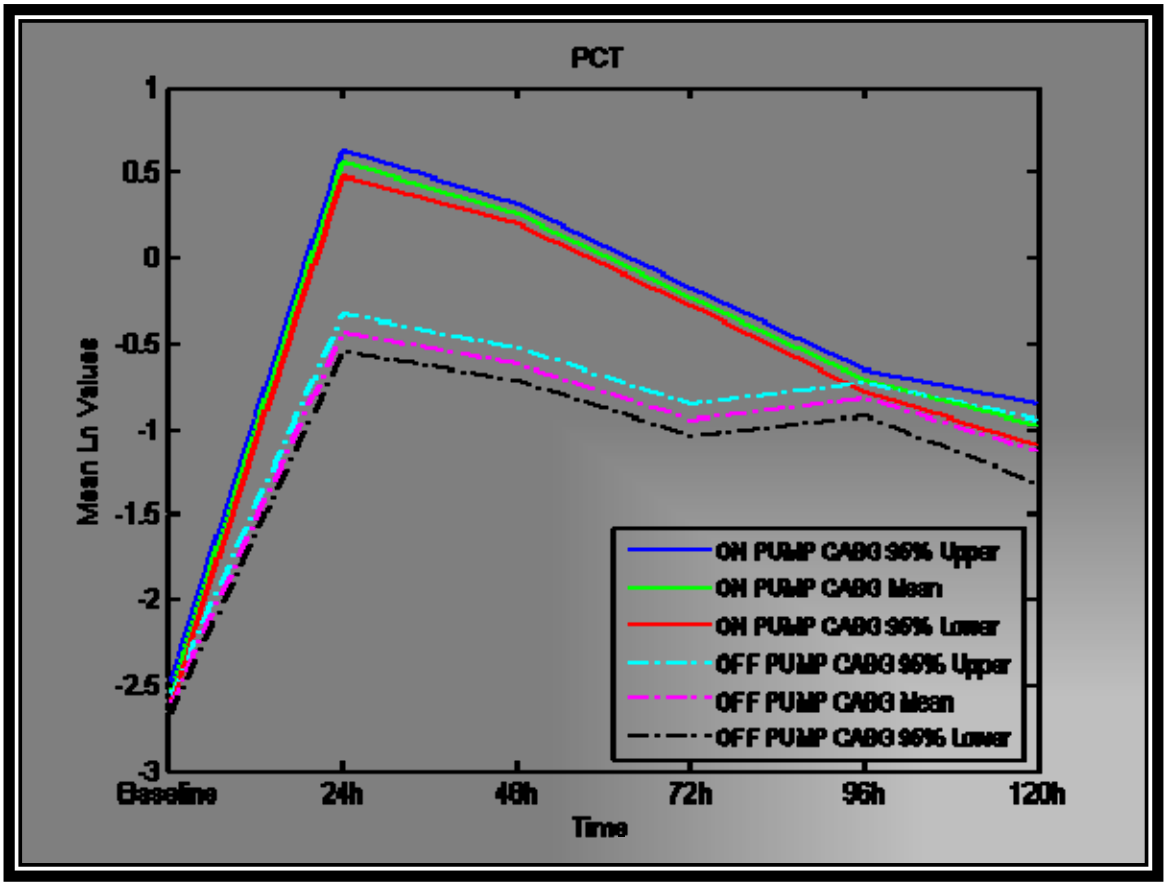


Figure 4.23 **The 95% confidence intervals using the mean lognormal values for PCT between on-pump and off-pump CABG surgical groups**

4.7.3 White cell count (WCC)

Figure 4.24 indicates the 95% confidence intervals using the mean lognormal values for WCC between on-pump and off-pump CABG surgical groups.

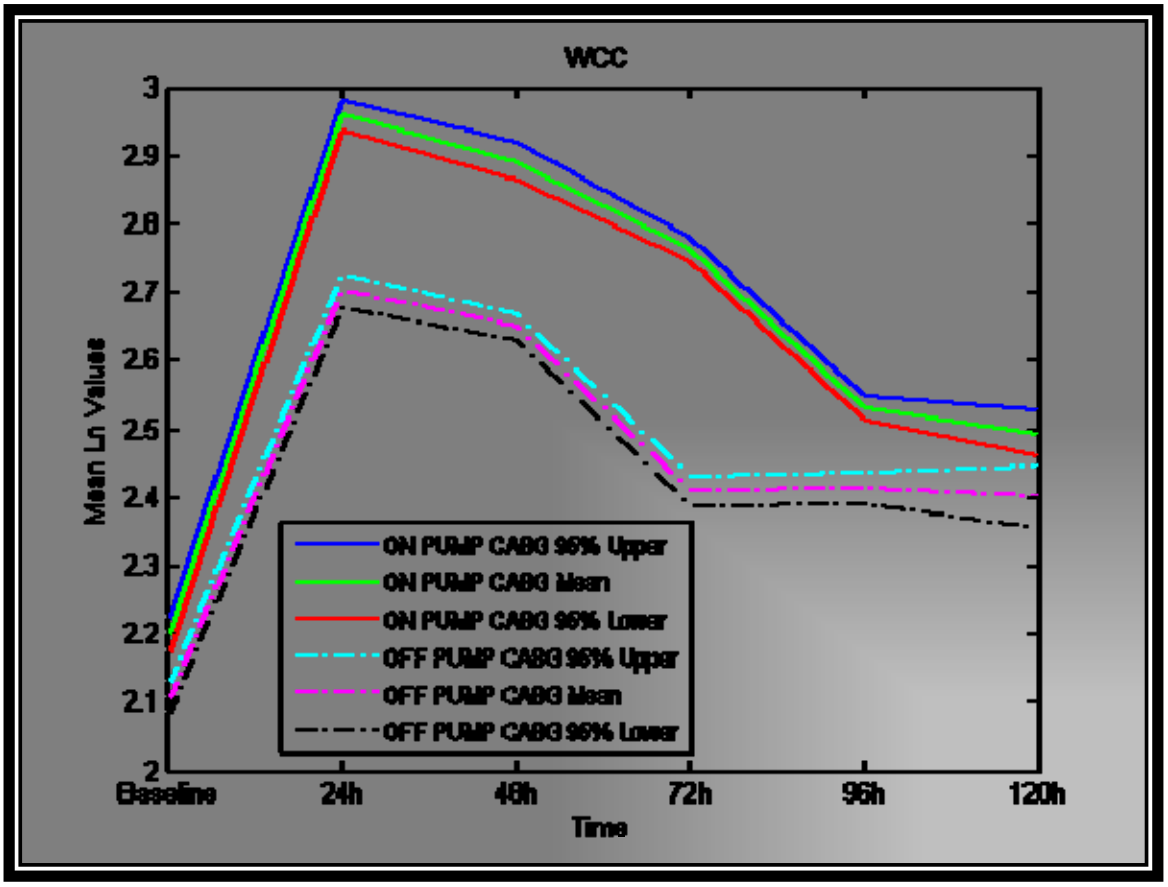


Figure 4.24 **The 95% confidence intervals using the mean lognormal values for WCC between on-pump and off-pump CABG surgical groups**

4.7.4 Neutrophils

Figure 4.25 indicates the 95% confidence intervals using the mean lognormal values for neutrophils between on-pump and off-pump CABG surgical groups.

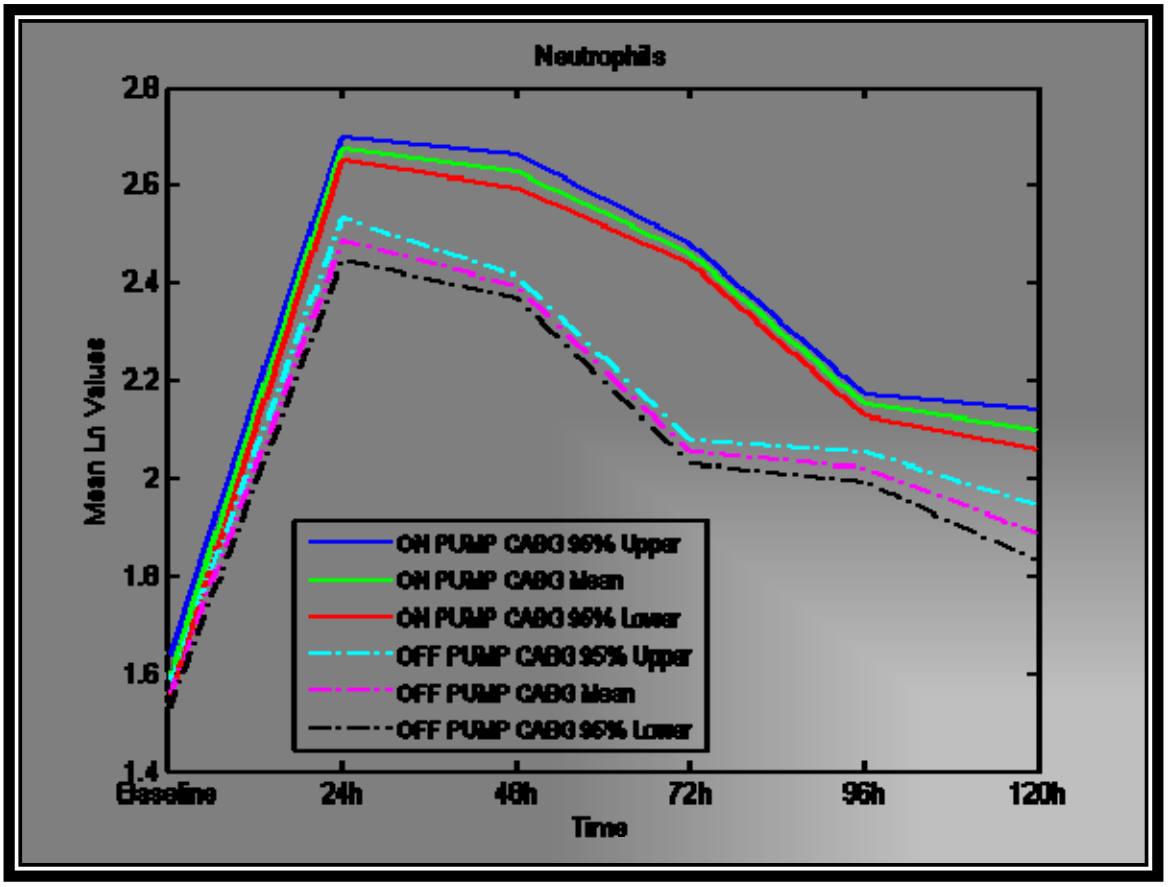


Figure 4.25 **The 95% confidence intervals using the mean lognormal values for neutrophils between on-pump and off-pump CABG surgical groups**

4.7.5 Lymphocytes

Figure 4.26 indicates the 95% confidence intervals using the mean lognormal values for lymphocytes between on-pump and off-pump CABG surgical groups.

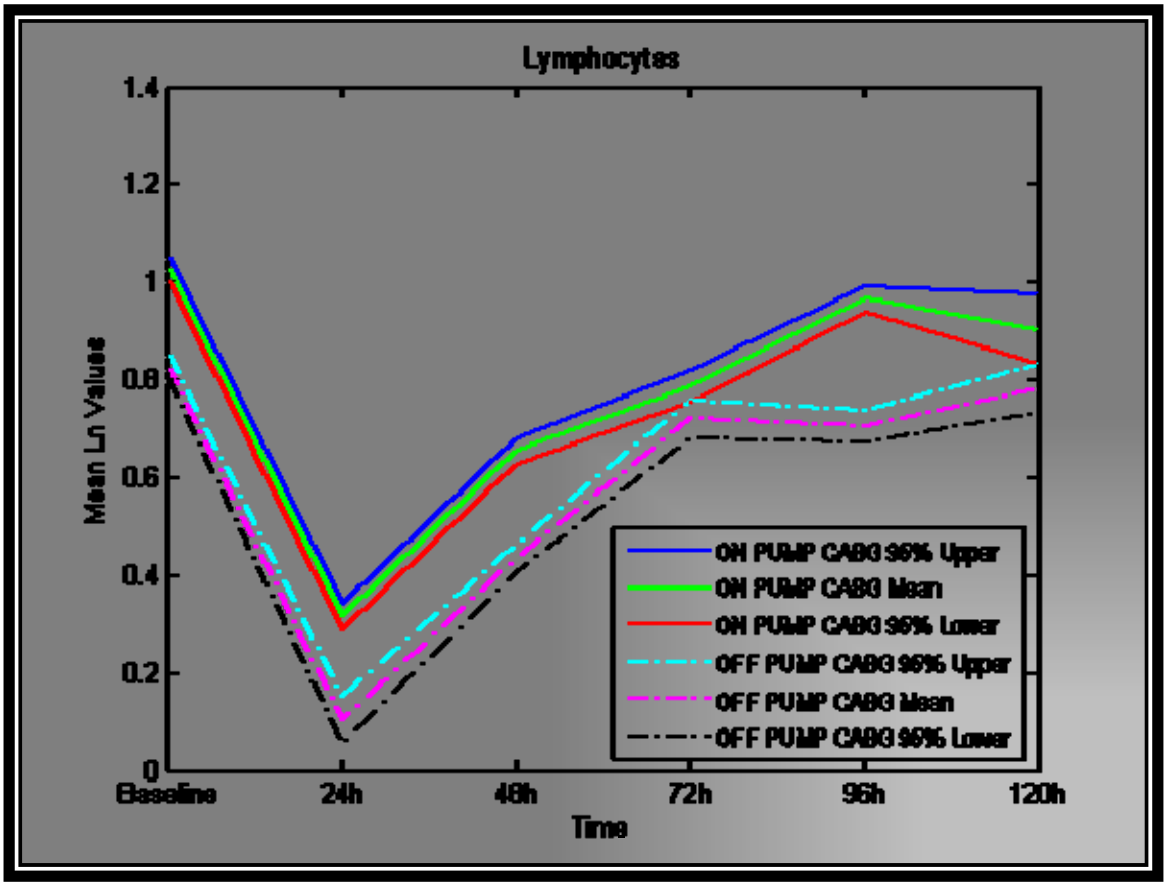


Figure 4.26 **The 95% confidence intervals using the mean lognormal values for lymphocytes between on-pump and off-pump CABG surgical groups**

4.7.6 Monocytes

Figure 4.27 indicates the 95% confidence intervals for the mean values of monocytes between on-pump and off-pump CABG surgical groups.

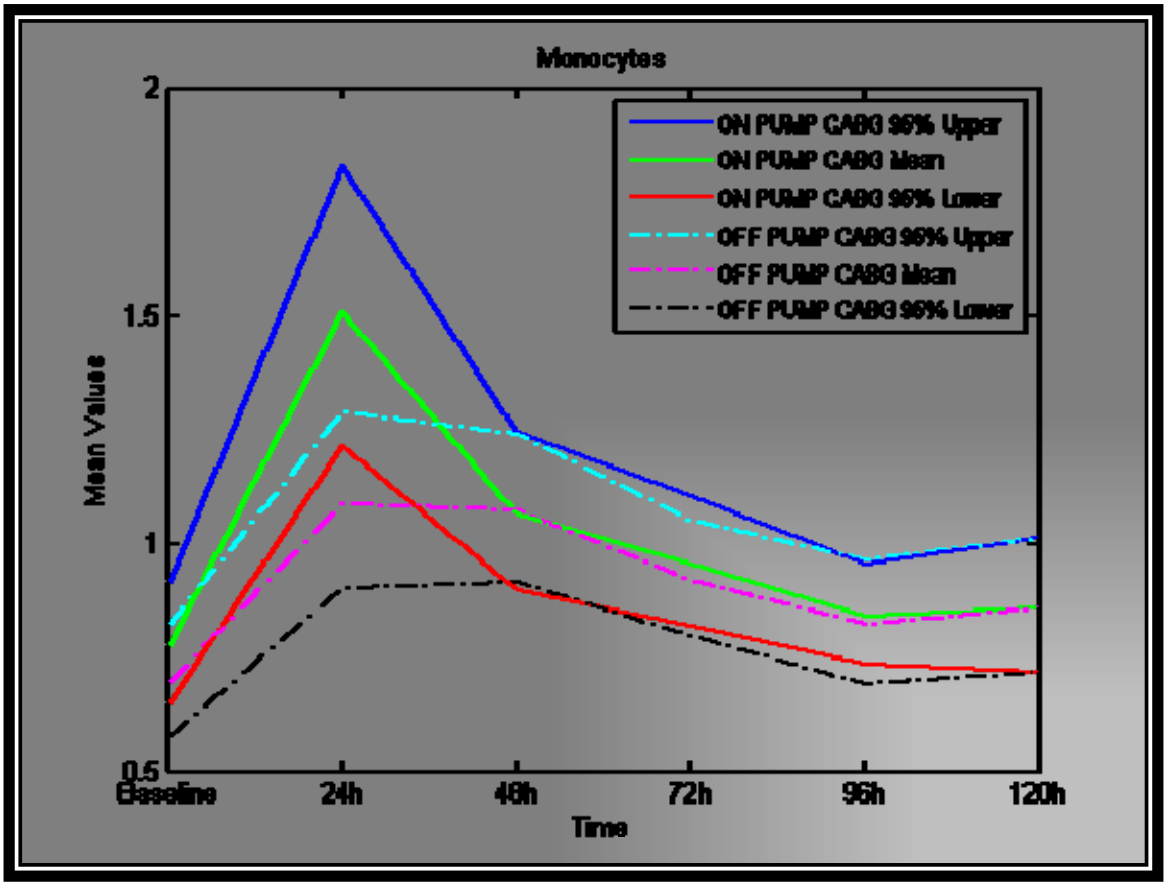


Figure 4.27 **The 95% confidence intervals for the mean values for monocytes between on-pump and off-pump CABG surgical groups**

4.7.7 Eosinophils

Figure 4.28 indicates the 95% confidence intervals of the mean proportion of the non-zero values for eosinophils between on-pump and off-pump CABG surgical groups.

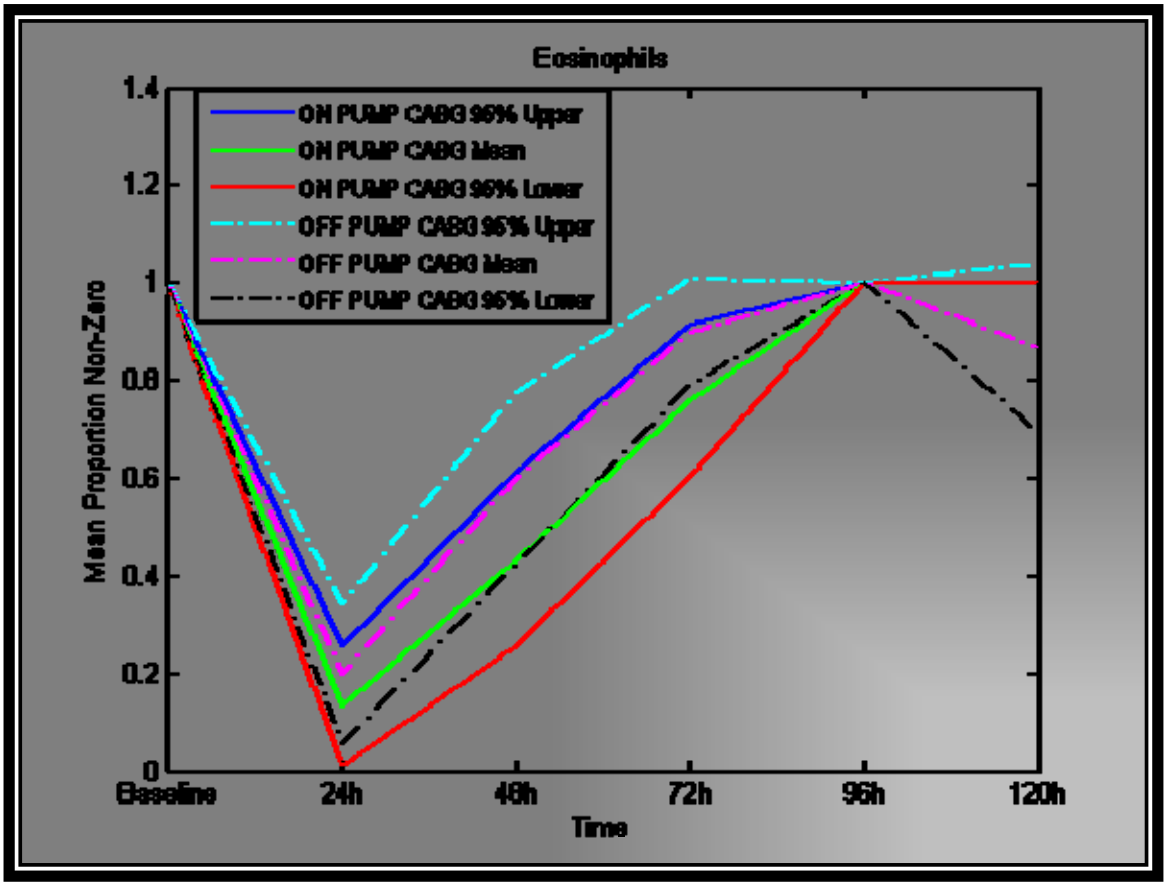


Figure 4.28 **The 95% confidence intervals of the mean proportion of the non-zero values for eosinophils between on-pump and off-pump CABG surgical groups**

4.7.8 Eosinophils

Figure 4.29 indicates the 95% confidence intervals of the mean lognormal non-zero values proportion of the non-zero values for eosinophils between on-pump and off-pump CABG surgical groups.

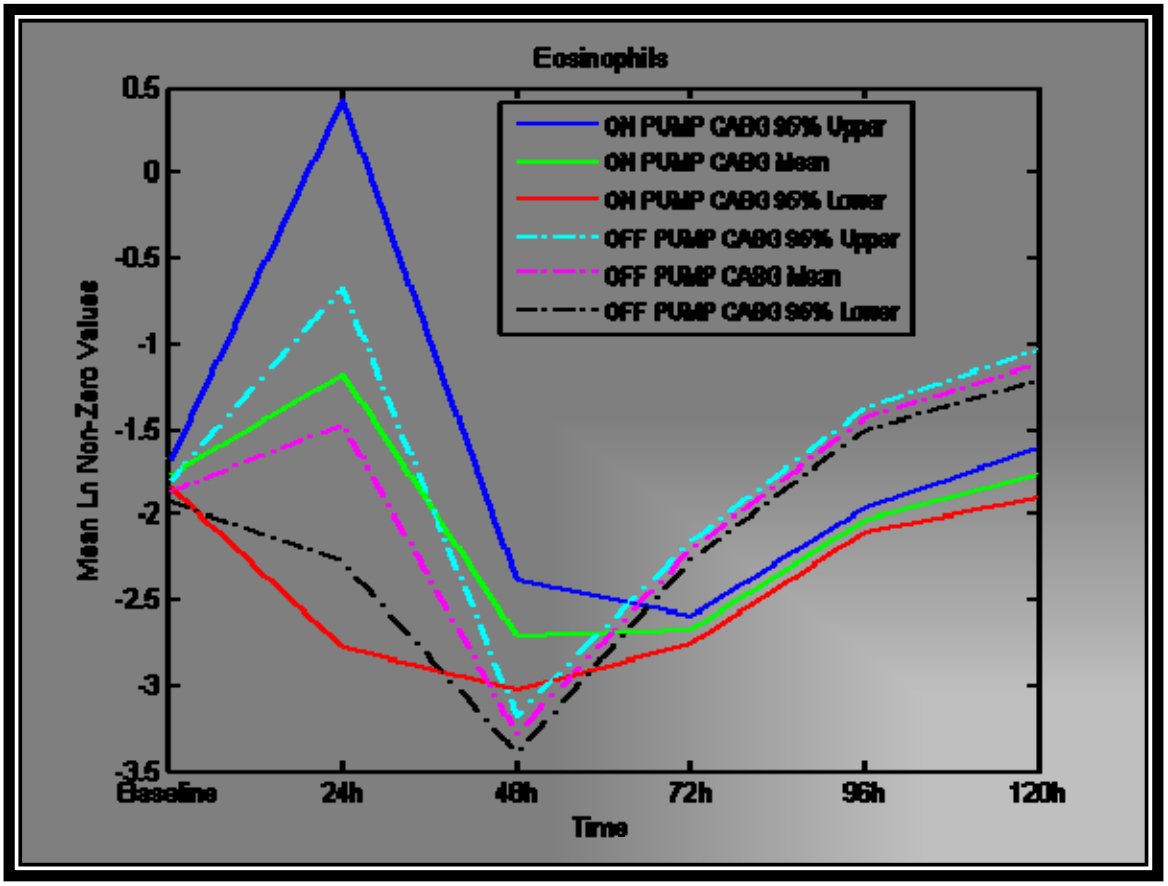


Figure 4.29 **The 95% confidence intervals of the mean lognormal non-zero values proportion of the non-zero values for eosinophils between on-pump and off-pump CABG surgical groups**

Scrutiny of both this and the previous graph makes it evident that the results are partially contradictory. Figure 4.28 includes all the "zero values" for eosinophils, which means that "zero values" indicated that no eosinophils were present when the count was done. Figure 4.29 excludes all the "zero values" for eosinophils.

4.7.9 Basophils

Figure 4.30 indicates the 95% confidence intervals using the mean proportion non-zero values for basophils between on-pump and off-pump CABG surgical groups.

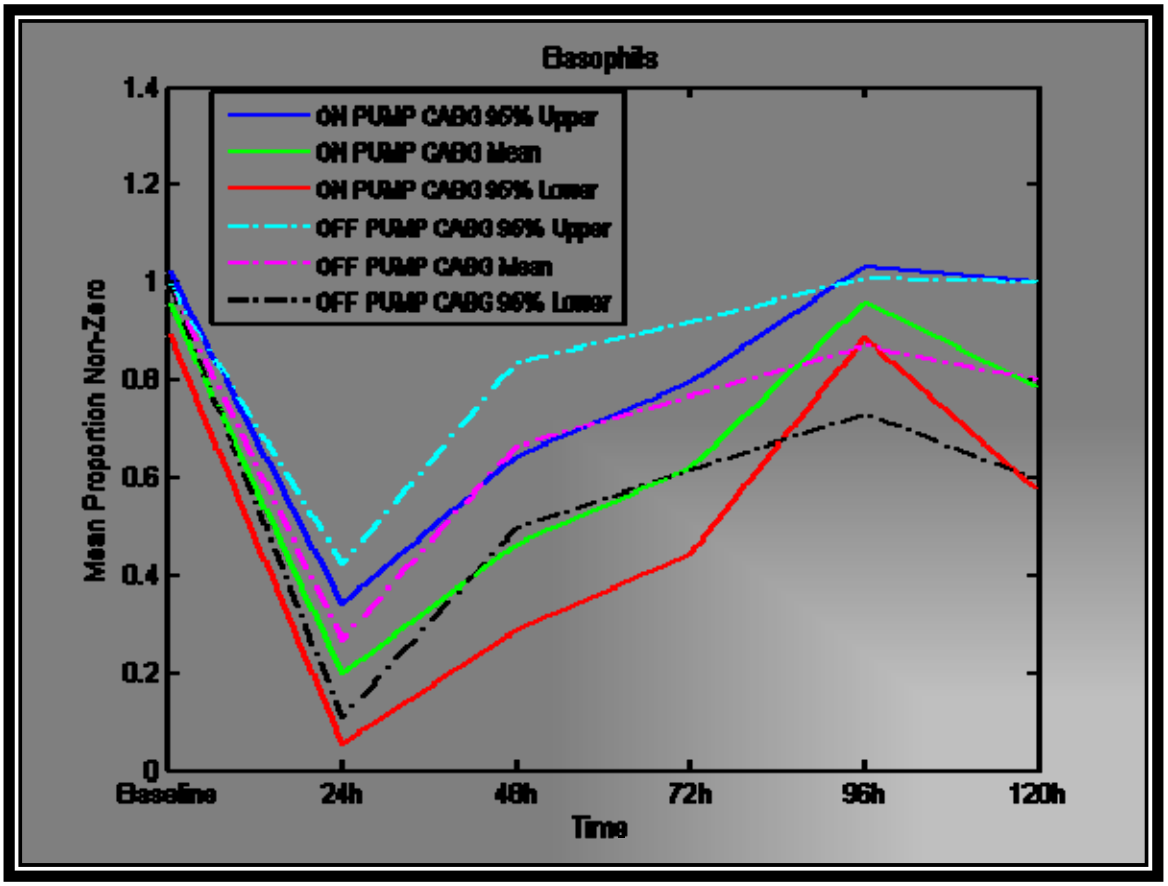


Figure 4.30 **The 95% confidence intervals using the mean proportion non-zero values for basophils between on-pump and off-pump CABG surgical groups**

4.7.10 Interleukin-6 (IL-6)

Figure 4.31 indicates the 95% confidence intervals of the mean proportion of the non-zero values for IL-6 between on-pump and off-pump CABG surgical groups.

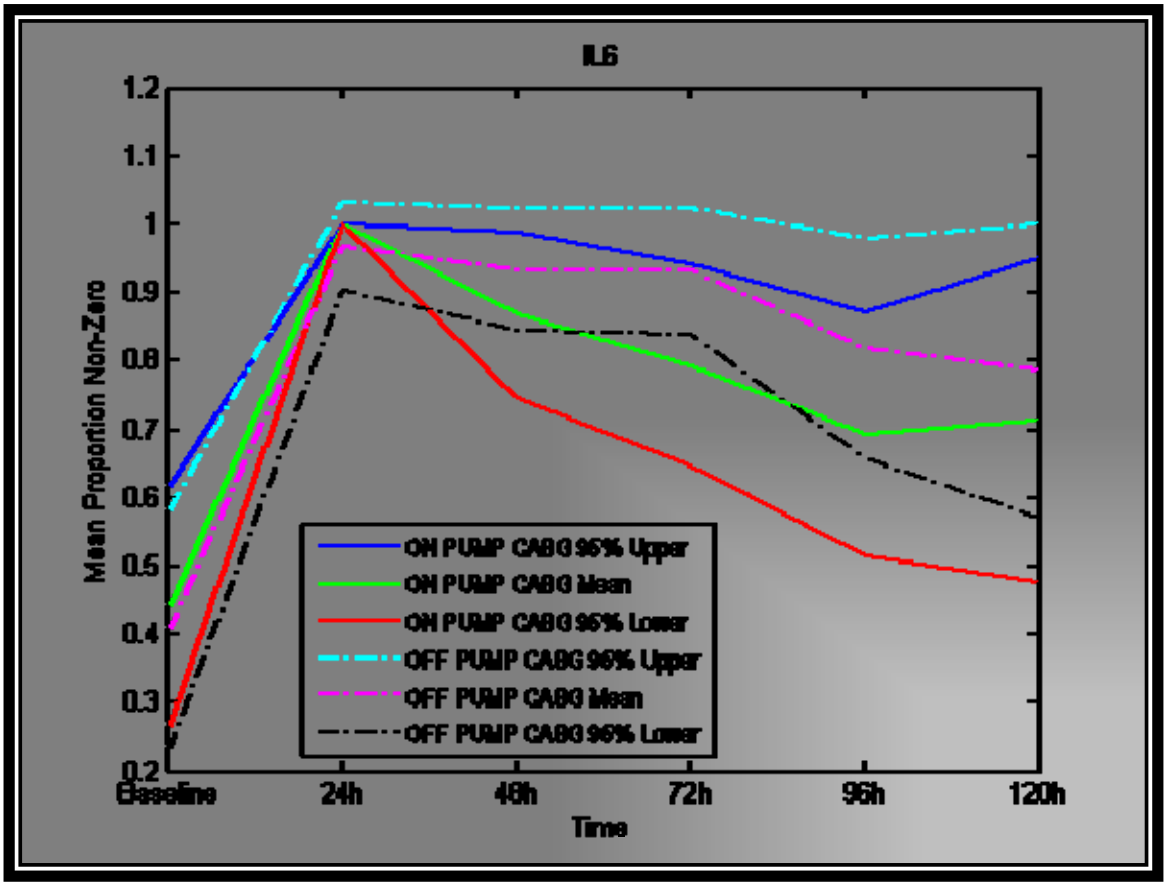


Figure 4.31 **The 95% confidence intervals of the mean proportion of the non-zero values for IL-6 between on-pump and off-pump CABG surgical groups**

4.7.11 Interleukin-6 (IL-6)

Figure 4.32 indicates the 95% confidence intervals of the mean proportion of the lognormal non-zero values for IL-6 between on-pump and off-pump CABG surgical groups.

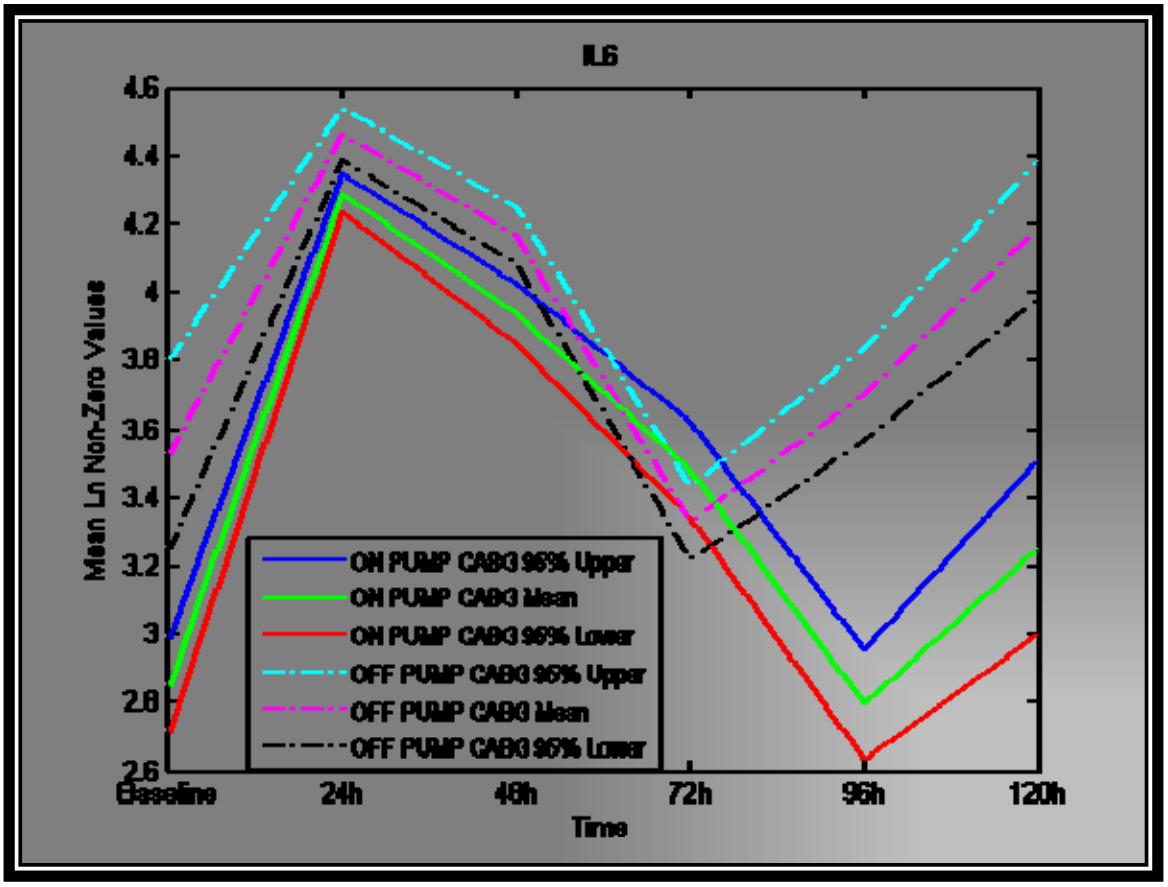


Figure 4.32 The 95% confidence intervals of the mean proportion of the lognormal non-zero values for IL-6 between on-pump and off-pump CABG surgical groups

Results in figures 4.31 and 4.32 are partially contradictory. Figure 4.31 includes all the zero values for IL-6, which means that 'zero values' indicated that no IL-6 was present and figure 4.32 excludes all the 'zero values' of the two groups.

4.7.12 Tumour necrosis factor alpha (TNF-α)

Figure 4.33 indicates the 95% confidence intervals using the mean lognormal values for TNF-α between on-pump and off-pump CABG surgical groups.

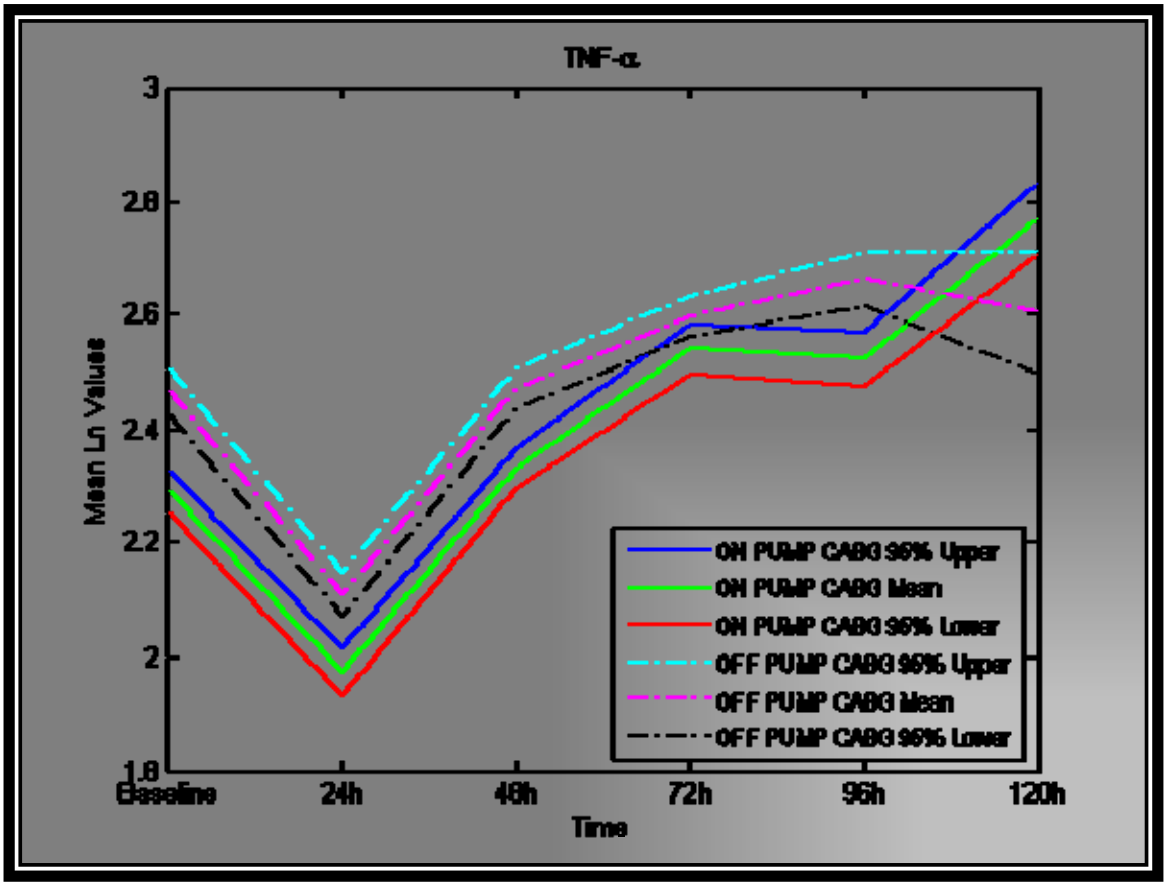


Figure 4.33 **The 95% confidence intervals using the mean lognormal values for TNF-α between on-pump and off-pump CABG surgical groups**

4.7.13 Regressions

In this section, the on-pump CABG surgical procedure was considered as a predictor of the dependent variables (inflammatory markers). More importantly, the risk factors were included as co-predictors to determine the effect of on-pump CABG surgery in the presence of the risk factors (taking into account the effects of the risk factors).

The risk factors included in the regressions were: age, gender, race, diabetes, cholesterol, hypertension, smoking, BMI and EuroSCORE %.

The regressions were done with time included as an additional predictor in the form of a cubic spline (including the variables time, time^2, and time^3 as predictors). When the profile graphs (above) are examined it becomes evident that the variables tend to change over time in a cubic curve, hence the decision to include time in this fashion. This produced results consistent with the previous section.

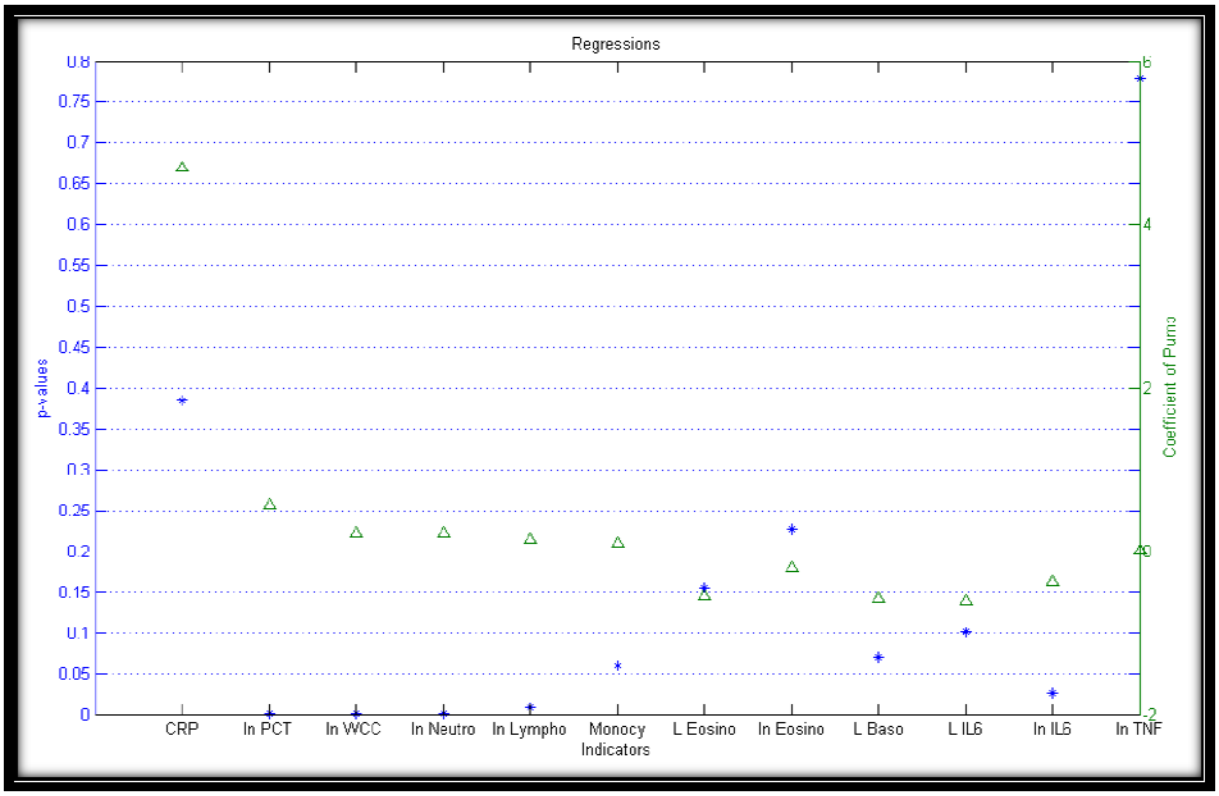


Figure 4.34 Lognormal and logistic regressions

Table 4.26 Lognormal and logistic regressions over time (baseline to 120 hours)

Indicator:	CRP	ln PCT	ln WCC	ln Neutrophils	ln Lymphocytes	Monocytes
p-value:	0.3850	0.0001	<0.00005	<0.00005	0.0086	0.0595
Beta:	4.7024	0.5666	0.2211	0.2259	0.1446	0.1066
Indicator:	**L Eosinophils**	**ln Eosinophils**	**L Basophils**	**L IL-6**	**ln IL-6**	**ln TNF-α**
p-value:	0.1560	0.2268	0.0702	0.1008	0.0266	0.7796
Beta:	-0.5540	-0.2010	-0.5842	-0.6079	-0.3642	0.0175

ln = normal logarithm; L = logistic regressions

In figure 4.34 and table 4.26 three types or regression are present. Where a variable name has no prefix, the OLS (ordinary least squares) regression was performed. Where a variable name is preceded by '*ln*' it implies that a lognormal regression was performed, *i.e.* the *ln* of the dependent variable was regressed on the predictors using OLS regression. Lastly, where a variable name is preceded by 'L', it implies that a logistic regression was performed. A logistic regression is used when the dependent variable can only assume values from 0 to 1, *e.g.* when it is a proportion.

Table 4.27 Lognormal and logistic regressions over time (baseline to 72 hours)

Indicator:	CRP	ln PCT	ln WCC	ln Neutrophils	ln Lymphocytes	Monocytes
***p*-value:**	0.1282	0.0001	0.0000	0.0008	0.0449	0.0784
Beta:	9.7689	0.6634	0.2247	0.2129	0.1279	0.1224
Indicator:	**L Eosinophils**	**ln Eosinophils**	**L Basophils**	**L IL-6**	**ln IL-6**	**ln TNF**
***p*-value:**	0.0831	0.8068	0.0330	0.4344	0.3938	0.7320
Beta:	-0.7170	-0.0526	-0.8122	-0.3427	-0.1562	-0.0239

ln = normal logarithm; L = logistic regressions

Tables 4.26 and 4.27 can be interpreted by first looking individually at the *p*-value and then the beta value for each variable per time interval, the reason being that, if a *p*-value is higher than the chosen threshold (usually 0.05) then the beta value is irrelevant. The beta value indicates the direction and strength of the relationship between the pump variable and the particular indicator in question.

A positive beta value indicates a positive relationship, meaning that the patients in the on-pump CABG group have higher values of the indicator, on average, than patients in the off-pump group. A negative beta value means that the patients in the on-pump CABG group have lower values of the indicator, on average, than the other patients.

The value of beta needs to be considered on the exponential scale for all indicators - CRP and monocytes excepted - where the scale is the same as that of the indicators. As an example, consider the PCT indicator: it has a very small *p*-value, which indicates a strong relationship between the pump and the PCT value. The beta value is 0.5666. This can be converted to a percentage change as follows: $(e^{0.5666} - 1) * 100\% = 76.2265\%$. This means that patients on the pump have about 76% higher values of PCT than those not on the pump, all other factors considered.

When narrowing the time-frame from basal to 72 hours post-operatively (table 4.27), the variable IL-6 is no longer significant. This confirms the information displayed in the graphs (figure 4.23 and figure 4.24), which indicated the negative relationship between on-pump CABG surgery and IL-6 to be significant only after 72 hours.

Figure 4.34 is a graphic representation of table 4.26. For each variable (inflammatory marker), calculations were done to determine whether the *p*-value (**blue star**) was low (preferably below $p=0.05$ on the top-left y-axis). If this was the case, further investigations were done in an attempt to determine whether the coefficient value (**green triangle**) was above or below zero on the right y-axis. If it was indeed above zero (and significant) then there was a positive relationship between on-pump CABG surgery and the variable (inflammatory markers). The off-pump CABG patients formed a baseline and the on-pump CABG patients were compared with the off-pump CABG patients to establish the precise effect of undergoing on-pump CABG surgery specifically with regard to the indicator in question. A positive coefficient (beta) value indicates that the on-pump CABG patients have higher values of the indicator than do the off-pump CABG patients.

4.8 Pre- and post-operative inflammatory markers - *p* values

For the next section, the approximate *p*-values were obtained for the gap between the means of the two CABG groups using the Satterthwaite adjusted *t*-test (table 4.28) for two CABG groups. In almost all cases, this produces results that agree with the graphs presented in Chapter 4. Exceptions occur because this test is more conservative than the confidence intervals given above. The highlighted cells have *p*-values of <0.1.

Table 4.28 ***p*** **values between on-pump and off-pump CABG surgical groups**

Variable	Transformation	Baseline	24 hours	48 hours	72 hours	96 hours	120 hours
CRP	None	0.27140	0.62356	0.49205	0.76571	0.19895	0.42409
PCT	ln	0.81017	0.00811	0.00966	0.01823	0.72387	0.73144
WCC	ln	0.32357	0.00761	0.01520	0.00004	0.13014	0.41097
Neutrophils	ln	0.78954	0.17422	0.04764	0.00003	0.18611	0.12872
Lymphocytes	ln	0.03024	0.17350	0.04829	0.64280	0.01982	0.48277
Monocytes	None	0.42842	0.03599	0.93676	0.71584	0.82357	0.99929
Eosinophils	Proportions	1.00000	0.49682	0.20285	0.15618	1.00000	0.16432
Eosinophils	ln non-zero	0.67682	0.88222	0.38325	0.06465	0.01535	0.07025
Basophils	Proportions	0.32558	0.54949	0.12207	0.23177	0.26675	0.92778
IL-6	Proportions	0.79762	0.32558	0.39832	0.13333	0.31889	0.67653
IL-6	ln non-zero	0.23611	0.50353	0.47128	0.72975	0.05352	0.09729
TNF-α	ln	0.25575	0.40147	0.31438	0.70912	0.40561	0.48907

ln= normal logarithm

4.9 Clinical data

Two patients in the off-pump CABG group died. Both patients, (1) UM00508999 and (2) UM00456515, underwent off-pump CABG surgery. A third patient, UM00364680 (no mortality), also underwent off-pump CABG surgery. Both pre-operatively and post-operatively, this patient presented with extremely elevated IL-6 levels.

CHAPTER 5: DISCUSSION

5.1 Introduction

In 2007 the Heart and Stroke Foundation of South Africa, predicted that in South Africa approximately 33 (thirty-three) people per day would die of a heart attack and that for every woman who died, approximately two men would die. It was moreover predicted that, despite the high death toll caused by AIDS in South Africa, death from a chronic disease - thus also heart disease - would increase from 565 deaths per day in the year 2000, to 666 deaths per day in the year 2010. These untimely deaths caused by cardiovascular disease in people of working age (aged 35 to 64 years) were also expected to increase by 41% between 2000 and 2030, which will bring yet another negative impact to bear on the South African economy (Steyn, 2007).

Although the inflammatory processes and the activation thereof during CABG surgery have been studied intensively in the past, many of the complex interactions of this process still remain elusive (Wan *et al.,* 1999). Despite a significant inflammatory response in either on-pump CABG or off-pump CABG surgery, surgical outcomes have improved significantly in recent years, and apart from the pre-existing inflammatory state associated with atherosclerosis, there are also, additionally, many major external contributory causative factors that activate the inflammatory response during CABG surgery (on-pump and off-pump CABG surgery). The surgical procedure itself (especially the sternotomy), altered shear stresses, contact of the blood with the artificial surfaces of the bypass circuit or with air in the pericardial space, aortic cross-clamping, reperfusion injury, and hypothermia all contribute significantly to the final inflammatory response (Ascione *et al.,* 2000).

The inflammatory response was discussed in some detail in Chapter 2, and the various mechanisms, such as complement activation, endotoxin release, leukocyte activation, the expression of adhesion molecules, and the release of endogenous substances - including oxygen-free radicals, macrophages, neutrophils, and also cytokines - have been mentioned (Ascione *et al.,* 2000; Wan *et al.,* 1999). It can therefore be postulated that by avoiding or attenuating the activation of these elements during CABG, the inflammatory response and a possible systemic inflammatory response (SIR) may be reduced.

The use of CABG surgery, especially in the on-pump CABG setting, produces an intense and unpredictable inflammatory response that could potentially entail a significant risk (Day & Taylor, 2005). The inflammatory response triggered by off-pump CABG surgery is however similar to, though less acute than the inflammatory processes seen in on-pump CABG surgery. Although several studies have claimed that the inflammatory response is less pronounced in the off-pump CABG group, meta-analyses of the published data fail to establish this clearly (Vallely *et al.*, 2001; Chassot *et al.,* 2004).

5.2 On-pump versus off-pump CABG surgery

5.2.1 Demographic data

The on-pump CABG group comprised thirty patients, their average age being 59.13 years (range 43-75 years). There were 27 (90%) males and three (10%) females, of whom 24 (80%) were white, three (10%) were black and three (10%) were coloured.

The mean age of the 30 patients receiving off-pump CABG was 61.2 years (range 47 - 80 years), which is higher than that of the patients receiving on-pump CABG surgery. The latter phenomenon is supported by literature as it is an established fact that older patients are usually considered for off-pump CABG surgery (Larmann & Theilmeier, 2004). Twenty-three (76.67%) of the group were males and seven (23.33%) were females, of whom 26 (86.67%) were white, three (10%) were coloured and one (3.33%) was black.

Comparison of the two CABG groups revealed that there were no significant differences between the two groups. Because this was a prospective longitudinal observational pilot study, the principal investigator accepted that these two CABG groups were reasonably comparable.

5.2.2 Pre-operative risk factors and EuroSCORE

The pre-operative risk factors (table 4.2) were surprisingly similar between the two CABG groups as was the EuroSCORE (the average for on-pump CABG group being 6.09% versus the 5.49% for the off-pump CABG group; table 4.4).

The EuroSCORE is described as a method of calculating predicted operative mortality for patients undergoing cardiac surgery and is a predictor of mortality. Operative mortality is a good measure of

the quality of cardiac surgical care, as long as patient risk factors are taken into consideration (Available from http://euroscore.org (EuroSCORE, [s.a.])). According to the EuroSCORE, patients are divided into low-risk patients (value ≤5) with a predicted mortality below 1%, moderate-risk patients (mortality around 3%) and high-risk patients (predicted mortality 10-11%) (Alvarez, Colmenero, Martin, Prades, Moreno, Gonzalez-Molina, Moreno, Azpitarte, 2003).

Table 5.1 EuroSCORE risk predictor for on-pump and off-pump CABG surgery

On-pump CABG			Off-pump CABG		
EuroSCORE	**n**	**Percentage**	**EuroSCORE**	**n**	**Percentage**
Low risk (0 - 5)	19	63.33%	**Low risk (0 - 5)**	21	70.00%
Moderate risk (≥5 - 10)	8	26.67%	**Moderate risk (≥5 - 10)**	5	16.67%
High risk (≥10)	3	10.00%	**High risk (≥10)**	4	13.33%

In the on-pump CABG group the major risk factor was unstable angina (UA) in 26 patients (86.67%), and in the off-pump CABG group there were 24 patients (80%) with UA.

The other major risk factor in the on-pump CABG group was MI in twenty-one patients (70%), and in the off-pump CABG group there were 15 patients (50%) with MI (the definitions of the EuroSCORE are available from the Society of Thoracic Surgery Adult Cardiac Surgery Database, 2006).

5.2.3 Post-operative outcomes

5.2.3.1 Complications

One patient (3.33%) in the on-pump CABG group had a sternal wound infection. In both CABG groups there was one patient (3.33%) who developed septicaemia, one patient (3.33%) who developed pulmonary complications and one patient (3.33%) who developed gastro-intestinal (GI) complications. Pulmonary complications can result from prolonged ventilation, pulmonary embolism or pneumonia (Jensen & Yang, 2007). GI complications may be due to GI bleeding requiring transfusion, pancreatitis, cholecystitis or mesenteric ischaemia during exploration (Akpinar, Săgbaş, Güden, Kemerta, Sönmez, Bayindir, Demiroglu, 2000). In the off-pump CABG group, one patient (3.33%) developed multisystem failure. This can be defined as when one or more organ systems suffer compromised functions.

In the on-pump CABG group there were 20 (66.67%) patients who developed SIRS, while this occurred in 16 (53.33%) of the off-pump CABG group. A patient was diagnosed with SIRS when two or more of the following criteria were met:

- temperature >38°C or <36°C,
- heart rate >90 beats/minute,
- respiratory rate >20 breaths/minute or $PaCO_2$ <4.3 kPa, or
- WCC <4 x 10^9/l or >12 x 10^9/l or >10% immature forms (Takenaka *et al.,* 2006).

According to table 4.3, more than half of the patients met the above-mentioned criteria and were diagnosed with SIRS.

5.2.3.2 Duration of hospital stay

The average duration of intensive care (ICU) stay for the on-pump CABG group was 3.07 days (range 2-6 days) and for the off-pump CABG group the average duration of ICU stay was 3.03 days (range 1-6 days) (p=0.004). The average hospital stay for the on-pump CABG group was 9.13 days (range 5-18 days) and the average hospital stay for the off-pump CABG group were 8.77 days (range 5-20 days) (p=0.013).

5.2.3.3 Ventilation times

The average ventilation time for the on-pump CABG group was 14.03 hours (6 - 55 hours) and for the off-pump CABG group this was 11.55 hours (4 - 36 hours) (p=0.062).

5.2.3.4 Mortalities

In the on-pump CABG group there were no mortalities; however, in the off-pump CABG group there were two mortalities. The two mortalities are to be discussed later.

5.3 Pre-operative inflammatory markers: on-pump versus off-pump CABG surgical group

5.3.1 C-reactive protein (CRP)

Pre-operatively, the 95th percentile upper and lower limit of the mean lognormal values for CRP (figure 4.22) were similar for both CABG groups and, as expected, for patients with ACS Thus, pre-operatively the baseline CRP values for both CABG groups were similar.

5.3.2 Procalcitonin (PCT)

Pre-operatively, the 95th percentile upper and lower limit of the mean lognormal values for PCT (figure 4.23) were similar for both of the CABG groups and that those for patients with ACS were as expected. Thus, pre-operatively, the baseline PCT values for both CABG groups were similar.

5.3.3 Leukocytes

Judging by the mean lognormal values and the mean lognormal zero values of all the leukocytes, it is evident that all the leucocytes were already elevated pre-operatively in both CABG groups, as expected from patients with ACS due to the already existing atherosclerotic process and the consequent triggering of the inflammatory response (figures 4.24 to 4.30). However, pre-operatively, a significant difference was detected for the lymphocytes between the two CABG groups (p=0.03024).

5.3.4 Interleukin-6 (IL-6)

In figure 4.31, i.e. the graph for the mean proportion non-zero values, it is evident that there are no significant measurable values for IL-6 pre-operatively between the two CABG groups.

In the graph indicating the mean lognormal non-zero values (figure 4.32) it is evident that the measurable values of IL-6 are higher for the off-pump CABG group than for the on-pump CABG group, except at 72 hours post-operatively. The differences appear to be barely significant pre-operatively (baseline) to 48 hours post-operatively, while it is very significant at 96h and 120h post-operatively.

5.3.5 Tumour necrosis factor alpha (TNF-α)

Pre-operatively, a difference in baseline TNF-α values between the on-pump and off-pump CABG surgical groups (figure 4.33) were noticed, with a tendency of higher values in the off-pump CABG group. The difference is however not significant (p=0.25575).

5.4 Post-operative inflammatory marker results: on-pump versus off-pump CABG surgical group

5.4.1 C-reactive protein (CRP)

Although the mean measurable levels for CRP were elevated post-operatively in both CABG groups (figure 4.22) from 24 hours to 72 hours post-operatively, current results reflect no real significant differences between the two CABG groups at 24 hours (p=0.62356), 48 hours (p=0.49205), 72 hours (p=0.76571), 96 hours (p=0.19895) and 120 hours (p=0.42409) post-operatively. The elevated CRP levels may have resulted from the surgical procedure and the trauma itself - without the presence of an infection (Celebi *et al.*, 2006; Vanek, *et al.*, 2002).

5.4.2 Procalcitonin (PCT)

From the mean lognormal results indicated for PCT in figure 4.23 it is evident that those patients undergoing on-pump CABG surgery had significantly higher measurable PCT values with a difference detected between the two CABG groups at 24 hours (p=0.00811), 48 hours (p=0.00966) and 72 hours (p=0.01823) post-operatively. No differences were detected between the two CABG groups at 96 hours (p=0.72387) and 120 hours (p=0.73144) post-operatively. These findings are in line with the findings of other studies (Meisner *et al.,* 1996; Reinhart & Karzai, 2000).

5.4.3 Leukocytes

The post-operative FBC values from the current study are in line with the findings of other studies: an elevated WCC response (Larmann & Theilmeier, 2004), elevated neutrophils (Menasche & Edmunds, 2003; Anderson *et al.,* 2007), elevated lymphocytes (Kotami, *et al.,* 2000; Blacher *et al.,* 2005), and elevated monocytes (Greilich *et al.,* 2008; Menasche & Edmunds, 2003). Post-operatively these markers were elevated in both of the CABG groups, but a significant elevation was noted in the on-pump CABG group for the WCC response and neutrophils.

5.4.4 White cell count (WCC)

The current study indicated that the patients undergoing on-pump CABG surgery had significantly higher WCC values post-operatively over the time horizon in question. Both surgical groups had significant elevated WCC responses (figure 4.24) post-operatively at 24 hours (p=0.00761), 48 hours (p=0.01520) and 72 hours (p=0.00004). This can be explained as having resulted from the blood coming into contact with the artificial surfaces of the bypass circuit, reperfusion injury and other external contributory factors (Larmann & Theilmeier, 2004; Takenaka *et al.,* 2006). Thereafter, the WCC response decreased similarly in both groups from 48 hours to 120h post-operatively, although the WCC response was still appreciably higher in the on-pump CABG group than in the off-pump CABG group.

5.4.5 Neutrophils

Although the mean lognormal measurable values (figure 4.25) for neutrophils were significantly higher in the on-pump CABG group from 24 hours to 120h post-operatively, no differences were apparent between the two CABG groups at 24 hours (p=0.17422), 96 hours (p=0.18611) and 120h (p=0.12872) post-operatively. However, significant differences were detected at 48 hours (p=0.04764) and 72 hours (p=0.00003) post-operatively between the two CABG groups. The neutrophil levels displayed the same trend as the WCC response and the explanation is the same as that mentioned for the WCC response.

5.4.6 Lymphocytes

Comparison of the mean lognormal measurable values (figure 4.26) for lymphocytes reveals that those patients undergoing on-pump CABG surgery had higher measurable lymphocyte values post-operatively than did the off-pump CABG group. No differences between the two CABG groups were noticeable at 24 hours (p=0.17350), 72 hours (p=0.64280) and 120 hours (p=0.48277) post-operatively. Yet, a significant difference was detected at 48 hours (p=0.04829) and at 96 hours (p=0.01982) post-operatively.

5.4.7 Monocytes

The mean measurable monocyte levels (figure 4.27) were elevated post-operatively in both CABG groups, while there was a difference between the two CABG groups at 24 hours (p=0.03599) post-operatively. No differences were subsequently detected between the two groups at 48 hours (p=0.93676), 72 hours (p=0.71584), 96 hours (p=0.82357) and 120 hours (p=0.99929) post-operatively. The elevated monocyte levels at 24 hours post-operatively may have been due to the surgical procedure and the trauma itself.

5.4.8 Eosinophils

From the graph indicating the mean proportion of the non-zero eosinophil values (figure 4.28) it is evident that on-pump CABG surgery has no effect on the measurable values of eosinophils. No differences were detected between the two CABG groups for eosinophils at 24 hours (p=0.49682), 48 hours (p=0.20285) and 72 hours (p=0.15618) post-operatively. The opposite happened at 120 hours (p=0.16432) post-operatively: all the on-pump CABG patients had measurable values, and there were no significant differences between the two CABG groups.

Figure 4.29 reflects that the mean lognormal non-zero measurable values for eosinophils were higher in the on-pump CABG group than in the off-pump CABG group. The reason for this lies in the on-pump CABG surgical procedure itself. No differences were detected between the two CABG groups at 24 hours (p=0.88222), or at 48 hours (p=0.38325). However, differences were detected between the two CABG groups at 72 hours (p=0.06465), 96 hours (p=0.01535) and at 120 hours (p=0.07025) post-operatively.

5.4.9 Basophils

From the graph (mean proportion non-zero values for basophils) as indicated in figure 4.30 it is evident that there is no difference between the two CABG groups with regard to measurable values. Furthermore no differences were detected between the two groups at 24 hours (p=0.54949), 48 hours (p=0.12207), 72 hours (p=0.23177) nor at 96 hours (p=0.26675) and 120 hours (p=0.92778) post-operatively. Overall, the basophils are thus not significantly different between the CABG surgical groups post-operatively.

5.4.10 Interleukin-6 (IL-6)

In figure 4.31, the mean proportion non-zero measurable IL-6 values for patients undergoing on-pump CABG surgery were not significantly different from IL-6 values of patients undergoing off-pump CABG surgery. Figure 4.32 reveals that the measurable values of IL-6 were higher for the off-pump CABG group than for those undergoing on-pump CABG surgery, except, that is, at 72 hours post-operatively. The differences between the two CABG groups were barely significant at 24 hours (p=0.50353), 48 hours (p=0.47128), and 72 hours (p=0.72975), and very significant at 96 hours (p=0.05352) and 120 hours (p=0.09729) post-operatively.

Studies done by Franke, Wan, Corbi and Fransen indicated an elevation in IL-6 levels in the off-pump CABG surgical group. These studies indicated that the IL-6 levels were higher intra-operatively, between 4 to 6 hours post-operatively and up to two days post-operatively in the off-pump CABG group. However, no significant differences were indicated between the on-pump and off-pump CABG groups in these studies. In the studies done by Franke and Wan the IL-6 levels were not measured further than respectively one to two days, post-operatively. The release of IL-6 is probably related rather to tissue trauma and the surgical procedure itself than to ischaemia. Wan and colleagues also indicated that IL-6 levels are higher in the coronary sinus than in the peripheral blood, thereby supporting other studies that suggest that the myocardium is an important source of IL-6 (Corbi *et al.,* 2000; Franke *et al.,* 2005; Fransen *et al.,* 1998; Wan *et al.,* 1999; Wan *et al.,* 2004).

Although there are many different findings regarding elevated levels of IL-6, the majority of findings of previous studies, for example in a study done by Larmann & Theilmeier, 2004, support elevated levels of IL-6 in patients undergoing on-pump CABG surgery.

One patient in the off-pump CABG group already had elevated pre-operative inflammatory markers, which was to be expected in a patient with ACS and the attendant risk factors. Post-operatively, this patient's inflammatory markers elevated significantly, especially the IL-6 levels. The elevated post-operative IL-6 levels can be explained as follows (see figure 5.1): an excess lipid accumulation is present in obese and severely obese patients, and this may lead to increased endoplasmic reticulum (ER) activity.

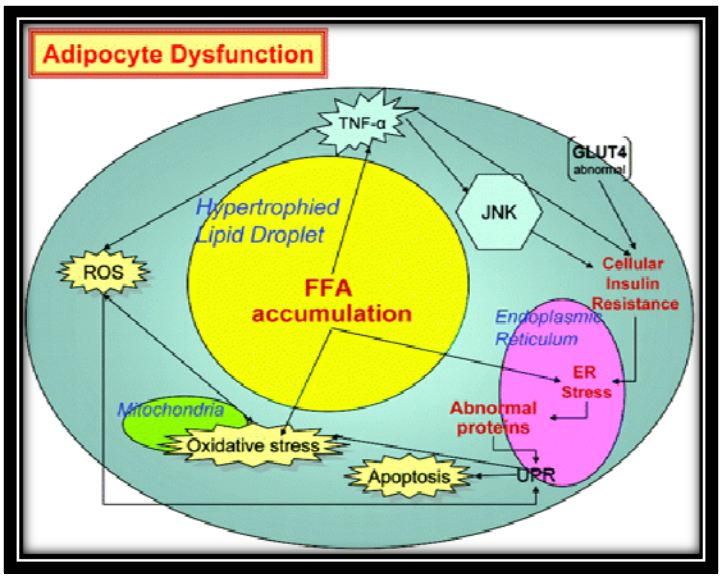


Figure 5.1 **Aspects of adipocyte dysfunction owing to nutrient excess (Adapted from De Ferranti & Mozaffarian, 2008).**

The ER is thus not able to properly fold nascent proteins. If this unfolded protein response (UPR) process continues, it may result in apoptosis. ER stress and the presence of excess free fatty acids (FFA) cause oxidative stress in the mitochondrion. This oxidative stress again produces reactive oxygen species (ROS) and the FFAs produce IL-6 and TNF-α, which, in turn, act on the C-Jun N-Terminal Kinase (JNK) causing cellular insulin resistance (Ferranti & Mozaffarian, 2008).

5.4.11 Tumour necrosis factor alpha (TNF-α)

The mean lognormal measurable values for TNF-α as indicated in figure 4.33 make it evident that the off-pump CABG group had higher TNF-α values at between 24 hours and 48 hours and also at 96 hours post-operatively. This could be in line with the findings of a study done by Diegler and colleagues (Diegeler *et al.*, 2000) where early post-operative increases of TNF-α soluble receptors were found in the off-pump CABG group.

Although the TNF-α values were slightly higher in the off-pump CABG group, there were no significant differences between the on-pump and off-pump CABG groups at 24 hours (p=0.40147), 48 hours (p=0.31438), 72 hours (p=0.70912), 96 hours (p=0.40561), or 120 hours (p=0.48907) post-operatively.

5.4.12 Regression results

In this section only those inflammatory markers with a *p*-value of (p <0.05) were considered, in that these are the cases that are statistically significant (figure 4.34). There were five such cases:

In the case of PCT, WCC response, neutrophils and lymphocytes the beta values were all positive. This means that there was a significant positive relationship between on-pump CABG surgery and the variables (inflammatory markers), taking into account all the risk factors and time. Thus, those patients undergoing on-pump CABG surgery had more elevated values for these variables (inflammatory markers) than those undergoing off-pump CABG surgery.

With regard to IL-6, we note that whenever the values are measurable they tend to be lower for the people undergoing on-pump CABG surgery.

The beta values can be interpreted on the exponential scale in a relative sense. Take PCT for example: ($e^{0.5666}$ - 1) * 100% ≈ 76%, those patients undergoing on-pump CABG surgery have PCT values that are generally about 76% higher than those of the people undergoing off-pump CABG surgery, after adjusting for risk factors and time.

It is worth noting that all the methods applied (confidence interval profiles, Student's *t*-tests and regressions) in this chapter yielded much the same results. The regression results give the conclusions drawn in this chapter a great deal of credibility.

5.5 Clinical data

The first patient, UM00508999, was a 59-year-old white male who underwent off-pump CABG surgery. This patient had a total EuroSCORE risk percentage of 1.56% (low-risk group). After a stable post-operative course, this patient developed acute respiratory distress syndrome (ARDS) and passed away while still in the ICU at 96h post-operatively, as a result of respiratory failure.

The second patient, UM00456515, was an 80-year-old white male with three-vessel disease, who also underwent off-pump CABG surgery. This patient had a total EuroSCORE risk percentage of 10.99% (high-risk group). This patient post-operatively developed kidney failure, respiratory failure,

cardiac failure with disrythmia and became overall hemodynamically unstable, and eventually passed away because of multi-organ failure and septic shock after six days in the ICU.

CHAPTER 6: CONCLUSION

6.1 Conclusion

The data reported in this pilot study support the concept that a detectable and important activation of inflammation occurs both in on-pump and in off-pump CABG surgery (Chong *et al.*, 2003).

In the on-pump CABG group there is an early and significantly more pronounced cellular response than in the off-pump CABG group. This was also recognised in previous reports in which significantly elevated levels of WCC (Abdelhadi *et al.*, 2004; Larmann & Theilmeier, 2004), neutrophils (Levy & Tanaka, 2003; Dacey *et al.*, 2003), lymphocytes (Kotami *et al.*, 2000; Blacher *et al.,* 2005) and PCT (Aouifi *et al.*, 1999; Fransen *et al.,* 1998) were detected in the on-pump CABG group.

Although both on-pump and off-pump CABG surgical procedures have similar cellular and inflammatory activation mechanisms, the cellular response induced post-operatively in the on-pump CABG surgical group is considerably more markedly elevated than the response in the off-pump CABG group. The reason for the increase in the total WCC, neutrophil, lymphocyte and PCT levels can be attributed to an inflammatory response being triggered by the on-pump CABG surgical procedure. When the endothelium is mechanically injured due to the (1) blood coming in direct contact with the artificial surface of the bypass circuit, (2) pulsatile flow being converted to laminar flow, (3) ischaemia, and (4) hypothermia, an inflammatory reaction is triggered that causes profound physiological alterations. Consequently the endothelial cells, leukocytes, platelets, the complement system as well as the coagulation cascades (Larmann & Theilmeier, 2004) are activated causing the inflammatory response to be further aggravated post-operatively.

Complement plays an important role in the body's humoral defence mechanisms and it is also a potent initiator and mediator of inflammation (Larmann & Theilmeier, 2004). Complement activation is further associated with acute phase reaction and leukocyte activation (Gibson *et al.*, 2007; Day & Taylor, 2005).

The current study has shown that, compared with the on-pump group, IL-6 was significantly elevated in the off-pump surgical group (96 - 120 hours). Vallely postulates that on-pump CABG surgery using the bypass circuit plays a secondary role in the stimulation/activation and release of IL-6 post-

operatively, whereas the surgical and tissue trauma caused during off-pump CABG surgery directly causes monocytes, macrophages and endothelial cells to release IL-6 (Vallely *et al.,* 2001; Volk *et al.,* 1998). In studies done by Wan, Corbi, Franke and Fransen, IL-6 levels in the off-pump CABG group peak intra-operatively and post-operatively (4 - 8 hours) up to one to two days post-operatively. However, these particular studies indicated no significant differences regarding IL-6 between the on-pump and off-pump CABG groups (Corbi *et al.,* 2000; Wan *et al.,* 1999; Fransen *et al.,* 1998; Franke *et al.,* 2005; Wan *et al.*, 2004) as was also demonstrated in the present study. Precisely why the response is different between the two groups remains unclear, as surgical trauma seems to be of similar magnitude and - if anything - less in the off-pump group.

Despite the demonstrable inflammatory responses in both CABG groups, no difference in clinical outcomes was observed.

In-conclusion: inflammatory responses evoked by on-pump and off-pump CABG procedures will be an area of interest for future research for some time to come, but certainly is not the only factor determining surgical outcomes. The impact of intra-operative events (*e.g*. prolonged hypotension) needs to be further elucidated and then also in greater detail so as to attempt to determine the relationship of these events on the extent of inflammatory responses and clinical outcomes irrespective of whether the procedure is performed with or without cardiopulmonary bypass.

6.2 Limitations

The small size of the patient population used in this pilot study may explain why some results showed no statistical significance.

6.3 Future investigations and recommendations

As it is not known whether a larger patient population would have made a significant difference to the results of the study, it is suggested that a larger patient population be used for the continuation of the study.

It is further suggested that future investigations use a larger patient population that is either case-matched or studied in a prospective randomised trial.

It is also recommended that future investigations focus on the inflammatory process mechanisms between the on-pump and the off-pump CABG surgical groups.

CHAPTER 7: REFERENCES

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APPENDICES

- **APPENDIX A:** Informed consent form
- **APPENDIX B:** Information sheet
- **APPENDIX C:** Ethical approval
- **APPENDIX D:** Control reference ranges and precision:
  - CRP
  - PCT
  - FBC
  - TNF-α
  - IL-6

APPENDIX A: INFORMED CONSENT FORM

Appendix A.1 **Endothelial function as a predictor of post-intervention outcomes in coronary disease (English version of consent form)**

Date: _____

CONSENT TO PARTICIPATE IN RESEARCH

You have been asked to participate in a research study.

You have been informed about the study by ……. .

You have been informed about any available compensation or medical treatment if injury occurs as a result of study-related procedures;

You may contact Prof FE Smit (082 774 1087) and Prof WMJ van Den Heever-Kriek (082 770 5356) at any time if you have questions about the research or if you are injured as a result of the research.

You may contact the Secretariat of the Ethics Committee of the Faculty of Health Sciences, UFS at telephone number (051) 4052812 if you have questions about your rights as a research subject.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to terminate participation.

If you agree to participate, you will be given a signed copy of this document as well as the participant information sheet, which is a written summary of the research.

The research study, including the above information has been verbally described to me. I understand what my involvement in the study means and I voluntarily agree to participate.

_____ _____
Signature of Participant Date

_____ _____
Signature of Witness Date

_____ _____
Signature of Translator Date

Appendix A.2 Endoteel funksie as 'n voorspeller van post-intervensie uitkomste in koronêre siekte (Afrikaans version of consent form)

Datum: _____

TOESTEMMING TOT DEELNAME AAN NAVORSING

U is versoek om aan 'n navorsingsstudie deel te neem.

U is oor die studie ingelig deur…….

U is ingelig oor die moontlike kompensasie en mediese behandeling wat sal intree indien komplikasies van u deelname aan die studie intree;

U kan Prof FE Smit (082 774 1087) / Prof WMJ van Den Heever-Kriek (082 770 5356) enige tyd kontak indien u vrae oor die navorsing het of as gevolg van die navorsing beseer is.

U kan die Sekretariaat van die Etiekkomitee van die Fakulteit Gesondheidswetenskappe, UV by telefoonnommer (051) 405 2812 kontak indien u enige vrae het oor u regte as 'n proefpersoon.

U deelname aan hierdie navorsing is vrywillig, en u sal nie gepenaliseer word of voordele verbeur as u weier om deel te neem of besluit om deelname te staak nie.
As u instem om deel te neem, sal 'n ondertekende kopie van hierdie dokument sowel as die inligtingsblad, wat 'n geskrewe opsomming van die navorsing is, aan u gegee word.

Die navorsingstudie, insluitende die bogenoemde inligting is verbaal aan my beskryf. Ek begryp wat my betrokkenheid by die studie beteken. Ek verstaan ook dat my pasiënt inligting konfidensieel hanteer sal word en dat my deelname vrywillig is en ek teen enige tyd kan onttrek.

_____ _____
Handtekening van deelnemer **Datum**

_____ _____
Getuie **Datum**

_____ _____
Handtekening van Vertaler **Datum**

Appendix A.3 Tumello ya ho nka karolo dipatlisisong (Sesotho version of consent form)

Letsatsi: _____

O kopilwe ho nka karolo thutong ya dipatlisiso.

O ile wa tsebiswa ka thuto ena ke …….

O ka nna wa ikopanya le Prof FE Smit (082 774 1087) / Prof WMJ van Den Heever-Kriek (082 770 5356) nako e nngwe le e nngwe ebang o nale dipotso mabapi le dipatlisiso kapa ebang o ka tswa kotsi ka baka la dipatlisiso.

O ka nna wa ikopanya le Mongodi wa Ethics Committee ya Faculty of Health Sciences, UFS nomorong ya mohala ya (051) 4052812 ebang o nale dipotso ka ditokelo tsa hao jwaloka eo ho etswang dipatlisiso ka yena.

Ho nka karolo ha hao dipatlisisong tsena ke boithaopong ba hao, mme o keke wa fumantshwa kotlo kapa wa lahlehelwa ke menyetla ya hao ebang o ka hana kapa wa nka qeto ya ho kgaotsa ka ho nka karolo.

Ha o dumela ho nka karolo, o tla nehwa khopi e saennweng ya tokomane ena hammoho le leqhephe la ba nkang karolo e leng le ngotsweng kgutsufatso ya dipatlisiso.

Thuto ya dipatlisiso ho kenyellwa lesedi le ngotsweng ka hodimo, di ile tsa hlaloswa ho nna ka molomo.

Ke utlwisisa hore ho nka karolo ha ka thutong ena ho bolelang. Ke boetse ke utlwisisa hore tlhahiso leseding e mabapi le dintlha tse amang botho ba ka, e tla nkwa e le sephiri le hore ho nka karolo ha ka ke boithaopo le hore nka nna ka ikgula nako e nngwe le e nngwe.

_____	_____
Tshaeno ya motho ya	**Letsatsi**
_____	_____
Letsatsinkang karolo	**Letsatsi**
_____	_____
Tshaeno ya mofetoledi	**Letsatsi**

APPENDIX B: INFORMATION SHEET

Appendix B.1 English version of the information sheet

Dear Patient

We, the department of Cardiothoracic Surgery and Cardiology, are doing research on endothelial function as a predictor of post-intervention outcomes. Research is just the process to learn the answer to a question.

Invitation to participate
We are asking/inviting you to participate in a research study. If you grant us permission you have to sign an informed consent form so that we have evidence that you were willing to participate in the research project.

What is involved in the study?
This is an uncontrolled longitudinal study. Sixty patients with acute coronary syndrome (ACS) will be recruited from the Cardiology clinic for participation. Due to the fact that every test performed except the atherosclerotic evaluation is routine practice for a patient with ACS the patient won't be subjected to numerous amounts of tests.

The following tests will be performed:
A) Physical Examination
After a confirmed diagnosis of acute coronary syndrome and granted consent a complete medical history and physical examination will be performed by a qualified medical doctor located at cardiothoracic surgery (Routine practice for ACS).

B) Cardiology evaluation
The patient will be submitted for a cardiac echocardiograph and an angiogram at the catheter lab on the second floor, Universitas Hospital (Routine practice for ACS).

C) Biochemical markers
The biochemical markers will be done as requested by the medical practitioner overseeing the patient and will include the following tests. All these tests will be performed in accordance with the standard operating procedures as accredited by the NHLS, Universitas hospital (Routine practice for an ACS patient).

- Cholesterol
- Triglycerides
- Micro-albuminuria
- Fasting glucose & Insulin
- Lipoproteins
  - High-Density Lipoprotein (HDL)
  - Low-Density Lipoprotein (LDL)
- Pro-BNP
- Troponin T
- CK & CKMB
- Homocysteine

D) Inflammatory markers

The following inflammatory markers will be done by the NHLS, Universitas hospital in accordance to their standard operating procedures. This is the only extra blood we will draw from the patient for measurement. The blood samples will be drawn at specific increments (Table B.1).

Table B.1a List of time schedules and blood draw tubes necessary

With admission (baseline):	**2 x purple; 1 x purple on ice; 3 x yellow; 1 x random urine; 1 x grey tube**
24 hours after intervention	**1 x purple; 2 x yellow**
48 hours after intervention	**1 x purple; 2 x yellow**
72 hours after intervention	**1 x purple; 2 x yellow**
96 hours after intervention	**1 x purple; 2 x yellow**
120 hours after intervention	**1 x purple; 2x yellow**

The following tests form part of the inflammatory markers:

- C-Reactive Protein
- Procalcitonin
- Tumor Necrosis Factor-α
- Interleukin-6
- Full Blood Count

Risks

The research project is very safe. All the procedures are non-invasive and no adverse effects are predicted. Patients participating in this study will be well monitored and can at any time discontinue participation in the study. The study will be discontinued prematurely if the researcher or any of the study leaders feels that a patient's confidentiality might be breached or if any unethical procedures occur.

Benefits

We know that SIRS mediators are activated in the endothelium and that this response can be abnormal in arthrosclerosis. It is also known that medical treatment with statins and ACE inhibitors modifies this response and can improve or normalise endothelial function. SIRS occurs more often post-intervention in some patients (e.g. metabolic syndrome, patients with pre-op elevated TNF, micro albumin urea). Outcomes in inflammatory markers and clinical SIRS might differ between On-pump and off-pump procedures.

If we can correlate the pre-intervention endothelial function to outcomes to acute coronary syndrome we might be able to develop a rational approach to intervention and choice of intervention.

Your participation will enable us to attempt to try and identify a relationship between clinical data, blood tests and endothelial function/atherosclerosis load will be made.

Participation is voluntary

Refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled; the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Confidentiality
Efforts will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Ethics Committee for Medical Research and the Medicines Control Council.

If results are published, this may lead to individual/cohort identification.

Contact details of researcher(s) - for further information/reporting of study-related adverse events.

a) **Prof FE Smit**
Head: Department Cardiothoracic Surgery
UFS
Cell: 082 774 1087

b) **Dr L Botes**
Senior Lecturer
CUT
Cell: 083 709 0312

Contact details of REC Secretariat and Chair - for reporting of complaints/problems.

a) **Prof BB Hoek**
Chairman: Ethical Committee
UFS
Phone: 051 405 3177

Appendix B.2 Inligtingsdokument (Afrikaans version of the information sheet)

Beste Pasiënt

Ons, die Departement Kardiotorakschirurgie, is besig om navorsing oor endoteel funksie as ‘n voorspeller van post-intervensie uitkomste in koronêre siekte te doen. Navorsing is slegs die proses waardeur ‘n antwoord op ‘n vraag verkry word. In hierdie studie wil ons leer of daar `n verskil is in die uitkomste van pasiënte wat presenteer met akute koronêre sindroom en die met verskwakkende of chroniese stabiele angina?

Uitnodiging om deel te neem
Ons versoek/nooi u uit om aan die navorsingstudie deel te neem. Indien u aan ons toestemming verleen moet u `n toestemmingsvorm teken sodat ons `n bewys het dat u gewillig was om aan die studie deel te neem.

Wat behels die studie?
Hierdie in `n ongekontrolleerde longitudinale studie. Sestig pasiënte met akute koronêre sindroon (AKS) sal gewerf word vanaf die Kardiologie kliniek vir deelname aan die studie. Al die toeste wat gedoen word, behalwe vir die aterosklerotiese evaluasie, is roetine toetse vir `n pasiënte met AKS. Die pasiënt sal dus nie onderworpe wees aan groot hoeveelhede toetse nie.

Die volgende toetse sal uitgevoer word:

A) Fisiese ondersoek
Nadat `n bevestigde diagnose van akute koronêre sindroom (AKS )en toestemming van die pasiënt sal `n volledige mediese geskiedenis en fisiese ondersoek gedoen word deur `n gekwalifiseerde mediese dokter by kardiotorakschirurgie (Roetine prakryk vir AKS).

B) Kardiologie evaluasie
`n Kardiale echokardiogram en angiogram sal van die pasiënt geneem word in die kateter lab op die tweede vloer in Universitas Hospitaal (Roetiene prakryk vir AKS).

C) Biochemiese merkers
Die biochemiese merkers sal gedoen word soos aangevra deur die mediese dokter wat die pasiënt behandel en sal die volgende toetse insluit. Al die toetse sal uitgevoer word soos gestipuleer deur die standaard operasionele prosedure geakkrediteer deur die NHLS, Universitas hospital (Roetiene praktyk vir AKS).

- Cholesterol
- Trigliseriede
- Mikroalbumienurie
- Vastende glukose & Insulien
- Lipoproteïene
  - HDL
  - LDL
- Pro-BNP
- Troponin T
- CK & CKMB
- Homocysteine

D) Inflammatoriese merkers

Die volgende inflammatoriese merkers sal gedoen word deur die NHLS, Universitas hospital volgens hulle standaard operasionele prosedures. Dit is die enigste ekstra bloed wat getrek moet word by die pasiënt. Die bloed sal toenemend getrek word.

Table B.1b: Tyd skedules en bloedtrek buise nodig (Afrikaans version of the Information Sheet)

Met opname (basaal)	2 x pers; 1 x pers op ys; 3 x geel; 1 x lukraak uriene; 1 x grys buis
24 ure na intervensie	1 x pers; 2 xgeel
48 ure na intervensie	1 x pers; 2 x geel
72 ure na intervensie	1 x pers; 2 x geel
96 ure na intervensie	1 x pers; 2 x geel
120 ure na intervensie	1 x pers; 2 x geel

Die volgende toetse word gedoen as deel van inflamatoriese merkers:

- CRP
- PCT
- TNF-α
- IL-6
- FBC

Risiko's

Hierdie projek is baie veilig. Al die prosedures is nie-indringend en geen nadelige gevolge word voorspel nie. Pasiënte wat aan die studie deelneem sal gemonitor word en kan enige tyd onttrek vanuit die studie. Die studie sal onmiddellik gestaak word indien die navorser of enige ander studieleier voel dat `n pasiënt se konfidensialiteit verbreek word of enige onetiese prosedures plaasvind.

Voordele

Ons weet dat SIRS middellaars geaktiveer word in die endoteel en dat hierdie respons abnormaal kan wees met aterosklerose. Dit is ook bekend dat mediese behandeling met statiene en ACE inhibitore hierdie respons verander en dat dit endoteel funksie kan verbeter of normaliseer. In sommige pasiënte vind SIRS meestal post-intervensie plaas (bv. metaboliese sindroom, pasiënte met pre-operatiewe verhoogde TNF en mikroalbumienurie waardes). Die kliniese uitkomste van SIRS gebasseer op inflammatoriese merkers mag verskil tussen KVO (met gebruik van die hart-long apparaat en daarsonder) en perkutane koronêre ingreep. Indien `n korrelasie tussen pre-intervensie endoteel funksie en uitkomste in twee verskillende gevalle (stabiele angina en akute koronêre sindroom) getref kan word, kan dit ons instaat stel om `n rasionale benadering tot intervensie en keuse van intervensie te maak.

U deelname sal ons instaat stel om te poog om die verhouding tussen kliniese data, bloedtoetse en endoteel funksie/aterosklerose lading te identifiseer.

Deelname is vrywillig

Weiering om deel te neem sal geen boete of verlies van voordele waarop die deelnemer andersins geregtig is behels nie; die proefpersoon kan te eniger tyd aan deelname onttrek sonder boete of verlies van voordele waarop die proefpersoon andersins geregtig is.

Vertroulikheid

Daar sal gepoog word om persoonlike inligting vertroulik te hou. Volkome vertroulikheid kan nie gewaarborg word nie. Persoonlike inligting kan bekend gemaak word as die wet dit vereis.

Organisasies wat u navorsingsrekords mag ondersoek en/of kopieer vir kwaliteitsversekering en data-analise sluit groepe soos die Etiekkomitee vir Mediese Navorsing en die Medisynebeheerraad in.

As resultate gepubliseer word kan dit lei tot individuele/groepsidentifikasie.

Kontakbesonderhede van navorser(s)

Vir verdere inligting/rapportering van studieverwante newe-effekte.

a) **Prof FE Smit**
Hoof: Departement Kardiotorakschirurgie
UFS
Sel: 082 774 1087

b) **Dr L Botes**
Senior Dosent
SUT
Sel: 083 709 0312

Kontakbesonderhede van REC Voorsitter vir raportering van klagtes/problem

a) **Prof BB Hoek**
Voorsitter: Etiese komitee
UFS
Telefoon: 051 405 3177

APPENDIX C: ETHICAL APPROVAL

UNIVERSITEIT VAN DIE VRYSTAAT
UNIVERSITY OF THE FREE STATE
YUNIVESITHI YA FREISTATA

Direkteur: Fakulteitsadministrasie / Director: Faculty Administration
Fakulteit Gesondheidswetenskappe / Faculty of Health Sciences

Research Division
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Ms H Strauss 2007-04-18

PROF FE SMIT
DEPT OF CARDIOTHORACIC SURGERY
FACULTY OF HEALTH SCIENCES
UFS

Dear Prof Smit

ETOVS NR 51/07
RESEARCHER: PROF FE SMIT
PROJECT TITLE: ENDOTHELIAL FUNCTION AS PREDICTOR OF POST INTERVENTION OUTCOMES IN CORONARY DISEASE.

- You are hereby informed that the The Ethics Committee approved the above-mentioned on 17 April 2007.
- The following documents are used by the Ethics Committee as guidance documents: Declaration of Helsinki, ICH, GCP and MRC Guidelines on Bio Medical Research. Clinical Trial Guidelines 2000 Department of Health RSA; Ethics in Health Research: Principles Structure and Processes Department of Health RSA 2004; the Constitution of the Ethics Committee of the Faculty of Health Sciences and the Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines.
- Any amendment, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.
- The Committee must be informed of any serious adverse event and/or termination of the study.
- A progress report should be submitted within one year of approval of long-term studies and a final report at completion of both short term and long term studies.
- Please refer to the ETOVS reference number in correspondence to the Ethics Committee secretariat.

Yours faithfully

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for PROF BB HOEK
CHAIR: ETHICS COMMITTEE

CC Prof WMJ van den Heever-Kriek, Central University of Technology Free State

APPENDIX D: CONTROL REFERENCE RANGES AND PRECISION

Appendix D.1 C-reactive protein (CRP)

Table D.1 summarizes the control precision run on the Beckman LX 20 analyzer for CRP on Vigil control level 2 and 3.

Table D.1: Control precision for CRP

	CRP Synchron Vigil control level 2	CRP Synchron Vigil control level 3
	49.1	79.7
	51.7	77.7
	49.8	79.7
	52.8	83.2
	52.1	80.1
	51.4	78.8
	53.0	80.5
	50.4	83.2
	51.1	79.9
	51.7	80.2
n	10	10
Average	50.6	79.1
SD	1.8184	2.1260
CV	4%	3%

n = total; SD = standard deviation; CV = correlation coefficient

Table D.2 summarizes the control reference ranges for CRP on Vigil control level 2 and 3.

Table D.2: Synchron CRP control reference ranges, lot number and expiry date (NHLS, Universitas hospital, 2009)

	Control reference ranges	Lot number	Expiry date
Vigil control level 2	45.5 - 61.4 mg/l	M806332	31/08/2010
Vigil control level 3	71.3 - 91.3 mg/l	M806333	31/08/2010

Appendix D.2 Procalcitonin (PCT)

Table D.3 summarizes the control precision run on the Kryptor analyzer for PCT on control level 1 and 2.

Table D.3: Batch control precision for PCT (NHLS, Universitas hospital, 2009)

	PCT Kryptor Control level 1	PCT Kryptor Control level 2
	0.2806	10.0600
	0.2595	9.5520
	0.2707	10.110
	0.2610	9.7430
	0.2730	10.340
	0.2744	9.9160
	0.2621	9.7470
	0.2779	10.010
	0.2596	9.8370
	0.2750	10.130
n	10	10
Average	0.2694	9.9445
SD	0.0081	0.2314
CV	3%	2%

n = total; SD = standard deviation; CV = correlation coefficient

Table D.4 summarizes the control reference ranges Lot no and expiry date for PCT on Control level 1 and 2.

Table D.4 PCT control reference ranges, lot number and expiry date (NHLS, Universitas hospital, 2009)

	Control reference ranges	Lot number	Expiry date
Level 1	0.2080 - 03120 ng/ml	25017A	20/06/2010
Level 2	7.864 - 11.80 ng/ml	25017A	20/06/2010

Appendix D.3 Full blood count (FBC)

Table D.5 summarizes the control precision run on the Sysmex XE 2100 analyzer for FBC on control level 1, 2 and 3.

Table D.5 Control precision for FBC (NHLS, Universitas hospital, 2009)

LEVEL 1			
	SD	MEAN	CV
Platelets 10^9/l	4	64	6.3
WCC 10^9/l	0.088	2.85	3.1
Neutrophils 10^9/l	0.061	1.33	4.6
Lymphocytes 10^9/l	0.04	0.97	4.1
Monocytes 10^9/l	0.025	0.27	9.3
Eosinophils 10^9/l	0.023	0.28	8.2
Basophils 10^9/l	0.068	1.8	3.8
LEVEL 2			
	SD	MEAN	CV
Platelets 10^9/l	7	225	3.1
WCC 10^9/l	0.137	6.9	2
Neutrophils 10^9/l	0.096	3.9	2.8
Lymphocytes 10^9/l	0.097	2	4.9
Monocytes 10^9/l	0.071	0.8	8.9
Eosinophils 10^9/l	0.06	0.71	8.5
Basophils 10^9/l	0.11	4.65	2.4
LEVEL 3			
	SD	MEAN	CV
Platelets 10^9/l	11.1	536	2.1
WCC 10^9/l	0.317	17.31	1.8
Neutrophils 10^9/l	0.241	9.64	2.5
Lymphocytes 10^9/l	0.142	3.75	3.8
Monocytes 10^9/l	0.116	1.89	6.1
Eosinophils 10^9/l	0.157	2.04	7.7
Basophils 10^9/l	0.246	13.52	1.8

SD = standard deviation; CV = correlation coefficient

Table D.6 summarizes the control Lot no for FBC on Control level 1, 2, and 3.

Table D.6 FBC control lot number and expiry date (NHLS, Universitas hospital, 2009)

	Lot no	Expiry date
Level 1	QC 91000810	02/07/2009
Level 2	QC 91000811	02/07/2009
Level 3	QC 91000812	02/07/2009

Table D.7 summarizes the control reference ranges for FBC on Control level 1, 2, and 3.

Table D.7 FBC control reference ranges (NHLS, Universitas hospital, 2009)

	Neutrophils 10^9/l	WCC 10^9/l	Monocytes 10^9/l	Eosinophils 10^9/l	Basophils 10^9/l	Lymphocytes 10^9/l
Level 1	1.04 - 1.56	2.65 - 3.25	0.07 - 0.61	0.14 - 0.42	0.41 - 3.25	0.62 - 1.44
Level 2	2.75 - 3.71	6.43 - 7.25	0.31 - 1.25	0.39 - 1.04	1.0 - 8.08	1.71 - 2.57
Level 3	7.80 - 10.56	16.59 - 18.71	1.02 - 3.08	1.01 - 3.05	2.87 - 23.27	3.51 - 5.27

Appendix D.4 Tumor necrosis factor-α (TNF-α)

Table D.8 summarizes the control precision for TNF-α on the low control and the high control.

Table D.8 Control precision for TNF-α

	Low control (Reference range 32 +/- 12 pg/ml) (BioSource, 2006) Lot no 8G3/1	High control (Reference range 81 +/- 18 pg/ml) (BioSource, 2006) Lot no 8G2/1
	38.811	78.700
	39.881	96.228
	41.507	71.723
	43.647	98.786
	45.858	83.167
	43.627	79.650
	41.506	74.340
	44.842	92.215
	53.683	95.087
	43.187	102.367
N	10	10
SD	4.136320841	10.97249523
Average	43.6549	87.2263
CV	9%	13%

n = total; SD = standard deviation; CV = correlation coefficient

Appendix D.5 Interleukin-6 (IL-6)

Table D.9 summarizes the control precision for IL-6 on the low control and the high control

Table D.9 Control precision for IL-6

	Low control (Reference range 32 +/- 12 pg/ml) (BioSource, 2006) Lot no 7E2/2	High control (Reference range 81 +/- 18 pg/ml) (BioSource, 2006) Lot no 7D3/2
	125.057	431.709
	114.874	451.585
	112.567	417.723
	115.997	438.323
	131.955	415.825
	115.035	396.215
	125.085	383.306
	119.382	421.533
	129.492	422.492
	139.43	435.85
SD	8.826646516	20.1213453
Average	122.8874	421.4561
CV	7%	5%