The University of Hull

Pre-treatment of patients undergoing cardiopulmonary bypass with hyperbaric oxygen. A prospective randomised double-blind evaluation of inflammatory response and neurocognitive outcome.

Being the thesis submitted for the degree of Doctor of Medicine (MD)

By

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Abstract

Over the past few decades, implementation of findings from scientific research and incorporation of technological advances into practice have produced a significant reduction in the morbidity and mortality from coronary revascularisation. Despite these improvements subtle neurocognitive impairment, from diffuse cerebral injury due to micremboli during cardiopulmonary bypass, continues to occur following cardiac surgery.

Previous human and animal studies have shown that hyperbaric oxygen can reduce some of the mediators of inflammation. Evidence from animal experiments also suggest that pre-treatment with hyperbaric oxygen can induce cerebral and spinal ischaemic tolerance. Using the prospective randomised double-blind method we set out to test the hypothesis that pre-treatment with hyperbaric oxygen could reduce inflammatory mediators and neurocognitive dysfunction following cardiopulmonary bypass.

64 patients undergoing elective coronary revascularisation were either randomised to **Group-A (air, 1.5 atmospheres absolute, n-31)** or **Group-B (hyperbaric oxygen, 2.4 atmospheres absolute, n-33)**. Both groups were comparable in terms operative and perioperative factors. Inflammatory markers were measured prior to anaesthesia and, 2 hours and 24 hrs after bypass. Neurocognitive assessment was performed 48hours before the operation and 4 months after the operation. Neurocognitive impairment was defined as $\geq$ 1standard deviation (SD) decline in postoperative score in more than 20% of the neurocognitive tests. ANOVA, t-test and Chi-square tests was used as appropriate for statistical analysis.

There was no difference in immediate postoperative clinical outcome. However Analysis of variance revealed that the postoperative peak rise in the inflammatory markers soluble

Abstract (continued)

endothelial selectin (sE-selectin), heat shock protein-70 (HSP-70) and cluster differentiation antigen-18 (CD-18) which was significant in group-A was not so in group-B. Analysis of data from the neurocognitive tests also revealed that the overall neurocognitive dysfunction was significantly higher in group-A compared to group-B.

From our findings we concluded that pre-treatment with hyperbaric oxygen may have a potential beneficial role in reducing neurocognitive impairment and reducing some of the inflammatory mediators following cardiopulmonary bypass. Larger multi-centre prospective randomised trials are needed to further evaluate this form of therapy.

List of contents **Page**

List of contents (Continued)

List of Figures **Page**

List of tables

Statement of originality

All the work described in this thesis is the original work of the author. Any assistance with the work is indicated in the acknowledgments.

Acknowledgement

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Introduction

1. Introduction

1.1 Statement of hypothesis.

Despite advances in surgical and anaesthetic techniques, perioperative care, cardiopulmonary bypass technology and pharmacotherapy, neurocognitive dysfunction continues to occur in a significant proportion of patients following cardiopulmonary bypass. It is widely believed to be a manifestation of primary ischaemic cerebral damage during surgery resulting from either microemboli or episodes of hypoperfusion. Inflammatory cytokines, activated leucocytes and free radicals play an important role in the secondary damage, which follows the ischaemia. Alongside continuing technological research aimed at minimising the primary injury, attention has now focussed on interventional strategies aimed at limiting secondary brain injury resulting from the inflammatory response.

Hyperbaric oxygen therapy has been used previously to treat patients with brain injury resulting from trauma, ischaemia, air-embolism, carbon-monoxide poisoning and radiation. Though this form of therapy has thus far been limited to treatment post-injury, studies have indicated a potential role as a pre-injury treatment modality in certain settings. Recent animal studies have shown that pre-treatment with hyperbaric oxygen can be used to induce ischaemic tolerance in the central nervous system, inhibit or reduce certain inflammatory cytokines and leucocyte adhesion molecules, and augment anti-oxidant enzymes and proteins. Before discussing the methodology, results and outcome of the study, evidence will be presented from the literature on, neurocognitive dysfunction and inflammatory response following cardiopulmonary bypass, effect of hyperbaric oxygen on cerebral ischaemic tolerance and the effect of hyperbaric oxygen on inflammatory mediators.

Using the prospective randomised double blind method, I have compared two groups of patients who either received air **(group-A)** (1.5 atmospheres absolute for 60 minutes X 3 sessions) or hyperbaric oxygen **(group-B)** (100% O_2 at 2.4 atmospheres absolute for 60 minutes X 3 sessions) prior to surgery. I have attempted to investigate the hypothesis that pre-treatment with hyperbaric oxygen may reduce the neurocognitive dysfunction and inflammatory response following cardiopulmonary bypass.

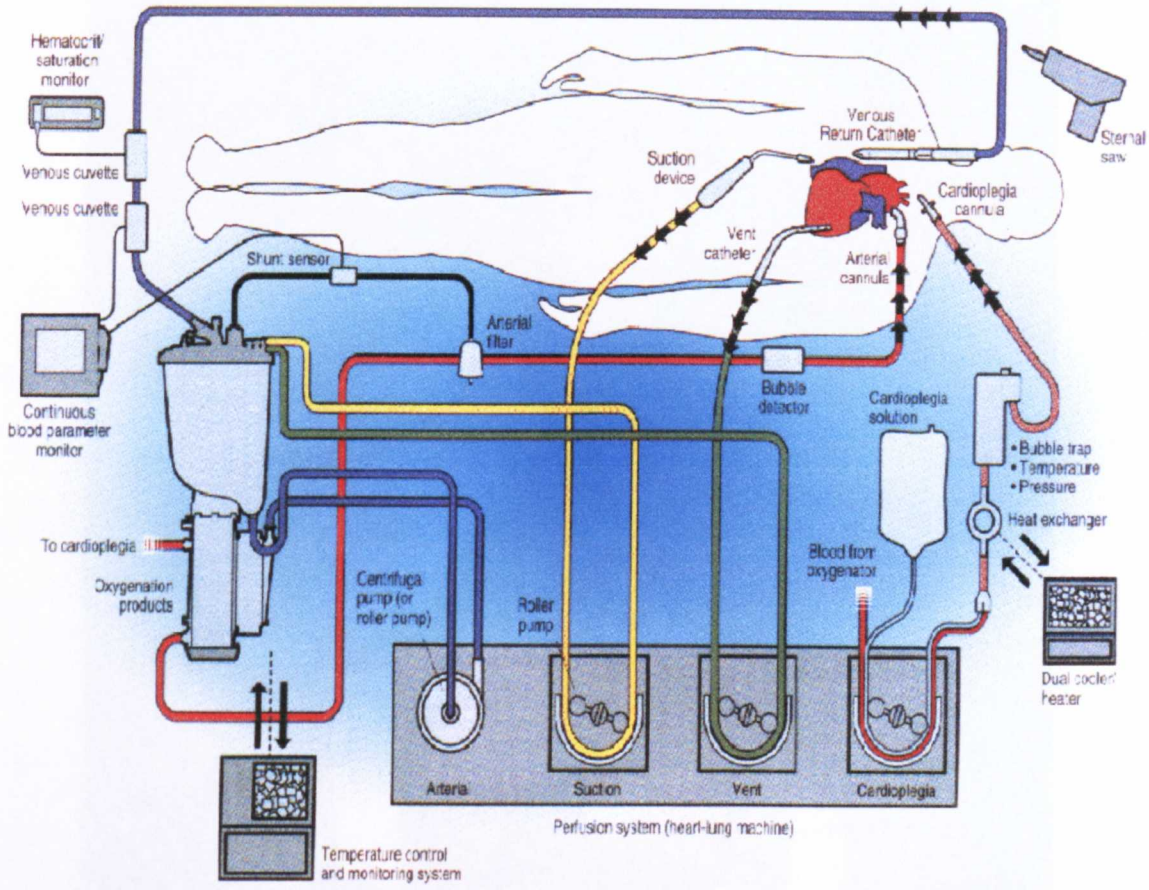
1.2 Evolution of cardiopulmonary bypass.

The first documented use of cardiopulmonary bypass on a human was in 1951 when the technique was used for 40 minutes to repair an ostium primum atrial septal defect in a 6 year old child who unfortunately, did not survive **(Dennis et al, 1951).** It was not until 1953, that Gibbon successfully used the technique for 26 minutes on a 26 year old woman to repair an atrial septal defect **(Gibbon, 1954).** This was followed by the successful use of the technique by Melrose and his associates in London the following year **(Cleland and Melrose, 1955)**. At about the same period **Lillehei** and his associates **(1955)** embarked on a different approach by attempting to achieve physiological bypass. They connected the patient's circulation in parallel to another human (usually the mother) instead of an artificial oxygenators. However, following the death of some healthy supporting subjects this technique was abandoned. The first successful series of operations, using cardiopulmonary bypass were reported by **Kirklin** and his associates **(1955)** at the Mayo clinic.

Though technological advances have facilitated the incorporation of safety features and more biocompatible materials the basic principle of cardiopulmonary bypass has remained the same. Venous blood, siphoned from the right atrium or vena cavae into a reservoir is then, with the aid of a roller or centrifugal pump, passed through a heat exchanger, oxygenator and filter before being returned to the patient via the aorta or a major artery (Figures - 1 and 2). Thus the venous return to the right atrium is bypassed away from the heart and lung into the cardiopulmonary bypass circuit which maintains the set temperature, oxygenates the blood and returns it to the arterial tree at a set flow rate and pressure. The two commonly used pumps in the bypass circuit are the roller pump and the centrifugal or vortex pump. Due to its simplicity and low cost roller pumps are more commonly used compared to the vortex pump.

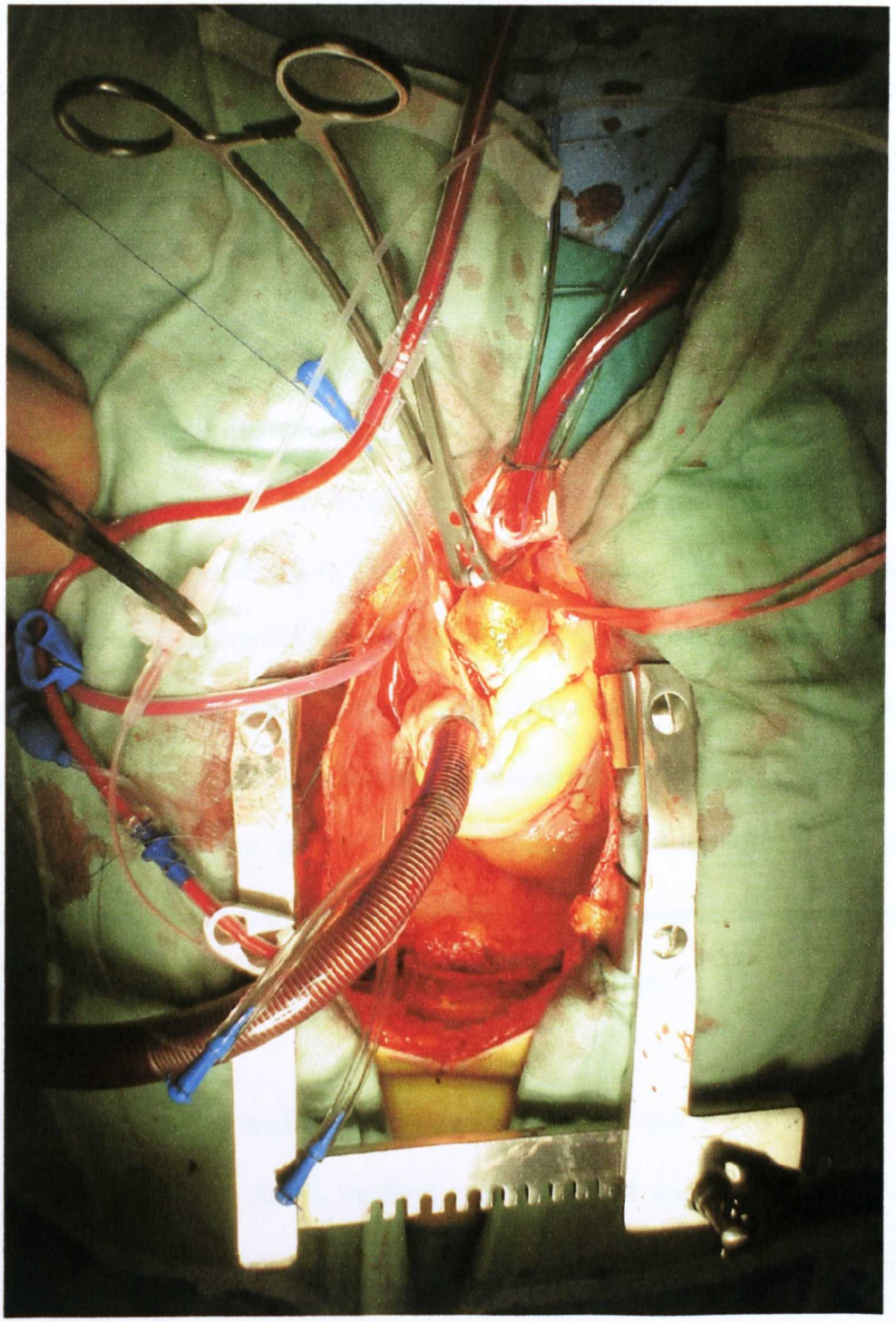
Though the degree of leucocyte activation was believed to be equivalent with either pump types **(Macey et al, 1997)**, a more recent study revealed greater leucocyte and compliment activation with the centrifugal pump **(Baufreton et al, 1999)**. Historically three main types of oxygenators have been used the Melrose rotating disc, the bubble and the membrane oxygenator. The rotating disc devise, which is no longer in use, consisted of series of spinning discs partially immersed in a bath of heparinised blood enclosed in an oxygen chamber. This technique was clumsy, needed a large amount of prime, caused severe haemolysis, denatured proteins and triggered a severe inflammatory response. The bubble oxygenator is a simple device consisting of a chamber of heparinised blood through which oxygen and carbon dioxide is passed allowing direct contact with blood. In a membrane oxygenator the blood is separated from oxygen by a partially permeable membrane, which allows free gas exchange without direct blood-gas contact which further reduces leucocyte activation **(Gillinov et al, 1993)**. The absence of air-blood interphase in the oxygenator minimises air bubbles, and reduces platelet activation and inflammatory response. There are three types of membranes that can be used in the membrane oxygenator, hollow-fibre, flat-sheet and silicone envelope. The hollow-fibre membrane, due to its smaller contact surface area, has been found to be more biocompatible and associated with a less pronounced inflammatory response **(de Vroege et al, 1997)**. We used roller pump with hollow-fibre membrane oxygenator, in the bypass circuit, for all patients in the study.

Figure - 1. A modern cardiopulmonary bypass circuit.



(Reproduced with permission from the Perfusion Department – Cardiothoracic Surgery, Castle Hill Hospital)

Figure - 2. A cannulated and cross-clamped heart.



1.3 Brain injury following cardiopulmonary bypass.

Advances in bypass technology, bio-monitoring, pharmacotherapy and perioperative management have significantly reduced the mortality following cardiopulmonary bypass. With improved survival, the focus of research has turned to the prevention and limitation of organ dysfunction and morbidity associated with the procedure. The reported incidence of brain injury varies widely between studies, it is estimated to range between 20 - 80% in patients. Cerebral injury can result in not just debilitating physical and mental changes, but can also have a profound and devastating socio-economic impact on the patient's life. The severity of brain injury can be described quantitatively as the structural damage and qualitatively as the functional deficit. Thus a small left cortical stroke in the pre-central gyrus, despite its small size, can have disastrous consequences like expressive dysphasia, dominant hand weakness, and ocular incordination. Conversely, a larger involvement of the anterior right frontal lobe may cause a lesser degree of functional deficit and may go unnoticed by the clinician, despite causing a greater level of tissue destruction. Thus, the functional impairment depends on the region of the brain involved, the magnitude of neuronal damage and the compensatory capacity of the remaining healthy neurons.

Clinically brain injury after cardiac surgery can be classified into 2 types, the less common type-1 injury which includes stroke, transient ischaemic attack and coma, which occurs in 2-6% of patients **(Cardiac surgical database 1999-2000)** and Type-2 injury which includes confusion, memory impairment and neurocognitive impairment. Type-2 injury is more common and occurs in 20-80% of patients following cardiopulmonary bypass **(Saveague et al 1982, Aberg et al 1984, Smith et al 1986, Shaw et al 1987, Treasure et al 1989, Newman et al 1995, Mahanna et al 1996, Plourde et al 1997, McKhann et al 1997,**

Braekken et al 1998, Browne et al 1999, Hall et al 1999, Taggart et al 1999, Borger et al 2001, Fearn et al 2001, Stroobant et al 2002, Van Dijk et al 2002, and Selnes et al 2003). Longitudinal follow-up studies have shown that neurocognitive dysfunction can persist even at 5 years after the operation **(Newman et al 2001)**.

1.4 Factors contributing to brain injury.

Increasing age **(Gardner et al 1985)**, diabetes mellitus **(Selnes et al, 1999)**, hypertension **(McKhann et al, 1997)**, atherosclerotic vascular disease **(Blauth et al 1992 and Hammon et al 1997)**, recent myocardial infarction **(Tuman et al, 1992)**, previous cerebrovascular accidents, chronic debilitating neurologic illness, lower education level, living alone, postoperative complications **(Ho et al, 2004)** and the duration of cardiopulmonary bypass **(Kilo et al 2001 and Van Dijk et al 2002)**, have all been identified as predictors of postoperative cognitive decline. The rapid improvements in health care along with the more sedentary lifestyle have resulted in a longer lifespan despite more concurrent illness. Thus the patients undergoing surgery are older and sicker than before. Some of the measures to limit brain injury include, preoperative assessment of carotid arteries, epiaortic doppler prior to cannulation, hypothermia, arterial line filters to reduce microemboli, leucocyte depleting filters, pH and blood gas management strategies tempered to the procedure, and optimising mean arterial blood pressure and flow rates during bypass.

1.5 Types and sources of cerebral emboli during CPB.

Cerebral emboli during cardiopulmonary bypass can be air or particulate. Air can enter the blood stream if, the chambers of the heart are opened, during cannulation or decannulation, accidentally by blowing through the tubes meant to vent the heart and during perfusionist interventions **(Taylor et al 1999 and Borger et al 2001)**. Biological aggregates include microscopic thrombi, platelet aggregates, atherosclerotic debris (Figure – 3), and liquified fat which give rise to small capillary and arteriolar dilatations (SCADS) in stained specimens of the brain following cardiopulmonary bypass (Figure – 4) **(Moody et al 1990 and Moody et al 1995)**. Thrombi can be either dislodged from the heart or can arise from the CPB circuit due to inadequate heparinisation, platelet aggregates are formed due to contact activation in the bypass circuit, atherosclerotic emboli arise mainly from aortic plaques and liquified fat is released by the activation of endothelial lipoprotein lipase, in addition to that from the bone marrow and soft tissues entering the operation site and being drawn into the cardiotomy reservoir by the pump suckers. Studies using Doppler recording **(Stump et al 1996 and Clark et al 1995)** have revealed that the microemboli load is higher during aortic manipulation, cannulation, application and removal of clamps, decannulation and during perfusionist interventions. Though anecdotal, due to the very high current manufacturing standards, embolisation of PVC debris from the bypass tubing, and silicone antifoam have been recorded during the early days of cardiac surgery.

Figure - 3. Atherosclerotic plaques within the aorta.

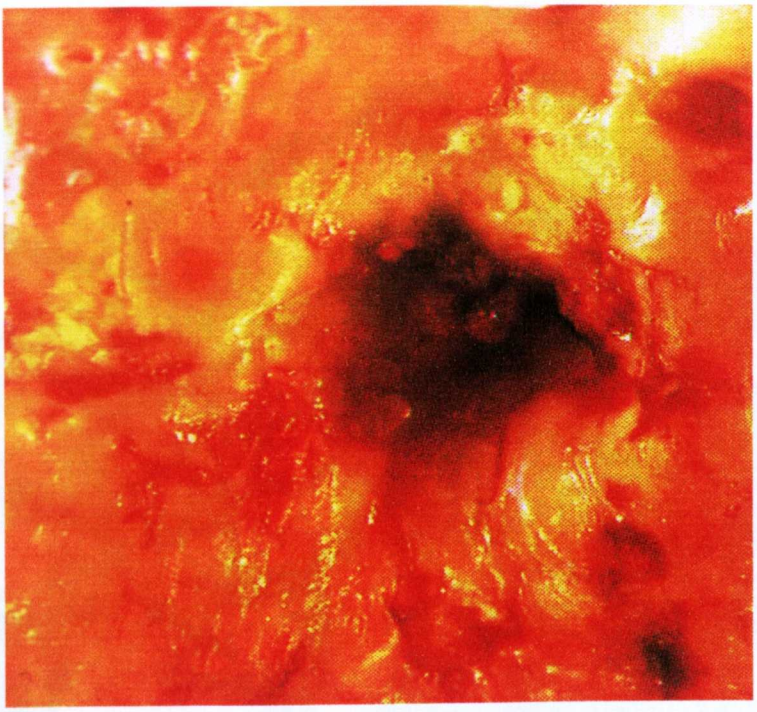
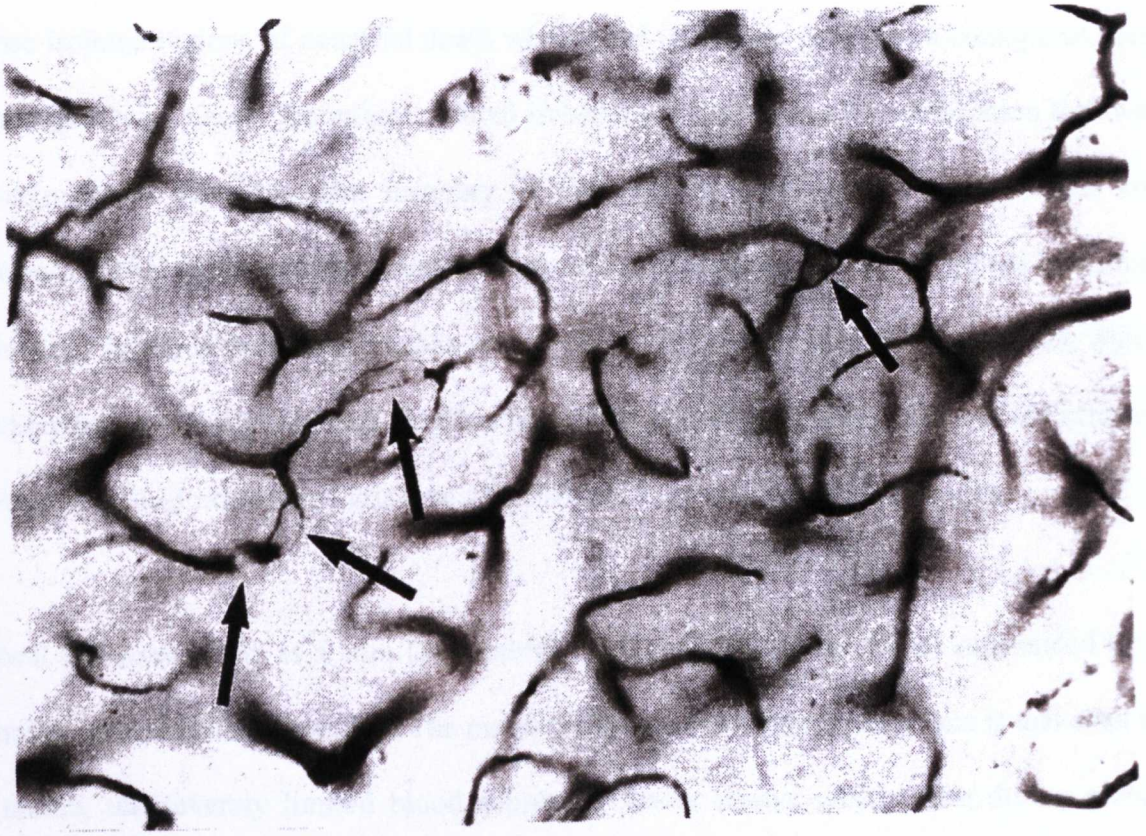


Figure - 4. Small capillary and arteriolar dilatations (SCADS) in the brain – "foot prints" of fat emboli in the brain.



1.6 The pathophysiology of brain injury.

The brain can be subjected to either one or both, of the two types of ischaemia, global and focal. The ischaemia can again be either temporary or permanent. Global ischaemia leads to diffuse isolated regions of neuronal death while focal ischaemia produces a contiguous mass of damaged brain tissue, the infarct. Global ischaemia is generally of brief duration followed by recirculation and long-term recovery. It can only be sustained for brief periods and prolonged global ischaemia is almost certainly lethal. A cardinal feature of global ischaemia is the delay between the actual episode and the final cell death **(Pulsinelli et al 1982)**, which is dependent on the severity and duration of ischaemia and the susceptibility of the affected neurons. A typical example of this type of ischaemia is the cardiac arrest situation.

In focal ischaemia there is a core (the umbra) of densely ischaemic tissue, surrounded by a penumbra of less ischaemic tissue. The major difference from global ischaemia is that even in the umbra, the severely limited blood supply is almost always greater than during global ischaemia, and in the penumbra, the limited supply from neighbouring vessels and leptomeningeal branches produces a gradation in the severity of ischaemia from the core to its outer boundary. The cells in the core are destined to die but the fate of cells in the penumbra are dependent on the severity of ischaemia, extent of blood supply from neighbouring vessels, extent of secondary damage mediated by leucocytes and the susceptibility of the neurons. Microemboli causing multiple areas of focal ischaemia is thought to be the commonest cause of brain injury in cardiac surgery **(Moody et al 1995, Taylor et al 1999 and Borger et al 2001)**.

Most of our current understanding of the pathophysiology of brain injury is based on the findings from experimental animal studies conducted on rats and gerbils, and observational human studies. In this section the author will cover the general pathophysiologic changes that occur following ischaemic injury and then go into focal ischaemia in more detail.

1.6.1 Stages of neuronal cell death.

Cell death can be broadly subdivided into 3 consecutive stages, the initial event, the free interval and the stage of secondary cell death.

The changes occurring during the initial event are the direct result of the abrupt cessation of energy production from oxidative phosphorylation, due to lack of oxygen and glucose following ischaemia. The depletion of adenosine triphosphate (ATP) leads to ion pump failure and the consequent massive efflux of K^+ and influx of Na^+, Ca^{++} and Cl^- accompanied by the osmotically obliged H_2O **(Silver and Erecinska 1990, and Ericinska and Silver 1994)**. Acidosis and free radical production by the mitochondrial chain occurs along with the activation of phospholipases, proteases, and deoxyribonucleases by the high intracellular Ca^{++} **(Siesjo, 1991)**. Some of the metabolites formed are biologically active while others are putative mediators of secondary damage.

The free interval is the repair phase when recirculation is established, production of ATP is resumed, membranes re-polarise and synaptic activity resumes. However due to the continued lower level of metabolic activity and blood flow, recovery of protein synthesis is still slow and may never recover in cells destined to die **(Thilmann et al, 1989)**. Recirculation also triggers a series of reactions and cascades involving the arachidonic acid, cyclooxygenase and lipoxygenase pathways, which result in protein degradation, formation and release of chemo

attractants, activation of leucocyte adhesion molecules, leucocyte sequestration and free radical damage to cellular structures **(Siesjo et al 1994 and Gido, Kristian and Siesjo 1997)**.

During the phase of delayed cell death a secondary depletion of energy stores due mitochondrial failure occurs. Sustained oxidative damage to the cell membrane proteins from free radicals **(Pahlmark et al 1993 and Gido, Kristian and Siesjo 1997)** and a gradual increase in Ca^{++} influx contribute to the mitochondrial failure **(Siesjo et al, 1995)**.

1.6.2 Pathophysiologic changes specific to focal ischaemia.

The pathophysiologic progression following the injury differs in the core and the penumbra. During the initial ischaemic phase, the core undergoes rapid anoxic depolarisation with Na^{+} and Ca^{++} influx and K^{+} efflux **(Harris and Symon 1984 and Gido, Kristian and Siesjo 1997)**. The ATP levels drop to nearly one-fourth of baseline **(Folbergrova et al 1995 and Sun et al, 1995)**. With reperfusion the extracellular K^{+} initially returns to baseline but continues to rise over the next 24 hours, ATP levels initially improve to about two-thirds of baseline before declining again due to mitochondrial dysfunction despite O_2 levels returning to normal **(Folbergrova et al 1995 and Sun et al, 1995)**. The limited free radical release in the core occurs mainly during the period of reperfusion **(Solenski et al, 1997)**. The severity of the initial ischaemic damage sustained by the neurons in the core precludes recovery and they eventually die.

Changes in the penumbra during the ischaemic phase are less dramatic. Because the ATP levels are maintained between half to two-thirds of basal value **(Folbergrova et al 1995 and Sun et al, 1995)** there is no anoxic depolarisation with the massive ion shift as seen in the

core, however sporadic transient depolarisation occurs **(Ginsberg and Pulsinelli, 1994)**. These sporadic intraischaemic depolarisations are believed to originate from the stimulation of NMDA and non-NMDA type glutamate receptors by the glutamate released from the core **(Mies, Kohno and Hossmann, 1994)**. The steadily increasing free radical release during ischaemia is also further elevated with the onset of reperfusion **(Solenski et al, 1997)**. Inhibition of these intraischaemic depolarisation with glutamate antagonists **(Back et al 1996 and Mies, Kohno and Hossmann 1994))** and inhibition of neuronal nitric oxide synthetase **(Shimizu-Sasamata et al, 1998)** have been shown to reduce the eventual size of the infarct. With reperfusion, the intraischaemic depolarisations stop, followed by recovery of mitochondrial function, glucose utilisation, and ATP production - reliable indicator of the fate of the neurons **(Belayev et al 1997 and Zhao, Belayev and Ginsberg 1997)**.

1.7 Inflammatory response to cardiopulmonary bypass.

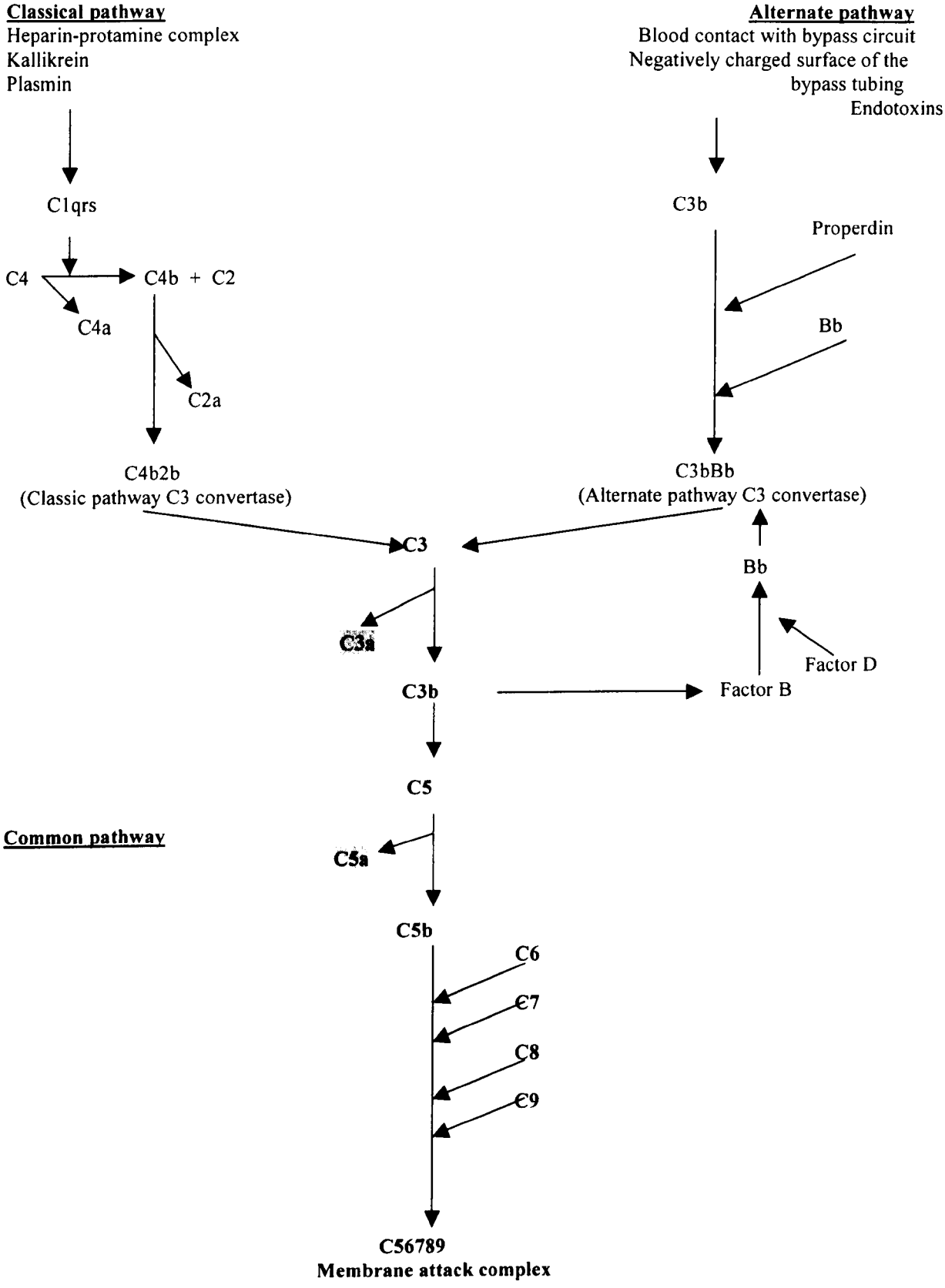
The normal inflammatory response following injury is primarily a self regulated and spontaneously terminated generalised defence mechanism. However unlike sepsis (**Denizot et al, 1998**) or other major surgery **(Boldt et al 1998 and Toft et al 1998)**, the use and the duration of cardiopulmonary bypass **(Whitten et al 1998 and Harig et al 2001)**, causes a massive inflammatory response that is not necessarily initiated by primary organ injury but can potentially lead to organ damage or dysfunction. Material dependent, and independent factors contribute towards triggering the inflammatory response. Material dependent factors include contact activation of leucocyte and platelets from exposure to the nonphysiologic materials and conditions in the extracorporeal circuit, while material independent factors are mainly, surgical trauma **(Corbi et al, 2000)**, ischaemia-reperfusion injury **(Galea et al 1998, Kalawski et al 1998 and Massoudy et al, 2001)** and endotoxin release **(Boelke et al 2000, Neuhof et al 2001 and Rothenburger et al, 2001)**. Methods to limit the inflammatory response include the incorporation of technical modifications in the bypass circuit and pharmacotherapy. Modification of the bypass circuit by incorporating heparin-bonded tubings **(Bozdayi et al 1996 and Moen et al 1997)** and leucocyte depleting filters **(Lazar et al 1995)** are two techniques which have been tried to reduce the inflammatory response. Pharmacotherapeutic interventions like aprotonin **(Gilliland et al 1999, Asimakopulos et al 2000 and Harig et al 2001)** a serine protease inhibitor, epsilon-aminocaproic acid **(Greilich et al, 2003)** an antifibrinolytic agent, and steroids **(Schurr et al, 2001)** have been shown to reduce the inflammatory response in some studies, but not in others **(Boldt et al 1995, Defraigne et al 2000 and Wei et al 2002)**.

Once initiated, the inflammatory process is mediated by cytokines, complement system, products from the clotting and fibrinolytic cascades, products from arachidonic acid, cycloxygenase, lipoxygenase and kinin-kallikrein pathways, platelet activating factor, endothelin-1, leucocyte-endothelial activation, leucocyte-endothelial adhesion molecules, lysosomal enzyme release and free radical generation. The inflammatory response slows down capillary and venous blood flow, increases blood viscosity and promotes stasis with margination of leucocytes. Following margination, leucocyte-endothelial adhesion is mediated by the interaction between surface adhesion molecules and their complementary ligands. Two opposing forces affect leucocyte-endothelial adhesion, the strength of the bond between the complimentary adhesion molecules, and the counteracting shear force generated by blood flow. Leucocyte activation and adhesion are followed by transmigration, degranulation, free radical release and tissue damage.

1.7.1 The complement cascade.

The complement cascade consists of sequentially activated plasma proteins (Figure – 5), which generate a number of biologically active split products and ultimately give rise to the membrane attack complex (Figure-5). Exposure of blood to the cardiopulmonary bypass circuit activates the alternate pathway **(Chenoweth et al 1981 and Kirklin et al 1983)**, heparin-protamine complexes activate the classical pathway **(Cavarocchi et al 1985 and Kirklin et al 1986)**, while endotoxin release can potentially activate both pathways **(Jansen et al, 1992)**. C3a and C5a are split products released from both pathways, which serve as anaphylatoxins and amplifiers of the inflammatory response. C3a is a potent stimulator of platelet aggregation while C5a stimulates neutrophil adhesion and aggregation, both cause histamine release from basophils, increase vascular permeability and stimulate leucocyte release of lysosomal enzymes and free radicals.

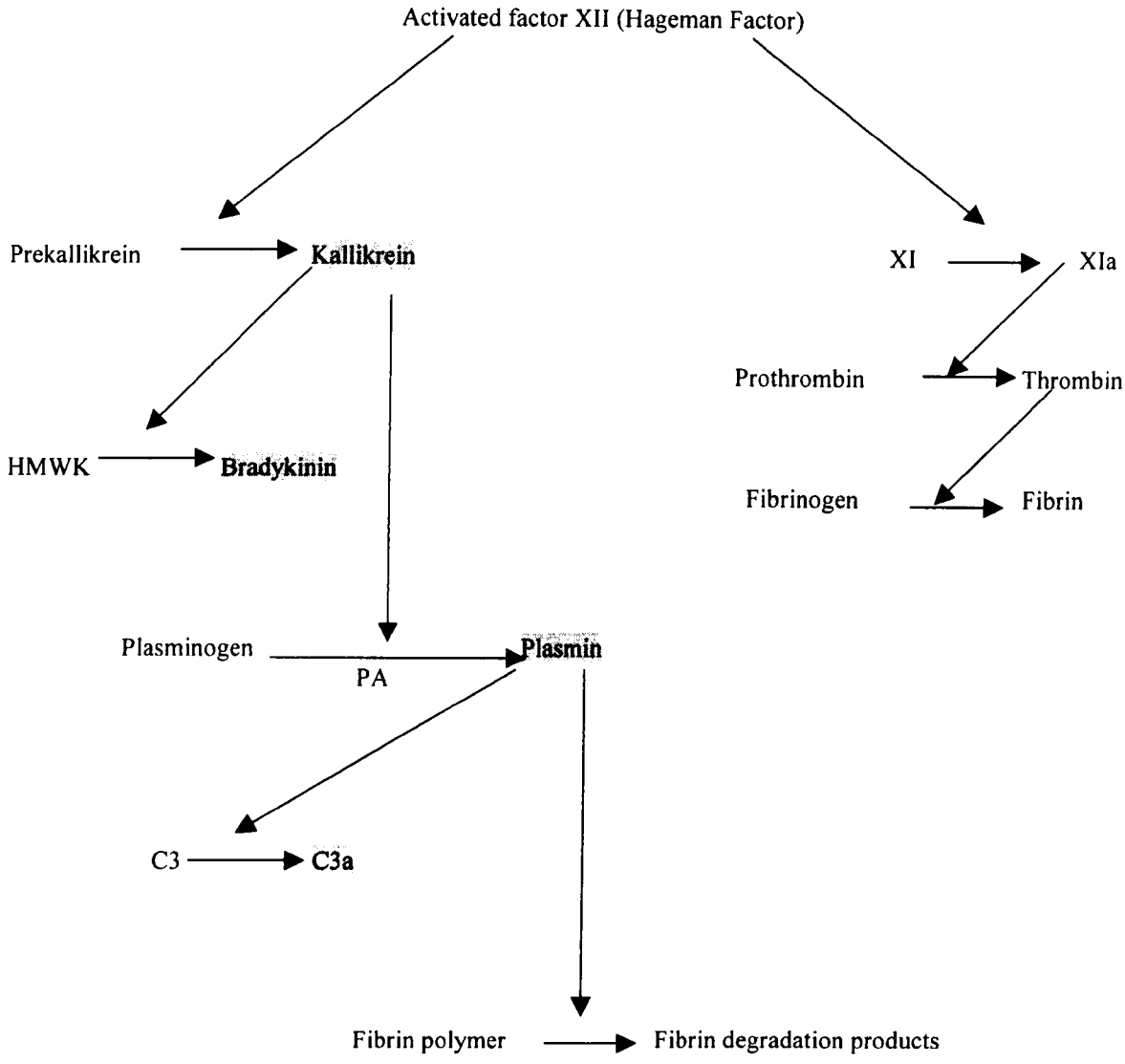
Figure – 5. Complement cascade activation during cardiopulmonary bypass.



1.7.2 Arachidonic acid metabolites, platelet activating factor, endothelin-1 and factor XIIa.

The serum levels of prostanoids, thromboxane A_2, leukotrienes, prostaglandins and prostacyclin rise following cardiopulmonary bypass **(Hashimoto et al 1992, Gadaleta et al 1994 and Singh et al 1995)** due to generation from arachidonic acid metabolism and release from pleural and pericardial fluid. The high levels of these vasoactive molecules, is also sustained partly due to reduced catabolism by the lungs as a consequence of shunting during cardiopulmonary bypass **(Cugno et al, 2001)**. Thromboxane-A_2 is a strong vasoconstrictor and promotor of platelet aggregation while the prostaglandins E_1, E_2 and prostacyclin counteract these effects. Leukotrienes increase vascular permeability and are potent chemo attractants. An increase in leukotriene-B_4 and a reduction in leukotriene-C_4 has been noted after major surgery and also during cardiopulmonary bypass **(Utoh et al, 1989)**. Platelets, endothelium and injured cells release the phospholipid, platelet activating factor. They are potent chemo attractants and stimulate leucocyte and platelet activation and aggregation. Endothelin-1, a peptide released from endothelial cells, has been implicated as a mediator of organ damage **(Matheis et al, 1995)**. It is a potent vasoconstrictor, increases response to serotonin and stimulates thromboxane-A_2 production. The clotting factor XII is activated by the bypass circuit and in turn activates cascades (Figure – 6) that generate kallikrein, bradykinin, plasmin and thrombin, all of which are promoters of neutrophil activation.

Figure – 6. Cascades triggered by factor XIIa



HMWK – High molecular weight kininogen

PA – Plasminogen activator

1.7.3 Cytokines.

Cytokines are low molecular weight proteins produced by leucocytes, macrophages and certain other cells like fibroblasts, that serve as chemo attractants, mediators and regulators the inflammatory response. Cytokine release is stimulated by a number of factors including ischaemia-reperfusion injury, endotoxin release, other cytokines and complement activation during cardiopulmonary bypass. Cytokines in turn stimulate the expression of surface adhesion molecules on endothelial cells, leucocytes and platelets. Pro-inflammatory cytokines (Table – 1) include tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8), while anti-inflammatory cytokines include interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1ra), tumour necrosis factor soluble receptor 1 and 2 (TNFsr-1 & TNFsr-2). Levels of TNF-α, IL-6 and IL-8 increase following cardiopulmonary bypass **(McBride et al 1995, Denizot et al 1998, Whitten 1998, Roth-Isigkeit et al 1999, Grunenfelder et al 2000, Neuhof et al 2001, Tassani et al 2002, Franke et al 2002)**, and increase in systemic levels of proinflammatory cytokines have been associated with poor outcome in critically ill patients **(Torpy, Bornstein and Chrousos 1998)**.

Table – I. Pro-inflammatory cytokines involved in the inflammatory response following cardiopulmonary bypass.

Cytokines	Source	Action
IL-1	Macrophages/Monocytes and other cells	Inflammatory response and immune activation
IL- 6	Fibroblasts, macrophages And T cells	Inflammatory response, B-cell growth factor and antibody production
IL-8	Macrophages, T cells and other cell types	Inflammatory response and chemo attractant neutrophil activation
TNF- α	Macrophages and fibroblasts	Inflammatory response and immune activation neutrophil aggregation and activation, release of proteolytic enzymes from mesenchymal cells
C3a	Plasma Complement cascade	Vasodilatation, increased vascular permeability,
C5a	Plasma Complement cascade	Vasodilatation, increased vascular permeability, chemo attractant
XIIa	Plasma	Inflammatory response amplification, activation of kallikrein –kinnin and coagulation cascades
Kallikrein	Plasma	Inflammatory response amplification, activation of bradykinins and plasmin
Bradykinins	Plasma	Inflammatory response, vasodilatation increased vascular permeability, chemo attractant

1.7.4 Leucocyte-endothelial adhesion molecules – Selectins.

The rolling and tethering of leucocytes is mediated by binding of the cell surface glycoproteins called selectins, to complementary ligands. There are three types of selectins, leucocyte selectin (L-selectin), platelet selectin (P-selectin) and endothelial selectin (E-selectin) (Figure-7). Once shed from the surface of their parent cells, the soluble forms are called sL-selectin, sP-selectin and sE-selectin.

L-selectins, also known as cluster differentiation antigen -62L (CD62L) (Figure – 7, Table – 2) are found on the surface of circulating neutrophils, monocytes, and lymphocytes. L-selectin on leucocyte surface transiently increases following activation before it is rapidly shed to give the soluble form sL-selectin. Complementary ligands that bind to L-selectin include glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1), cluster differentiation antigen-34 (CD34), P-selectin glycoprotein ligand-1 (PSGL-1), peripheral lymph node addressin (PNAd), mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1). Temporal studies on L-selectin expression have revealed either a transient increase **(McBride et al 1995 and Blume et al, 1997)** or no change during cardiopulmonary bypass **(LeDeist et al 1995 and 1996, Galinanes et al 1996 and Kalawski et al 1998)**.

P-selectin, also known as cluster differentiation antigen -62P (CD62P, PADGEM, GMP-140) (Figure – 7, Table – 3) is stored in the alpha-granules of resting platelets **(Stenberg et al 1985)** and in the Weibel-Palade bodies of endothelial cells **(Bonfanti et al, 1989)**. Hence, P-selectin is expressed rapidly on the cell surface following stimulation without the time-lag required for protein synthesis. Through complementary ligands that bind to it, such as PSGL-1, Sialyl-Lewis X, Sial-Lewis A, and other oligo and polysaccharides P-selectin, supports the binding of leucocytes to platelets and endothelial cells. Soluble P-selectin (sP-selectin) levels

increase following cardiopulmonary bypass **(Komai et al 1994, Menasche et al 1995, Blume et al 1997 and Wei et al 2002, 2003).**

E-selectin, also known as cluster differentiation antigen -62E (CD62E) (Figure – 7, Table – 3) supports the adhesion of leucocytes to the endothelium. It is expressed transiently on the surface of activated endothelial cells and bind to conjugated linoleic acid (CLA), E-selectin ligand-1 (ESL-1), PSGL-1, Sialyl-Lewis, other oligo and polysaccharides. Unlike P-selectins, it does not exist in cell granules and there is a time-lag between endothelial cell stimulation and expression of E-selectin on the cell surface. Due to its minimal expression in resting endothelium, increase in E-selectin and its soluble form sE-selectin are very good markers of endothelial activation. sE-selectin levels rise after cardiopulmonary bypass **(Weerwind et al 1995, Blume et al 1997, Galea et al 1998, Paret et al 2000, Grunenfelder et al 2000, Schurr et al 2001 and Wei et al 2002,2003).**

1.7.5 Leucocyte-endothelial adhesion molecules - Surface immunoglobulins.

These are transmembrane glycoproteins of the immunoglobulin family that bind to integrins. Intercellular adhesion molecule -1 (ICAM-1) (Figure – 7, Table – 3) is mainly expressed on endothelial cells, but also on leucocytes and some other cell types, they serve as ligands for β2 – integrins, leucocyte function associated antigen-1 (LFA-1) and macrophage-1 antigen (Mac-1). ICAM-1 expression is induced by IL-1, TNFα, and lipopolysaccharides. Soluble ICAM – 1 levels increase following cardiopulmonary bypass **(Menasche et al 1994, Weerwind et al 1995, Blume et al 1997, Galea et al 1998, Kalawski et al 1998, Grunenfelder et al 2000, Schurr et al 2001, Wei et al 2002 and 2003, and Eikemo et al 2004).**

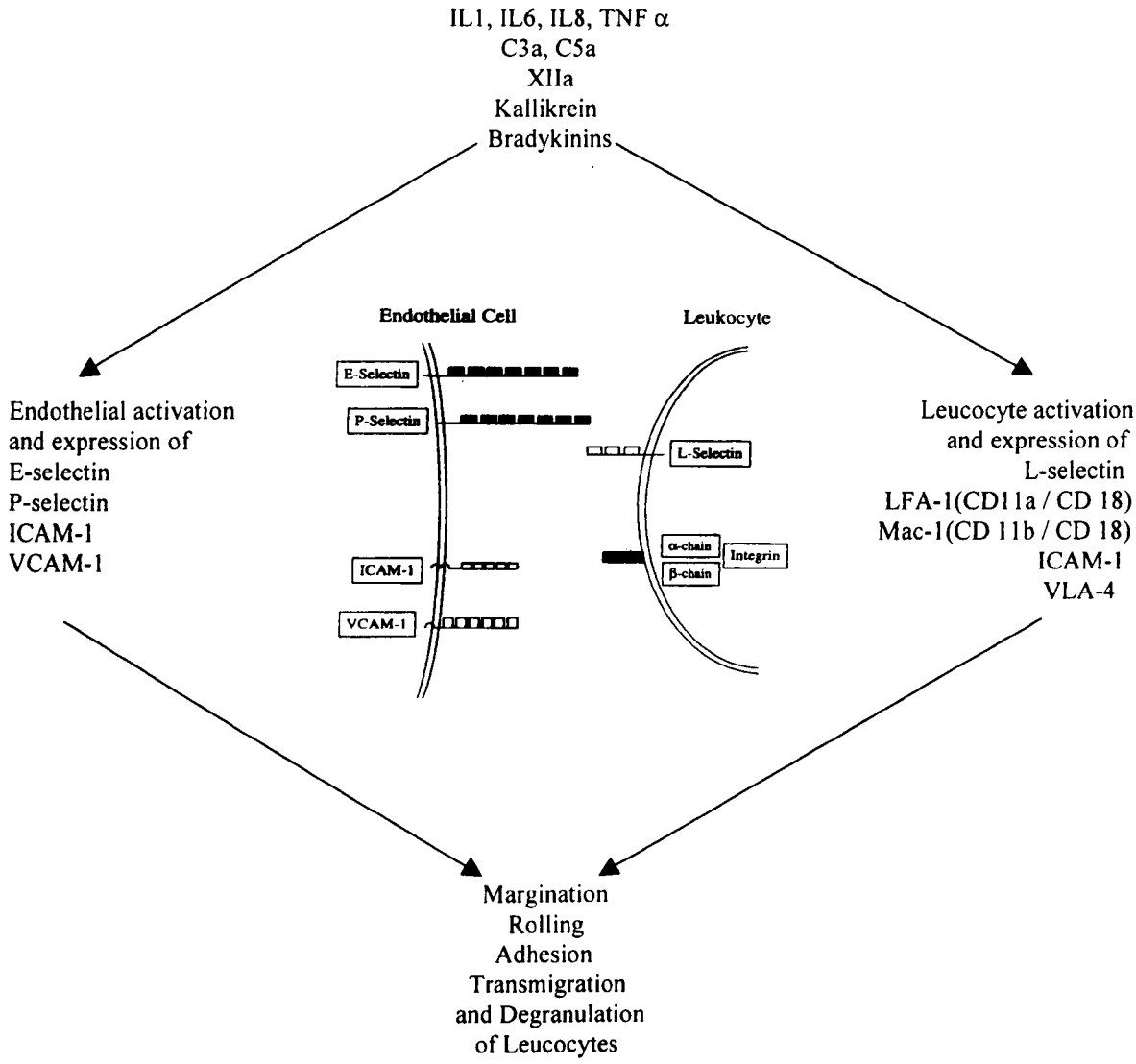
Vascular cell adhesion molecule – 1 (VCAM-1) (Figure – 7, Table – 3) is expressed by macrophages, endothelial cells, fibroblasts, synovial cells and dendritic cells. They mediate the adhesion of monocytes and lymphocytes to endothelium. Inducers of endothelial expression of VCAM-1 include IL-1, and TNFα. Endothelial VCAM-1 interacts with its complimentary ligand, beta 1- integrin on the leucocyte surface. Soluble VCAM-1 level increases following cardiopulmonary bypass **(Boldt et al 1995, Blume et al 1997, Kalawski et al 1998 and Eikemo et al 2004)**.

1.7.6 Leucocyte-endothelial adhesion molecules – Integrins.

These are transmembrane glycoprotein adhesion receptors found on most cell types (Figure – 7, Table -2). After the rolling and tethering mediated by selectins, binding of leucocyte integrins to their endothelial and tissue ligands (ICAM-1, VCAM-1, factor X, coagulation proteins, E-cadherin, complement iC3, fibrinogen, fibronectin, vitronectin, laminin, collagen, etc) mediate adhesion, transendothelial migration, degranulation and phagocytosis. Each integrin is a non-covalently bound dimer of α and β subunits. Integrins are subclassified based on the β chain into β1, β2 (CD-18), β3 and β7 integrins. αL (CD11a), αM (CD11b), αX (CD11c) and αD are the different alpha subunits that covalently bind with β2 (CD-18) subunit. LFA-1 a dimer of αL/β 2 (CD11a/CD-18), and Mac-1 a dimer of αM/β 2 (CD11b/CD-18), are β2 integrins confined to leucocytes which play an important role in the inflammatory response **(Plow and Zhang, 1997)**. Mac-1 plays an important role in neutrophil binding to fibrinogen and degranulation, while LFA-1 may play a more important role in transmigration **(Lu et al, 1997)**. Both CD11b and CD-18 expression increases

following cardiopulmonary bypass **(Gillinov et al 1993, LeDeist et al 1996, Macey et al 1997, Paugam et al 1997, and Asimakopoulos et al 2000)**. The β1 subunit covalently binds to α1, α2, α3, α4, α5 and α6 subunits to form β1 integrins also known as very late antigens (VLA-1, VLA-2, VLA-3, VLA-4, VLA-5 and VLA-6 respectively). The β1 integrin VLA-4 (α4β1) is expressed in lymphocytes and eosinophils but not by neutrophils, it interacts with endothelial VCAM-1 and mediates selectin-independent rolling of lymphocytes and eosinophils. By serving as a leucocyte receptor to fibronectin, they also mediate leucocyte infiltration into inflamed tissue. β1 integrin level is not altered significantly by cardiopulmonary bypass **(Asimakopoulos et al, 2000)**.

Figure – 7. The leucocyte endothelial interaction.



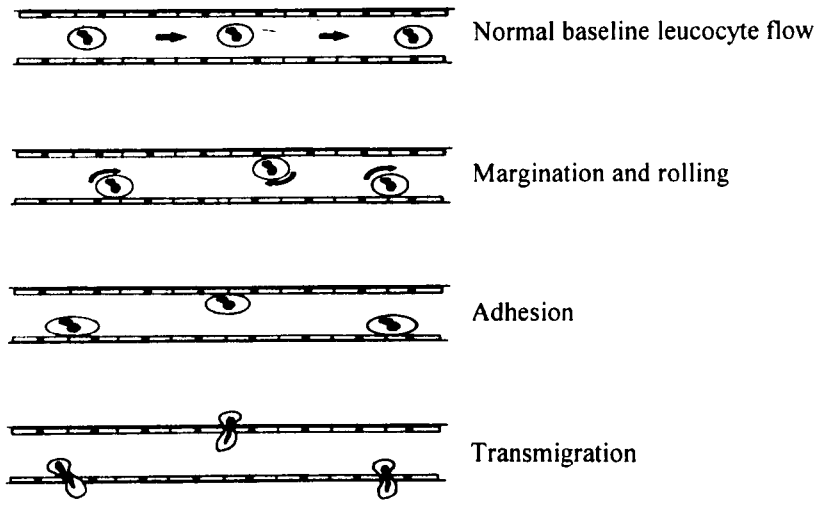


Table – 2. Leucocyte adhesion molecules.

Adhesion molecules	Complementary ligands / receptors
L-selectin (CD62L)	GlyCAM-1, CD34, PSGL-1, PNAd, MAdCAM-1
LFA-1(CD11a/CD-18)	ICAM-1, ICAM-2, ICAM-3, ICAM-4
Mac-1(CD11b/CD-18)	ICAM-1, ICAM-2, Complement iC3b, collagen, factor X, Coagulation proteins, fibrinogen
VLA-4 (CD49d)	VCAM-1, fibronectin

Table – 3. Endothelial adhesion molecules.

Adhesion molecules	Complementary ligands / receptors
E-selectin (CD62E)	CLA, ESL-1, PSGL-1, Sialyl-Lewis, other oligo and Polysaccharides
P-selectin (CD62P)	PSGL-1, Sialyl-Lewis, other oligo and polysaccharides
ICAM-1	LFA-1(CD11a/CD-18), Mac-1(CD11b/CD-18)
VCAM-1	VLA-4 (CD49d)

1.7.7 Heat shock protein 70 (HSP-70).

Heat shock proteins are a group of highly conserved proteins that are present in virtually all species and are induced by stress. Cells respond to stress such as metabolic disturbances, ischaemia and trauma, by mounting a stress response that incorporates the induction of numerous genes encoding proteins which may protect the cell. HSP-70 is a 70 kilodalton stress response protein that functions as a chaperone that assists in the folding, transport and assembly of other proteins in the cytoplasm, mitochondria and endoplasmic reticulum. HSP-70 expression in the cerebral cortex and especially the penumbra has been shown to increase following ischaemia in rodent model **(Lee et al 2001 and Kokubo et al 2003)**. A recent study revealed a similar increase in HSP-70 in the CSF following traumatic injury in human subjects **(Lai et al, 2004).** Similarly studies have shown that HSP-70 mRNA synthesis increases several fold in the myocardium when subjected to ischaemic stress **(Taggart et al 1997, Schmitt et al 2002, Giannessi et al 2003, Rafiee et al 2003 and Dybdhal et al 2004)**.

1.8 Evolution of hyperbaric oxygen therapy.

The history of compressed air therapy dates back to 1662 when Henshaw a British clergyman built a sealed chamber he called the "Domicilium" to treat diseases. The air pressure in the chamber was controlled by valved organ bellows which could either raise or lower it. Based on intuition rather than scientific rationale he used higher pressure to treat acute diseases and lower pressure to treat chronic diseases. Though this was before the discovery of oxygen in 1775 by Priestley, oxygen concentration was not a consideration in any of the subsequent compressed air chambers until much later. In 1879 the first semi-scientific study into compressed air therapy was conducted by Fontaine a French surgeon who conducted over 20 operations in a hyperbaric chamber **(Fontaine, 1879)**. He used a nitrous oxide – oxygen mixture for anaesthesia and noted that hernias were more easily reducible and patients were less cyanotic while coming out of anaesthesia. The first systematic use of compressed air therapy to treat decompression illness was conducted by Moir, a British engineer, who in 1889 assumed responsibility for the first attempt at excavating a tunnel under the Hudson river between Manhattan and New Jersey **(Moir, 1896)**. The last of the great compressed air enthusiasts was Orville J Cunningham a professor of anaesthetics at the University of Kansas who built a huge multiplace chamber which was 88 feet long and 10 feet in diameter **(Jacobson, Morsch and Rendell-Baker, 1965)**. He used the chamber to treat a multitude of diseases mostly without scientific rationale and in 1930 the American Medical Association, having failed to receive any scientific evidence, forced him to close.

The advent of hyperbaric oxygen therapy in clinical medicine started in 1955 when Churchill-Davidson used it to potentiate the effect of radiation therapy in cancer patients **(Churchill – Davidson, Sanger and Thomlinson, 1955)**. The same year Ite Boerema a professor of

surgery at the University of Amsterdam conceived the idea of "drenching" tissues with oxygen by increasing the ambient pressure surrounding the patient. Ensuing animal experiments proved so promising that he proposed the use of hyperbaric oxygen to prolong the duration of safe circulatory arrest during cardiac surgery. He conducted a variety of operations under hyperbaric oxygen including the surgical correction of transposition of great vessels, tetralogy of Fallot, and pulmonary stenosis **(Boerema et al, 1956)**. In 1961 Brummelkamp and his group discovered that hyperbaric oxygen inhibited anaerobic infections and used it to treat gas gangrene **(Brummelkamp, Boerema and Hoogendyk, 1961)**. The following year Smith and his group from Glasgow were the first to use the technique to treat carbon monoxide poisoning in humans **(Smith et al, 1962)**. Since then the application of this form of therapy has continued to expand. A typical modern multiplace chamber is depicted in the next two pages (Figures – 8 and 9). Currently hyperbaric oxygen is used in the treatment of, decompression sickness, gas embolism, carbon monoxide poisoning, gas gangrene, necrotising soft tissue infections, osteomyelitis, radiation induced necrosis and tissue damage, crush injuries, compartment syndrome, thermal burns and brain injury.

Figure – 8. A modern multiplace hyperbaric chamber.

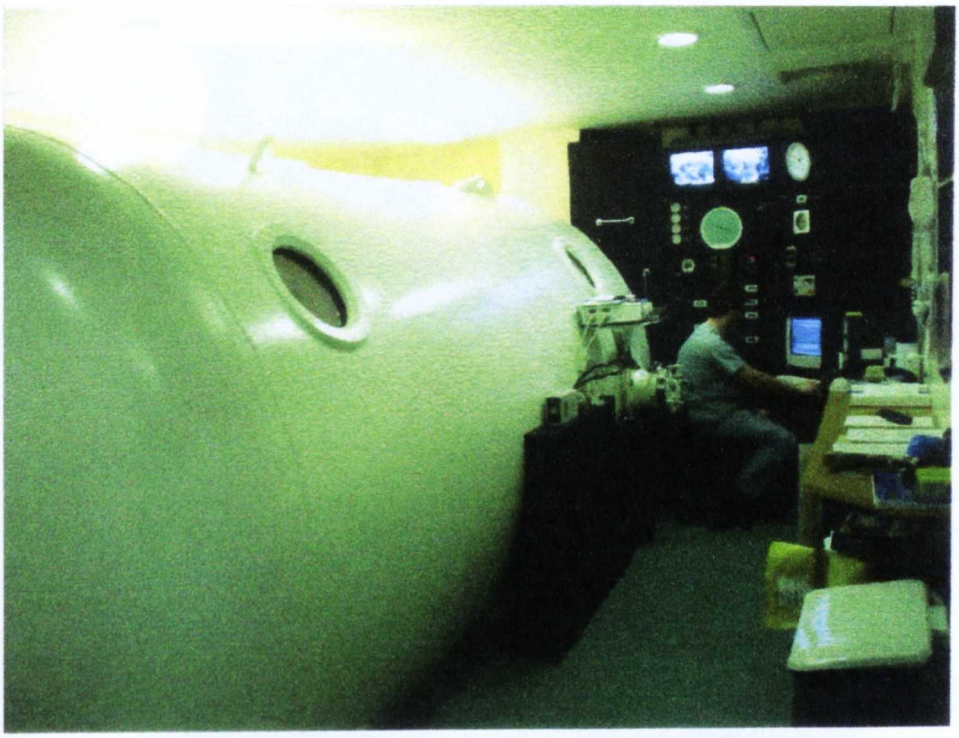
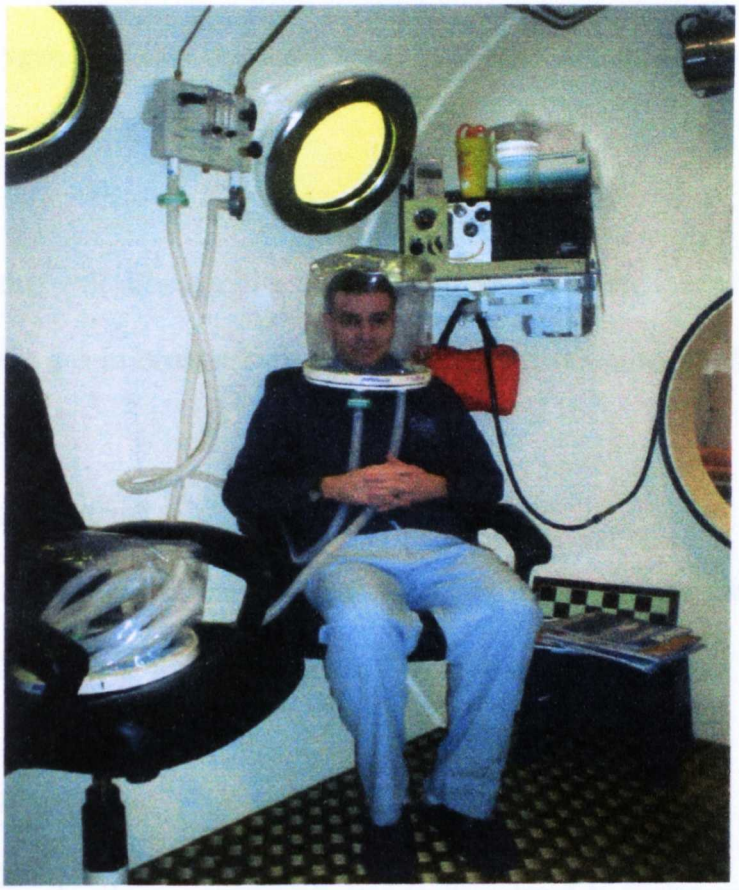


Figure – 9. Hyperbaric oxygenation



1.9 Understanding oxygen solubility in plasma under hyperbaric conditions.

As with any other liquid, the four basic gas laws and the solubility coefficient are applicable when calculating oxygen solubility in plasma under hyperbaric conditions.

1.9.1 Henry's Law.

The amount of gas that will dissolve in a liquid at a given temperature is proportional to the partial pressure of that gas in contact with the liquid and the solubility coefficient of the gas for that particular liquid.

$p \alpha PR$ (when T is constant)

p – partial pressure of the gas in the liquid, P - partial pressure of the gas in air, R – the solubility constant of the gas at that temperature, T – absolute temperature

1.9.2 Charles' law.

For any gas a change of volume or pressure is directly related to a change in temperature. Therefore lower the temperature, the lower the gas volume and pressure.

$PV \alpha T$

P – partial pressure of the gas, V – volume of the gas, T - absolute temperature

1.9.3 Boyle's law.

At a constant temperature the volume of a gas will vary inversely to the pressure to which the gas is subjected and vice versa.

$V \alpha 1/P$ (when T is constant)

V – volume of the gas, P – partial pressure of the gas at that temperature, T – absolute temperature

1.9.4 Dalton's law.

In a mixture of gases, the sum of the partial pressures of the gases in the mixture equals the total pressure.

$P_t = P_1 + P_2 + P_3$

P_t – total pressure, P_1 – partial pressure of gas 1, P_2 – partial pressure of gas 2, P_3 – partial pressure of gas 3

1.9.5 Bunsen's solubility coefficient.

This determines the amount of gas in solution in a liquid. Bunsen's solubility coefficient has been determined for all combinations of gases and liquids. Multiplying the partial pressure by the coefficient will indicate the actual amount of gas dissolved. The solubility coefficient for oxygen in plasma is 0.0031mls/torr.

1.9.6 Pressure in atmospheres and atmosphere absolute (ATA).

The normal atmospheric pressure equates to 14.7 pounds per square inch or 1.03 kilograms per square centimetre. Thirty three feet (10.06 metes) of sea water equates to one atmosphere pressure. During hyperbaric oxygen therapy the ambient pressure in the chamber is described in atmospheres absolute (ATA). This means that one always includes the atmospheric pressure at sea level plus the added pressure when describing the ambient pressure in the chamber. Thus if one descends 33 feet in sea-water, the absolute atmospheric pressure at that depth is 2ATA.

1.9.7 Pressure conversion table.

	Atmospheres	Bar	Pounds/inch^2	Kilograms/cm^2
1 Atmospheres	1	1.01	14.7	1.03
1 Bar	0.99	1	14.5	1.02
1 lb/inch^2	0.68	0.07	1	0.07
1 Kg/cm^2	0.99	1.02	14.5	1
1 Kilopascal	0.01	0.01	0.15	0.01
1 Torr	0.001316	0.001	0.02	0.001

1.9.8 Oxygen content in blood under normal atmospheric pressure.

Maximum oxygen content of blood = O_2 bound to Hb + O_2 dissolved in plasma

=(Hb% X 1.34ml/gm) + (PaO_2 X 0.0031ml/dl/torr)

=(15g/dl X 1.34ml/gm) + (100torr X 0.0031ml/dl/torr)

= 20 + 0.3

= 20.3 ml/dl

1.34ml/gm – is the amount of oxygen bound by 1gm of Hb, 0.0031ml/torr – is the solubility coefficient of O_2 in plasma

1.9.9 Oxygen content in blood at 2.4 atmospheres absolute (ATA) hyperbaric oxygen therapy.

Maximum oxygen content of blood = O_2 bound to Hb + O_2 dissolved in plasma

=(Hb% X 1.34ml/gm) + (PaO_2 X 0.0031ml/torr)

=(15gm/dl X 1.34ml/gm) + (2193torr X 0.0031ml/dl/torr)

= 20 + 6.7ml

= 26.7 ml/dl

1.34ml/gm – is the amount of oxygen bound by 1gm of Hb, 0.0031ml/torr – is the solubility coefficient of O_2 in plasma

The normal arteriovenous oxygen difference at rest is about 4.6ml/dl. This is the amount of O_2 removed by the tissues from each 100ml of blood passing through it. Thus under hyperbaric conditions, the dissolved O_2 in plasma should be sufficient to meet tissue requirements without using the Hb-bound-O_2. The normal O_2 diffusion distance is 63μm at the arterial end of capillaries and 36μm at the venous end. At 2.4 ATA hyperbaric oxygen therapy the diffusion distance increases to 247μm at the arterial end and 64μm at the venous end.

1.10 Pre-treatment with hyperbaric oxygen and induction of ischaemic tolerance in the central nervous system.

Hyperbaric oxygen has been used to treat brain injury from radiation **(Chuba et al, 1997)**, acute ischaemia **(Nighoghossian et al, 1995)**, trauma **(Sukoff and Ragatz, 1982 and Rockswold et al 2001)** and the delayed neuropsychologic sequelae after carbon monoxide poisoning **(Thom et al 1995 and Coric et al 1998)**. Application of the technique in modern cardiac surgery has mainly been limited to treating accidental air embolism during cardiopulmonary bypass **(Winter, Alvis and Gage 1971 and Ziser et al, 1999)**. In 1966 Moor and his group demonstrated by clinical and histologic examination of the brain that, in dogs, hyperbaric oxygen could extend the safe period of circulatory arrest by 1-2 minutes under normothermic conditions and by 2-3 minutes under moderate (28-30^{0}C) hypothermia **(Moor et al, 1966)**. Reduction in infarct size and neurologic deficit **(Yin et al 2002 and Schabitz et al 2004)** following focal ischaemia, improved glucose utilisation after freeze trauma **(Contreras et al, 1988)** and improved survival **(Krakovsky et al, 1998)** after global ischaemia, have been demonstrated in rats treated with hyperbaric oxygen after the injury. However of more relevance to this study were the remarkable findings from recent animal studies that showed hyperbaric oxygen could induce ischaemic tolerance in the central nervous system.

In 1996 Wada and his group demonstrated that repeated pre-treatment with hyperbaric oxygen could induce cerebral ischaemic tolerance against global ischaemia in gerbils **(Wada et al, 1996)**. In their study male Mongolian gerbils were exposed to 1 hour of 100% O_2 at 2 atmospheres absolute every alternate days for 5 days. 48 hours after the final hyperbaric

exposure both the common carotid arteries were occluded for 5 minutes followed by recirculation. Histologic examination 7 days after ischaemia revealed significantly greater (p-0.001) amount of intact pyramidal cells in the CA1 area of the hippocampus in the hyperbaric group (n=10) compared to the control group (n=10), 54.9% vs 8% respectively. Interestingly, in a sub-study they also found a significantly greater (p-0.01) rise in HSP-72 in the CA1 area following hyperbaric oxygen (n=5) compared to controls (n=5). In 2000 Prass and his group demonstrated that tolerence against permanent focal cerebral ischaemia could be induced in wild-type SV129 strain of mice pretreated with hyperbaric oxygen **(Prass et al, 2000)**. The animals in their study were exposed to 1 hour of 100% O_2 at 3 atmospheres absolute for 5 days. 24 hours after the final hyperbaric exposure, the middle cerebral artery was occluded for 24 hours, before the brain was analysed for infarct volume size. There was a significant (p-0.001) reduction (27%) in the mean infarct volume size in the hyperbaric group (n=26) compared to the control group (n=26). In 2002 Dong and his group demonstrated significantly better neurologic outcome and preservation of anterior horn cells in the spinal cord of New Zealand white rabbits pretreated with hyperbaric oxygen **(Dong et al, 2002)**. The animals in their study were exposed to 1 hour of 100% O_2 at 2.5 atmospheres absolute for 5 days. Spinal cord ischaemia was induced 24 hours after the final hyperbaric exposure by occluding the aorta for 20 minutes followed by reperfusion. 48 hours after reperfusion hind-limb motor function assessment was followed by histologic examination of the spinal cord. The neurologic outcome (p-0.004) and histopathologic outcome (p-0.001) was significantly better in the hyperbaric group (n=7) compared to the control group (n=7). In 2003 Miljkovic-Lolic and his group compared the effect of a single session of hyperbaric oxygen (100% O_2 at 3 atmospheres absolute) immediate pre-ischaemia (n=13) or post-ischaemia (n=12) to controls (n=15). Neurologic outcome was better and infarct volume smaller in the hyperbaric groups while brain myeloperoxidase activity was significantly higher in the control group **(Miljkovic**

et al, 2003). The importance of these studies were that they proved that pre-treatment with hyperbaric oxygen could induce ischaemic tolerance in the mammalian central nervous system.

1.11 Role of inflammatory mediators in ischaemic brain injury.

The most significant evidence correlating leucocyte accumulation to damage came from human SPECT and CT scan studies, which showed a striking correlation between the amount and duration of leucocyte accumulation to infarct size and neurologic outcome **(Akopov, Simonian and Grigorian, 1996)**. In rodents, following ischaemia, TNFα **(Buttini et al, 1996)** and IL1β **(Zhang et al, 1998)** levels increase within 30 minutes, endothelial ICAM-1 is expressed as early 15 hours **(Lindsberg et al, 1996)**, E-selectin and P-selectins is expressed as early as 2-4 hours **(Haring et al 1996, Zhang et al 1998 and Huang et al 2000)** and the induction of CD11/CD-18 complex occurs. Similarly, a significant increase in soluble ICAM-1, E-selectin and P-selectin levels and an upregulation of endothelial surface ICAM-1, VCAM-1 and E-selectin occurs in the acute stage after ischaemia in humans **(Frijns et al 1997, Stanimirovic et al 1997 and Shyu et al 1997)**. The early and prolonged TNFα increase has been found to be unrelated to the size of infarct or to the severity of neurologic impairment in humans **(Intiso et al, 2004)**. IL1β, IL8 and IL17 mRNA expression by human peripheral blood mononuclear cells rises significantly following an ischaemic stroke, more notably, there appears to be a correlation between the number of mRNA expressing monocytes and the severity of the neurologic impairment **(Kostulas et al, 1999)**.

Thus, inhibiting cytokine function may have potential beneficial effects following ischaemia. This was evidenced by the effect of IL1β receptor antagonist **(Garcia, Liu and Relton 1995)** in reducing neutrophil accumulation, and the improvement in the number of perfused capillaries noted following TNFα blockade **(Hallenbeck, 1995)**. One hour following reperfusion, leucocyte-endothelial adhesion occurs in cerebral venules, capillaries and arterioles **(Ritter et al, 2000),** reducing or blocking leucocyte accumulation reduces infarct size greatly. A near two-thirds reduction in infarct size was noted in rats and mice treated with anti-neutrophil antibodies **(Connolly et al, 1996 and Matsuo et al, 1994)**. Antibodies against E-selectin **(Huang et al, 2000)** and P-selectins **(Connolly et al, 1997)** significantly increased ischaemic cortical blood flow and also produced a significant reduction in neurologic deficits, mortality, and infarct volume. By inhibiting ICAM-1 with antibodies **(Matsuo et al 1994, Zhang et al 1995, Chopp et al 1996 and Connolly et al 1996),** and by genetic modification to create null mice **(Connolly et al 1996 and Soriano et al 1996)**, a reduction in infarct size of up to 50% and 75% respectively, was possible in animal studies. A similar benefit was noted following transient but not permanent ischaemia in CD-18 null mice **(Prestigiacomo et al, 1999)**, but the extent of the benefit was reduced in normal rats treated with CD11/CD-18 antibodies **(Chopp et al, 1996)**. Free radicals are generated by neutrophils through nicotinamide adenine diphosphate (NADPH) oxidase activity, a 50% reduction in infarct size was noted in transgenic mice lacking the enzyme **(Walder et al, 1997)**. Inhibition of the enzyme inducible nitric oxide synthetase by aminoguanidine was noted to limit extension of infarct 24 to 72 hours after ischaemia **(Iadecola et al 1996 and Nogawa et al 1998)**, however this protection was counteracted by the nitric oxide precursor argenine **(Iadecola, Zhang and Xu 1995)**.

From the pathophysiology of ischaemic brain injury it is clear that the secondary damage during reperfusion, in both global and focal ischaemia, is partly mediated by inflammatory cytokines, leucocyte sequestration, degranulation and free radical release. Reducing cytokine release, leucocyte-endothelial activation, and concurrently inducing antifree-radical enzymes and stress response proteins could potentially reduce the severity of secondary brain injury.

1.12 Effect of hyperbaric oxygen on inflammatory mediators - limitation of secondary injury – the possible mechanisms involved in inducing ischaemic tolerance.

1.12.1 Effect of hyperbaric oxygen on cytokine release.

From previous animal and human studies it appears that hyperbaric oxygen may have a beneficial effect on all the factors mentioned above. In the rat model of zymosan (a component of yeast cell wall) induced **(Luongo et al, 1998)** and hemorrhagic shock **(Yamashita and Yamashita, 2000)**, hyperbaric oxygenation inhibited TNFα secretion, leucocyte sequestration, and plasma nitric oxide levels. TNFα secretion induced by intestinal ischaemia-reperfusion injury was reduced compared to controls (n=9) in rats exposed to hyperbaric oxygen (n=9) during ischaemia **(Yang et al, 2001)**. In studies on human monocyte-macrophages, induction of IL1β and TNFα, by lipopolysaccharides, lipids and phytohemagglutinins, was down regulated by hyperbaric oxygen **(Granowitz et al 1999 and Benson et al 2003)**. A similar reduction in TNFα, IL1 and IL6 levels was noted in patients (n=7) suffering from perianal Crohn's disease who underwent the therapy **(Weisz et al, 1997)**.

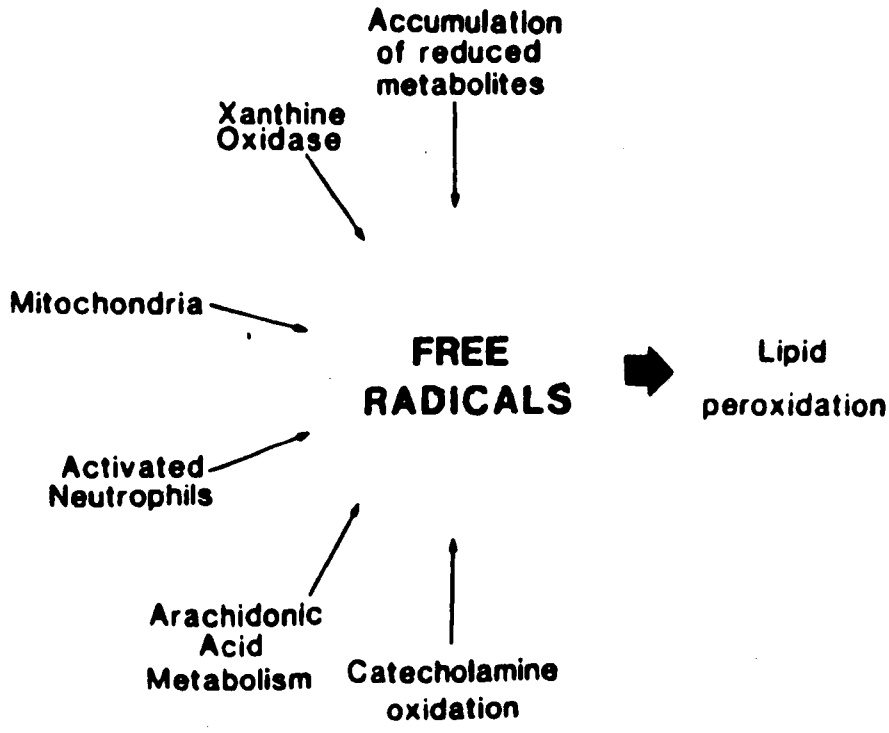
1.12.2 Effect of Hyperbaric oxygen on leucocyte-endothelial adhesion molecules.

Animal and human studies have shown that hyperbaric oxygen can exert an inhibitory effect on leucocyte sequestration and adhesion molecules. By using intra vital microscopy, Zamboni and his colleagues demonstrated that leucocyte sequestration following ischaemia could be significantly reduced in rat gracilis muscle by subjecting it to hyperbaric oxygen after the event **(Zamboni et al 1993, Zamboni, Wong and Stephenson 1996)**. A similar response to hyperbaric oxygen was noted in a rat model of intestinal ischaemia-reperfusion **(Tjarnstrom et al, 1999)**. The treatment has been shown to reduce the surface expression of the endothelial adhesion molecule E-selectin and ICAM-1 **(Buras et al, 2000)**, and inhibit neutrophil beta-2 integrin function **(Chen et al 1996, Thom et al 1997 and Kalns et al 2002)** in in-vitro studies. In vivo studies suggest that hyperbaric oxygen reduces new CD-18 synthesis on circulating neutrophils postoperatively following major surgery **(Ueno et al, 1999)**. Treatment during and after ischaemia, decreased ischaemia-reperfusion induced ICAM-1expression in rat musculocutaneous flap **(Hong et al, 2003)**.

1.12.3 Effect of Hyperbaric oxygen on lipid peroxidation.

Hyperbaric oxygen reduces lipid peroxidation, a indirect marker of leucocyte degranulation and free radical damage (Figure – 10). Hyperbaric oxygen inhibited lipid peroxidation and reduced brain myloperoxidase titre in rats exposed to carbon monoxide **(Thom, 1993)**. A reduction in leucocyte adhesion and lipid peroxidation, with improvement in ATP levels following hepatic ischaemia-reperfusion was seen in rats pretreated with hyperbaric oxygen (n=8) compared to controls (n=8) **(Chen et al, 1998)**. A similar effect on myeloperoxidase and lipid peroxidation was seen in rats treated after zymosan induced shock **(Cuzzocrea et al, 2000)**.

Figure – 10. Sources of free radicals causing lipid peroxidation.



1.12.4 Effect of hyperbaric oxygen on antioxidant defence.

Hyperbaric oxygen stimulates antioxidant defenses and apoptosis suppressor proteins. Hyperbaric oxygen has been shown to induce the antioxidant enzyme heme oxygenase-1 in human lymphocytes **(Speit et al, 2000)**. Significant reduction in infarct size owing to enhancement of enzymatic activity and gene expression of catalase, was demonstrated in the myocardium of rats exposed to hyperbaric oxygen **(Kim et al, 2001)**. In 2001 Wada and his group conducted a further study on male Mongolian gerbils. Histologic and immunocytochemical examination of the hippocampus not only confirmed their previous finding that animals exposed to 1 hour of 100% O_2 at 2 atmospheres absolute every alternate days for 5 days (n=5) had a better preservation (27.3% more, p-0.05) of CA1 pyramidal neurons, but also an almost 2 fold increase (p-0.05) in anti-oxidant enzyme manganese superoxide dismutase (Mn-SOD) and a 4 fold increase in the apoptosis suppressor protein Bcl-2, compared to controls (n=5) **(Wada et al, 2001)**. Inducible nitric oxide synthase (iNOS) expression, myeloperoxidase level in the lungs, and plasma nitric oxide level was significantly reduced in pre-treated rats compared to controls following exposure to lipopolysaccharide **(Pedoto et al, 2003)**.

Despite the promising findings of the studies mentioned above, the actual mechanism by which hyperbaric oxygen induces tolerance against ischaemic injury is still incompletely understood and continues to be investigated. As mentioned earlier, after the primary ischaemic injury, secondary injury is mediated by inflammatory cytokines, local release of chemo attractants from the injured tissue, leucocyte-endothelial activation, degranulation with release of proteases and oxidising enzymes which generate nitric oxide and free radicals causing further damage. The stage of repair and regeneration follows, after a variable latent

period, depending on the type of tissue and the severity of injury. The pyramidal neurons in the central nervous system unlike many other tissues have very little, if any, capacity to regenerate. Any intervention aimed at limiting brain damage should ideally be focussed on preventing the primary injury and limiting the secondary injury. Induction of tolerance to ischaemia by limiting secondary damage can be achieved by modulating any or all of the four factors, namely, inhibiting or reducing inflammatory cytokines, inhibiting or reducing leucocyte adhesion, inducing anti oxidant enzymes and generating stress response proteins which suppress apoptosis.

Hyperbaric oxygen has been shown to modulate all the four factors mentioned above. More importantly, pre-ischaemic hyperoxia was found to improve post-ischaemic myocardial and neuronal recovery. In the myocardium, contractility, coronary blood flow and infarct size was reduced in treated rats compared to controls **(Tahepold et al, 2002)**. The mechanism is thought to be a reduction in the nuclear factor kappa B activation (a redox sensitive transcriptional regulator of ICAM-1) during ischaemia-reperfusion **(Tahepold et al, 2003)**. In the brain, a reduction in leucocyte sequestration, infarct volume and neurologic deficit was also noted in treated rats subjected to temporary middle cerebral artery occlusion **(Atochin, et al 2000)** and decompression sickness **(Martin and Thom, 2002)**. Given the results of all these studies, it appears that pre-treatment with hyperbaric oxygen could have a potential role in reducing the inflammatory response and neurologic dysfunction associated with cardiopulmonary bypass.

1.13 Measuring brain injury - neurocognitive testing.

Clinical, biochemical and various imaging techniques can be used to identify and quantifying brain injury. A routine clinical examination identifies the functional deficit while neurocognitive testing allows assessment of psychomotor skills, concentration, comprehension, association, logic and language. The reliability of biochemical markers of brain injury like S100β protein and neuron-specific enolase in cardiac surgical patients have recently been debated owing to it's poor correlation to neurologic outcome **(Westaby et al, 2000)** and the high risk of false positivity due to contamination from extracerebral sources **(Johnsson et al 2000 and Anderson et al 2000)**. Though a correlation between magnetic resonance image findings to neurocognitive outcome has been demonstrated in some studies **(Goto et al 2001 and Kohn 2002)**, the high cost and limited availability have precluded their widespread use in clinical practice. At the present time clinical examination combined with neurocognitive testing continues to remain the most widely used and accepted methods of assessing brain injury following cardiac surgery.

1.13.1 Neuropsychology and neurocognitive testing.

The outward expression of brain function is behaviour. Though the term brain damage is useful as a classification concept for a broad range of behavioural dysfunction, to the patient, the concept of brain damage only becomes meaningful in terms of their specific behavioural dysfunction, the implications on daily functioning, and the potential for recovery. Neuropsychology is the applied science that measures and analyses the behavioural dysfunction resulting from brain injury. Behaviour can be conceptualised into three broad functional domains: **cognition**, the information handling aspect; **emotion**, which deals with feelings and motivation; **execution**, which controls behavioural expression. Cognitive

functions have received more attention in neuropsychology because they are easily conceptualised and measured, and correlate to neuroanatomic domains. Cognitive function can be further classified into: **reception**, which is the ability to acquire, classify, select, and integrate information; **learning and memory**, which deals with information storage and retrieval; **thinking**, which deals with the mental organization and reorganization of information; and **expression**, which include communication and, or acting on information. Neurocognitive testing on patients, who do not demonstrate focal neurologic deficits on standard clinical examination, may still reveal significant cognitive changes that can profoundly impair them **(Bashein et al 1988, Callahan, Hendrie ahd Tierney 1995 and Ahlgren et al 2003)**.

The concept of intelligence has limited application in neurocognitive testing and when examining a patient an examiner can use the patients best level of educational and vocational achievement or previous test performance, as a general standard against which to compare current observations and test performance. Data from neurocognitive tests provide a sensitive index of the extent of the patient's mental efficiency. Many of the tests used in neuropcognitive evaluation were originally developed for the examination of normal cognitive functioning and were subsequently recalibrated for neuropsychology use in the course of research on brain dysfunction. A neurocognitive test battery typically includes measurement of attention, concentration, comprehension, learning, retention, memory, association, motor coordination, constructional ability and psychomotor speed. The sensitivity and specificity of neurocognitive tests makes it suitable to identify and assess the severity of cognitive dysfunction following brain injury. Repeated testing provides a reliable indication of cognitive improvement or deterioration following surgery. Neurocognitive evaluation has

been used as a tool to assess cognitive outcome following cardiac surgery since 1957 **(Boshes et al, 1957)**.

1.13.2 The guidelines for neurocognitive testing in patients following cardiac surgery.

The "Conference on CNS Dysfunction after Cardiac Surgery: Defining the Problem" was held on the 10th and 11th of December 1994 at Fort Lauderdale, Florida. This landmark conference proposed a Consensus Statement outlining the criteria and guidelines for assessment of central nervous system (CNS) outcomes after cardiac surgery **(Murkin et al, 1995)**. Prior to this conference the main problem with neurocognitive assessment in cardiac surgery was the diversity of test batteries used, the discrepancy in follow-up intervals and the variety of definitions of neurocognitive decline. The consensus statement and recommendations are listed below-

1, A spectrum of postoperative CNS dysfunction both acute and persistent occurs in a proportion of patients after cardiac surgical procedures, including brain death, stroke, subtle neurologic signs, and neuropsychologic impairment.

2, A number of patients presenting for a cardiac operation have preexisting CNS abnormalities. A patient's neurologic and neuropsychologic state needs to be assessed at a time prior to operation to provide accurate baseline information.

3, The individual change in performance from baseline to a time after operation is essential to any evaluation of the impact of the operation or any intervention associated with it.

4, When indicated, design should incorporate the use of a control or comparison group.

5, Because of the time constraints and the physical limitations of the patient performing a neuropsychologic assessment in the context of a cardiac operation, care must be taken to select appropriate tests. Selection of tests should take the following issues into consideration:

a. The cognitive domain of the test
b. The sensitivity and reliability of the test
c. The time taken to perform the test
d. The degree to which learning may occur in the test
e. The availability of parallel forms of the test
f. The physical effort required to perform the test
g. The overall balance of the cognitive domains assessed in the battery

6, The tests should be free from sex, race, and ethnic bias and structured to avoid floor and ceiling effect.

7, Because of the multifocal nature of the potential lesion locations, no single test will always detect postoperative neurobehavioural dysfunction.

8, Care must be taken in performing the assessments, as neurobehavioral performance can be influenced by environmental, psychiatric, physiologic, and pharmacologic factors.

9, As the performance of neuropsychologic tests can be influenced by mood state and mood state variations, it is important that mood state assessments be performed concurrently with the neuropsychologic assessments.

10, To ensure objectivity and reliability of the assessment, we encourage that the testing of each patient be performed by the same or equivalently qualified and trained individuals and that the tests minimise subjectivity and be performed in a standardised manner. The examiner should be blinded to any treatment group.

11, A comprehensive and concise neurologic examination should be performed by a suitably qualified and trained individual.

12, As the incidence of postoperative neurobehavioral dysfunction is highest in the immediate postoperative period and then declines, care must be taken to perform at least one assessment when the performance is more stable. Ideally, this should be at least 3 months postoperatively.

13, Investigators should be aware that new events may occur in the days after the operation.

14, Cognitive testing can be associated with improvement in performance on repeated testing, recognised as "practice effect". This improvement needs to be taken into consideration in any analyses of the data. In addition, study design incorporating procedures to minimise practice effects (eg, providing sufficient practice trials on each test at each assessment period) is encouraged.

15, The minimum "core" neurocognitive test battery proposed by the conference.

- Rey auditory verbal learning test
- Trail-making A
- Trail-making B

- Grooved pegboard

16, Definitions of significant cognitive decline as proposed by the conference.

- Decline of 1 standard deviation or more in 20% of tests.
- Decline of 20% or more in 20% of tests.
- Impairment rating (IR): Russell
- Impairment rating : Halstead-Reitan
- Impairment rating : Heaton
- Clinical rating

All the guidelines mentioned above were followed during the conduct of this study. We used the first definition in section 16 as our index to identify patients with significant cognitive decline.

Materials and methods

2.1 Aim of the study.

To test the hypothesis that pre-treatment with hyperbaric oxygen could reduce inflammatory mediators and neurocognitive dysfunction following cardiopulmonary bypass.

2.2 Design of the study.

2.2.1 Ethical consent.

A full study protocol was submitted to the, Local Regional Ethics Committee - Hull and East Riding and to the NHS Research and Development Department of the Hull and East Yorkshire NHS Trust. Full ethical approval was obtained from both organisations prior to the start of the trial.

2.2.2 Research staff.

The group involved in the study comprised two consultant cardiothoracic surgeons, the research director of the hyperbaric chamber, a professor of vascular surgery, a clinical psychologist and staff from the department of psychology, an immunologist and staff from the department of immunology and the author of this thesis.

The author was responsible for designing the study, securing ethics approval, recruitment and obtaining informed consent from the patients, implementing the protocol, co-ordination of staff, transportation of patients, occasionally accompanying patients in the chamber and diving with them when needed, assisting during surgery, collection and preparation of samples, data collection, statistical analysis and presentations at speciality forums.

2.2.3 Patients selection.

Only patients scheduled to undergo coronary artery bypass grafting with cardiopulmonary bypass support, under the care of two consultant cardiothoracic surgeons, Mr Steven C Griffin and Mr Alex RJ Cale were considered for the trial. Though the unit had four consultant cardiothoracic surgeons at the time of the trial, in order to standardise operative factors only patients of the two consultants who used similar surgical and bypass techniques were recruited for the study. At the time of the study (January 2003 – January 2004), their combined total number of elective coronary artery bypass operations amounted to 300 per year.

2.2.4 Exclusion criteria.

1. Unstable / emergency patients were not considered for the study due to the logistic difficulty of organising the neurocognitive examination at short notice and also the risk attached with the limited access while in the chamber.

2.

Due to the nature of the chamber patients suffering from claustrophobia were not considered.

3. The nature of the study precluded inclusion of patients with a history of cerebrovascular disease / transient ischaemic attack / dementia / any neurologic dysorders and symptomatic carotid stenosis.

4. Due to the nature of the neurocognitive test battery used, patients with visual impairment, hearing difficulty, poor English language skills and learning difficulties were not considered.

5. Patients with previous pneumothorax / pulmonary bullae / middle ear problems impeding pressure equalisation were not considered.

6. Patients over the age of 80 generally find neurocognitive testing difficult to perform and the results become difficult to interpret, hence they were excluded.

2.2.5 Statistical considerations in the study design.

The power calculation for the study was based on the findings from a non-randomised pilot study performed in our unit prior to the start of this trial. 20 consecutive elective CABG patients consented to take part in the pilot study. The first 10 patients (pilot-control) had routine coronary revascularisation while the subsequent 10 patients (pilot-HBO) had pre-treatment with hyperbaric oxygen prior to their operation. The pre-treatment protocol and neurocognitive assessments were similar to the subsequent double-blind trial. 6 patients in the pilot-control group had significant neurocognitive dysfunction compared to 2 patients in the pilot-HBO group. Thus the observed effectiveness of treatment was 67%. Though the patients matched well in terms of age and co-morbidity, allowing for possible unaccountable biologic variations between the groups we assumed a success rate of 50% (p_1). The consensus following discussion with the practicing consultants within the department, was that for the therapy to be cost effective and clinically viable enough to change current practice a minimum clinically important difference of at least 35% was needed. The sample size was calculated for 80% power of detecting a clinically important difference of 35% between the groups at 5% (p-value – 0.05) significance.

To use the Altman's nomogram, the formula for calculating standardised difference (σ_d) is;

$\sigma_d = p_1 - p_2$ / square root of [P(1-P)]

From the pilot study,

$p_1 = 0.5$, $p_2 = 0.5 + 0.35 = 0.85$, $P = p_1 + p_2 / 2 = 0.675$.

Thus the standardised difference σ_d was 0.75.

From the Altman's nomogram with the standardised difference set at 0.75 and the α error set at 0.05, the minimum number of patients needed for 80% power was 56 that is, 28 patients in each group. Allowing for the risk of dropouts and operative complications the total target was set at 64 patients.

2.2.6 Patient recruitment and randomisation.

All elective patients admitted for coronary artery bypass grafting under the care of the two previously mentioned consultant surgeons were considered for the study. Eighty-two patient who met the criteria for inclusion in the trial were approached by the author. The purpose of the study was explained to the patient and their families and they were shown a 5 minute video overview of the working of the hyperbaric chamber and the cardiopulmonary bypass procedure. Following this they were given the patient information leaflet and any questions answered. If the patient then decided to take part in the study they were asked to provide a written consent. Patients were shown the hyperbaric chamber if they wished to see it.

Sixty-eight agreed to take part, giving an 82.9% recruitment rate. Four patients had their operations cancelled due to emergencies or lack of intensive care unit (ICU) beds prior to consenting and randomisation. Computer generated randomisation was used and 64 envelopes containing the randomisation number was entrusted with a secretary not involved in the study at the BUPA hospital. The envelopes were picked by the secretary just before the start of the first treatment session. Of the 64 patients recruited, 31 were randomised to group-A and the other 33 to group-B.

2.2.7 Selection of inflammatory markers and neurocognitive tests.

In selecting the inflammatory markers our aim was to gain an insight into the inflammatory response at 3 levels, initiation by inflammatory cytokines, leucocyte-endothelial interaction and tissue stress response. All the analysed inflammatory markers, TNF α, IL6, IL8 **(McBride et al 1995, Denizot et al 1998, Whitten 1998, Roth-Isigkeit et al 1999, Grunenfelder et al 2000, Neuhof et al 2001, Tassani et al 2002, Franke et al 2002)**, sE-selectins **(Weerwind et al 1995, Blume et al 1997, Galea et al 1998, Paret et al 2000, Grunenfelder et al 2000, Schurr et al 2001 and Wei et al 2002,2003)**, sP-selectins **(Komai et al 1994, Menasche et al 1995, Blume et al 1997 and Wei et al 2002, 2003)**, ICAM-1 **(Menasche et al 1994, Weerwind et al 1995, Blume et al 1997, Galea et al 1998, Kalawski et al 1998, Grunenfelder et al 2000, Schurr et al 2001, Wei et al 2002 and 2003, and Eikemo et al 2004)**, CD-18 **(Gillinov et al 1993, LeDeist et al 1996, Macey et al 1997, Paugam et al 1997, and Asimakopoulos et al 2000)** and HSP-70 **(Taggart et al 1997, Schmitt et al 2002, Giannessi et al 2003, Rafiee et al 2003 and Dybdhal et al 2004)** have been analysed in previous human studies on inflammatory response to cardiopulmonary bypass.

The minimum four "core" tests recommended by the 1994 consensus conference were the, Rey auditory verbal learning test, trail making test – A, trail making test – B and the grooved peg board test. In addition they recommended pre-test mood assessment and preoperative intelligence assessment. One of the limitations of using just the mimimum four "core" tests is that the tests did not examine all the neuropsychologic domains. In view of this drawback the consensus conference agreed that the minimum "core" test battery could be expanded to include additional tests. However for comparability of studies and data over time it was

recommended that the minimum "core" tests were retained in future studies **(Murkin et al 1995)**. In 1989 The National Institute of Mental Health (NIMH) Workshop on Neuropsychological Assessment Approaches **(Butters et al 1990)**, developed a neurocognitive test battery suitable for early detection of neurologic complications. At the Consensus Conference a modified version of the NIMH list, useful for assessing cognitive changes after cardiac surgery was produced **(Blumenthal et al 1995 and Stump 1995)**. This list was based on the reliability, validity, availability of normative data, sensitivity of the relevant cognitive domains, availability of alternative forms of the tests, and use in prior studies. In choosing the additional tests from this recommended list our aim was to perform a global assessment of all psychological domains within an optimum time period without mentally fatiguing the patient. In addition to the "core" tests we included the, digit span forwards, digit span backwards and the adult information processing table – A. Table – 4 gives the list of neurocognitive tests used and their properties **(Coughlan and Hollows 1985, Holdnack 2001 and Lezak, Howieison and Loring 2004)**.

Table – 4. Neurocognitive tests and their properties.

Test	Validated psychological domain assessed	Anatomical domain	Patient effort	Estimated time in minutes	Test-retest reliability
Hospital anxiety - depression scale	Mood	Limbic system	Attention Writing	5	0.78 – 0.86
Wechsler test of adult reading	Verbal IQ Performance IQ Memory	Left hemisphere	Attention Speech	5	0.9 -0.94
Raven's coloured matrices	Non-verbal IQ	Right hemisphere	Attention Pointing	10	0.75 – 0.85
Rey auditory verbal learning test	Verbal learning Short-term verbal memory Retention Delayed verbal memory Auditory recognition	Left tempero-pareital cortex	Attention Speech	10	0.61 – 0.86
Adult information processing table-A	Information processing rate	Widespread	Attention Writing	5	0.84 – 0.97
Grooved pegboard	Perception Dexterity Hand-eye coordination	Widespread	Attention Hand-movements	5	0.8 – 0.9
Digit span Forwards Backwards	 Very short-term memory Working memory	Left frontal	Attention Speech	5	0.66 – 0.89
Trail making Test – A Test - B	 Action planning Visuo-motor perception Response inhibition Complex conceptual reasoning Double tracking	Frontal	Attention Drawing	5	0.8 - 0.94

2.2.8 Data collection.

All demographic, preoperative, intraoperative, postoperative, neurocognitive and lab data were prospectively collected by the author. Angina and dyspnoea severity were stratified according to the Canadian Cardiovascular Society (CCS) angina class (Table – 5) and New York Heart Association (NYHA) Functional grade (Table – 6) symptoms. Operative risk was compared using both the Parsonnet (Table – 7) and European System for Cardiac Operative Risk Evaluation Score (EuroSCORE) (Table – 8) risk stratification systems. Intraoperative data which included the number of grafts, duration of cardiopulmonary bypass, and duration of myocardial ischaemia were collected during the procedure by both the perfusionist and the author. Postoperatively all patients were visited twice a day by the author and any notable postoperative events documented. All neurocognitive and lab data were collected and tabulated at point of analysis.

Table – 5. Canadian Cardiovascular Society (CCS) angina classification.

Angina Class	Symptoms
Class - 0	Asymptomatic
Class - 1	Angina with strenuous exercise
Class - 2	Angina with moderate exercise
Class - 3	Angina with mild exertion - walking 1-2 level blocks at normal pace - climbing 1 flight of stairs at normal pace
Class - 4	Angina at any level of physical exertion

Table -6. New York Heart Association (NYHA) functional grading.

Grade	Symptoms
Grade - I	Cardiac disease without limitation of physical activity
Grade - II	Slight limitation of physical activity - comfortable at rest, but ordinary physical activity results in fatigue, palpitations, dyspnoea or angina
Grade - III	Marked limitation of physical activity - comfortable at rest, but less than ordinary physical activity causes fatigue, palpitations, dyspnoea or angina
Grade - IV	Inability to carry on any physical activity without discomfort or symptoms at rest

Table – 7. The Parsonnet risk stratification score.

Factors		Definition	Score
Patient factors	Gender	Female	1
	Morbid obesity	Body mass index > 35	3
	Diabetes	Any history of diabetes except latent diabetes of pregnancy	3
	Hypertension	Any history of blood pressure greater than 140/90 mm Hg on two occasions, or lower if on medications	3
	Left ventricular function	Good (≥50%) Fair (30-49%) Poor (<30%)	0 2 4
	Age	70-74 years 75-79 years >80 years	7 12 20
	Re-operation	Second operation Third (or more)	5 10
	Intra-aortic balloon pump	Prior to surgery (other than prophylactic insertion)	2
	Left ventricular aneurysm	Aneurysmectomy	5
	Recently failed intervention	< 24 hours of operation > 24 hours of operation	10 5
	Renal	Dialysis dependency	10
	Catastrophic state	e.g. acute structural defect, cardiogenic shock, acute renal failure	10-50
	Other rare circumstances	e.g. paraplegia, pacemaker dependency, congenital heart disease in adults, severe asthma	2-10
Surgical factors	Mitral valve surgery	Systolic pulmonary artery pressure < 60 mm Hg Systolic pulmonary artery pressure ≥ 60 mm Hg	5 8
	Aortic valve surgery	Aortic valve pressure gradient ≤ 120 mm Hg Aortic valve pressure gradient ≥ 120 mm Hg	5 7
	Coronary artery bypass grafting at the time of valve surgery		2

Risk stratification

Additive score	Predicted operative mortality	Risk group
0-4	1%	Low risk
5-9	5%	Elevated risk
10-14	9%	Significantly elevated risk
15-19	17%	High risk
> 19	31%	Very high risk

Table – 8. The European System for Cardiac Operative Risk Evaluation (EuroSCORE)

	Factors	Definition	Score
Patient factors	Age	Per 5 years or part therof over 60	1
	Gender	Female	1
	Chronic pulmonary disease	Long-term use of bronchodilators or steroids for lung disease	1
	Extra-cardiac arteriopathy	Any one or more of the following, claudication, carotid occlusion > 50%, previous or planned surgery on the abdominal aorta, limb arteries or carotids	2
	Neurological dysfunction	Disease severely affecting ambulation or day to day functioning	2
	Previous cardiac surgery	Previous surgery requiring opening of the pericardium	3
	Serum creatinine	> 200 μmol / L pre-operatively	2
	Active endocarditis	Patient still under antibiotic treatment for endocarditis at the time of surgery	3
	Critical pre-operative state	Ventilation before arrival in the anaesthetic room, pre-operative inotropic support, intra-aortic balloon pump, preoperative acute renal failure (anuria or oliguria < 10ml / hr)	3
Cardiac factors	Unstable angina	Angina requiring IV nitrates until arrival in the operating room	2
	Left ventricular dysfunction	Moderate (ejection fraction 30-50%) Poor < 30%	1 3
	Recent myocardial infarction	< 90 days	2
	Pulmonary hypertension	Systolic pulmonary artery pressure > 60 mm of Hg	2
Surgical factors	Emergency	Carried out on referral before the beginning of the next working day	2
	Other than isolated coronary artery bypass grafting	Major cardiac operation other than or in addition to coronary artery bypass grafting	2
	Surgery on the thoracic aorta	Ascending, arch or descending aorta	3
	Post-infarct ventricular septal rupture		4
Risk stratification			
Additive scores equate to approximate percentage predicted mortality			

2.2.9 Pre-treatment protocol for each group.

Patients in both groups had 3 sessions in the chamber 24hrs, 12hrs and 4 hrs prior to bypass. Each session for **Group – A** consisted of (Figure-11), slow pressurisation over 10 minutes to 1.5 atmospheres absolute (ATA) [approximately 10 feet of sea water (Fsw)], followed by 30 minutes wearing a hood supplying normal atmospheric oxygen – 5 minutes "air" break with hood off – followed by a further 30 minutes with the hood back on. At the end of the second 30 minutes slow depressurisation to normal atmospheric pressure was accomplished over 25 minutes. Clearly this is not a true placebo as air at this pressure is equivalent to a surface oxygen of 30%. However, this was necessary for three reasons, firstly, in order to preserve the double blind nature of the trial, this slightly higher than atmospheric pressure was used so that patients in this group heard similar pressurisation sounds and felt similar ear pressure changes as patients in group-B. Secondly, 1.5 meters was the lowest pressure at which the hoods worked and allowed for the two sensations of pressurisation noises and middle ear equalising. Thirdly, if we had used diluted air to make a true placebo, i.e. the patient breathed 21% O_2 at the 1.5 ATA, the increase in nitrogen in the air mix would have increased the risk of decompression illness that was minimised by the safe protocol we used, i.e. true air at depth. Each session for **Group – B** consisted of (Figure-12) slow pressurisation over 10 minutes to 2.4 ATA [approximately 40 feet of sea water (Fsw)], followed by 30 minutes wearing a hood supplying 100% oxygen – 5 minutes "air" break with hood off – followed by a further 30 minutes with the hood back on. At the end of the second 30 minutes slow depressurisation to normal atmospheric pressure was accomplished over 25 minutes.

Patient safety, compliance, practicality, logistics and available evidence were taken into consideration while designing the pre-treatment protocol. The important aspect of incorporating patient safety in the control group has been mentioned earlier. Though animals studies have demonstrated beneficial effect 1 pretreatment session **(Chen, Banick and Thom 1996, Chen et al 1998, Kim et al 2001, Miljkovic-Lolic et al 2002, Pedoto et al 2003)**, for optimum effect most studies used 3 **(Dennog et al 1999, Wada et al 2001, Dong et al 2002)** to 5 **(Wada et al 1996, Dong et al 2002)** sessions. Patients who volunteered for the trial were admitted 24 hours prior to the operation instead of the normal overnight admission. This early admission was needed to complete the 3 sessions. If we had opted for 5 sessions the patients would have had to be in hospital 48 hours prior to surgery. One had to consider the fact that prior to major heart surgery not many patients would have been willing to spend such a long period away from their families. In addition, by minimising preoperative ward stay we were also trying to minimise the patients risk of hospital acquired infections. Moreover, with the perpetual shortage of bed space within the National Health Service (NHS) and it was ethically unjustified for us to with hold an acute ward bed for 48 hours for the purpose of research.

Figure – 11. Dive profile, group-A.
(Fsw - feet of sea water)

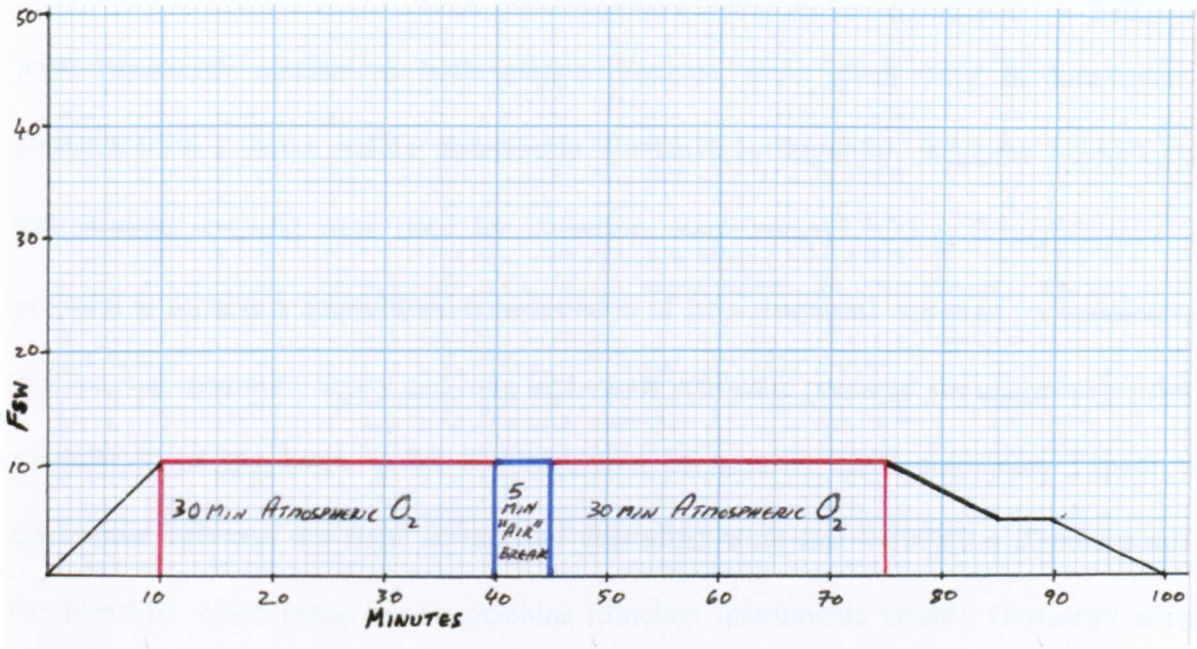
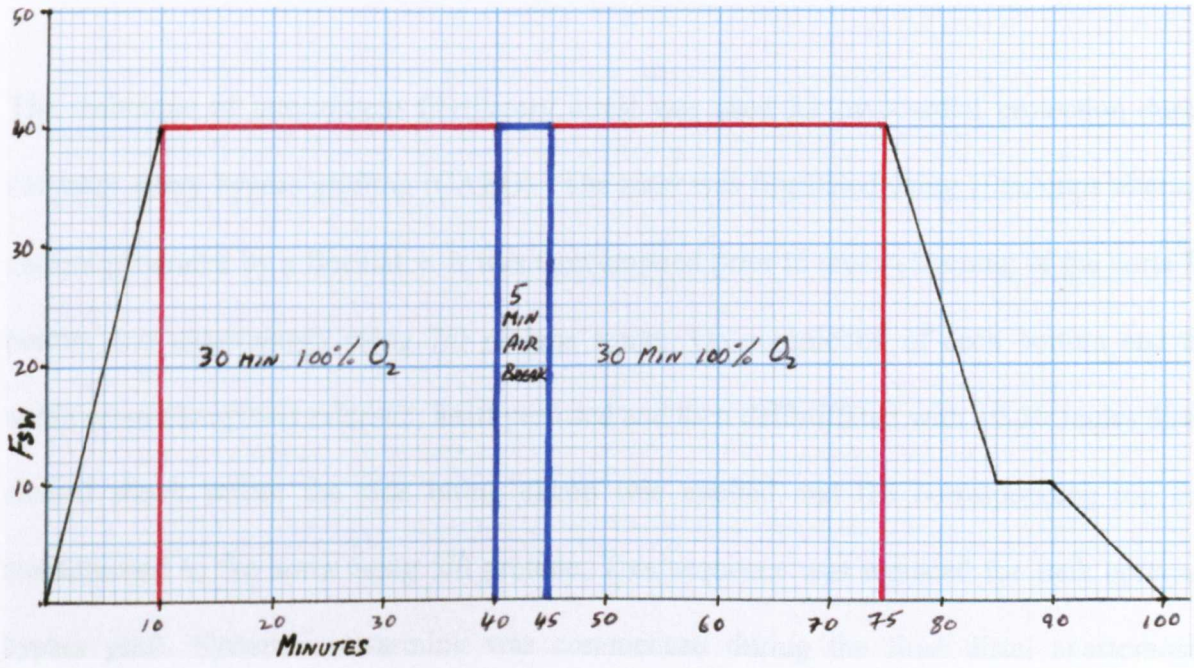


Figure – 12. Dive profile, group-B.
(Fsw - feet of sea water)



2.3 Anaesthetic, cardiopulmonary bypass and operation technique.

The anaesthetic technique including premedication, induction and maintenance of anaesthesia were essentially similar in both groups. Patients were given 1mg of lorazepam as premedication 2 hours before anaesthesia. Fentanyl 5-10μgm/kg, propofol 1-2mg/kg and pancuronium 8-10mg were used for induction. Remifentanyl 0.25 – 0.5μgm/kg/min and propofol to achieve a target blood concentration of 1.5 – 2μgm/ml was used for maintenance. The internal mammary artery and long saphenous vein were prepared simultaneously prior to cannulation of the heart. After administration of heparin, cardiopulmonary bypass was established between the right atrium and ascending aorta and ventilation discontinued. A Stockert SIII roller pump bypass machine (Stockert Instrumente GmbH, Germany) using a hollow fiber membrane oxygenator with integral hardshell venous cardiotomy reservoir and a 38μm arterial line filter was used in all cases. Pump flow was maintained between 2.4 – 2.2 litres/minute/square meter. Moderate systemic hypothermia of 35°C was maintained during bypass in all cases with rewarming commenced during the last distal-end anastomosis.

The technique of intermittent fibrillatory arrest was used for myocardial protection during coronary artery bypass grafting (CABG). The heart was fibrillated using 10mAmps alternate current generated by a fibrillator. It was then emptied prior to cross-clamping of the aorta for bottom end anastomosis using 7/0 prolene suture. On completion of each bottom end the aortic cross-clamp was released, heart perfused and then defibrillated with 10-20 Joules direct current shock before the side biting clamp was applied and the corresponding top end anastomosed to the aorta using 5/0 prolene. This sequence was repeated for each coronary bypass graft. Systemic rewarming was commenced during the final distal anastomosis.

Weaning from the bypass machine was attempted only after the patient had fully rewarmed and ventilation commenced. Once the patient was hemodynamically stable the heart was decannulated and the heparin was reversed with protamine sulphate. Hemostasis was then secured the sternum was closed using wires and the subcutaneous tissue and skin closed with absorbable vicryl sutures. The mediastinum and pleura were drained using plastic tube closed wound drainage system.

2.4 Mood assessment - Hospital anxiety and depression scale (HADS)

This is a questionnaire (Figure – 13) on which the patient indicates their feelings on questions related to anxiety and depression. There are a total of 14 questions, 7 relate to anxiety while the other 7 relate to depression. The patient is asked to tick the immediate reaction to each question from one of the four given choices. The score for each question ranges from 0 to 4. It is thus a self-reported measure of the patient's subjective levels of anxiety and depression. It is a sensitive assessment of the patient's mood (limbic system). A higher score indicates a higher level of anxiety or depression. All patients were requested to complete the questionnaire before the preoperative and postoperative neurocognitive assessments.

Figure – 13. The hospital anxiety and depression scale (HADS) questionnaire and score sheet.

HAD Scale

Name: Date:

Doctors are aware that emotions play an important part in most illnesses. If your doctor knows about these feelings he will be able to help you more.
This questionnaire is designed to help your doctor to know how you feel. Read each item and place a firm tick in the box opposite the reply which comes closest to how you have been feeling in the **past week.**
Don't take too long over your replies: your immediate reaction to each item will probably be more accurate than a long thought-out response.

Tick only one box in each section

I feel tense or 'wound up':
- Most of the time
- A lot of the time
- Time to time. Occasionally
- Not at all

I still enjoy the things I used to enjoy:
- Definitely as much
- Not quite so much
- Only a little
- Hardly at all

I get a sort of frightened feeling as if something awful is about to happen:
- Very definitely and quite badly
- Yes, but not too badly
- A little, but it doesn't worry me
- Not at all

I can laugh and see the funny side of things:
- As much as I always could
- Not quite so much now
- Definitely not so much now
- Not at all

Worrying thoughts go through my mind:
- A great deal of the time
- A lot of the time
- From time to time but not too often
- Only occasionally

I feel cheerful:
- Not at all
- Not often
- Sometimes
- Most of the time

I can sit at ease and feel relaxed:
- Definitely
- Usually
- Not often
- Not at all

I feel as if I am slowed down:
- Nearly all the time
- Very often
- Sometimes
- Not at all

I get a sort of frightened feeling like 'butterflies' in the stomach:
- Not at all
- Occasionally
- Quite often
- Very often

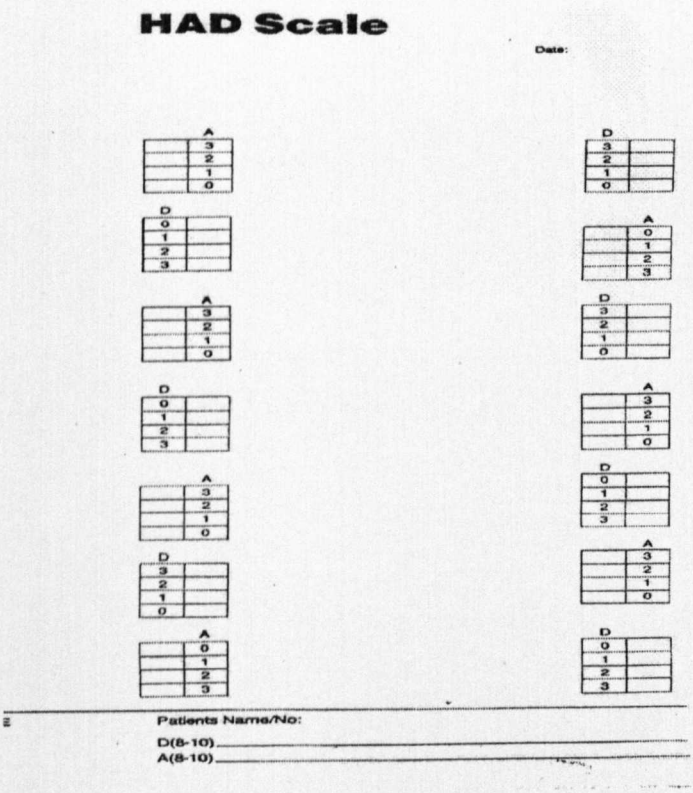
I have lost interest in my appearance:
- Definitely
- I don't take so much care as I should
- I may not take quite as much care
- I take just as much care as ever

I feel restless as if I have to be on the move:
- Very much indeed
- Quite a lot
- Not very much
- Not at all

I look forward with enjoyment to things:
- As much as ever I did
- Rather less than I used to
- Definitely less than I used to
- Hardly at all

I get sudden feelings of panic:
- Very often indeed
- Quite often
- Not very often
- Not at all

I can enjoy a good book or radio or TV programme:
- Often
- Sometimes
- Not often
- Very seldom



HAD Scale

Date:

A	
	3
	2
	1
	0

D	
0	
1	
2	
3	

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	3
	2
	1
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D	
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1	
2	
3	

Patients Name/No:

D(8-10)

A(8-10)

2.5 Assessment of intellect.

2.5.1 Wechsler test of adult reading (WTAR).

This test estimates global intellectual function and is not expected to change after cardiac operation **(Smith et al, 1986)**. In this test patients read out loud a list of irregularly pronounced words (Figure – 14). The words on the list are deliberately irregulary pronounced to minimise the patients ability to apply standard pronounciation rules and access previous learning of words. The accuracy on this test which is the number of words correctly pronounced out of 50 correlates highly with IQ. The raw score is converted to standardised scores based on, age, number of years of education and social background. The standardised score indicates the verbal IQ, performance IQ, full-scale IQ and memory. A higher score indicates a higher IQ.

Figure – 14. The word list used in the Wechsler test of adult reading (WTAR).

wtar **WORD CARD**

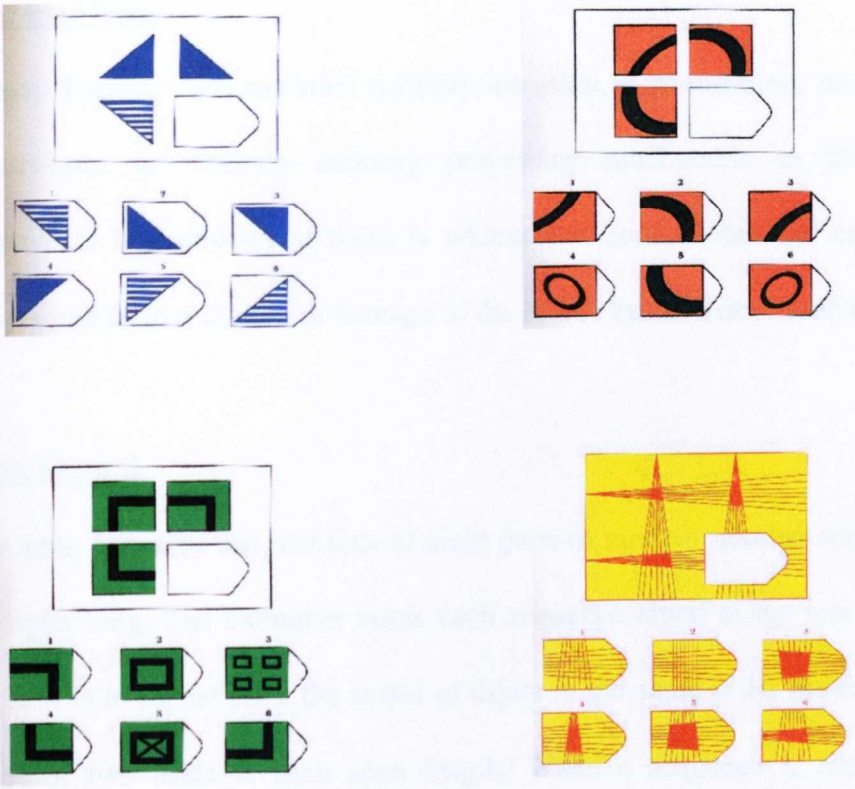
WECHSLER® TEST OF ADULT READING™

1. again
2. address
3. cough
4. preview
5. although
6. most
7. excitement
8. know
9. plumb
10. decorate
11. fierce
12. knead
13. aisle
14. vengeance
15. prestigious
16. wreathe
17. gnat
18. amphitheatre
19. lieu
20. grotesque
21. iridescent
22. ballet
23. equestrian
24. porpoise
25. aesthetic
26. conscientious
27. homily
28. malady
29. subtle
30. fecund
31. palatable
32. menagerie
33. obfuscate
34. liaison
35. exigency
36. xenophobia
37. ogre
38. scurrilous
39. ethereal
40. paradigm
41. perspicuity
42. plethora
43. lugubrious
44. treatise
45. dilettante
46. vertiginous
47. ubiquitous
48. hyperbole
49. insouciant
50. hegemony

2.5.2 Raven's coloured progressive matrices.

This multiple-choice paper and pencil test consists of a series of visual pattern matching and analogy problems pictured in non-representational designs (Figure – 15). It requires the patient to conceptualise, spatial design and numerical relationships ranging from the very obvious and concrete to the very complex and abstract. This is a "culture fair" test that requires neither language nor academic skills for success. This is a non-verbal, non-motor, visual IQ and memory test with standardised scores for older patients. There is no time limit and it consists of 60 items grouped into 5 sets, A, B, C, D and E. Each item contains a pattern problem with one part removed from 6 (subsets A and B) to 8 (subsets C,D and E) pictured inserts of which one contains the correct pattern. Each set involves a different theme with increasing order of difficulty. Patients point to the pattern piece they select or write its number on an answer sheet. The coloured background makes it interesting and obviates the need for much explanation. The raw scores are converted to a percentile rank based on age. A higher score indicates a higher visual IQ.

Figure – 15. Examples of the Raven's coloured progressive matrices.



2.6 The neurocognitive test battery used in this study.

2.6.1 The span tests.

These tests (Figure – 16) examine auditory attention, concentration, auditory memory, and very short-term or working memory processing attributable to the left hemisphere. Performance is impaired when there is widespread cortical damage especially to the left hemisphere and frontal cortex, or damage to the limbic system, or reticular formation.

Digit span forward

The digit span forwards test, consists of eight pairs of random number sequences that are two to nine digits long. The examiner reads each sequence aloud at the rate of one per second. Patients task is to repeat back the series of digits in the same order presented. The patient is only allowed two trials at each span length. When a sequence is repeated correctly the examiner reads the next longer sequence of numbers, continuing until the patient fails a pair of sequences or repeats a 9-digit sequence correctly. Digit span forward score is the longest string that can be repeated correctly. It is sensitive to organic brain damage and a higher score indicates a better performance.

Digit span backward

The digit span backwards test consists of seven pairs of random number sequences that two to eight digits long. The examiner reads each sequence aloud at the rate of one per second. Patients task is to say the series of digits in the exactly reverse order presented. The patient is only allowed two trials at each span length. When a sequence is correctly repeated in the reverse order, the examiner reads the next longer sequence of numbers, continuing until the patient fails a pair of sequences or repeats an eight-digit sequence correctly. Digit span

backward score is the longest string that can be repeated backwards correctly. It is sensitive to organic brain damage and a higher score indicates a better performance.

The requirement to store data and juggle them around mentally is an effort that involves temporal ordering and mental double-tracking that calls on working memory compared to the more passive very short-term memory required for the digit span forwards test.

Figure - 16. The forward and backward span tests.

DISCONTINUE RULE
Digits Forward & Backward:
Score of 0 on both trials of any item.
For both Digits Forward & Backward, administer both trials of each item even if Trial 1 is passed. Administer Digits Backward even if examinee scores 0 on Digits Forward.

SCORING RULE
Each Trial: 0 or 1 pt. for each response
Item score = Trial 1 + Trial 2

Digits Forward Trial Item/Response	Trial Score	Item Score (0,1 or 2)
1. 1 1-7		
2 6-3		
2. 1 5-8-2		
2 6-9-4		
3. 1 6-4-3-9		
2 7-2-8-6		
4. 1 4-2-7-3-1		
2 7-5-8-3-6		
5. 1 6-1-9-4-7-3		
2 3-9-2-4-8-7		
6. 1 5-9-1-7-4-2-8		
2 4-1-7-9-3-8-6		
7. 1 5-8-1-9-2-6-4-7		
2 3-8-2-9-5-1-7-4		
8. 1 2-7-5-8-6-2-5-8-4		
2 7-1-3-9-4-2-5-6-8		

Digits Forward Total Score (Maximum = 16)

Digits Backward Trial Item/Response	Trial Score	Item Score (0,1 or 2)
1. 1 2-4		
2 5-7		
2. 1 6-2-9		
2 4-1-5		
3. 1 3-2-7-9		
2 4-9-6-8		
4. 1 1-5-2-8-6		
2 6-1-8-4-3		
5. 1 5-3-9-4-1-8		
2 7-2-4-8-5-6		
6. 1 8-1-2-9-3-6-5		
2 4-7-3-9-1-2-8		
7. 1 9-4-3-7-6-2-5-8		
2 7-2-8-1-9-6-5-3		

Digits Backward Total Score (Maximum = 14)

Forward	+	Backward	=	(Maximum = 30)

2.6.2 Information processing A.

This paper and pencil test (Figure – 17) measures sustained attention, visual scanning, the speed of information processing, inhibition of rapid response, and attention shifting rather than intelligence. Failure in cancellation tasks appear to be associated with spatial neglect in right hemisphere lesion, and impaired temporal processing of information in patients with left hemisphere lesions.

The patient is presented with a sheet of paper with 21 blocks of 5 X 5 two-digit numbers. The task is to cross out the second highest number in each row and to cross out as many as possible in 4 minutes. The patient is first assessed for motor speed (the number of 11's crossed off as fast as the patient can in 20 seconds) and given a practice run on the practice sheet. The "task A total" is the total number of items crossed off in 4 minutes. "Errors %" is the the number of errors / task A total. Speed is the number of 11's cancelled in 20 seconds. The raw score is adjusted for error % and speed to give the adjusted score. A higher score indicates a better performance.

Figure - 17. The information processing- table A.

AMIPB INFORMATION PROCESSING A FORM 2

NAME _____ DATE _____

AGE _____ DOB _____ REF. NO. _____

Demonstration	Motor Speed		
38, 25, 79	11	11	11
97, 22, 18, 65	11	11	11
85, 27, 20, 48, 52	11	11	11
72, 50, 23, 74, 16	11	11	11
	11	11	11
92, 18, 54, 77, 21			
46, 39, 38, 16, 72	11	11	11
17, 54, 83, 11, 80	11	11	11
26, 87, 66, 39, 48	11	11	11
54, 56, 51, 63, 22	11	11	11
	11	11	11

Demonstration			
11	11	11	11
11	11	11	11
11	11	11	11
11	11	11	11
11	11	11	11

	Score	%ile range
Task A Total	____	_____
Errors %	____	_____
Speed	____	_____
Adjusted	____	_____

11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11
11	11	11

28, 16, 49, 21, 72	90, 13, 15, 62, 26	84, 19, 50, 91, 44
64, 27, 93, 91, 80	18, 29, 88, 15, 28	18, 17, 51, 27, 82
15, 87, 26, 13, 70	49, 93, 62, 51, 52	93, 45, 26, 54, 82
13, 14, 15, 11, 17	83, 29, 38, 84, 22	10, 44, 15, 82, 20
27, 92, 25, 58, 36	98, 71, 48, 43, 44	98, 77, 33, 71, 49
19, 72, 83, 16, 14	92, 14, 28, 11, 73	46, 10, 43, 19, 28
84, 22, 27, 54, 53	26, 29, 14, 82, 71	47, 52, 58, 61, 43
55, 54, 36, 17, 18	67, 82, 93, 11, 17	65, 18, 28, 66, 22
22, 31, 82, 47, 51	25, 14, 82, 55, 64	88, 71, 47, 72, 85
41, 16, 20, 18, 36	65, 86, 27, 51, 25	92, 41, 56, 27, 28
83, 10, 42, 44, 16	27, 22, 14, 83, 11	12, 69, 75, 77, 41
72, 75, 69, 58, 70	65, 66, 22, 14, 91	21, 91, 16, 14, 58
11, 15, 46, 99, 30	16, 70, 56, 31, 93	50, 94, 27, 16, 26
32, 37, 41, 66, 55	45, 57, 98, 66, 81	17, 28, 88, 15, 72
90, 16, 91, 18, 92	80, 26, 73, 66, 82	22, 92, 51, 14, 85
44, 90, 22, 80, 43	63, 15, 81, 35, 14	26, 37, 24, 97, 72
45, 27, 24, 26, 60	95, 28, 50, 20, 26	38, 74, 23, 21, 92
62, 54, 18, 73, 28	25, 17, 54, 36, 83	29, 94, 65, 32, 50
42, 80, 93, 66, 68	22, 18, 11, 19, 14	83, 42, 11, 72, 90
28, 27, 44, 16, 93	93, 95, 81, 92, 99	44, 55, 62, 42, 26
19, 41, 27, 82, 65	61, 62, 41, 51, 90	19, 62, 17, 57, 16
72, 14, 11, 31, 85	11, 14, 12, 82, 66	17, 24, 38, 62, 57
27, 28, 41, 92, 30	18, 17, 84, 48, 82	25, 15, 91, 70, 52
67, 52, 65, 39, 38	16, 83, 99, 95, 41	59, 93, 99, 26, 14
26, 47, 29, 73, 22	52, 78, 68, 10, 15	51, 52, 41, 55, 80
86, 76, 91, 11, 15	41, 11, 39, 92, 14	42, 32, 93, 66, 15
26, 14, 23, 81, 42	92, 31, 82, 65, 73	81, 27, 24, 55, 36
65, 91, 42, 82, 71	53, 41, 17, 24, 67	22, 66, 31, 60, 82
35, 80, 26, 29, 14	30, 81, 26, 36, 14	65, 63, 26, 54, 22
80, 62, 32, 53, 55	80, 26, 23, 55, 53	64, 17, 25, 11, 91
38, 39, 45, 40, 36	19, 72, 27, 67, 80	67, 57, 68, 36, 21
62, 65, 88, 74, 82	26, 38, 49, 55, 57	19, 93, 52, 83, 49
94, 91, 85, 86, 60	41, 82, 35, 63, 80	11, 93, 66, 25, 31
74, 83, 20, 11, 91	26, 81, 31, 41, 11	56, 32, 48, 64, 68
55, 60, 41, 27, 16	27, 24, 28, 39, 22	83, 32, 93, 66, 74

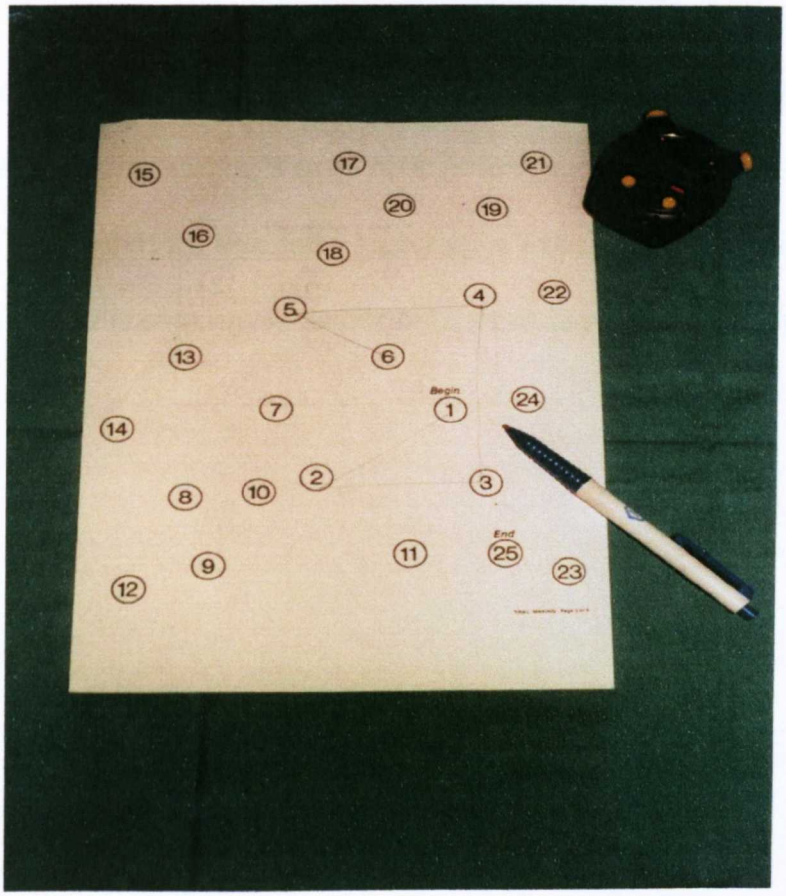
2.6.3 Trail making tests.

These are sensitive tests of frontal lobe function and assess visual attention, concentration, scanning, shifting and visuo-motor perception, coordination, tracking and response inhibition.

Trail-making test A.

The test comprises circled numbers from 1 to 25 distributed around the paper (Figure – 18). After a practice run the patient is timed while he joins the numbers in numerical ascending order without taking the pencil from the paper. The score is the time in seconds to complete the task. Patients are corrected if they go wrong and have to go back an item and carry on from there. The score is the time taken to complete the task and a higher score indicates a poorer performance.

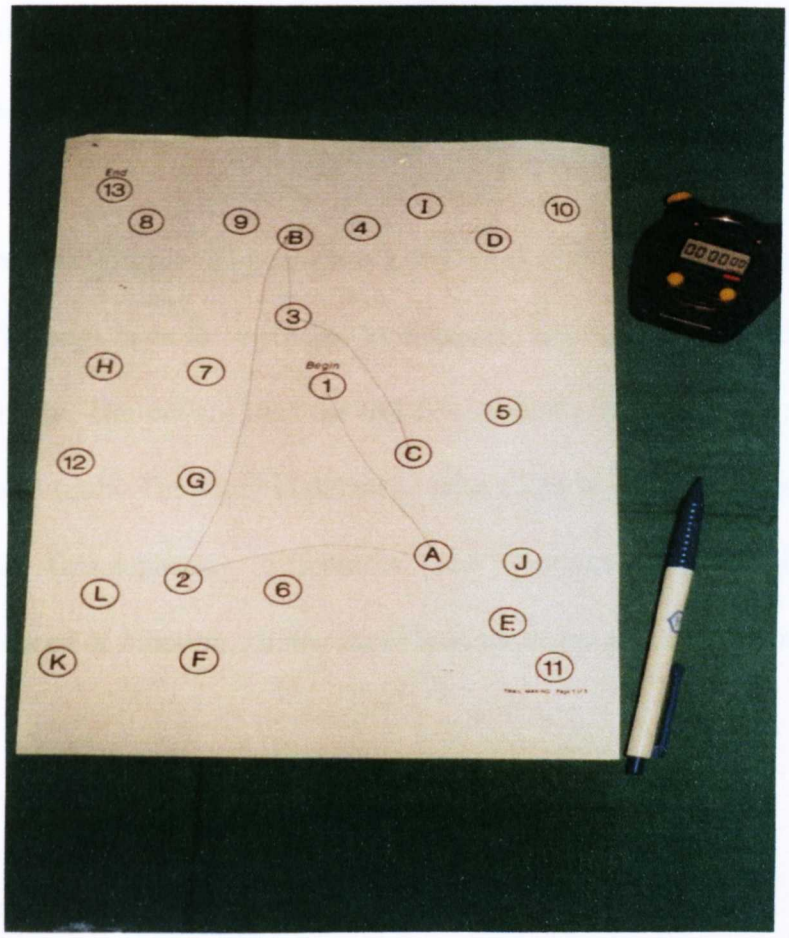
Figure - 18. The trail making – A test.



Trail-making test B

The test comprises circled numbers 1 to 13 and the letters A to L scattered around the page (Figure – 19). The patient is timed whilst he or she joins the numbers alternately in numerical and alphabetical order i.e. 1-A-2-B-3-C-4-D and so on until L-13. Again patients are corrected if they go wrong and have to go back an item and carry on from there. The score is the time taken to complete the task and a higher score indicates a poorer performance. Unlike test A, this test also calls on complex double or multiple conceptual tracking.

Figure - 19. The trail making – B test.

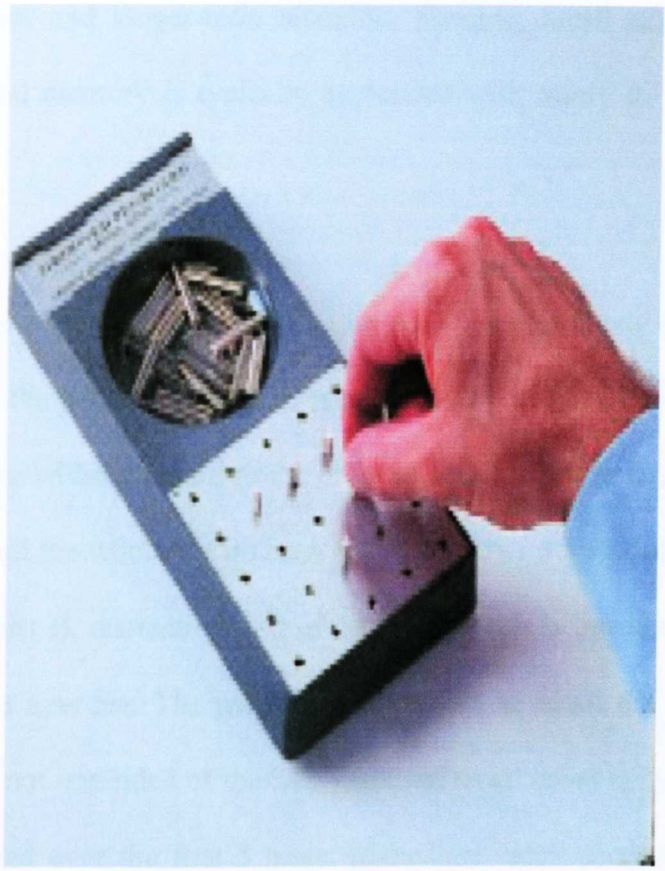


2.6.4 Grooved -pegboard test.

This timed test of manual dexterity and fine motor coordination (Figure – 20) discriminates differences in right and left hemispheric performance. It requires less visual scanning and cognitive planning than the trail-making tests.

The patient moves 25 grooved pegs onto a pegboard with 25 slotted holes angled in different directions. The pegs have to be rotated in different directions and twisted in different angles to fit in the holes. The patient tries the test first with the dominant, then non-dominant hand alternately for 3 trials. The score is the mean time taken in seconds to move the 25 pegs. The between-hand (hemispheric) differences and practice improvement reflect overall hemispheric speed of function. Higher score indicates a poorer performance.

Figure - 20. The grooved peg board test.



2.6.5 Rey auditory verbal learning test (AVLT)

This is a test of verbal learning and memory (Tables – 4, 5, 6 and 7). It tests immediate memory, short-term and longer-term retention, learning, recall and recognition. Impaired verbal learning and memory is typically associated with injury to the left tempero-pareital cortex.

The subject is read a first list of 15 words (list A) at approximately one word per second. At the end of the list the patient is asked to recall as many words as possible, these are marked off by the examiner without regard to the order of recall. The list is repeated 5 times and the patient tries to recall the whole list on each occasion. After 5 initial attempts to recall the first list a second list (list B, distraction list) of different words is introduced, again the patient is asked to recall this new list. The patient is then asked to recall the first list again only this time the patient is not reminded of the list. The total recall score is the total number of words immediately recalled over the first 5 trials. Immediate recall score is the number of words recalled from the first list immediately after the distraction list. Delayed recall score is the number of words from the first list recalled 30 minutes after the distraction list. Recognition score is the number of words recognised from the original list of 15 words among others in a list of 50 words. A higher score indicates a better performance. To avoid practice effect parallel forms of the word lists and recognition lists are used during repeat testing.

Table – 9. Rey auditory verbal learning test (AVLT) - word lists.

Normal list		Parallel lists				Alternate list
A	**B**	**AC**	**BC**	**A/JG**	**B/JG**	**C**
Drum	Desk	Doll	Dish	Violin	Orange	Book
Curtain	Ranger	Mirror	Jester	Tree	Armchair	Flower
Bell	Bird	Nail	Hill	Scarf	Toad	Train
Coffee	Shoe	Sailor	Coat	Ham	Cork	Rug
School	Stove	Heart	Tool	Suitcase	Bus	Meadow
Parent	Mountain	Desert	Forest	Cousin	Chin	Harp
Moon	Glasses	Face	Water	Earth	Beach	Salt
Garden	Towel	Letter	Ladder	Knife	Soap	Finger
Hat	Cloud	Bed	Girl	Stair	Hotel	Apple
Farmer	Boat	Machine	Foot	Dog	Donkey	Chimney
Nose	Lamb	Milk	Shield	Banana	Spider	Button
Turkey	Gun	Helmet	Pie	Radio	Bathroom	Log
Colour	Pencil	Music	Insect	Hunter	Casserole	Key
House	Church	Horse	Ball	Bucket	Soldier	Rattle
River	Fish	Road	Car	Field	Lock	Gold

AC, BC, A/JG, B/JG are parallel forms to list A & B to avoid practice effect
C – alternate list to be used if ether the A or B list presentations are interrupted

Table - 10. Rey AVLT – recognition list for list A-B.

Bell	Home	Towel	Boat	Glasses
Window	Fish	Curtain	Hot	Stocking
Hat	Moon	Flower	Aren't	Shoe
Barn	Tree	Colour	Water	Teacher
Ranger	Balloon	Desk	Farmer	Stove
Nose	Bird	Gun	Rose	Nest
Weather	Mountain	Crayon	Cloud	Children
School	Coffee	Church	House	Drum
Hand	Mouse	Turkey	Stranger	Toffee
Pencil	River	Fountain	Garden	Lamb

Table - 11. Rey AVLT – recognition list for lists AC-BC

Nail	Envelope	Ladder	Foot	Water
Sand	Car	Mirror	Bread	Joker
Bed	Face	Screw	Desert	Coat
Pony	Toad	Music	Street	Captain
Jester	Silk	Dish	Machine	Tool
Milk	Hill	Pie	Head	Fly
Plate	Forest	Wood	Girl	Song
Heart	Sailor	Ball	Horse	Doll
Jail	Dart	Helmet	Soot	Stall
Insect	Road	Stool	Letter	Shield

Table – 12. Rey AVLT – recognition list for A/JB-B/JB

Rock	Star	Soap	Television	Violin
Corn	Peel	Frog	Hotel	Beach
Pear	Lock	Dog	Piano	Radio
Tree	Banana	Orange	Spider	Bus
Cork	Toad	Cousin	Bucket	Doctor
Bread	Uncle	Bathroom	Soldier	Chest
Sofa	Earth	Gloves	Scarf	Knife
Stair	Hospital	Field	Wife	Donkey
Ham	Grass	Armchair	Train	Hunter
Casserole	Lunchbox	Blanket	Suitcase	Chin

2.7 Test-retest interval.

All the neurocognitive tests were performed as per the recommendations of the 1994 conference on CNS dysfunction after cardiac surgery **(Murkin et al, 1995)**. A baseline test battery was performed 48 hours prior to the operation and the mean interval between the preoperative and postoperative testing was 4 months.

2.8 Definition of cognitive decline

The standard deviation index (SDI) method was used in this study. A patient was considered to have a major deterioration in a test, if the decline in score was greater than or equal to 1 standard deviation (SD) of the preoperative group mean scores. A patient was considered to have significant neurocognitive impairment if they showed $\geq$ 1SD deterioration in greater than 20% (2 tests in this study) of the test battery. This was one of the definitions of cognitive decline proposed by the 1994 conference **(Murkin et al, 1995)**. This definition has been validated and used in many previous studies analysing cognitive decline after cardiac surgery **(Harrison et al 1989, Newman et al 1993, Pugsley et al 1994, Patel et al 1993 and 1996, Toner et al 1996, Braekken et al 1998 and Zamvar et al 2002)**.

2.9 Inflammatory mediators measured in the study.

We measured the inflammatory cytokines - TNFα, IL6, IL8; the soluble adhesion molecules - sE selectin, sP selectin, sICAM-1, leucocyte bound adhesion molecule – CD-18, and the stress response protein – HSP-70.

2.10 Blood sampling, preparation and storage.

Blood for analysis was drawn form the central venous line at 3 time points. Just before skin incision, 2 hours and 24 hours after the end of cardiopulmonary bypass. During each sampling 4 mls of blood was drawn into purple-capped vacutainer tubes (K2E EDTA K2 Vacuette, Ref 454210, Greiner bio-one GmbH, Austria) placed on a test-tube rotator (Labinco BV, L28, Netherlands) until analysis for CD-18 within 4 hours. 15 ml from the same blood sample was drawn into three orange-capped vacutainer tubes (SST II BD Vacutainer, Ref 367954, BD Vacutainer systems, UK) allowed to stand for at least 30min before being centrifuged at 1500 times gravity for 15 minutes, the serum was pippeted out and stored at $-70^{O}C$ till analysis.

2.11 Tests used to measure the inflammatory markers.

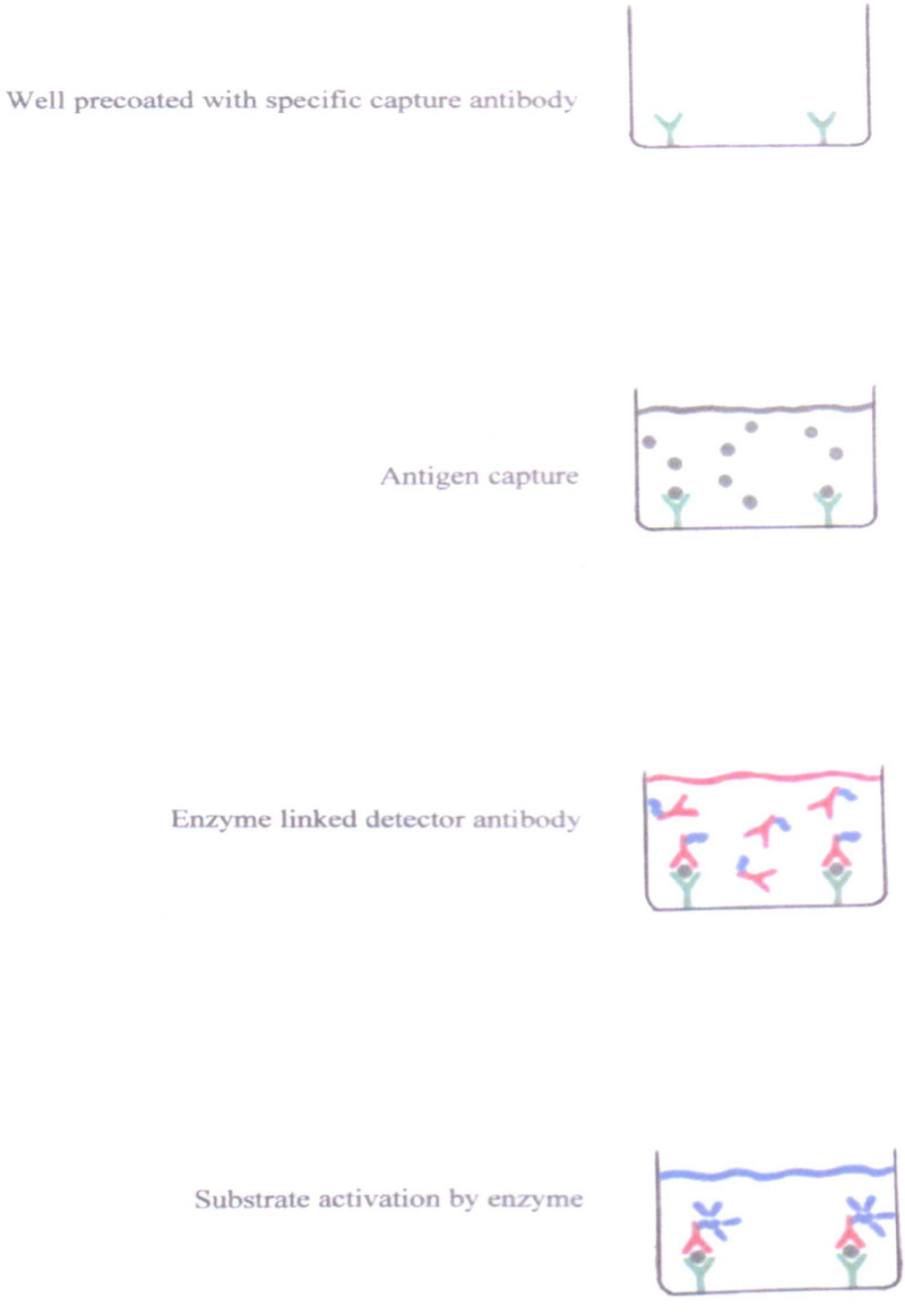
Serum levels of TNFα, IL6, IL8, sE selectin, sP selectin, sICAM-1 and HSP-70 levels were measured using the enzyme linked immunosorbent assay (ELISA) test, while leucocyte CD-18 levels were measured using immunofluorescein flow cytometry.

2.11.1 Enzyme linked immunosorbent assay (ELISA)

Antibody specific for an antigenic site on the target protein being assayed is coated onto wells in a 96-well microtitre plate (Figure – 21). The serum being tested for the protein is applied to the surface and any unbound material is removed by washing with a buffer solution. Enzyme-labelled (usually horseradish peroxidase or alkaline phosphatase) antibody specific for a second site on the captured target protein is then applied to the wells, any excess of the enzyme-labelled antibody is removed by washing with the buffer solution. A substrate of the enzyme, which changes colour on reaction is added. Finally after a designated time interval an acid stop solution is added which stops any further reaction and converts the colour. Spectrophotometric measurement of the intensity of colour change is an indicator of the amount of enzyme labelled antibody bound by the protein, and from this value one can determine the amount of protein in the serum being tested.

Quantitative ELISA kits from Biosource Intl, USA, were used for measuring IL6 (Ref no: KAC1261), IL8 (Ref no: KAC1301), TNFα (KAC1751), sE-selectin (KHS2011), sP-selectin (KHS2021), sICAM-1 (KHS5411). For HSP-70 quantitative ELISA kits (EKS-700) from Stressgen Biotechnologies, Canada, were used. An Anthos 2010 (Anthos Labtec Instruments GmbH, Austria) was used for the spectrophotometric measurement with Stingray software (Stingray software Inc, USA) for analysis.

Figure – 21. The enzyme linked immunosorbent assay (ELISA) test.

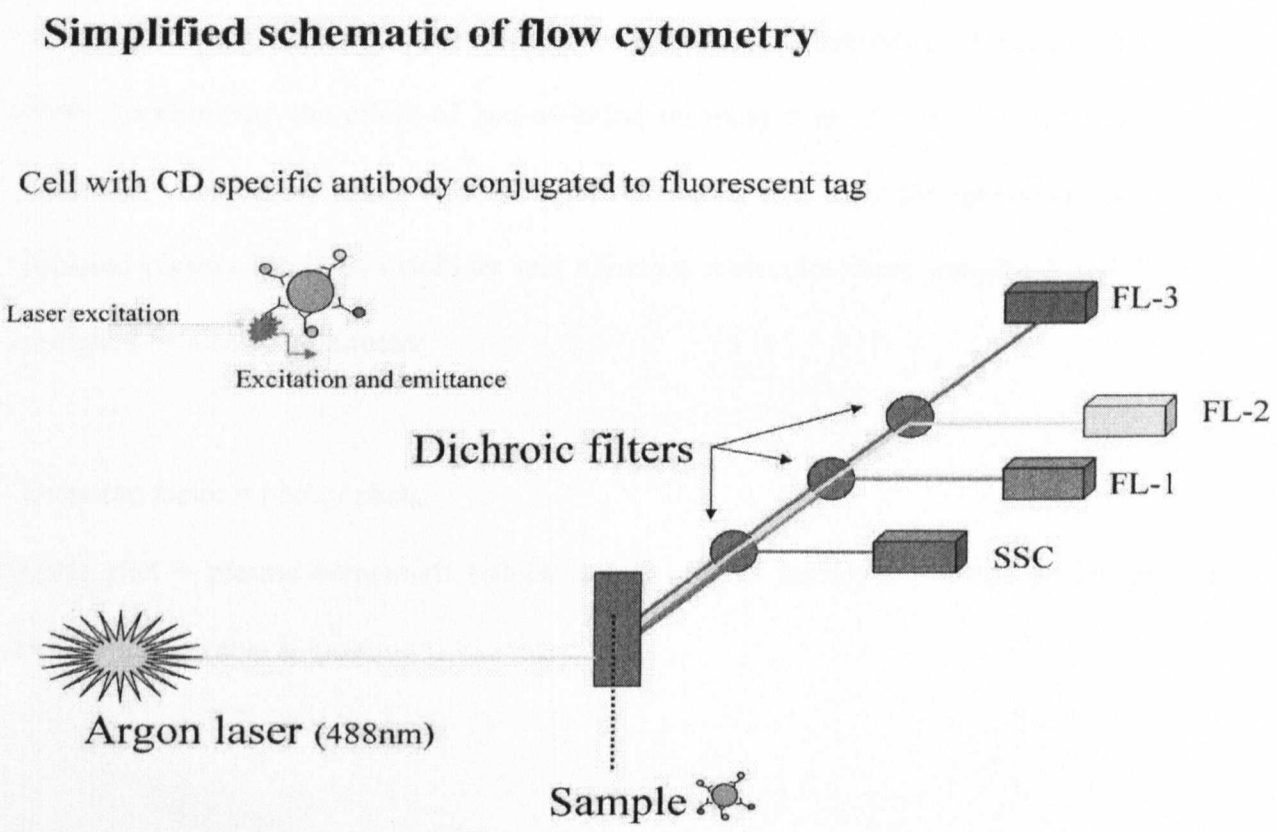


2.11.2 Flow cytometry

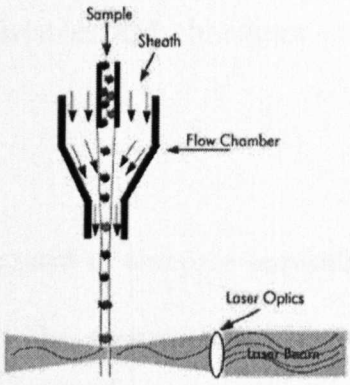
Flow cytometry is a rapid automated counting method that allows analysis of an extremely high number of cells within a very short period of time (Figure – 22). Cells are mixed with fluorescein isothiocyanate conjugated antibody specific for the surface antigen being assayed. Counting is accomplished in an automated cell counter by passing a single file of cells suspended in a stream of sheath fluid past a probe beam. The sheath fluid is pumped much more rapidly than the sample so the cells are constrained to the centre of the sheath fluid. This process of hydrodynamic focussing allows a reproducible delivery of the fluid being analysed. At the measuring point the stream of cells intersect a beam of argon laser at 488 nm that is directed through the stream of cells and measured by a detector. The probe beam is scattered by the passage of each cell which is sensed by the detector and converted into a signal that is proportional to the magnitude of the cell variable being detected (eg: fluorescence, cell size). 10000 cells are counted and displayed as a histogram or scattergram that depicts the distribution of cell sizes and shapes in the sample analysed using CELLQuest software.

We used rat antibodies against human CD-18 (Ref-MCA503, Serotec Inc, USA) for the quantitative flow cytometric estimation of leucocyte CD-18. The equipment used was a FACSCalibur (BD Biosciences Inc, USA) with CellQuest software (BD Biosciences Inc, USA).

Figure – 22. Flow cytometry.



The Flow Chamber



2.12 Correction for hemodilution.

One problem faced by any study measuring serum markers following cardiopulmonary bypass, is the effect of hemodilution on the measured levels. It has been demonstrated before that not correcting for this factor can lead to erroneous interpretation **(Roth-Isigkeit et al 1999)**. To eliminate the effect of hemodilution resulting from the priming solution in the pump and from intravenous fluids administered during and after the operation, the actual measured plasma levels of cytokines and adhesion molecules from samples 2 and 3 were multiplied by a correction factor

Correction factor = $phct_{ti}$ / $phct_{to}$

Where phct = plasma hematocrit (which is 100 – RBC hct%); ti = value at the time of sampling; to = value at baseline.

2.13 Statistical analysis

SPSS version 11 for Windows (SPSS Inc, Chicago) was used for statistical analysis. Demographic, pre-operative, intra-operative and post-operative data, were analysed using the unpaired t-test for continuous variables and chi-square or Fisher test, as appropriate, for discrete variables.

Nonparametric ANOVA test was used to compare sequential change during the three time points for inflammatory markers. The postoperative change in inflammatory markers was tested for correlation to age, cardiopulmonary bypass duration and cross-clamp time, using the Spearman's correlation.

Inter-group comparison of IQ scores (Weschler test of adult reading (WTAR) and Raven's matrices) and hospital anxiety and depression scale (HADS) scores were done using the unpaired t-test, while the paired t-test was used to compare preoperative and postoperative changes in HADS score within each group.

The group standard deviation (SD) for each neurocognitive test was calculated. A patient was considered to have major deterioration in a particular test if the score deteriorated, by $\geq$ 1SD of the group mean, from the preoperative score. A patient was considered to have significant neurocognitive impairment if there was a major deterioration in more than 2 (>20%) of the tests. Chi-square test was used to calculate statistical significance of the difference in neurocognitive impairment between the two groups. This was one of the methods recommended by the "Conference on CNS Dysfunction after Cardiac Surgery: Defining the Problem" held on the 10^{th} and 11^{th} of December 1994 at Fort Lauderdale, Florida **(Murkin et al, 1995)**. Multivariate logistic regression analysis including the variables, age, gender, left ventricular function, cardiopulmonary bypass time, cross-clamp time, IQ level, anxiety and depression score, and postoperative change in individual inflammatory markers were used to test for correlation and try to identify predictors of neurocognitive dysfunction.

Results

3 Results.

Of the 64 patients recruited for the study, 1 patient from group-A and 2 patients from group-B failed to attend the follow up neurocognitive assessment. 1 patient from group-A had postoperative laparotomy for bowel obstruction followed by prolonged ventilation. 1 patient from group-B had intraoperative complications with a prolonged period of hypotension. Both these patients were dropped from the study as the effect of these complications would have interfered with the performance and outcome during neurocognitive testing. Thus the final strength was 29 patients in group-A and 30 patients in group-B. For ease of interpretation the results have been displayed in a series of 12 tables which follow.

Table 13 - Intergroup comparison of demographic and preoperative factors – Unpaired t-test / chi-square test.

Demographic & preoperative data	Group-A	Group-B	p-value
Age Mean ± SEM SD 95% CI	 65.9 ± 1.7 9.2 62.6 – 69.2	 66.2 ± 1.5 8.3 63.3 – 69.1	 0.9
Gender ratio Male Female	 83.9 % 16.1 %	 87.5 % 12.5 %	 0.7
Body mass index Mean ± SEM SD 95% CI	 26.7 ± 0.6 3.2 25.5 – 27.9	 27.3 ± 0.8 4.5 25.7 – 28.9	 0.5
Hypertension	67.7 %	62.5 %	0.7
Diabetes mellitus	12.9 %	15.6 %	0.8
Canadian Cardiovascular Society angina class **I** **II** **III** **IV**	 3.3 % 67.7 % 29 % 0	 18.7 % 31.3 % 50 % 0	 0.009
Unstable angina	6.5 %	6.3 %	0.9
New York Heart Association dyspnoea grade **I** **II** **III** **IV**	 29 % 58.1 % 12.9 % 0	 40.6 % 31.3 % 28.1 % 0	 0.1
Left main stem disease	29 %	18.8 %	0.3
Coronary disease severity **Single** **Double** **Triple**	 12.9 % 32.3 % 54.8 %	 3.1 % 21.9% 75 %	 0.2
Previous myocardial infarction	9.7%	31.3%	0.03
Left ventricular function **Good** **Moderate** **Poor**	 87.1% 9.7% 3.2%	 81.2% 12.5% 6.3%	 0.9
Arrhythmia	3.2%	3.1%	0.9
Renal dysfunction	0	0	
Peptic ulcer	3.2%	3.1%	0.9

Cerebrovascular accident	0	0	
Parsonnet score Mean ± SEM SD 95% CI	 6.5 ± 1.2 6.5 4.1 – 8.9	 7.2 ± 1 5.5 5.2 – 9.2	 0.6
Euroscore Mean ± SEM SD 95% CI	 3.3 ± 0.4 2.4 2.5 – 4.1	 3.5 ± 0.3 1.9 2.9 – 4.1	 0.7

SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

Table 14 – Intergroup comparison of intraoperative and postoperative factors – Unpaired t-test / chi-square test.

Intraoperative & postoperative data	Group-A	Group-B	p-value
Number of grafts 1 2 3	 16.1% 35.5% 48.4%	 0 37.5% 62.5%	0.07
Myocardial ischaemia time (minutes) Mean ± SEM SD 95% CI	 23.5 ± 1.5 8.5 20.6 – 26.4	 24.8 ± 1.4 8.1 22.1 – 27.5	0.5
Cardiopulmonary bypass time (minutes) Mean ± SEM SD 95% CI	 50.1 ± 4.3 23.9 41.7 – 60.5	 52.1 ± 3 17 46.2 - 58	0.7
Ventilation time (hours) Mean ± SEM SD 95% CI	 7.7 ± 5.3 28.6 3 – 12.4	 4.2 ± 1 5 2.2 – 6.1	0.5
Postoperative inotrope	12.9%	15.6%	0.8
Postoperative arrhythmia	25.8%	18.8%	0.5
Postoperative myocardial infarction	0	0	
Postoperative renal dysfunction	0	3.1%	0.3
Postoperative Confusion Transient ischaemic attack	 3.2% 3.2%	 0 0	0.2
Major sepsis	0	0	
Multiorgan failure	0	0	
Intensive care unit stay (days) Mean ± SEM SD 95% CI	 1.4 ± 0.4 2.3 0.6 – 2.2	 1.1 ± 0.1 0.3 0.9 – 1.3	0.4
Postoperative duration of stay (days) Mean ± SEM SD 95% CI	 9 ± 1.6 9 5.9 - 12.1	 6.4 ± 0.1 0.8 6.2 – 6.6	0.1
Mortality	0	0	

SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

Table 15 – Change in levels of inflammatory markers in group-A - ANOVA test.

Group – A Inflammatory markers	Preoperative	2hrs post-bypass	24hrs post-bypass	Total value	F-ratio	p-value
sE-selectin						
Mean ±SEM	23.2 ± 2.4	36.9 ± 6.4	24 ± 3.6	28.1 ± 2.6	3	0.05
SD	12.6	33.3	17.4	23.5		
95% CI	18.3 - 28.1	23.7 - 50.1	16.6 - 31.3	22.9 – 33.4		
Median	21.7	33	21.1			
Interquartile range	14.9 – 33.1	21.6 - 40	16.6 – 27.8			
sP-selectin						
Mean ±SEM	180.6 ± 22.1	172 ± 25.2	121.8 ± 13	159.2 ± 12.3	2.2	0.1
SD	117	128.5	65.1	109.6		
95% CI	135.3 – 226	120.1 – 224	94.9 - 148.7	134.6 – 183.7		
Median	152.8	126.2	99.7			
Interquartile range	102 – 224.4	91.3 – 193.3	82.4 – 157.4			
IL-6						
Mean ±SEM	3 ± 1.1	76.5 ± 6.4	76.9 ± 5.7	51.2 ± 4.9	77.5	0.0001
SD	5.6	32.1	28.9	43		
95% CI	0.8 – 5.2	63.3 – 89.7	65.2 – 88.6	41.5 – 60.9		
Median	0.76	100	100			
Interquartile range	0.3 – 2.7	38.4 - 100	56.7 - 100			
IL-8						
Mean ±SEM	11.6 ± 2	61.9 ± 11.4	22.3 ± 13.2	31.3 ± 4.5	15.8	0.0001
SD	10.6	57.1	13.2	39.7		
95% CI	7.4 – 15.8	38.4 – 85.5	16.9 – 27.6	22.3 – 40.2		
Median	8.4	38.7	19.9			
Interquartile range	5.2 – 13.5	23.97 – 93.6	12.9 – 27.9			
ICAM-1						
Mean ±SEM	342.2 ± 26	267.2 ± 15.5	336.1 ± 18.2	316.2 ± 12.5	3.9	0.02
SD	137.4	77.5	91.1	110.6		
95% CI	288.9 – 395.5	235.2 – 299.2	298.5 – 373.7	291.3 – 341.1		
Median	336.1	252.7	322.1			
Interquartile range	219.1 - 421	214.1 – 329.4	258.7 – 414.6			
CD-18						
Mean ±SEM	63.6 ± 3.6	83.8 ± 3.4	80.5 ± 4.7	75.5 ± 2.4	8.1	0.001
SD	19.6	17.8	22.9	21.8		
95% CI	56.2 – 71.1	76.8 – 90.9	70.8 – 90.2	70.7 – 80.4		
Median	64.5	85.1	78.3			
Interquartile range	49.5 – 80.3	66.6 – 96.6	60.3 – 95.1			

TNFα						
Mean ±SEM	21.1 ± 2.5	47.3 ± 7.1	22.4 ± 3	30.1 ± 3	10.2	0.0001
SD	13	36.4	15.4	26.4		
95% CI	16.1 – 26.2	32.6 – 62	16.2 – 28.7	24.2 – 35.9		
Median	20.5	35.8	22.1			
Interquartile range	13.8 – 25.7	22.4 – 63.7	14.3 – 28.1			
HSP-70						
Mean ±SEM	8.1 ± 1	19.7 ± 4.5	8.9 ± 1	12 ± 1.7	6.3	0.007
SD	3.1	12.7	3	8.9		
95% CI	5.7 – 10.5	9.1 – 30.4	6.7 – 11.2	8.4 – 15.6		
Median	6.6	13	8.4			
Interquartile range	6.1 – 9.2	11.8 – 33.8	6.7 – 10.3			

SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

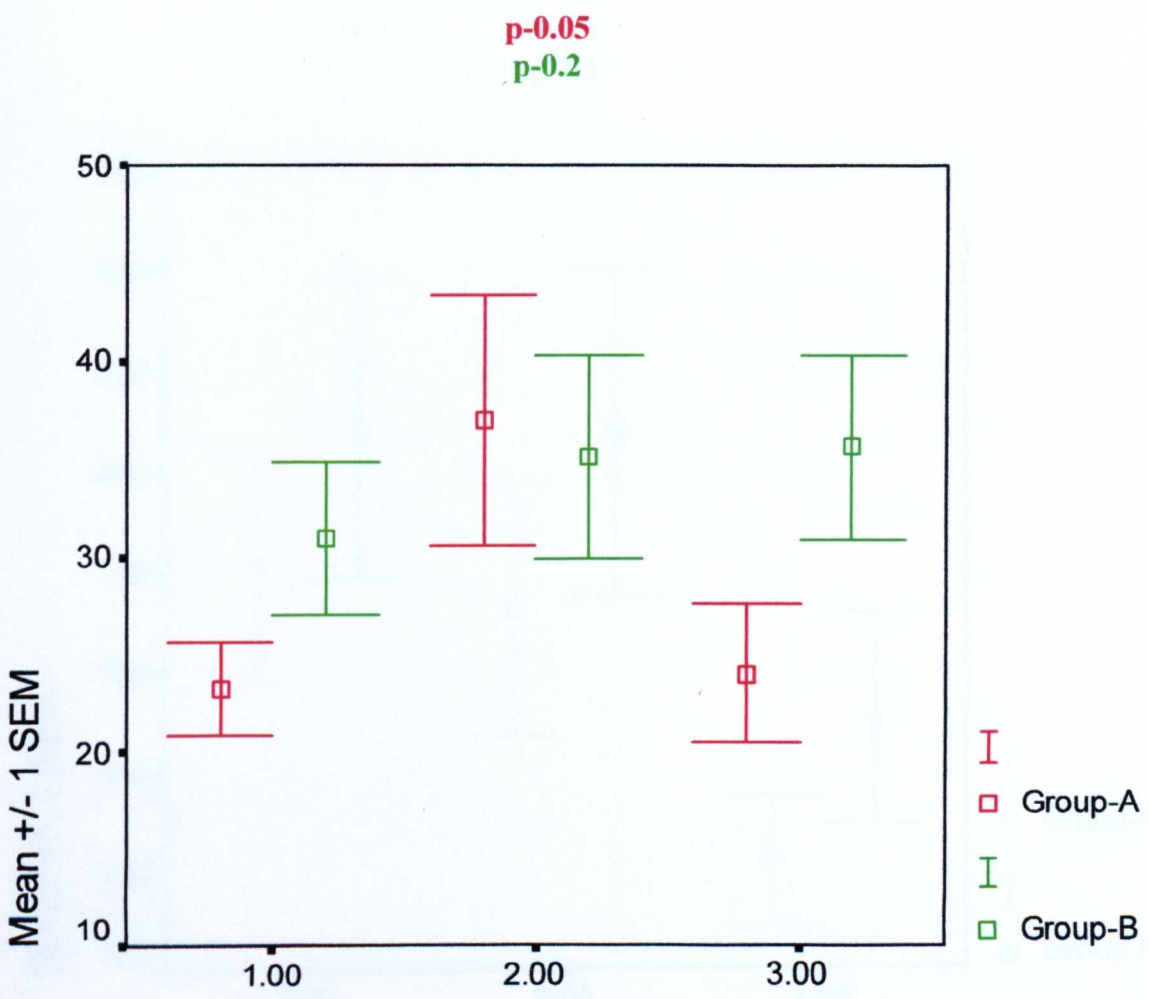
Table 16 - Change in levels of inflammatory markers in group-B – ANOVA test.

Group – B Inflammatory markers	Preoperative	2hrs postobypass	24hrs post-bypass	Total value	F-ratio	p-value
sE-selectin						
Mean ±SEM	30.9 ± 3.9	43.2 ± 5.4	34.4 ± 4.4	36 ± 2.7	1.9	0.2
SD	21.5	28.2	23.5	24.7		
95% CI	22.8 – 38.9	32.1 – 54.4	25.3 – 43.5	30.6 – 41.3		
Median	23	35.5	24.2			
Interquartile range	14.9 – 37.2	22 – 61.3	15.5 – 53.6			
sP-selectin						
Mean ±SEM	208.5 ± 30	208.5 ± 32.7	135.6 ± 21.2	184.5 ± 16.6	2.2	0.1
SD	164.6	169.8	112.2	153.3		
95% CI	147.1 – 270	141.3 – 275.7	92 – 179.1	151.4 – 217.6		
Median	155.5	183.2	107			
Interquartile range	95.8 – 282.8	84.6 – 242.8	55.4 – 171.3			
IL-6						
Mean ±SEM	3.2 ± 1	81.8 ± 6.1	68.7 ± 5.9	49.7 ± 4.7	79.2	0.0001
SD	5.5	31.6	31.3	43.1		
95% CI	1.1 – 5.2	69.3 – 94.3	56.6 – 80.8	40.4 – 59		
Median	1.1	100	67			
Interquartile range	0.4 – 2.6	76.5 - 100	51.9 - 100			
IL-8						
Mean ±SEM	8.6 ± 1.4	60.3 ± 9.2	24.2 ± 2.2	30.5 ± 3.9	24.1	0.0001
SD	7.5	48.8	11.8	36		
95% CI	5.8 – 11.4	41.4 – 79.3	19.6 – 28.8	22.8 – 38.2		
Median	6.1	49.6	20.8			
Interquartile range	2.4 – 10.4	30.5 – 72.4	14.5 – 35.1			
ICAM-1						
Mean ±SEM	372.2 ± 23.8	294.3 ± 22.6	431.4 ± 42	367 ± 18.5	4.9	0.01
SD	130.6	117.6	222.1	170.6		
95% CI	323.5 – 421	247.7 – 340.8	345.3 – 517.6	330.2 – 403.8		
Median	330.6	255.9	362.2			
Interquartile range	277.7 – 471.1	214.6 – 362.2	279.5 – 522.2			
CD-18						
Mean ±SEM	63.3 ± 4.1	77.5 ± 4.7	71.9 ± 4.8	70.9 ± 2.7	2.5	0.1
SD	21.9	25.1	24.8	24.4		
95% CI	54.8 – 71.8	67.7 – 87.2	62.1 – 81.7	65.6 – 76.2		
Median	63.1	77.8	73.6			
Interquartile range	47.2 – 81.2	58.5 – 93.7	46.6 – 91.8			

TNFα Mean ±SEM SD 95% CI Median Interquartile range	 27.5 ± 3.4 18.6 20.5 – 34.4 27.2 18.3 – 32.7	 54.8 ± 9.3 48.1 35.8 – 73.9 39.4 21.1 – 78.7	 26.4 ± 3.1 16.5 20 – 32.8 25 18.5 – 34.8	 35.8 ± 3.6 33.1 28.7 – 42.9	7.6	0.001
HSP-70 Mean ±SEM SD 95% CI Median Interquartile range	 7.6 ± 1.4 3.1 3.7 – 11.4 7.4 5 – 10.2	 14 ± 2.8 6.9 6.8 – 21.2 13.7 6.8 - 21	 11.9 ± 2 5 6.7 – 17.2 11.3 7.3 – 16.1	 11.4 ± 1.4 5.7 8.5 – 14.3	2	0.2

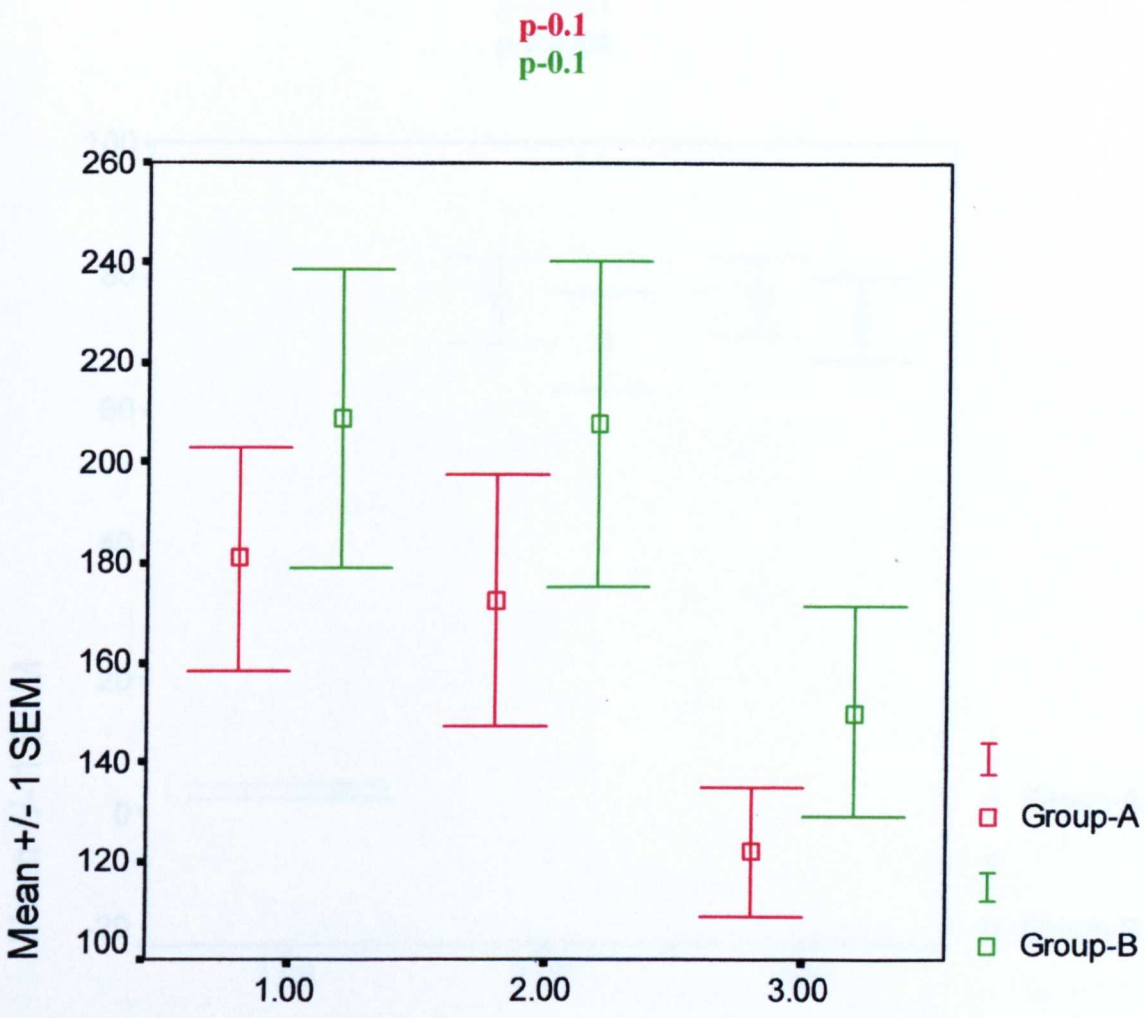
SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

Figure – 23. Comparison of trend in sE-selectin (nanograms/ml)



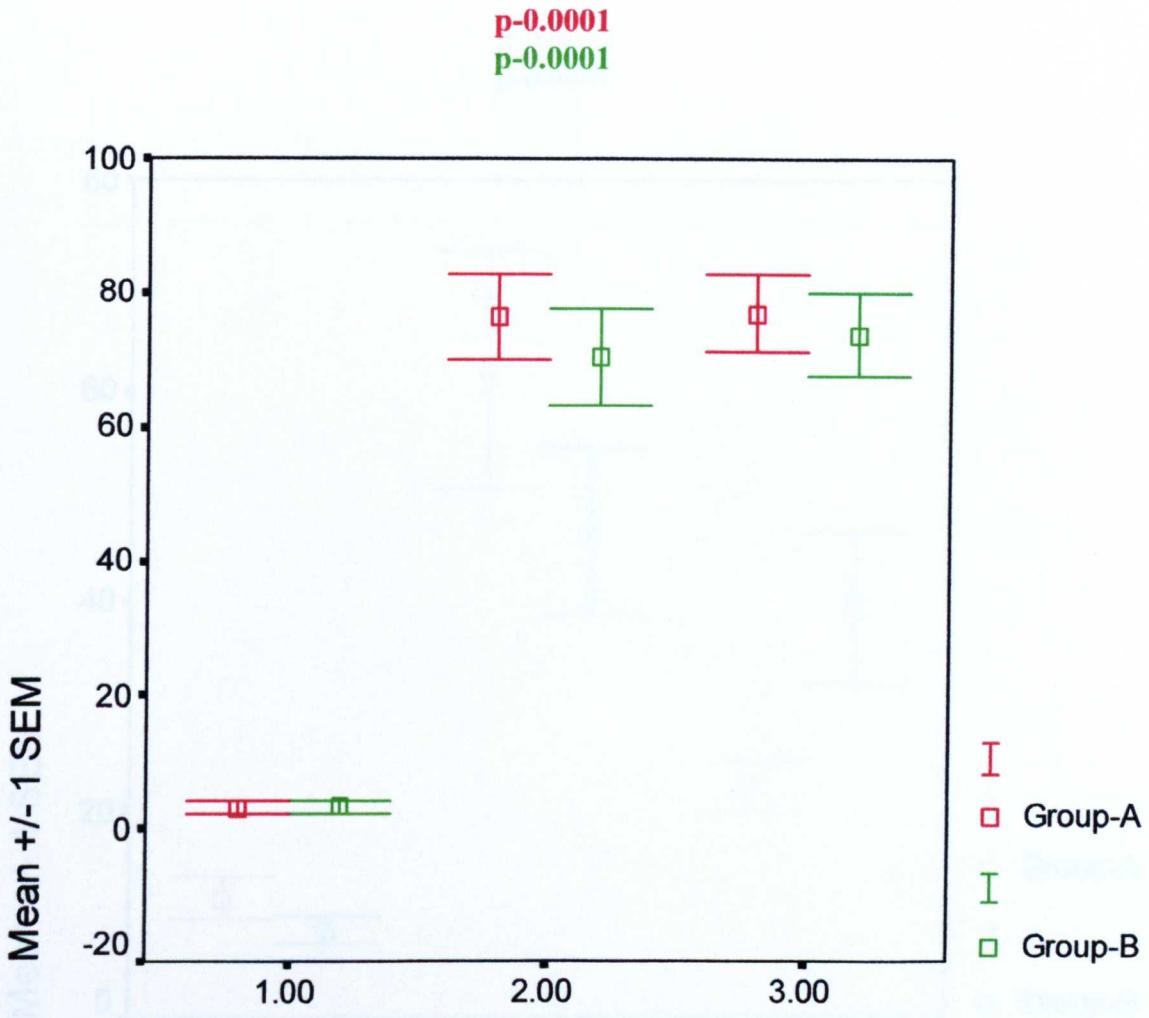
1 - Preoperative, 2 - 2 hours post-bypass, 3 - 24 hours post-bypass.

Figure – 24. Comparison of trend in sP-selectin (nanograms/ml)



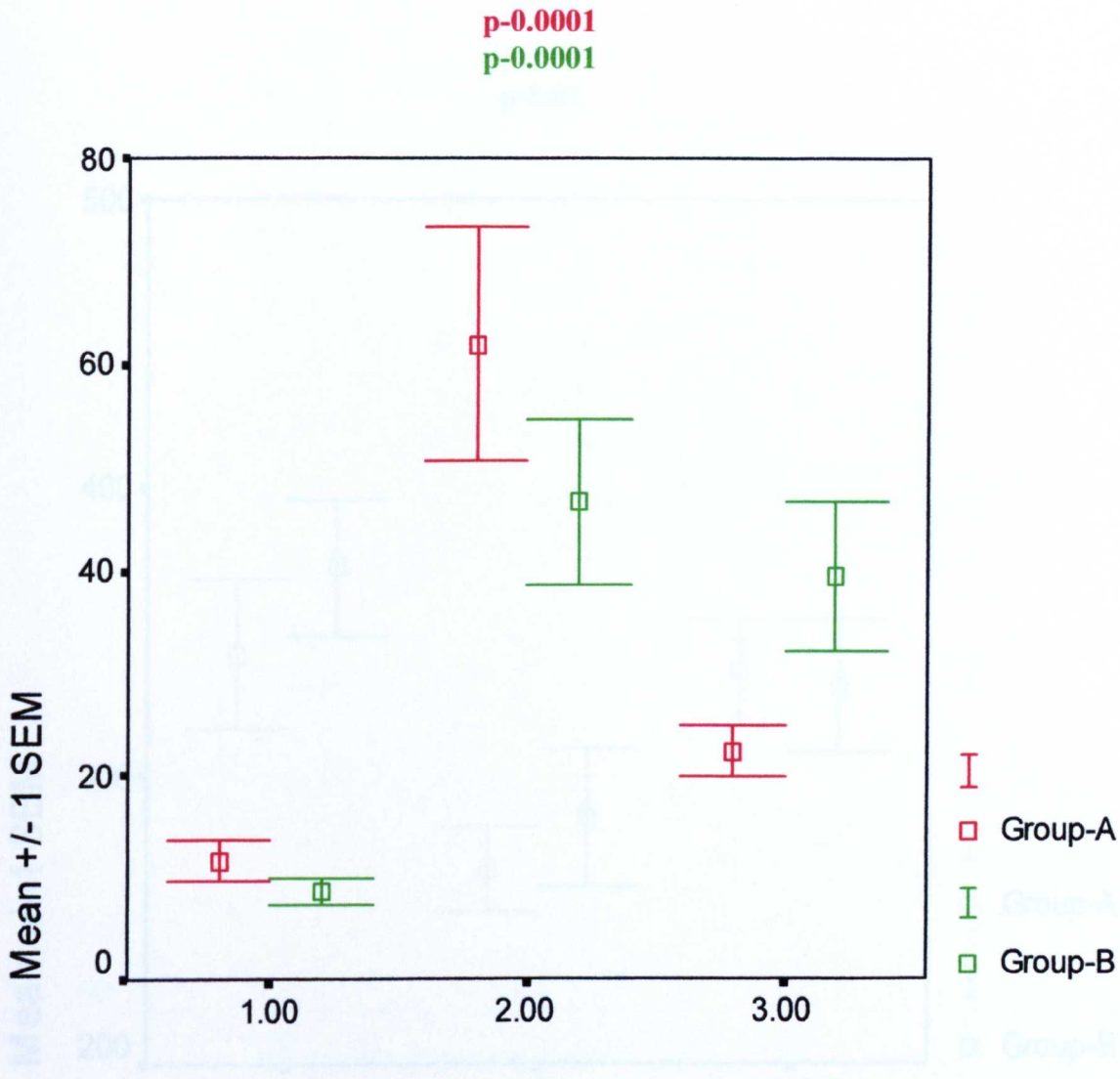
1 - Preoperative, 2 - 2 hours post-bypass, 3 - 24 hours post-bypass.

Figure – 25. Comparison of trend in IL6 (picograms/ml)



1 - Preoperative, 2 - 2 hours post-bypass, 3 - 24 hours post-bypass.

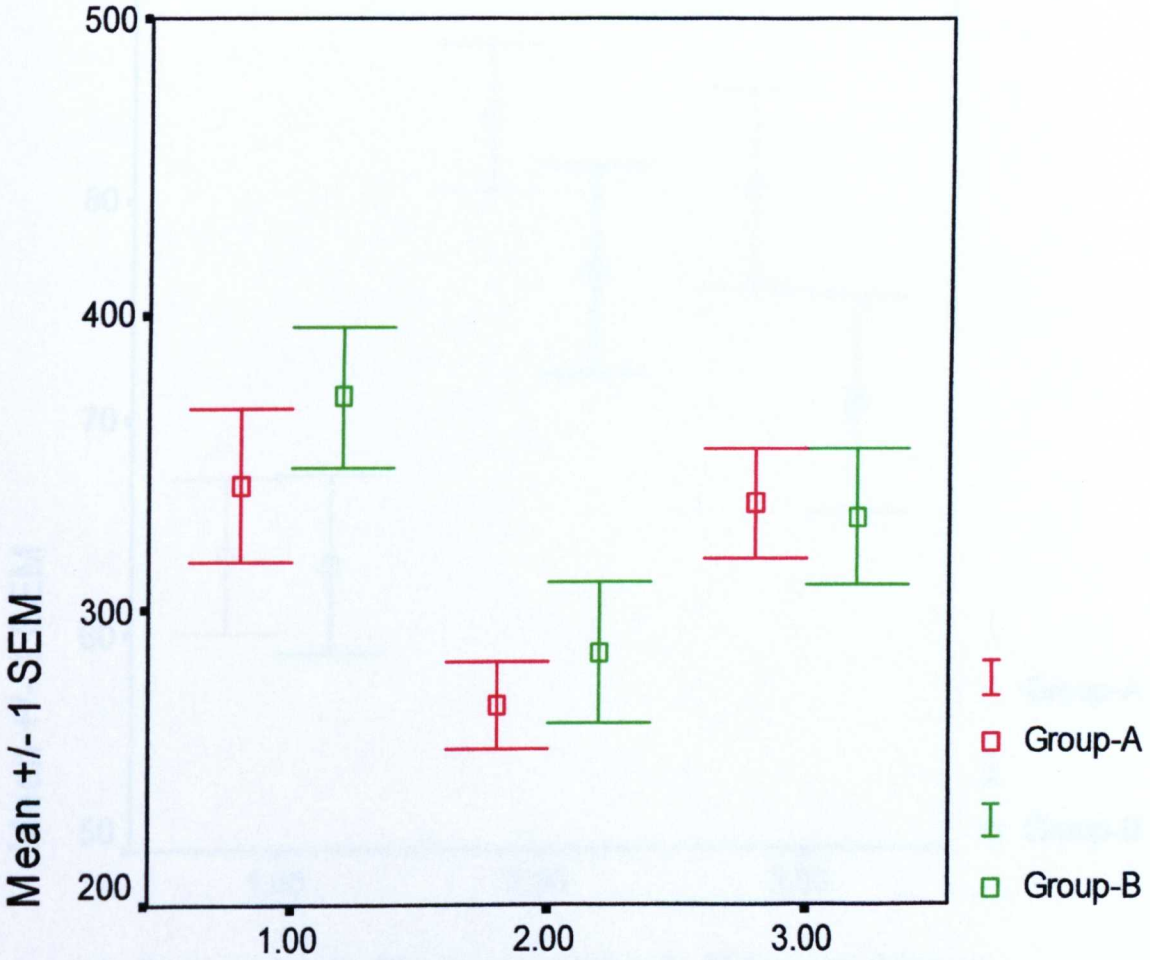
Figure – 26. Comparison of trend in IL8 (picograms/ml)



1 - Preoperative, 2 - 2 hours post-bypass, 3 - 24 hours post-bypass.

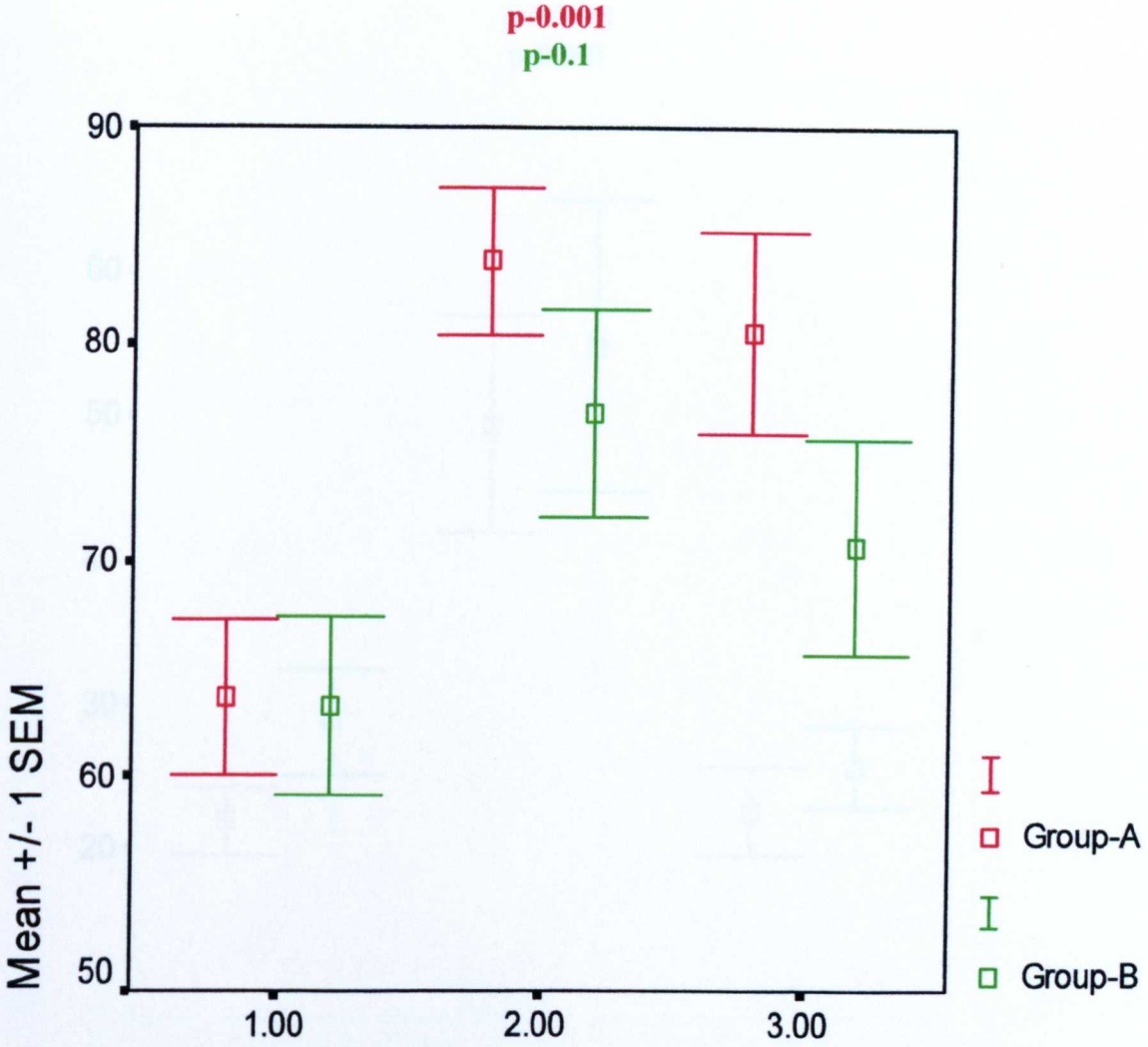
Figure – 27. Comparison of trend in ICAM-1 (nanograms/ml)

p-0.02
p-0.01



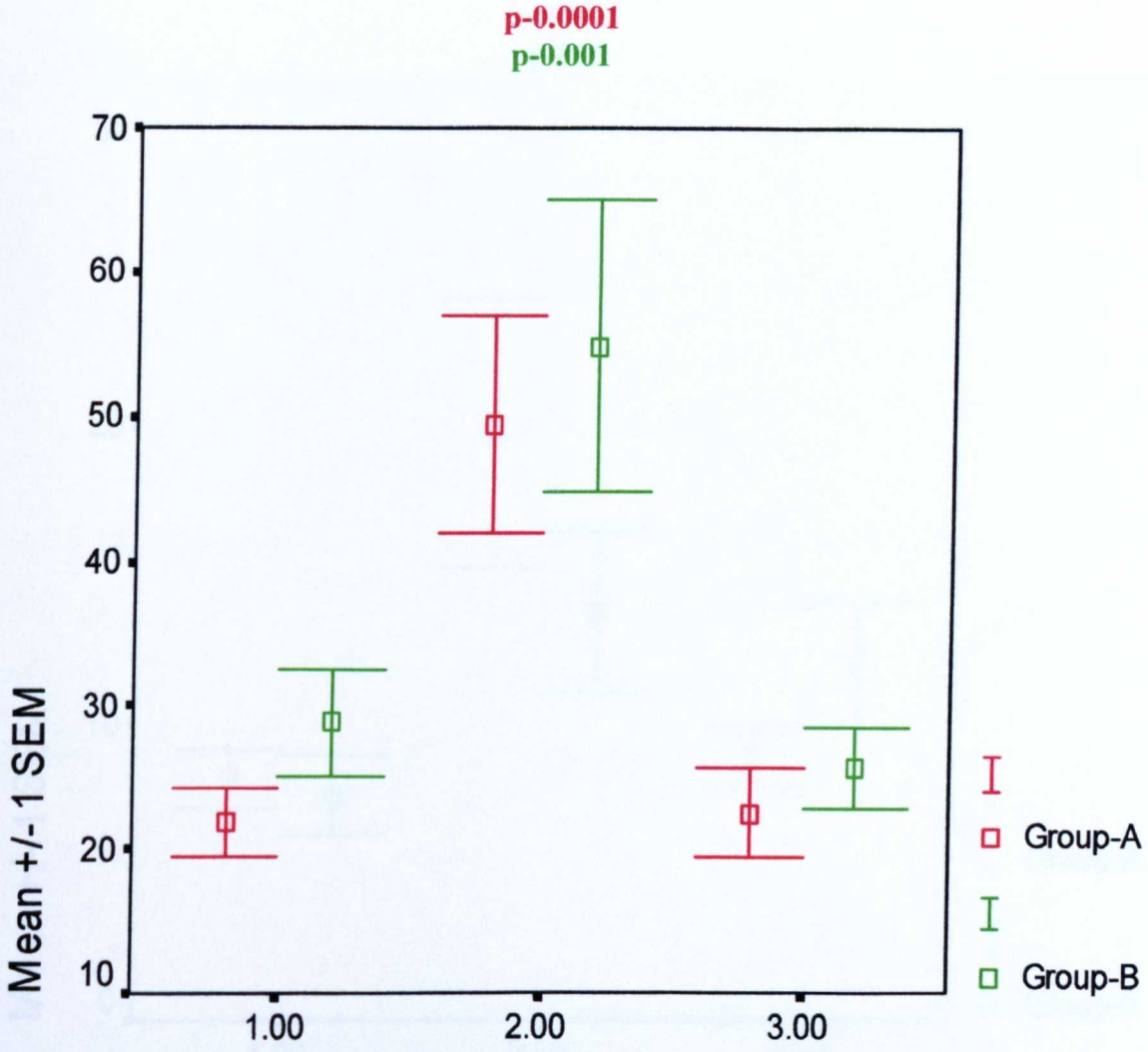
1 - Preoperative, 2 - 2 hours post-bypass, 3 - 24 hours post-bypass.

Figure – 28. Comparison of trend in CD-18 (mean fluorescence)



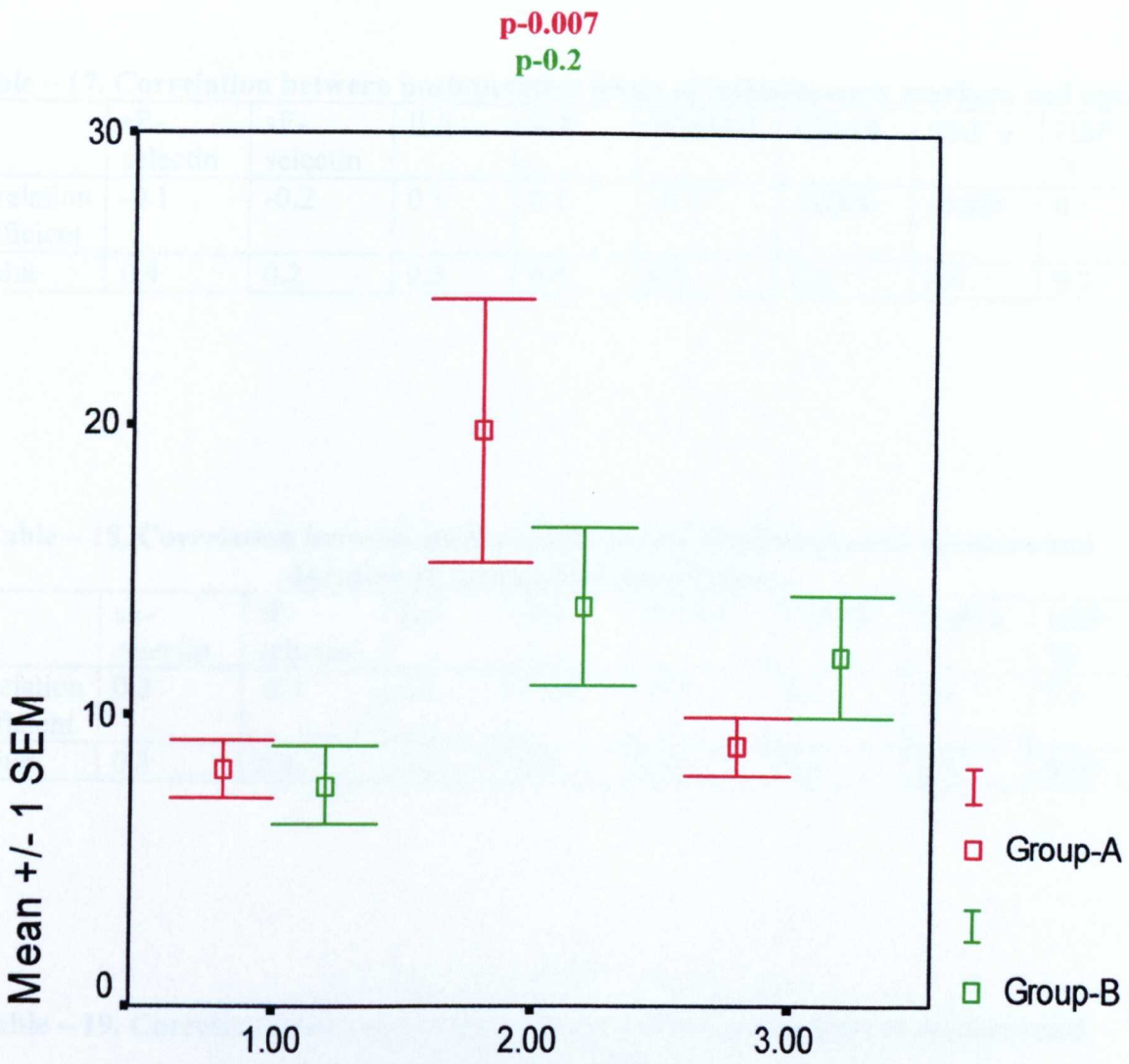
1 - Preoperative, 2 - 2 hours post-bypass, 3 - 24 hours post-bypass.

Figure – 29. Comparison of trend in TNF alpha (picograms/ml)



1 - Preoperative, 2 - 2 hours post-bypass, 3 - 24 hours post-bypass.

Figure – 30. Comparison of trend in HSP-70 (nanograms/ml)



1 - Preoperative, 2 - 2 hours post-bypass, 3 - 24 hours post-bypass.

We further analysed the data to see if there was any correlation (Spearman's) between the rise in inflammatory markers and age (Table-13), gender, duration of cardiopulmonary bypass (Table-14) and duration of cross clamp (Table-15). The only statistically significant correlation was between the postoperative rise in HSP 70 and the duration of cardiopulmonary bypass and cross-clamp time.

Table – 17. Correlation between postoperative levels of inflammatory markers and age.

	sE-selectin	sP-selectin	IL6	IL8	ICAM-1	CD-18	TNF α	HSP-70
Correlation coefficient	-0.1	-0.2	0.1	0.1	-0.1	-0.009	-0.005	0.1
p-value	0.4	0.2	0.3	0.4	0.3	0.9	0.9	0.7

Table – 18. Correlation between postoperative levels of inflammatory markers and duration of cardiopulmonary bypass.

	sE-selectin	sP-selectin	IL6	IL8	ICAM-1	CD-18	TNF α	HSP-70
Correlation coefficient	0.2	-0.1	0.1	0.04	-0.2	0.2	0.1	0.6
p-value	0.3	0.4	0.3	0.8	0.3	0.1	0.3	0.02

Table – 19. Correlation between postoperative levels of inflammatory markers and cross-clamp time.

	sE-selectin	sP-selectin	IL6	IL8	ICAM-1	CD-18	TNF α	HSP-70
Correlation coefficient	0.2	-0.1	0.2	0.1	-0.05	0.2	0.2	0.7
p-value	0.1	0.5	0.4	0.6	0.7	0.2	0.1	0.006

Figure – 31. Scatterplot of correlation between increase in HSP-70 and cardiopulmonary bypass time.

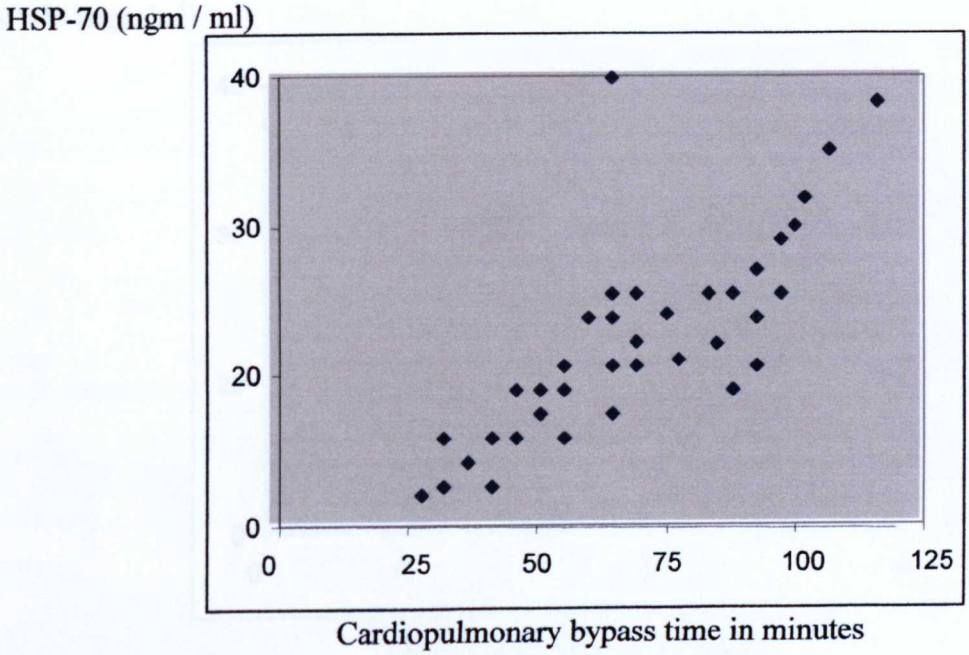


Figure – 32. Scatter plot of correlation between increase in HSP-70 and cross-clamp time.

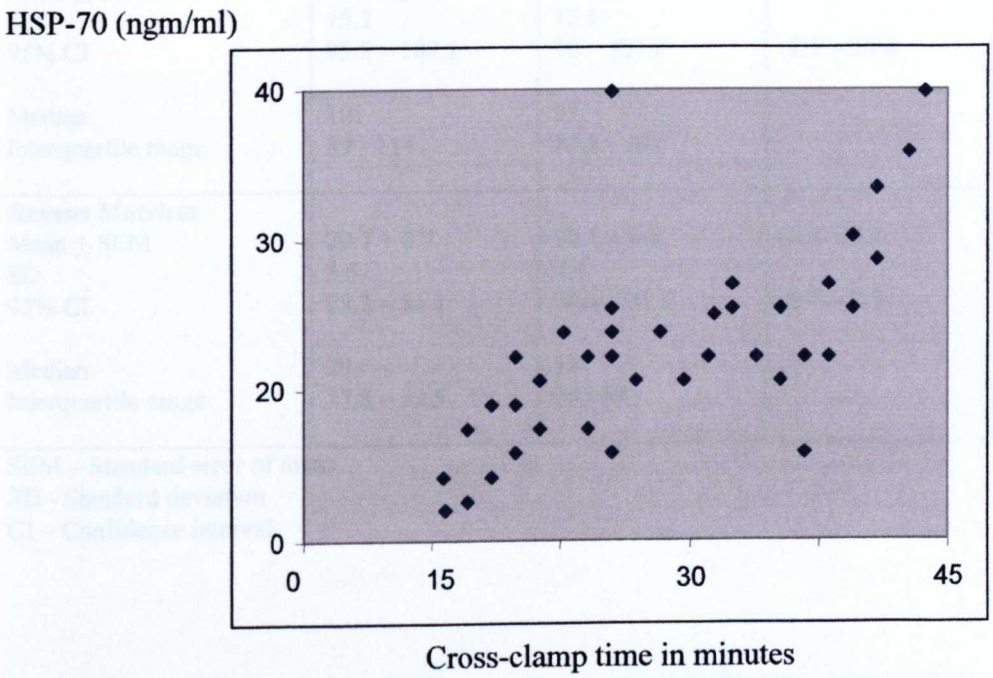


Table 20 - Intergroup comparison of baseline IQ – Unpaired t-test.

Test	Group-A	Group-B	Intergroup difference	p-value
Wechsler test of adult reading (WTAR) – standardised score				
Mean ± SEM	101.3 ± 2.8	95.8 ± 2.9	5.5 ± 4	0.2
SD	15.3	15.6		
95% CI	95.5 – 107.1	90 – 101.6	-2.5 – 13.6	
Median	101	97		
Interquartile range	89 - 114	89.8 - 108		
Ravens Matrices				
Mean ± SEM	29.7 ± 0.7	30.1 ± 0.8	-0.4 ± 1.1	0.7
SD	3.5	4.4		
95% CI	28.2 – 31.1	28.4 – 31.7	-2.6 – 1.7	
Median	29	31		
Interquartile range	27.8 – 32.5	26 - 34		

SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

Figure – 33. Comparison of mean baseline IQ scores.

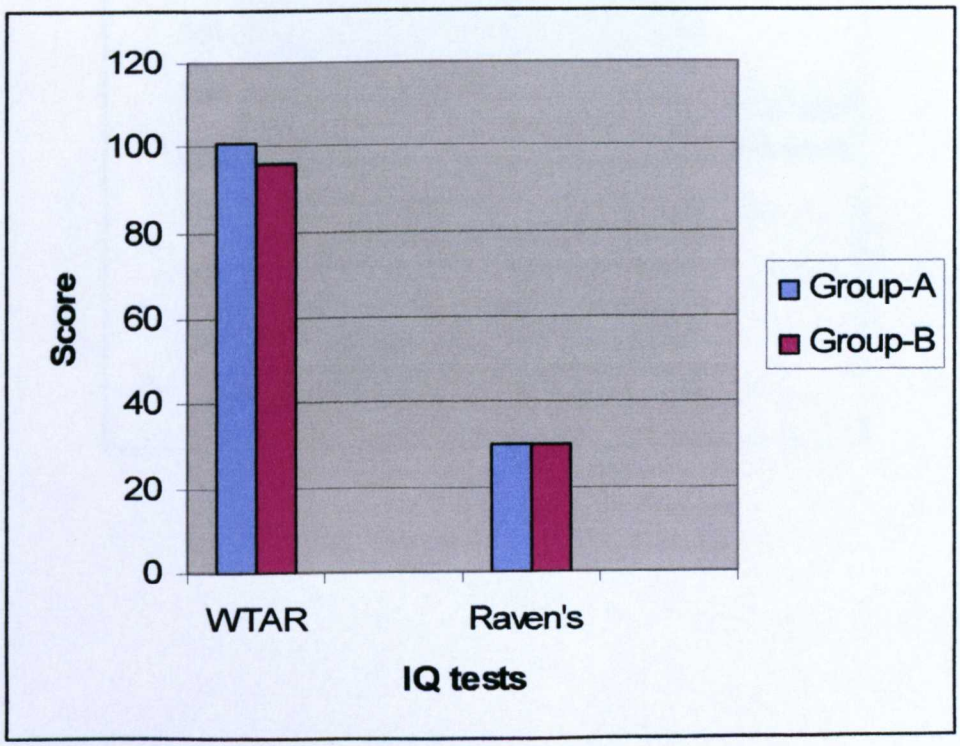


Table 21 – Intergroup comparison of neurocognitive test-retest interval (days) – Unpaired t-test.

Interval between neuropsychomertic assessment	Group-A	Group-B	Inter group difference	p-value
Mean ± SEM SD 95% CI	120.1 ± 3.6 19.3 112.8 – 127.5	126.3 ± 4.3 23.5 117.5 - 135	-6.1 ± 5.6 -17.4 – 5.1	0.3

SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

Figure – 34. Mean test retest interval in days

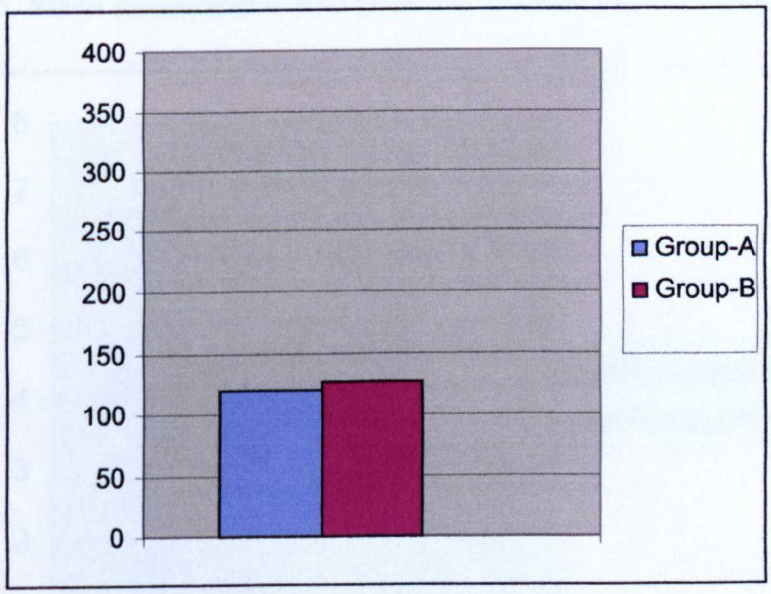


Table 22 – Preoperative and postoperative hospital anxiety-depression scale (HADS) scores in group-A – Paired t-test.

HADS component	Preoperative score	4 months postoperative score	Difference in scores	p-value
Anxiety				
Mean ± SEM	6.7 ± 0.8	4.9 ± 0.6	1.8 ± 0.6	0.008
SD	4.4	3.3	3.3	
95% CI	5 – 8.4	3.7 – 6.2	0.5 – 3.1	
Median	6	5		
Interquartile range	4 – 8.7	2 - 7		
Depression				
Mean ± SEM	4.6 ±0.6	3.3 ± 0.5	1.3 ± 0.6	0.03
SD	3.1	2.7	3.1	
95% CI	3.4 – 5.8	2.3 – 4.3	0.1 – 2.5	
Median	4	3		
Interquartile range	1.3 – 6.8	1 – 4.8		

SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

Figure – 35. Mean preoperative and postoperative HADS scores in group-A.

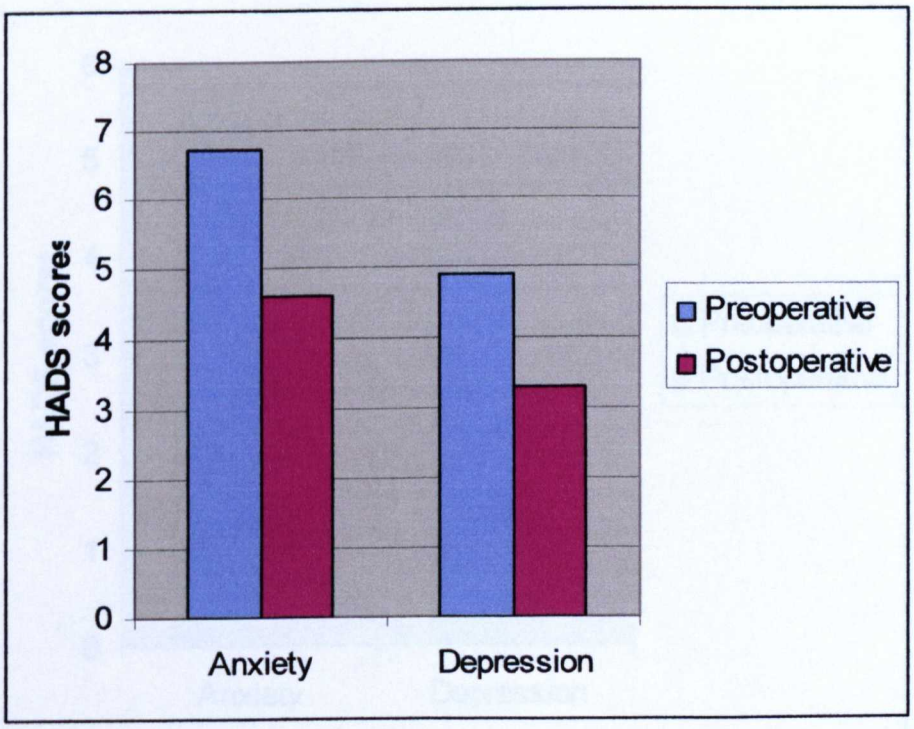


Table 23 – Preoperative and postoperative hospital anxiety-depression scale (HADS) scores in group-B – Paired t-test.

HADS component	Preoperative score	4 months postoperative score	Difference in scores	p-value
Anxiety				
Mean ± SEM	5.6 ± 0.8	4.6 ± 0.6	1 ± 0.6	0.1
SD	4.1	3.3	3.1	
95% CI	4.1 - 7	3.3 – 5.8	-0.2 – 2.2	
Median	5	3		
Interquartile range	2.5 - 8	2 - 4		
Depression				
Mean ± SEM	3.2 ± 0.4	2.3 ± 0.4	0.9 ± 0.4	0.03
SD	2.4	2.4	2.2	
95% CI	2.4 – 4.1	1.4 – 3.2	0.1 – 1.8	
Median	3	2		
Interquartile range	2 - 7	0.2 - 4		

SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

Figure – 36. Mean preoperative and postoperative HADS scores in group-B.

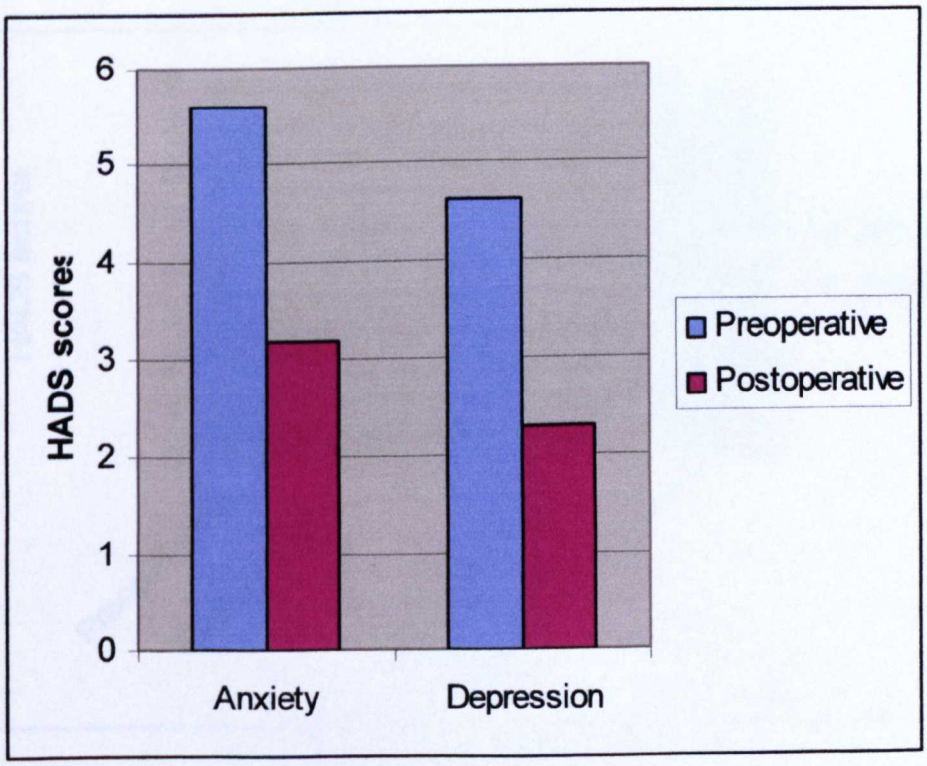


Table 24 - Intergroup comparison of preoperative and postoperative hospital anxiety depression scale (HADS)scores - Unpaired t-test.

HADS component		Group-A	Group-B	Intergroup difference	p-value
Anxiety	**Preoperative**				
	Mean ± SEM	6.7 ± 0.8	5.6 ± 0.8	1.2 ± 1.1	0.3
	SD	4.4	4.1		
	95%CI	5 – 8.4	4.1 - 7	-1.1 – 3.4	
	Postoperative				
	Mean ± SEM	4.9 ± 0.6	4.6 ± 0.6	0.4 ± 0.9	0.7
	SD	3.3	3.3		
	95% CI	3.7 – 6.2	3.3 – 5.8	-1.4 – 2.1	
Depression	**Preoperative**				
	Mean ± SEM	4.6 ±0.6	3.2 ± 0.4	1.4 ± 0.7	0.1
	SD	3.1	2.4		
	95% CI	3.4 – 5.8	2.4 – 4.1	-0.09 – 2.8	
	Postoperative				
	Mean ± SEM	3.3 ± 0.5	2.3 ± 0.4	1 ± 0.7	0.2
	SD	2.7	2.4		
	95% CI	2.3 – 4.3	1.4 – 3.2	-0.4 – 2.3	

SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

Figure – 37. Comparison of mean HADS scores in group-A and B.

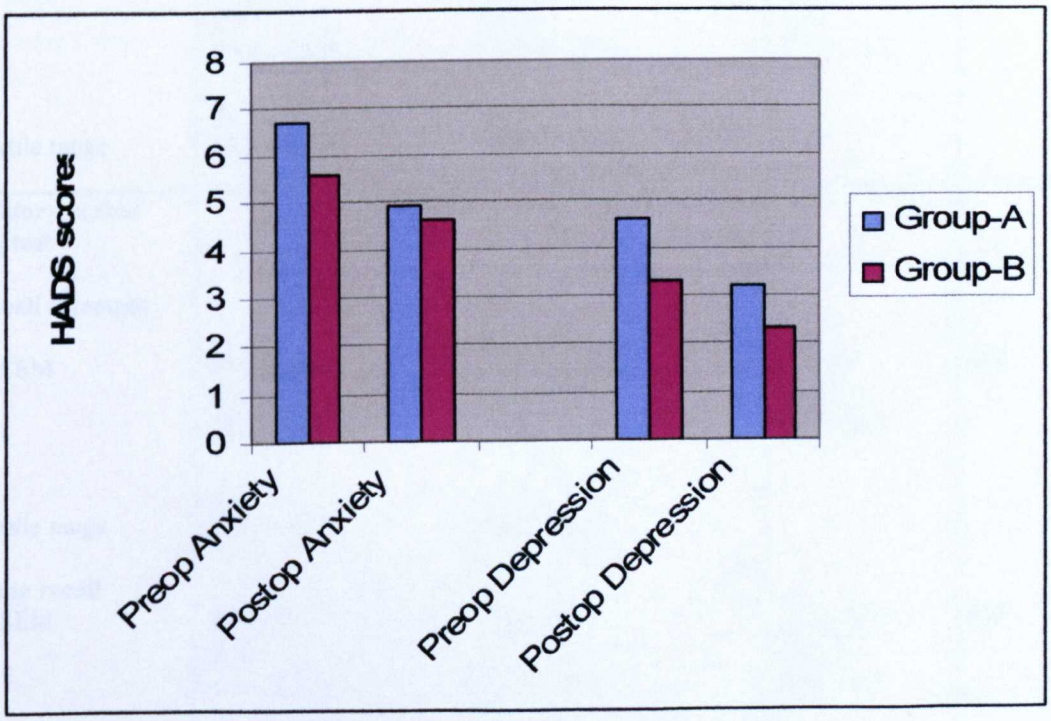


Table 25 - Preoperative and postoperative neurocognitive test scores in group-A - Paired t-test.

Neurocognitive test	Preoperative score	4 months postoperative score	Difference in scores	p-value
Trail making – A				
Mean ± SEM	41.7 ± 2.8	40.6 ± 2.9	1.1 ± 2	0.6
SD	14.9	15.7	10.7	
95% CI	36.1 – 47.4	34.6 – 46.6	-3 – 5.2	
Median	35	38		
Interquartile range	30.5 - 55	30 – 47.5		
Trail making – B				
Mean ± SEM	101 ± 8.1	107 ± 10.2	-6 ± 7.7	0.4
SD	43.5	54.7	41.2	
95% CI	84.5 – 117.6	86.2 – 127.8	-21.6 – 9.7	
Median	96	87		
Interquartile range	67 – 109.5	69 – 132.5		
Grooved pegboard				
Dominant hand				
Mean ± SEM	19.1 ± 0.4	19.1 ± 0.5	-0.04 ± 0.4	0.9
SD	2.2	2.6	2.3	
95% CI	18.2 – 19.9	18.1 – 20.1	-0.9 – 0.8	
Median	18.8	18.8		
Interquartile range	17.5 – 20.9	17 – 20.9		
Non-dominant hand				
Mean ± SEM	21 ± 0.4	20.4 ± 0.5	0.6 ± 0.3	0.03
SD	2.3	2.6	1.4	
95% CI	20.1 – 21.9	19.4 – 21.4	0.05 – 1.1	
Median	21	20		
Interquartile range	19.1 – 22.5	18.5 – 21.9		
Rey auditory verbal learning test				
Total recall attempts 1-5				
Mean ± SEM	41.2 ± 1.8	40.6 ± 1.9	0.6 ± 2	0.7
SD	9.6	10.4	10.6	
95% CI	37.6 – 44.9	36.6 – 44.5	-3.4 – 4.7	
Median	41	37		
Interquartile range	34.5 – 47.5	33 – 48.5		
Immediate recall				
Mean ± SEM	8.1 ± 0.6	7.8 ± 0.6	0.3 ± 0.5	0.5
SD	3.3	3	2.6	
95% CI	6.8 – 9.3	6.6 – 8.9	-0.7 – 1.3	
Median	7	8		
Interquartile range	5 - 11	5 - 10		

Delayed recall				
Mean ± SEM	8.4 ± 0.6	7.7 ± 0.6	0.7 ± 0.5	0.2
SD	3.5	3	2.5	
95% CI	7.1 – 9.7	6.6 – 8.7	-0.3 – 1.6	
Median	8	8		
Interquartile range	6 - 12	5 - 10		
Recognition				
Mean ± SEM	11.8 ± 0.5	12.1 ± 0.4	-0.3 ± 0.5	0.5
SD	2.8	2.3	2.7	
95% CI	10.7 – 12.9	11.2 - 13	-1.4 – 0.7	
Median	12	13		
Interquartile range	10 – 14.8	11 – 13.8		
Information processing Table A				
Mean ± SEM	59 ± 2.7	61.1 ± 2.8	-2.2 ± 2.5	0.4
SD	14.6	15.1	13.4	
95% CI	53.4 – 64.5	55.4 – 66.9	-7.3 – 2.9	
Median	60	63		
Interquartile range	48 – 69.5	49 – 73.5		
Digit span forwards				
Mean ± SEM	9.9 ± 0.5	9.5 ± 0.5	0.4 ± 0.5	0.4
SD	2.7	2.6	2.4	
95% CI	8.9 – 10.9	8.5 – 10.5	-0.5 – 1.3	
Median	10	10		
Interquartile range	7.3 - 12	8 - 11		
Digit span backwards				
Mean ± SEM	6.8 ± 0.5	6.3 ± 0.4	0.5 ± 0.4	0.2
SD	2.5	2.2	1.9	
95% CI	5.8 – 7.7	5.5 – 7.2	-0.3 – 1.2	
Median	6	6		
Interquartile range	5 – 8.8	5 - 8		

Table 26 - Preoperative and postoperative neurocognitive test scores in group-B - Paired t-test.

Neurocognitive test	Preoperative score	4 months postoperative score	Difference in scores	p-value
Trail making – A				
Mean ± SEM	43 ± 3	41.4 ± 2.8	1.6 ± 3.2	0.6
SD	16.3	15.5	17.6	
95% CI	36.7 – 49.1	35.5 – 45.3	-5 – 8.1	
Median	39.5	38		
Interquartile range	31 - 50	29.8 – 55.3		
Trail making – B				
Mean ± SEM	127.1 ± 9.6	108.7 ± 8.3	18.4 ± 7	0.01
SD	52.6	45.5	38.6	
95% CI	107.5 – 146.8	91.7 – 125.7	4 – 32.8	
Median	114	100		
Interquartile range	89.3 – 158.5	69.5 - 134		
Grooved pegboard				
Dominant hand				
Mean ± SEM	19.7 ± 0.5	19.1 ± 0.5	0.7 ± 0.4	0.1
SD	2.8	2.5	2.1	
95% CI	18.7 – 20.7	18.2 - 20	-0.1 – 1.5	
Median	19	19		
Interquartile range	17.5 – 21.6	17 - 20		
Non-dominant hand				
Mean ± SEM	21.1 ± 0.5	20.9 ± 0.7	0.2 ± 0.5	0.7
SD	3	3.7	2.6	
95% CI	20 – 22.1	19.5 – 22.2	-0.8 – 1.1	
Median	21	20		
Interquartile range	18.8 - 22	19 - 23		
Rey auditory verbal learning test				
Total recall attempts 1-5				
Mean ± SEM	37.8 ± 2.1	39.4 ± 2.1	-1.6 ± 2	0.4
SD	11.5	11	11	
95% CI	33.5 – 42.1	35.2 – 43.5	-5.7 – 2.5	
Median	36.5	37.5		
Interquartile range	29 – 47.3	32 – 45.3		
Immediate recall				
Mean ± SEM	7.5 ± 0.5	7.5 ± 0.6	0.07 ± 0.6	0.9
SD	2.7	3.5	3.4	
95% CI	6.5 – 8.6	6.2 – 8.8	-1.2 – 1.3	
Median	7	8		
Interquartile range	6 – 8.3	6 – 10.3		

Delayed recall				
Mean ± SEM	7.6 ± 0.7	7.9 ± 0.6	-0.3 ± 0.7	0.7
SD	3.6	3.2	3.7	
95% CI	6.3 – 8.9	6.7 – 9.1	-1.7 – 1.1	
Median	7.5	7		
Interquartile range	5 – 9.3	5.8 – 10.3		
Recognition				
Mean ± SEM	11.4 ± 0.5	12.4 ± 0.4	-0.9 ± 0.5	0.07
SD	2.7	1.9	2.6	
95% CI	10.4 – 12.5	11.6 – 13.1	-1.9 – 0.09	
Median	11	13		
Interquartile range	10 - 14	11 - 14		
Information processing Table A				
Mean ± SEM	52.9 ± 2.9	55.5 ± 2.5	-2.6 ± 2.9	0.4
SD	15.8	13.7	15.5	
95% CI	46.8 – 58.9	50.3 – 60.7	-8.5 – 3.2	
Median	50	53		
Interquartile range	43 - 61	47.5 – 60.5		
Digit span forwards				
Mean ± SEM	8.9 ± 0.4	9.2 ± 0.5	-0.3 ± 0.5	0.6
SD	2.3	2.5	2.8	
95% CI	8 – 9.8	8.2 – 10.1	-1.3 – 0.8	
Median	8.5	9		
Interquartile range	7.8 - 11	7.8 - 11		
Digit span backwards				
Mean ± SEM	5.9 ± 0.4	6.1 ± 0.4	-0.2 ± 0.3	
SD	2.1	2.1	1.7	0.5
95% CI	5.1 – 6.7	5.3 – 6.9	-0.8 – 0.4	
Median	6	6		
Interquartile range	4.8 - 7	5 - 8		

SEM – Standard error of mean
SD - Standard deviation
CI – Confidence interval

Table 27 – Test-retest neurocognitive score change in group-A.

Score change & number of patients	Trail making - A	Trail making - B	Grooved pegboard	Rey auditory verbal learning test	Information processing	Digit span forward	Digit span backward
Improvement (learning effect)	17	12	14	6	15	11	12
No change	0	0	1	0	3	6	4
Decline	12	17	14	23	11	12	13
Decline of ≥ 1 SD from their preop scores	2	3	6	19	3	6	10
Decline of ≥ 1 SD from their preop scores in ≥ 2 tests (significant decline)	16						

Table 28 – Test-retest neurocognitive score change in group-B.

Score change & number of patients	Trail making - A	Trail making - B	Grooved pegboard	Rey auditory verbal learning test	Information processing	Digit span forward	Digit span backward
Improvement (learning effect)	19	24	12	9	21	12	11
No change	0	0	0	0	0	8	11
Decline	11	6	18	21	9	10	8
Decline of ≥ 1 SD from their preop scores	4	2	4	10	4	5	5
Decline of ≥ 1 SD from their preop scores in ≥ 2 tests (significant decline)	9						

Applying the definition mentioned previously, 16 patients in group-A and 9 patients in group-B had significant neurocognitive dysfunction. Using the Chi-square test this difference in outcome was statistically significant (p-0.05).

Figure – 38. Number of patients showing significant (1SD) decline in individual neurocognitive tests at 4 months.

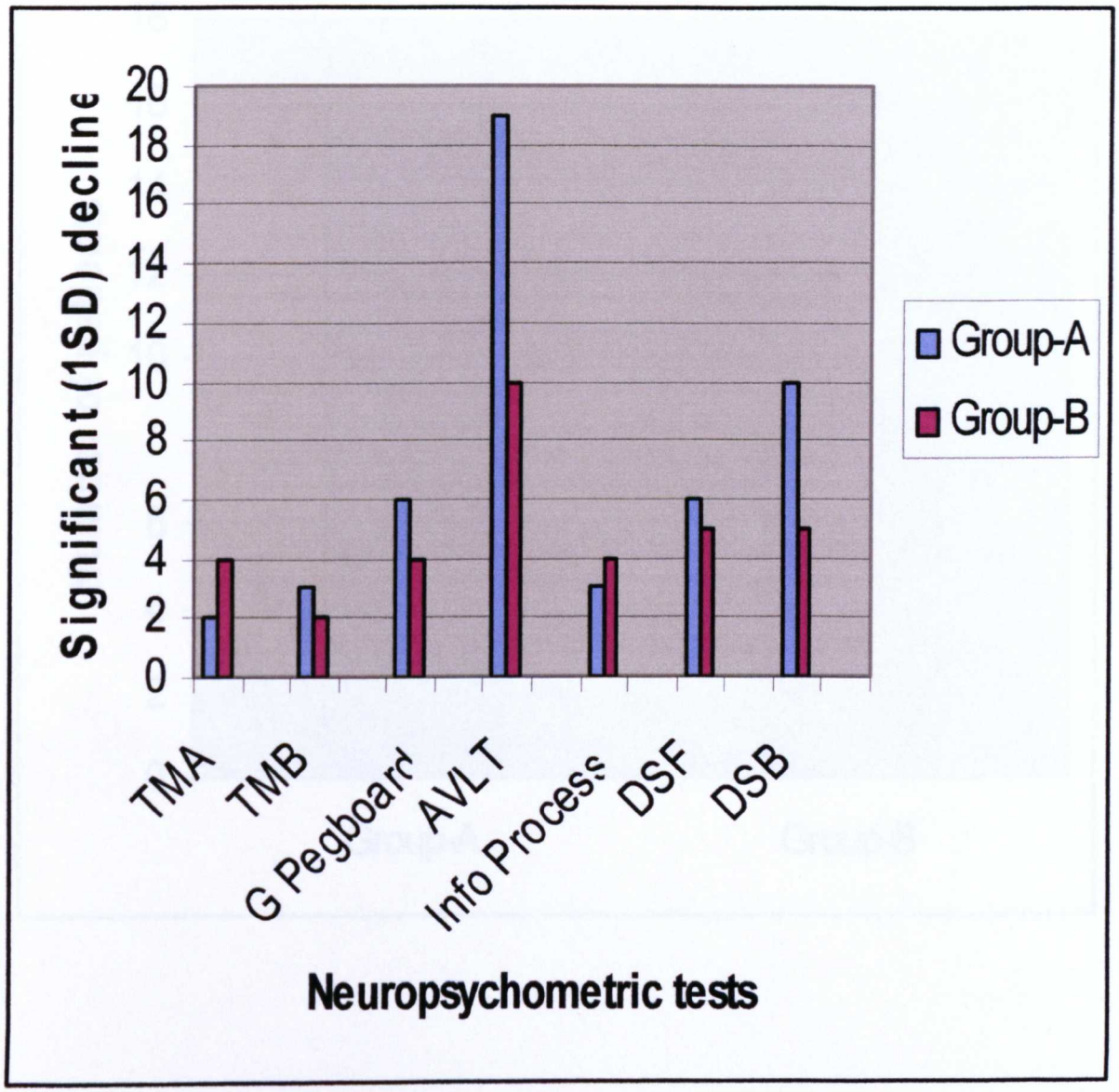


Figure – 39. Number of patients with neurocognitive decline at 4 months in each group (p-0.05).

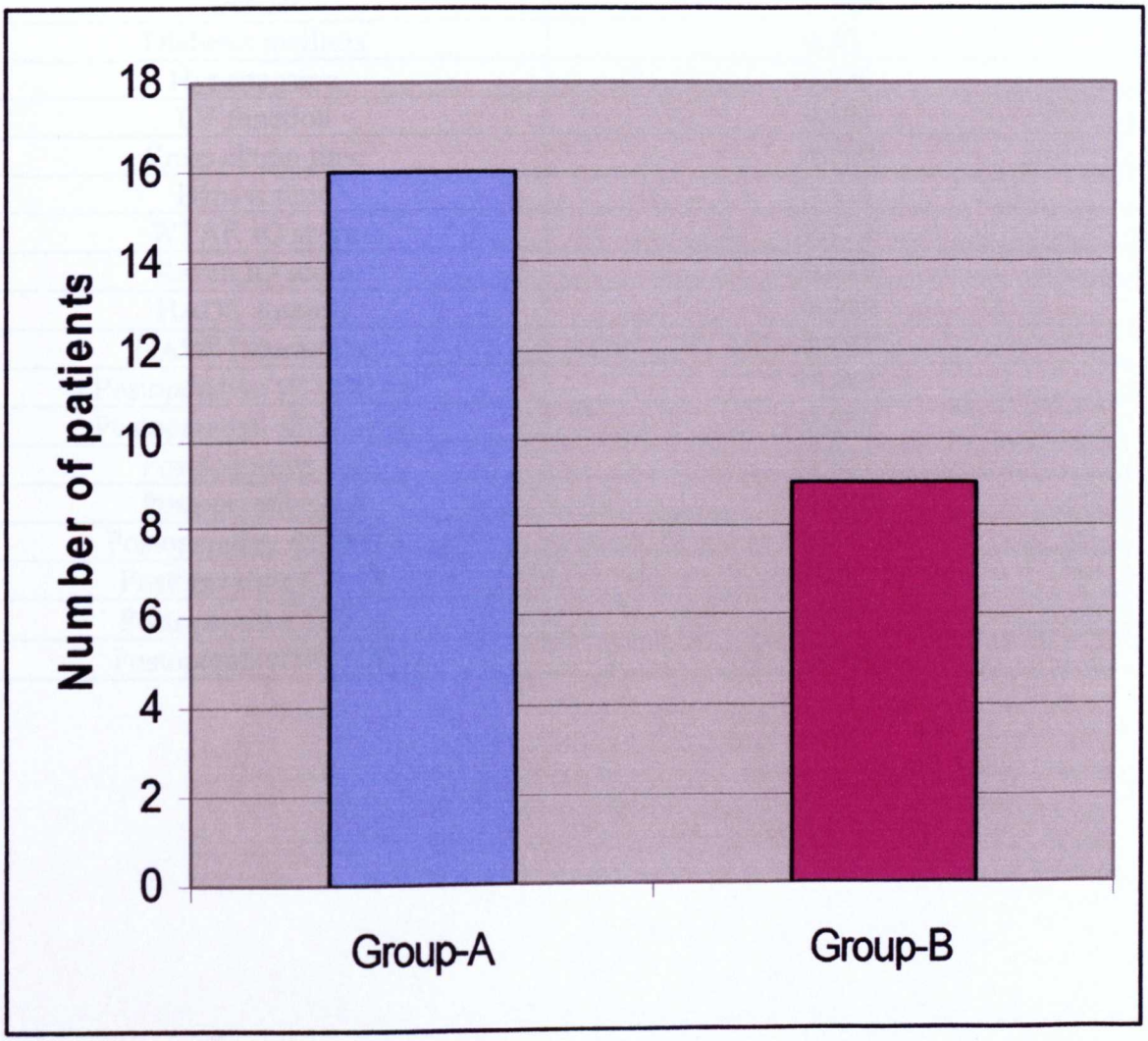


Table - 29. p-values for fitting logistic regression models to neurocognitive decline.

Variable	P-value
Age	0.329
Gender	0.389
Diabetes mellitus	0.327
Hypertension	0.339
LV function	0.396
Cross clamp time	0.200
Bypass time	0.328
WTAR IQ score	0.374
Raven IQ score	0.242
HADS Anxiety	0.495
HADS Depression	0.437
Postoperative sE-selectin	0.442
Postoperative sP-selectin	0.219
Postoperative IL6	0.898
Postoperative IL8	0.559
Postoperative ICAM14	0.770
Postoperative CD-18	0.253
Postoperative TNF α	0.830
Postoperative HSP-70	0.658

Discussion

4.1 Reason for the choice of study

Coronary artery bypass surgery is the most commonly performed operation worldwide. Despite advances, as previously stated there is a significant risk of brain injury inherent with the procedure. The procedure itself and the use of the cardiopulmonary bypass machine trigger an inflammatory response which plays an important role in progressive organ damage.

Hyperbaric oxygen therapy is relatively safe, has been used as adjunctive therapy in the treatment of certain types of brain injury. A review of some of the recently published work shows that cerebral **(Wada et al 1996, Prass et al 2000 and Miljkovic et al 2003)** and spinal **(Dong et al, 2002)** ischaemic tolerance can be induced by pre-treatment with hyperbaric oxygen in animals. Though the precise mechanism of this effect continues to be investigated, limitation of leucocyte induced secondary damage by the reduction in inflammatory cytokines and adhesion molecules is believed to play a major part. The potential clinical application, given this background and the relative safety of hyperbaric therapy were the main reasons for initiating this study.

The decision on which inflammatory markers to analyse was taken following a review of the published literature and the availability, reproducibility, sensitivity, specificity and the cost of the tests. The logic behind analysing cytokines, adhesion molecules and stress response proteins was to obtain a snapshot of different phases of the inflammatory cascade.

Neurocognitive examination is a time-tested method of identifying cognitive dysfunction resulting from brain injury. Despite the advent of functional MRI scan, when performed by trained individuals, neurocognitive examination is still considered to be a reliable and

reproducible way of identifying, assessing and measuring brain injury. In choosing the neurocognitive test battery, the aim was to perform a global assessment of different domains within an optimum time period without mentally fatiguing the patient. In keeping with the recommendations of the consensus conference on CNS dysfunction after cardiac surgery **(Murkin et al, 1995)**, the 4 core tests, trail making-A, trail making-B, grooved peg board and the auditory verbal learning test, were retained. In addition to the 4 core tests patients had to perform the information processing test, digit span forwards and digit span backwards. All the 14 recommendations of the conference were complied with during the conduct of the study.

4.2 Demographic and preoperative variables

The unpaired t-test and chi-square test were used to compare the demographic and preoperative clinical data (Table –13). Both groups were comparable in terms of age, gender ratio, body mass index, hypertension, diabetes mellitus, unstable angina, New York Heart Association (NYHA) dyspnoea, left main stem disease, coronary disease severity, left ventricular function, arrhythmias, renal dysfunction and peptic ulcer disease. None of the patients had any history of cerebrovascular disease. More patients in group B had Canadian Cardiovascular Society (CCS) class I (18.7%) and class III (50%) angina while the majority (67.7%) of patients in group-A had class II symptoms (p-0.009). In group-A, 9.7 % had a history of myocardial infarction, compared to 31.3 % had a history in group-B (p-0.03). There was no significant difference between the two groups in either of the commonly used risk-stratification scores, Parsonnet score or Euroscore. Thus overall the two groups were well matched except for angina class and previous myocardial infarction which were both significantly higher in group-B.

4.3 Intraoperative data and postoperative outcome

The unpaired t-test and chi-square test were used to compare the intraoperative data and postoperative clinical outcome (Table-14). More patients (62.5%) in group-B had 3 grafts compared to group-A (48.4%), however this difference was not statistically significant (p-0.07) and the myocardial ischaemia time and cardiopulmonary bypass time were comparable between the two groups. Again there was no significant difference in the postoperative inotrope requirement or incidence of arrhythmias, between the groups. One patient in group-B had renal dysfunction from which he fully recovered prior to discharge. One patient in group-A had an episode of acute confusion while another had a transient ischaemic attack, both patients recovered fully within 24 hours. There was no incidence of postoperative myocardial infarction, major sepsis, multiorgan failure or mortality in either group. Compared to group-A, the mean ventilation time (7.7 hours versus 4.2 hours), intensive care unit stay (1.4 days versus 1.1 days) and postoperative duration of stay (9days versus 6.4 days) were shorter in group-B, however the differences were not statistically significant. Thus the overall early postoperative outcome was comparable between the two groups.

4.4 Inflammatory markers

The postoperative trend during each assessed time points for all the measured inflammatory mediators were essentially similar in both groups (Tables-15 and 16). Levels of sE-selectin, IL6, IL8, CD-18, TNFα and HSP-70 peaked at 2 hours and declined at 24 hours. sP selectin level showed a declining trend while ICAM-1 initially declined at 2 hours before reaching preop levels at 24 hours. Analysis of variance revealed a significant postoperative rise in sE-selectin (p-0.05), CD-18 (p-0.001), and HSP-70 (p-0.007) in group-A, but not in group-B (p-

0.2, p-0.1, and p-0.2, respectively). The postoperative rise in IL6, IL8, ICAM-1 and TNFα were significant in both groups, while that of sP selectin was not significant in either group. As noted previously both groups were well matched in terms of preoperative, intraoperative and postoperative factors which could influence the inflammatory response. Most importantly the myocardial protection technique (intermittent fibrillatory arrest), surgical technique, the bypass circuit and pump used were the same in all patients. The duration of myocardial ischaemia, and duration of cardiopulmonary bypass were again similar in both groups. Thus despite comparable operative and perioperative factors the postoperative rise in adhesion molecules, sE-selectin and CD-18, and the stress response protein HSP-70 were statistically significant in group-A, but not so in group-B.

The main difference between this study and previously published work using hyperbaric oxygen as a modulator of inflammatory response is that our main trigger for inflammation was extracorporeal circulation and not, sepsis **(Weisz et al 1997, Granowitz et al 1999 and Benson et al 2003)**, shock **(Luongo et al 1998, Cuzzocrea et al 2000 and Yamashita et al 2000)**, ischaemia **(Zamboni et al 1996, Chen et al 1998, Tjarnstrom et al 1999, Yang et al 2001, Hong et al 2003)** or carbon monoxide poisoning **(Thom, 1993)**. In accordance with the National Institute for Health and Clinical Excellence (NICE) guidelines for the treatment of ischaemic heart disease, all the patients were on low dose aspirin, statin, beta-blockers, angiotensin converting enzyme (ACE) inhibitors and nitrates. Previous studies have shown that low dose asprin **(Cyrus et al 2002, Bates et al 2004, Hartel et al 2004)**, statins **(Nawami et al 2003, Omi et al 2003, Ascer et al 2004, Chello et al 2004, Zapolska-Downar et al 2004, Doo et al 2005, Pannu et al 2005, Tousoulis et al 2005)**, and ACE inhibitors **(Cominacini et al 2002, van Haelst et al 2003, Graninger et al 2004, da Cunha et al 2005)** can modulate inflammatory response. In addition there is a diurnal variation

(Maple et al 1998, Undar et al 1999, Kanabrocki et al 2001, Niehaus et al 2002, Osmanicik et al 2004) in baseline serum levels of inflammatory molecules, we used comparable timing of operation and blood sampling to minimise this effect. Thus pharmacotherapy, diurnal variation, species specific differences in inflammatory response and trigger sensitive differences in the activation, recruitment, and amplification of the various inflammatory cascades and molecules within the same species **(Boldt et al 1998 and Denizot et al 1998)** are important factors which could explain why the trend in certain markers like CD-18 correlated with previous work **(Ueno et al 1999 and Klans et al 2002)**, while others like TNFα **(Yamashita et al 2000, Yang et al 2001 and Benson et al 2003)** IL6 **(Weisz et al, 1997)**, and ICAM-1 **(Buras et al 2000 and Hong et al 2003)** did not. This difference persisted even after the preliminary results were corrected for haemodilution. Of note is the fact that despite the significantly higher postoperative levels in sE-selectin, CD-18, and HSP-70 in group-A, the overall trend in the rise and fall of all the measured markers were similar in both groups. Therefore in this study, the pooled data was further analysed for correlation between the postoperative levels of inflammatory markers and HSP-70 to, age, cardiopulmonary bypass duration and cross-clamp time (Tables – 17, 18 and 19). Though postoperative rise in inflammatory markers did not correlate to any of the factors mentioned above, HSP-70 showed a significant direct correlation to cardiopulmonary bypass time and cross-clamp time but not to age. This finding is consistent with previous animal **(Lee et al 2001 and Kokubo et al 2003)** and human studies **(Schmitt et al 2002, Rafiee et al 2003, Dybdhal et al 2004 and Lai et al 2004)** that have revealed HSP-70 to be an acute response protein induced by stressful stimuli.

4.5 Neurocognitive test – retest interval.

Analysis of the interval between preoperative and postoperative neurocognitive testing using the unpaired t-test, (Table-21) revealed no statistically significant difference between the two groups. The test-retest interval was thus comparable and neither group had a longer recovery period compared to the other.

4.6 Baseline intelligence quotient (IQ)

The Wechsler test of adult reading (WTAR) and Raven's matrices were used to measure the IQ of patients prior to the preoperative neurocognitive assessment. While the former is a measure of verbal IQ standardised to the age and education level of the patient, the latter is a measure of non-verbal IQ standardised for age. Comparison of the baseline IQ scores between the two groups using the unpaired t-test (Table-20) revealed that the two groups matched in terms of both verbal and non-verbal IQ level. Thus difference in intelligence levels which influences learning and performance was not a factor that affected this study.

4.7 Anxiety and depression scores

Both anxiety and depression can impair performance in neurocognitive tests. In order to assess their level of anxiety or depression, all patients were requested to complete the hospital anxiety and depression scale (HADS) questionnaire prior to each neurocognitive assessment. The preoperative and postoperative scores were compared within each group using paired t-test (Tables-22 and 23), and between the two groups using the unpaired t-test (Table-24). The intra-group comparison (Tables-22 and 23) of the preoperative and postoperative HADS

scores revealed that, both the anxiety and depression level of patients were higher during preoperative assessment in both groups. The postoperative reduction in both anxiety (p-0.008) and depression (p-0.03) scores were significant in group-A. In group-B, postoperative depression score was significantly lower (p-0.03), but the anxiety score, though lower was not statistically significant (p-0.1). Inter-group comparison (Table-24) of the preoperative and postoperative HADS scores revealed no statistically significant difference in the comparative level of anxiety or depression. Thus the mood state (anxiety or depression) during testing which could have a direct effect on the neurocognitive test score was not a factor which affected this study.

4.8 Neurocognitive test results.

The main problem during analysis and interpretation of neurocognitive test results of a group is the effect of practice and learning during repeat testing. This effect increases with more frequent testing and in order to minimise this confounding factor we limited the test battery to one postoperative reassessment. Just like the proportion of patients whose performance and scores decline on repeat testing, a proportion of patients will do the opposite and score higher. This phenomenon is due to the "practice" or "learning" effect, and it increases with the more number of times the patient is subjected to the same test battery. Though this effect can be reduced by using alternate forms of the same test and limiting the number of repeat tests, it cannot be completely eliminated. The result is that when the significance of the difference in group mean is calculated, the improvement in scores due to the "practice" effect off-sets the decline in scores of the patients who actually deteriorated, resulting in no statistical significance being demonstrated. The "learners" are patients who either suffered no ill effects from the procedure or those who cope and compensate remarkably well and would have

gained little by way of therapeutic intervention anyway. Thus the overall group mean loses its relevance in a clinical study aimed at preventing or reducing the deterioration caused by cardiopulmonary bypass. This effect is exemplified in the neurocognitive score results using the paired t-test (Tables-25 and 26). Using this test where the difference in overall group mean is analysed, a "significant improvement" was demonstrated in the grooved pegboard test using the non-dominant hand in group-A and the trail making test-B in group-B. On closer examination it becomes clear that this result is due to the high scoring "learners" overshadowing the patients who actually deteriorated.

A validated method **(Harrison et al 1989, Newman et al 1993, Pugsley et al 1994, Patel et al 1993 and 1996, Toner et al 1996, Braekken et al 1998 and Zamvar et al 2002)** of overcoming this effect and gaining a true insight into neurocognitive decline caused by cardiopulmonary bypass is the standard deviation index (SDI) method. This was also one of the definitions of cognitive decline proposed by the 1994 consensus conference on CNS dysfunction after cardiac surgery **(Murkin et al, 1995)**. According to this method a patient is considered to have major deterioration in a particular test if a test score deteriorates by ≥ 1 standard deviation (SD) from the preoperative score, and a significant neurocognitive impairment is defined as, major deterioration in more >20% of the tests (2 out of 7 in this study). Using this method, a significantly higher number of patients in group-A, 16 out of 29 (55%) compared to group-B, 9 out of 30 (30%), were found to have significant neurocognitive impairment (Tables-27 and 28). Using the Chi-square test this difference between the two groups was statistical significant at $p=0.05$.

The data was pooled and further analysed using multivariate logistic regression analysis to identify predictors and correlation between significant neurocognitive dysfunction and age,

gender, diabetes mellitus, hypertension, left ventricular function, cross-clamp time, cardiopulmonary bypass time, baseline IQ, increase in anxiety, increase in depression, and postoperative levels of inflammatory markers (Table-29). No definite correlation between any of the tested variables and the incidence of significant neurocognitive impairment was identified in this study. Previously identified predictors of neurocognitive decline include, increasing age **(Gardner et al 1985)**, diabetes mellitus **(Selnes et al, 1999)**, hypertension **(McKhann et al, 1997)**, atherosclerotic vascular disease **(Blauth et al 1992 and Hammon et al 1997)**, recent myocardial infarction **(Tuman et al, 1992)**, previous cerebrovascular accidents, chronic debilitating neurologic illness, lower education level, living alone, postoperative complications **(Ho et al, 2004)** and the duration of cardiopulmonary bypass **(Kilo et al 2001 and Van Dijk et al 2002)**. Unlike most of the previously mentioned studies which were non-randomised retrospective analysis of previously collected data, this was a prospective randomised double blind study with standardised data collection.

Chronic diabetes mellitus, hypertension and hyperlipidaemia play an important role in microangiopathic changes within the vasculature. A significant amount of data in earlier studies, were based on patients from an era when anti-platelet and lipid lowering agents were not part of routine pharmocotherapy for cardiac patients. All the patients in this study were on anti-platelet and lipid lowering medication. In addition, the effect of earlier diagnosis, better treatment and tighter control of diabetes mellitus, hypertension, hyperlipidaemia and peripheral vascular disease cannot be ignored. The role of early thrombolytic therapy in reducing embolic risk from mural thrombi could possibly be the reason why previous MI was not a risk factor in our study. Thus arguably, patients in this study were less prone to microangiopathic changes than the cohort of patients in the previously mentioned studies. This might explain why despite the higher mean age of patients in this study, age was not a

predictor of significant neurocognitive dysfunction after cardiopulmonary bypass. Due to the nature of the neurocognitive tests patients with a history of cerebrovascular or other neurologic diseases and learning difficulties had to be excluded from this study. Thus, it is impossible to say from this study whether these factors are predictor of neurocognitive decline after cardiopulmonary bypass in the general population. As per standard practice at the institution where this study was conducted, a 38μm arterial line filter was used to reduce microembolic load during cardiopulmonary bypass, in all patients. This could explain why unlike earlier studies, the duration of cardiopulmonary bypass did not correlate to the incidence of significant neurocognitive dysfunction in this study.

4.9 Strengths of the study.

This was a prospective randomised double blind trial. The overall recruitment rate was 83.9 % of the eligible patients. There were no crossovers between the groups. Both groups matched well in terms of demographic, preoperative, intraperative and postoperative factors. The anaesthetic, surgical and cardiopulmonary bypass factors were standardised. All patients underwent the same procedure using the same conduits and technique of anastomosis. Measurement of inflammatory markers were performed in an accredited immunology laboratory of the University of Hull using standardised techniques and using the same brand of reagents and analysis kits for all samples. Neurocognitive testing was performed by fully trained and qualified neuropsychologists from the University of Hull and all the recommendations of the consensus conference on CNS dysfunction after cardiac surgery **(Murkin et al, 1995)** were followed. Pre-treatment with hyperbaric oxygen was the only major difference between the two groups in this study.

4.10 Limitations of the study.

As mentioned earlier, of the 64 patients recruited for the study, there were 2 drop-outs from group-A, and 3 from group-B giving a final strength was 29 patients in group-A and 30 patients in group-B. This study was based on a small sample of low-risk patients at a single centre. It could be argued that this allows reproducibility of results and one cannot generalise the findings of this study. Only a prospective randomised controlled multicenter trial would provide the solution to this argument.

Operative and perioperative factors were standardised to the maximum extent possible, however, one factor which could have a bearing on neurocognitive outcome is the distribution of atherosclerotic plaques within the ascending aorta. Patients with cerebrovascular disease were excluded from the study and the surgeons externally palpated the aorta and avoided any areas of plaques during clamping of the aorta, however, this is not a foolproof method. Perhaps epi-aortic doppler scanning or trans oesophageal echocardiography would have been a better way of detecting aortic atherosclerosis.

All patients in this study had only one postoperative neurocognitive assessment, at 4 months. The primary reason was to minimise the "practice" effect associated with repeat testing. Secondly, earlier testing at say 6 weeks has the risk of being masked by continuing background interference from patient recovery and medications, while a more prolonged assessment at beyond 6 months has the risk of ongoing, new but unrelated, cerebral events interfering with the results. Thirdly, despite standardisation of the anaesthetic techniques and drugs used, possible variations in dosage, patient metabolism and elimination of the different drugs is inevitable. The time lapse between surgery and assessment would have certainly

allowed for any direct cerebral effects of the anaesthetic medications to be eliminated. Athough the outcome at 4 months is an indication of the long-term effects it may not necessarily reflect the permanent sequelae from the initial brain injury.

Considering the nature of this study (live human subjects as opposed to post mortem evaluation) it was unethical and inconceivable to conduct a histological evaluation of cerebral tissue samples. Recent studies **(Anderson et al 2000, Johnsson et al 2000, Westaby et al 2000)** have clearly proved that due to contamination mainly from haemolysed blood and adipose tissue, both S100β protein and neuron specific enolase are unreliable serum markers of brain injury after cardiac surgery. Examination of other tissue samples which would have given an insight into cellular level changes in enzymes induction or inhibition, free radical release or inhibition, antioxidant status and protein synthesis were not sampled due ethical reasons, financial and logistic limitations. However, previous animal studies have shown that pre-treatment with hyperbaric oxygen prior to ischaemia induces central nervous system ischaemic tolerance and better functional recovery **(Moor et al 1966, Wada et al 1996, Prass et al 2000, Dong et al 2002 and Miljkovic et al 2003)**. Other studies have shown that reducing levels of inflammatory mediators correlated with reduction in brain infarct volume following ischaemia **(Chopp et al 1996, Hallenbeck 1997, Prestigiacomo et al 1999 and Huang et al 2000)**. This study revealed that pre-treating patients with hyperbaric oxygen prior to cardiopulmonary bypass has a potential beneficial effect by reducing the postoperative rise in sE-selectin, CD-18 and HSP-70, and neurocognitive dysfunction, however, the actual mechanism underlying this benefit remains to be deciphered. As noted in the previous studies, reduction of inflammatory cytokines and adhesion molecules **(Weisz et al 1997, Granowitz et al 1999, Tjarnstrom et al 1999, Atochin et al 2000, Buras et al 2000, Yang et al 2001, Kalns et al 2002, Martin and Thom 2002, Benson et al 2003, Hong**

et al 2003 and Tahepold et al 2003), augmentation of antioxidant status **(Thom 1993, Chen et al 1998, Cuzzocrea et al 2000, Speit et al 2000, Kim et al 2001, Wada et al 2001 and Pedoto et al 2003)** and induction of apoptosis suppressor proteins **(Wada et al, 2001)** are potential mechanisms which contribute towards the preconditioning process. Perhaps future animal studies, and human studies incorporating muscle and nerve biopsies may shed more light on the other mechanisms involved in the process.

4.11 Potential for implementation into clinical practice.

Undeniably, further multi-centre prospective randomised controlled trials are warranted to confirm the findings of this study prior to clinical application. With the limited number of hyperbaric chambers in the country compared to cardiac surgical units, the major factor limiting implementation into clinical practice would be accessibility. This study used three hyperbaric oxygen treatment sessions. The cost of one hyperbaric oxygen session for this study was calculated to be £50. However, if one session could accomplish the same effect the technique becomes more cost-effective and clinically applicable. If the findings of this study can be confirmed by further studies, there is a definite scope for clinical application. Neurocognitive dysfunction does not necessarily prolong hospitalisation or add to the immediate cost of treatment but when the impact on a patient's behavioural outcome is considered the potential cost-effectiveness of this modality becomes clear.

4.12 Conclusion and future research.

In conclusion this study revealed that pre-treating patients with hyperbaric oxygen prior to cardiopulmonary bypass has a potential beneficial effect by reducing the postoperative rise in sE-selectin, CD-18 and HSP-70, and neurocognitive dysfunction. However, the actual mechanism underlying this beneficial effect still remains unclear. Pursuing cellular level changes in enzyme induction or inhibition, free radical release or inhibition, antioxidant status and protein synthesis may hold the answers to this and is an area for further research.

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Pretreatment with hyperbaric oxygen – Neurocognitive dysfunction and inflammatory response following cardiopulmonary bypass – A prospective randomised double blind trial. (Abstract Id: 23815) 2005 annual meeting (5-8 March 2005) Society of Cardio-Thoracic Surgeons (SCTS) of Great Britain and Ireland, London.

Trial application of a new modality to reduce neurocognitive dysfunction following cardiopulmonary bypass - Results of a prospective randomised double-blind study. (Abstract Id: 342) 85Th Annual meeting (10-13 April 2005) American Association of Thoracic Surgery (AATS), San Francisco, USA.

Hyperbaric preoxygenation a potential modulator of systemic inflammatory response to cardiopulmonary bypass – results of a prospective randomised double blind trial. (Abstract Id:21355) 3rd Annual meeting (12-15 September 2004) European Association of Cardio-Thoracic Surgery (EACTS) / European Society of Thoracic Surgery (ESTS), Leipzig, Germany.

Hyperbaric preoxygenation and neurocognitive dysfunction after cardiopulmonary bypass – results of a prospective randomised double blind trial.
(Abstract Id: 3651) 2004 Scientific Session (7-10 November 2004), American Heart Association (AHA), New Orleans, USA.

Antegrade-retrograde cold blood cardioplegia vs intermittent fibrillatory arrest – comparison of immediate postoperative outcome.
2003 Perfusion Congress (24 – 26 October 2003), Brighton, UK.

7 Appendix

7.1 Patient Information Leaflet.

A prospective randomised trial to assess the beneficial effect of preoperative hyperbaric oxygen on postoperative neuropsychological outcome and systemic inflammation in patients undergoing cardiopulmonary bypass.

REFERENCE NO OF STUDY OR PROTOCOL- ELSY ref 2690, LREC-07/02/128

We wish to invite you to take part in a research study. Before you decide whether to do so, please read the following information carefully and discuss it with friends, relatives and your GP if you wish. Please ask if there is anything that it not clear or if you would like more information. You will be given as much time as you want to make a decision.

What is the purpose of this study?

The cardiac bypass machine, sometimes called the heart lung machine, has revolutionised heart surgery.

Heart surgery is safer now than at any time in the past.

The advantages of the surgery in the light of your heart disease, outweighs any risks involved in the surgery. However, as with all surgical procedures, heart surgery has a complication rate. As far as the complications are concerned, the time patients spend in theatre on the bypass machine is associated with an increased likelihood of experiencing some of the complications. That is to say, the longer you are on the bypass machine the more chance you have of postoperative complications. For example brain injury ~ ranging from minor temporary problems to more long-term effects. Another complication involves the body mounting an inflammatory response to the bypass machine that causes further illness.

The team of doctors and nurses who are managing your operation are very well aware of the complications and are actively seeking to reduce their effects. They believe that the body's response to the bypass machine and the number of patients who have brain injury after the operation, can be reduced, by breathing high concentration of oxygen before the surgery. This study is designed to compare two groups of patient who are having heart surgery. One group will breathe high concentration oxygen the day before, and on the day of surgery, the other group will be managed in the normal manner.

Why have I been invited?

To complete the study, that is, to answer the question, "Is high dose oxygen beneficial before cardiac surgery?" We need to have **64** patients to study. The **64** patients will be divided into two groups. One group will be given the high dose oxygen – a process called hyperbaric oxygen - before surgery; the other group will have their surgery in the current normal manner. All of the patients in each group need to be similarly matched for age, sex and to have no illness or conditions that might affect the test results. Thus on the completion of the study we would have two groups of the same size, with similar patients in each group. To achieve this

we are inviting all eligible patients to join the study in the hope that we will eventual recruit the 64 volunteer patients we need.

What will happen if I decide to take part?

If you agree to join the study you will be randomly allocated to one of the study groups. I.e. either to the "Hyperbaric oxygen + heart surgery group"(Group – B) or the "Normal air + heart surgery group"(Group – A). Should you choose to enter this study, neither the doctors nor you, will be able to choose which group you are in. This is important to prevent the selection of patients from unfairly affecting the results.

Either as a patient in the "Hyperbaric oxygen + heart surgery group" or the " Normal air + heart surgery group", we will want to take blood samples from you at fixed time points in relation to your operation. There will not be any need to prick you for the blood samples as you will have a special tube in your vein which will allow us to take blood without you feeling any pain. Additionally, there are some memory and reasoning tests we will require you to do before your surgery and again about 4 months after surgery. These tests involve, for example, trying to memorise a few words, joining dots on a picture and placing pegs in a board whilst being timed. How long you take is not of interest, but any difference between your performance, before and after surgery, is of interest. The results of tests are not compared with the results of other patients. So don't worry about how well you might perform these tasks, but do try your best. Once enrolled in the study you will have the memory & reasoning tests on a mutually agreed day the week before your operation.

We will require you to attend the BUPA hospital in Anlaby, Hull twice the day before & once during morning of your operation. Please come prepared for admission to Castle Hill Hospital the day before your operation. Transport will be arranged to take you to & back from the BUPA hospital. During these oxygen or air breathing sessions you will be required to breathe pure oxygen or air in a specially built "hyperbaric" chamber. The chamber is quite large and able to hold up to 8 patients at any one time (The doctor will show you a photograph of the chamber). Whilst in the chamber you will be accompanied by a qualified nurse. The oxygen or air breathing period last for around a total of 60 minutes. Transport, to take you to the chamber and back to castle Hill Hospital will be provided. Whilst you probably have not heard of this form of oxygen or air breathing, the chamber at the BUPA hospital has carried out thousands of these oxygen and air breathing session without any notable complications.

What do I have to do?

If you agree to take part in the study, You will be given a date and time for the memory & reasoning tests (a week prior to the operation).

Once you have had the memory & reasoning tests you are free to go home. You are requested to return to ward 6, Castle Hill Hospital, at 8.45AM the day before your operation (unless the doctor has asked you to come in at some other time). The "oxygen" or "air" breathing sessions will be at 9AM and 6PM the day before your operation and at 7AM the morning of your operation day. This will not interfere with your routine care and medications.

Please make sure that you attend on time for the oxygen breathing sessions. These sessions are done in groups and a missed turn would mean waiting for a few hours before the next turn.

All patients in the study will be requested to attend ward 6 at Castle Hill Hospital (CHH) for a repeat memory & reasoning tests about 4 months after the operation. The doctor will ring you up after you have been discharged from hospital and give you the time & date for the test.

Do I have to take part?
Only if you wish to do so.
Participation is voluntary, you may refuse to participate or withdraw from the study at any time. But please let us know if you are unable fully to take part, as doing only parts of the study, rather than all of it, will affect the value of the research. You do not need to tell us why you do not want to take part. If you choose to withdraw or not to participate, your decision will in no way affect your future treatment. Sometimes the investigator or sponsor of the study may consider that it is in your interests to withdraw you or stop the study altogether.

Are there any risks involved?
Taking part in the study does not increase your operation risk in any way.
However, all the risks associated with heart surgery and general anaesthesia will remain the same.

Ear discomfort / ears "popping" can rarely occur in some people during the treatment. This is similar to the feeling during taking-off and landing in an aeroplane or diving underwater.

Are there any costs involved?
No

Confidentiality
In order to meet legal obligations, a member of the local research ethics committee may inspect your hospital records. Details of your treatment and your past relevant medical history as required for the study, will be recorded on a Case Record Form (CRF) the information from which will be entered onto computer in the cardiothoracic department research office. A CRF includes all information collected in the course of the research study. This information will be retained by the cardiothoracic department at Castle Hill Hospital (CHH) and may be passed on to the authorised regulatory authorities. Access to data will be limited to the named researchers directly involved in the study.

The records will identify you only by a number (not your hospital number) and your initials. All information in your notes and CRF will be treated in strict confidence. A copy of this Informed Consent Form will be kept with the CRF and you will be given a copy.

When you attend the BUPA hospital for the "oxygen" or "air" breathing sessions, your medical notes will be personally taken from Castle Hill Hospital by the researchers and brought back to Castle Hill Hospital at the end of each session. At no time will the notes be accessible to anyone other than the researchers and the above-mentioned personnel.

The information from this study will be retained until the data are analysed and published and for a further 3 years in the hospital.
By signing the attached consent form you give permission for the above to occur.

If you agree to participate in this study, your General Practitioner will be informed, unless you state otherwise.

Your rights

Your participation in this study is entirely voluntary and refusal to participate will not affect your treatment and care or any other medical treatment. You may, without giving reason, refuse to take part, or withdraw from the trial, and this will not in any way affect your continuing treatment by your doctor. Your doctor will give you any relevant updated information about hyperoxia that may occur during the study.

Who is organising and funding the research?

The study has been sponsored by the Cardiothoracic department of Castle Hill Hospital (CHH).

Trial-related injury

If you suffer from injury or illness, as a result of participation in this study, compensation and the usual NHS procedures for redress will be available to you.
If you suffer from illness or injury during the study, or have any questions about the research study, please contact.

Mr S C Griffin
Consultant Cardiothoracic Surgeon
Castle Hill Hospital
Castle Road
Cottingham
HU16 5JQ
Tel: 01482 623623
Fax: 01482 623257

For any further information regarding the study please contact,

Mr J Alex
Clinical Research Fellow
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Cardiothoracic Surgery
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HU16 5JQ
Tel: 01482 875875 - Bleep 645 / 01507 363541 / 07939595029

Thank you

7.2 Informed Consent.

A prospective randomised trial to assess the beneficial effect of preoperative hyperbaric oxygen therapy on postoperative neuropsychological outcome and systemic inflammatory response in patients undergoing cardiopulmonary bypass.

Protocol number/identifier – ELSY ref 2690, LREC –07/02/128

NAME OF LOCAL LEAD RESEARCHER: Mr S C Griffin

SUBJECT ID or HOSPITAL NO: _____

Please initial box

1) I confirm that I have read and understand the information sheet dated(version..) for the above study and have had the opportunity to ask questions. ☐

2) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected ☐

3) I understand that sections of any of my medical notes relating to my taking part in the study may be looked at by responsible individuals from the Hull & E Yorkshire NHS Trust / local regional ethics committee or from the appropriate regulatory authority(ies). I give permission for these individuals to have access to my records. ☐

4) I understand that samples of my blood will be stored and examined at the end of the study. I also understand I will have memory & reasoning tests before and after my operation. I give my permission for this. ☐

5) I agree to take part in the above study. ☐

Name of Subject (BLOCK CAPITALS)	Date	Signature
Name of Person taking consent	Date	Signature
Researcher/witness	Date	Signature

1 copy for subject; 1 for researcher; 1 to be kept with hospital notes

7.3 Letter to patient's general practitioner (GP)

Date

Dear Dr

Re: Patient Name and Address.
.....
.....

A prospective randomised trial to assess the beneficial effect of preoperative hyperbaric oxygen therapy on postoperative neuropsychological outcome and systemic inflammatory response in patients undergoing cardiopulmonary bypass.

I am writing to inform you that your patient has been enrolled into the above research study.

Numerous studies have shown that hyperoxia can reduce the mediators of systemic inflammatory reaction and also has a beneficial effect in improving neurological outcome following brain injury. This study is aimed at trying to establish if this beneficial effect can be extended to the field cardiac surgery where both these complications can occur after cardiopulmonary bypass. The study involves:

- 3 sessions of preoperative hyperbaric oxygen therapy at the hyperbaric unit in the BUPA Hospital, Anlaby Road, Hull.
- 3 blood sample analysis perioperatively.
- A neurological and neuropsychological examination preop, and another 4 months postop.
- The patient will receive normal intraop, postop and follow-up care as per unit protocol.
- This prospective randomised study involving 64 patients will take place in the cardiothoracic unit at Castle Hill Hospital from August 2002 till December 2003.

If you have any questions regarding any of the above, please feel free to contact me at Castle Hill Hospital, Tel: 01482 623623 Bleep 645

Yours sincerely

Mr Joseph Alex
Clinical Research Fellow
Cardiothoracic Surgery
Castle Hill Hospital

7.4 Glossary of terms.

ACE – Angiotensin converting enzyme
AFRCS – Associate Fellow of The Royal College of Surgeons
ANOVA – Analysis of variance
ATA – Atmospheres absolute
ATP – Adenosine tri phosphate
AVLT – Adult verbal learning test
Ca^{++} - Calcium ion
CABG – Coronary artery bypass grafting
CCS – Canadian Cardiovascular Society
CD – Cluster differentiation antigen
CI – Confidence interval
Cl^{-} - Chloride ion
CLA – Conjugated linoleic acid
cm – Centimeters
CNS – Central nervous system
CPB – Cardiopulmonary bypass
dl – Deciliters
DSB – Digit span backwards
DSF – Digit span forwards
EDTA – Ethylene diamino triphospho acetic acid
ELISA – Enzyme linked immunosorbent assay
ELSY – Hull and East Riding NHS trust approval
eNOS – Endothelial nitric oxide synthase
ESL-1 – E-selectin ligand-1
Fsw – Feet of sea water
G pegboard – Grooved pegboard
GlyCAM-1 – Glycosylation-dependent cell adhesion molecule-1
gm – Grams
H_2O – Water
HADS – Hospital anxiety depression scale
Hb – Haemoglobin
hct – Hematocrit
HMWK – High molecular weight kininogen
HSP – Heat shock protein-70
ICAM – Intrecellular adhesion molecule
ICU – Intensive care unit
IL – Interlukin
Info process – Information processing A
iNOS – Inducible nitric oxide synthase
IQ – Intelligence quotient
K^{+} - Potassium ion
kg – Kilograms
kP – Kilopascals
lb – Pounds
LFA – Leucocyte function associated antigen
LREC – Hull and East Riding Local Research and Ethics Committee
LV – Left Ventricular

Mac-1 – Macrophage-1-antigen
MAdCAM-1 – Mucosal vascular addressin cell adhesion molecule-1
MBBS – Batchelor of Medicine, Batchelor of Surgery
mg - Milligram
min – Minute
ml – Milliliters
Mn-SOD – Manganese superoxide dysmutase
MRI – Magnetic resonance imaging
mRNA – Messenger ribonucleic acid
Na^+ - Sodium ion
NADPH – Nicotinamide adenine diphosphate
ngm – Nanogram
NICE - National Institute for Health and Clinical Excellence
NMDA – N-methyl D-aspartate
NOS – Nitric oxide synthase
NSE – Neuron specific enolase
NYHA – New York Heart Association
O_2 – Oxygen
P – Partial pressure of a gas
p – Partial pressure of a gas dissolved in liquid
PA – Plasminogen activator
PaO_2 – Partial pressure of oxygen
PNAd – Peripheral lymph node addressin
PSGL-1 – P-selectin glycoprotein ligand-1
PVC – Poly vinyl chloride
R – Solubility constant of a gas at a given temperature
S100β - S100 beta protein
SCADS – Small capillary and arteriolar dilatations
SD – Standard deviation
SEM – Standard error of mean
sE-selectin – Soluble E-selectin
SOD – Superoxide dysmutase
sP-selectin – Soluble P-selectin
T – Absolute temperature of a gas
TMA – Trail making-A
TMB – Trail making-B
TNF - Tumour necrosis factor alpha
t-test – Students t-test
V – Volume of a gas
VCAM – Vascular cell adhesion molecule
WTAR – Wechsler test of adult reading
μgm – Microgram
μm – Micrometers
> - Greater than
≥ - Greater than or equal to
>/- - Greater than or equal to
+/- - Ranges from plus or minus