A clinical study of the effect of leucocyte depleting arterial line filtration on cerebral microemboli and neuropsychological outcome after coronary artery bypass surgery

by

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Abstract

Objective
A randomised clinical trial sought evidence as to whether leucocyte depleting arterial line filters had any effect on intra-operative cerebral microemboli and post-operative neuropsychological outcome in non emergency patients undergoing coronary artery bypass (CABG) surgery.

Methods
One hundred and ninety-two patients were randomised to the use of a Pall LG-6 leucocyte depleting (test) filter or either an Avecor Affinity or Pall AV-6 control filter. Cerebral microemboli during surgery were recorded by transcranial Doppler monitor over the right middle cerebral artery. Evidence of cerebral impairment was obtained by comparing patients' performance in a neuropsychological test battery (9 tests) administered 6-8 weeks post-operatively with their pre-operative scores.

Results
The groups proved well balanced in pre-operative variables. During cardiopulmonary bypass the median number and range of microemboli was 15 (3-180) in the leucocyte depleting group compared to 67 (5-846) and 55 (2-773) for the Avecor and AV6 groups respectively ($p<0.0001$). One hundred and sixty-one patients completed all the neuropsychological tests. The leucocyte depleting group showed better post-operative performance in all but one of the nine tests although the difference in a total change score did not reach significance ($p = 0.07$, 1 tailed t test).

Conclusion
Leucocyte depleting filtration during CABG reduced the number of cerebral microemboli recorded by transcranial Doppler and showed a strong trend towards improving neuropsychological performance post-operatively. These findings suggest that the use of such filters in CABG surgery may offer increased neuroprotection.

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Introduction

Cardiac surgery accompanied by cardiopulmonary bypass (CPB) may in some patients result in a global cerebral injury manifest as a decline in post-operative neuropsychological (NP) performance. This decline is defined as an impairment of concentration, memory, learning or the speed of mental and visuomotor responses (although not necessarily all of these). NP testing is currently the best method of assessing global cerebral injury, although a clear relationship has not been established between decline on NP testing and everyday functioning and quality of life. One report suggests that there is impaired driving performance as well as cognitive impairment after CABG surgery[1]. It is important to distinguish diffuse cerebral injury resulting in NP deficits from a gross cerebral injury resulting in stroke. These separate phenomena probably have differing aetiologies. The incidence of strokes is reported as between 1.5 and 5.2%[2] but NP deficits are far commoner with incidences of between 15% and 80%[3] reported in the literature. With improvements in techniques to protect the brain, NP outcome has improved and is now accepted to occur in about 20% of cases[4]. The incidence of such deficits is found to vary according to the methods and timing of measurement used in each study. Significantly, these deficits are not always a temporary problem but have been shown to persist in studies up to five years post surgery[5]. Although any resulting disability must be seen in the context of the overall benefits of cardiac surgery, there is certainly much scope for improvement.

A considerable number of investigations have attempted to elucidate the causes of post-operative neuropsychological deficits, yet most theories for causes and mechanisms of cerebral injury remain unproven. Much of the literature suggests that CPB is the principal cause. However, cardiac surgical patients are inherently prone to neurological and neuropsychological insult[6] and factors other than CPB, such as surgical trauma or anaesthesia, may be involved to a greater extent than is realised. Several recent studies

suggest that cerebral injury as measured by NP deterioration is similar in cardiac surgical patients whether they receive an operation with or without CPB[6;7]. Despite this, most evidence continues to implicate CPB and this thesis focuses on the cerebral injury associated with cardiac surgery and CPB. There are currently three inter-related causal theories of cardiopulmonary bypass induced global cerebral injury. These are altered cerebral perfusion, microembolism and the systemic inflammatory response[4]. Neutrophils have a pivotal role in the inflammatory response to bypass and may have a role in microemboli production. Despite a large body of evidence for this systemic inflammatory response, there are few studies of its relation to cerebral injury and so any link between the two is, at present, mainly speculative.

The improvement in NP outcome seen in recent years results from a number of refinements in anaesthetic, perfusion and surgical practice[3]. The conventional arterial line filter has made an important contribution to this improvement. Most commonly used is the 40μm screen arterial line filter which is positioned in the tubing returning blood from the CPB machine to the patient with the aim of filtering out potentially harmful bubbles or particulate debris. This filter has been shown conclusively to simultaneously reduce the number of microembolic events and reduce NP deficit[6;8]. In addition to this "conventional" filter, a leucocyte-depleting (LD) filter has more recently been designed for clinical use. This LD filter consists of a screen filter with an additional polyester mesh, which has the ability to trap leucocytes. It is claimed that this LD filter attenuates the inflammatory response and may reduce CPB induced cardiopulmonary damage. However any effect of the LD filter on microemboli has not been studied. There are therefore two possible ways in which the LD filter could modulate cerebral injury and NP outcome: via the inflammatory response and microemboli. The aim of this thesis is to investigate the effects of using a LD arterial line filter during CPB on neuropsychological outcome as the primary endpoint. Microemboli and

the inflammatory response are secondary and potentially mediating outcomes.

Original Hypothesis

The null hypotheses tested were: -

1) There will be no significant difference in the neuropsychological outcome when leucocyte-depleting filters are used.

2) There will be no significant difference in the microemboli count or inflammatory response when leucocyte-depleting filters are used.

Brief Outline of Methods Used

The study was a prospective, randomised, controlled clinical trial conducted in the Department of Cardiothoracic Surgery at University College London Hospitals (Middlesex Hospital). Following local ethics committee approval selected patients scheduled for non emergency coronary artery bypass surgery were invited to participate. If patients consented they were randomised into two groups to either receive conventional or leucocyte depleting arterial line filtration. Other anaesthetic, perfusion and surgical variables were standardised. Patients received a pre-operative and post-operative neuropsychological assessment, measurement of inflammatory markers peri-operatively and intra-operative measurements of microemboli counts in the middle cerebral artery.

Review of the Relevant Literature

The first six chapters of this thesis consist of a review of the relevant current literature. The specific areas discussed are: -

1) The assessment of neuropsychological function after cardiac surgery

2) Interventions which affect neuropsychological outcome following cardiac surgery

3) Mechanisms of cerebral injury during cardiac surgery

4) The role of neutrophils in the inflammatory response to cardiopulmonary bypass

5) The use of conventional and leucocyte depleting arterial line filters to reduce CPB induced injury

6) The role of S100b as a marker in cardiac surgery

Chapter 1

The assessment of neuropsychological function after cardiac surgery

1.1 Historical Note

The concept of brain injury has been a concern ever since cardiac surgery was first attempted by Souttar in 1925[9]. Describing closed digital mitral valvotomy, Souttar wrote "In view of the extreme danger to the brain from even the shortest check to its blood supply, any manipulations which are carried out must therefore be executed in the full flow of the bloodstream, and they must not perceptibly interfere with the contractions of the heart". However it is only since cardiac surgery became an extremely common and safer procedure[10] in the 1970s that attention was able to shift from mortality rates to more subtle measures of outcome such as neuropsychological (NP) function. This chapter describes the development of NP testing, explains how it is best carried out, summarises the main findings relating to cardiac surgery and discusses some of the complex methodological issues involved.

1.2 Methods of Testing

In a clinical setting a psychologist conducts the standard clinical NP assessment of an individual patient over one or two test periods totalling between about two and five hours. The patient is given a comprehensive battery of up to 25 standardised tests with the aim of identifying and localising a brain lesion, assessing the degree of cognitive disturbance in patients with known brain lesions or in order to distinguish between neurological and psychiatric symptoms[11]. The NP assessment of outcome following cardiac surgery differs from this standard assessment in several ways.

Firstly, assessments must be performed both prior to and then following surgery, rather than the single assessment thus enabling comparisons to be made between the patients own baseline score pre-operatively to post-operative performance. The purpose is to measure change in performance over time. Time constraints only allow about one hour for each of the pre-operative and post-operative tests. Therefore, in order to perform as many tests as possible and so be as

comprehensive as possible in testing different cognitive domains, relatively short tests must be selected. Investigators have used between 1 and 14 tests in various studies. The fact that the test will be repeated shortly afterwards demands that the test should have a minimal learning effect. Parallel forms of the same test may be used to reduce learning effects. If the tests were too easily learnt this would decrease the sensitivity of post-operative testing. Some degree of learning is inevitable with most tests and it is expected that the majority of patients without impairment will show an improvement in neuropsychological test performance post-operatively. As learning is inevitable, it is best taken into account using Z change scores (see later). It can therefore be seen that the battery of tests one uses to assess patients before and after cardiac surgery is a compromise between wishing to be as comprehensive as possible while completing testing in about one hour. This one hour limit is arbitrary but considered practical and reasonable for patients awaiting surgery.

The early incidence studies were designed to assess what affect cardiac surgery had on cognitive function. These early studies used a single group assessed on at least two separate occasions. This work established that a proportion of patients do show a post-operative neuropsychological decline but the incidence varied widely. While it has been suggested that such a variety of results could be due to an inherent problem of neuropsychological testing, the variability is no greater than that reported for post-operative strokes[12]– a related problem which is often perceived as a more reliable outcome measure as it has overt clinical signs and consequences. It is worth considering the reasons for variability between results of these incidence studies. The factors that will affect results are test, patient, study design and data analysis variables.

1.3 Test Variables

As has already been mentioned, NP testing in cardiac surgery is a compromise between being exhaustive in assessing cognition and being able to perform the tests in the time available. The

more tests that are used, the greater will be the probability of detecting a lesion because more domains are being tested. However the potential problem of using too many tests is that some may test the same domain and result in a deficit appearing worse than it actually is. The sensitivity of the tests used in neuropsychology test batteries has improved as the study of NP outcome has been refined. The early studies used intelligence tests or tests such as the Mini Mental State Exam. Although these are highly reliable they are relatively insensitive[13] and a number of tests have now been specifically chosen for use in cardiac surgery[14].

The way in which tests are administered is highly important but it is rarely mentioned in publications. To ensure reliability of assessments it is preferable that the same psychologist does the pre-surgery and post-surgery tests in the same environment. Factors such as noise and lighting may have effects upon test results. Also it is important that the patient can practise tests and so be fully aware of what is expected with each test and perform to the best of his/her ability.

1.4 Patient Variables

In addition to these test variables there are also a number of patient variables that could explain the difference in incidence between studies. There is considerable evidence that age is associated with NP outcome following cardiac surgery. Older patients have a worse outcome[15-18]. This may be because more microemboli are produced in older patients[19] or because cerebral blood flow autoregulation on CPB is impaired in the elderly[15]. This will be discussed further in chapter 2. Therefore the age of study populations is significant when comparing studies from different centres and also over time because the average age of the cardiac surgical patient has risen over the last twenty years.

Gender of patients may also be an issue. As CABG is much commoner in males, many studies have only had male participants but there is some evidence that women have a worse outcome. A relatively old study from 1981 involving forty nine valve replacement patients found that women had a poorer neuropsychological outcome[20]. A more recent study[21] also suggests that women have a worse neuropsychological outcome after cardiac surgery. Di Carlo et al[21] studied 110

patients, 30% of whom were female, having any type of cardiac surgery requiring cardiopulmonary bypass. Di Carlo et al did not use the standard battery of NP tests[14] which limits the validity of their results. However they found that female sex significantly predicted a greater cognitive decline six months after surgery.

The separate issue of whether valve replacement heart surgery (true "open heart" surgery because the heart chambers are opened) carries a greater risk of neuropsychological impairment remains unresolved. In a relatively small study, Braekken et al[22] in 1998 compared 26 patients having valve replacement to 14 patients undergoing CABG. No difference in neuropsychological outcome was found between the two groups at two months after surgery. Braekken did however find that in valve replacement patients, those with deficits had higher microemboli counts whereas this relationship was not seen in the CABG group. Neville at al (2001)[23] compared 193 patients undergoing CABG to 73 matched patients having valve replacement surgery. They measured neuropsychological performance with a standard battery of eleven tests and found no difference in outcome at five to seven days, one month and six months post surgery. This lack of difference in neuropsychological outcome was interesting as there was a significantly higher number of microemboli in the valve replacement group. On the other hand, Zimpfer et al (2002)[24] compared 30 patients having CABG with 30 age and sex matched patients having isolated tissue aortic valve surgery. A reduction in auditory evoked P300 potentials possibly indicating greater impairment was seen at 7 days and 4 months post surgery in the valve group compared to the CABG group. However the significance of these auditory evoked potentials in such a population is unknown and this limits the significance of this result. Zimpfer did not measure microemboli. Therefore although it is a consistent finding that there is a greater number of detectable cerebral microemboli during valve surgery compared to CABG surgery, there is no clear consensus as to whether this leads to worse neuropsychological outcome in the valve patients. The potential role of microemboli in causing NP deterioration

after CABG surgery is discussed later in Chapters 2 and 3.

Atrial fibrillation (AF) is another patient factor which has received recent attention. AF is very common after cardiac surgery, occurring in up to 20 to 40% of cases[25] and leads to ineffective beating of the atria. There is a well-recognised association between post-operative AF and strokes or transient ischaemic attacks[26]. In addition there is one study which has suggested that AF may be associated with a worse early post-operative neuropsychological decline. Stanley et al (2002)[27] prospectively observed 411 patients having elective CABG of whom 69 developed AF. Multivariate analysis controlling for age and other potential determinants of neuropsychological outcome showed that those patients with AF had a greater cognitive decline than those without AF.

There is a theory that suggests that patients with a higher intelligence should be less susceptible to the insult of cardiac surgery (the cognitive reserve theory) but there is no evidence of an association of intelligence with NP outcome. There is, however evidence that higher levels of education may protect against NP decline[15]. A recent study[5] examined NP function five years after surgery and found that the main predictors of cognitive decline at five years were increased age, decreased level of education and the cognitive function at discharge. Genetic factors may also have a role in determining NP outcome. One study by Tardiff et al[28] has shown an association between apolipoprotein E e-4 and NP decline. Tardiff et al found that in a relatively small group of 65 patients there was a higher level of cognitive deterioration post surgery in those patients who possessed the e4 allele. They also found that higher levels of education protected against cognitive decline in all patients and that this effect was more marked in those with the e4 allele. It is postulated that patients with this genotype may have impaired neuronal repair and maintenance because patients with the e4 allele also recover more slowly after closed

head injury or stroke. However, a more recent study by Steed at al[29] replicated Tardiff's study with a greater number of patients (n=111) and found that there was no association between cognitive decline and the presence or absence of apolipoprotein e4. The influence of this particular genetic factor therefore remains unresolved. Another genetic factor recently studied is polymorphism of the gene for the platelet glycoprotein 3a (GPIIIa) receptor. Polymorphism of this gene may affect thrombogenicity and risk for cerebrovascular thrombosis but this is, at present, controversial. Mathew et al[30] have shown that presence of the P^{A2} allele is associated with significantly greater early neurocognitive decline. However their study group consisted of only 70 patients and they only used a single NP test (the Mini Mental State Exam which, as mentioned, is relatively insensitive to change) at 4 days post surgery. The extent of the patients' cardiac and atherosclerotic disease may have a direct effect upon their neuropsychological outcome. For example, Hammon et al[31] found that the presence of a palpable aortic plaque was significantly associated with the incidence of new neuropsychological deficits. M. Newman et al[15] also found that severity of atherosclerotic disease of the aorta was associated with cognitive decline. Patients' pre-operative medications may also be important although, as far as the author is aware, there has been no study of the effect of medication on post-operative neuropsychological outcome.

Another patient factor which has been shown to affect NP outcome is endotoxin immunity. During cardiac surgery most patients are exposed to endotoxin[32] which is the lipopolysaccharide component of the cell wall of Gram negative bacteria. The origin of endotoxin is unclear but thought to arise from the translocation of bacteria across ischaemic gut mucosa[33]. Mathew at al[34] have recently found that patients with low anti-endotoxin core antibody were more likely to suffer from post-operative cognitive dysfunction.

Patients with severe cardiac disease have been shown to have a NP impairment before surgery

compared to age matched controls without cardiac disease[35]. Also, CABG patients have been shown to have MRI abnormalities suggestive of cerebrovascular disease pre-operatively. This then raises the issue of whether patients with cardiac disease are more at risk of cognitive decline after surgery because of their inherent disease rather than due to the surgery itself. (Conversely, a low pre-surgery NP performance could actually mask a further decline due to the statistical phenomenon of regression towards the mean; this issue is discussed later.) Finding suitable non-surgical controls to compare neuropsychological outcome is problematic. Selnes et al[36] compared 140 patients having CABG with 92 matched patients with coronary artery disease not having CABG. Both groups had baseline then 3 and 12 month NP testing. There were no differences between the groups at 12 months. However this was not a randomised study (it would be difficult to withhold CABG from half a study group) and it is not clear why the control group, if they were so well matched were not suitable for CABG surgery.

1.5 Study Design

Moving on from patient factors to issues of study design, there is the possibility that selective recruitment of patients may lead to the study population being unrepresentative of the general cardiac surgery population. Even if one aims to be as inclusive as is practical, a proportion of patients will inevitably decline consent to be studied leading to a possible sampling error. Borowicz et al[3] report a refusal rate of 61% and in the refusal group they found the stroke and mortality rate was 7.1% and 6.1% respectively compared to 2.8% and 1.1% in the consenting group. Although strokes and mortality are a different entity to neuropsychological decline this study by Borowicz illustrates the potential difference in the studied and non-studied populations. Apart from this consent example, researchers may deliberately select more healthy patients for their trial. The study for this thesis has deliberately not recruited patients over the age of 80 or patients with insulin-dependent diabetes. Both these factors increase the risk of NP deficit and have the potential to confound results. Another practical reason for not including such patients is the increased risk of complications which would make post-operative follow up and assessment

more difficult and reduce the number of patients able to be studied in a given time.

Selective attrition at follow up may also alter the reported incidence of NP deterioration after cardiac surgery. Patients who suffer a severe deterioration may become unable or may be unwilling to attend follow up and so lead to an underestimate of the incidence of deterioration. Borowicz found that patients who performed badly on the pre-operative baseline test were less likely to attend follow up assessment[3]. He also found that those who deteriorated more at 1 month were less likely to attend at 1 year. However, follow up data at the institution of this study does not support this[37].

The timing of NP assessment both pre- and post-operatively may have a considerable affect on performance. Patients who are tested pre-operatively, especially if it is on the day prior to surgery are frequently anxious. Although there is little evidence that anxiety depresses performance, it has become conventional to assess anxiety and depression contemporaneously to the NP assessment[14]. A study at UCLH[38] has found that patients are less anxious the day before surgery than they are a week before surgery but that this difference in anxiety did not alter the NP performance.

After surgery, the timing of testing undoubtedly influences the incidence of NP deterioration found. Higher NP deficit rates are reported in the first few days after surgery. However the lower incidence at longer term follow up shows that *some* of the deterioration found immediately post-operatively is of a temporary nature. In the first few days the patients' pain, tiredness or anaesthetic or analgesic drugs are likely to cloud the NP tests. A recent consensus decided that the ideal timing of the post op assessment would be at 3 months when any detected deficit would be a true, permanent deficit[14]. However many workers see their patients at 6 to 8 weeks post-surgery in order to coincide with the return of the patient for outpatient surgical review. There is some evidence that the early cognitive decline found at discharge seems to predict the late cognitive decline found at five years[37]. Any causal link, however, remains unproven and it could simply be that those patients who decline most in the early days of the post-operative period are

more susceptible to cerebral injury and so are the ones who will decline more with age, anyway. Whatever the validity of tests in the first few days after surgery, the fact remains that doing NP assessment so early after surgery is also practically difficult and may be distressing for the patient.

1.6 Methods of Analysis

In addition to patient factors and study design, the statistical analysis of NP data may have a significant influence on the incidence of neuropsychological deficit found. Methods of analysis have evolved in an effort to be more sensitive but there is no universally accepted method. Some investigators have been criticised for using as many methods as possible to analyse their results and then presenting the method which yields the most significant result. It is therefore important to define the type of analysis to be used when designing a trial and deciding on sample numbers. One has to choose a method of analysis to use before starting a trial and then keep to this plan.

The earlier, descriptive studies, which involved a single group of patients undergoing cardiac surgery, examined NP change from before to after surgery. The change in performance was analysed as group comparison or individual comparison. Group comparison consists of pooling data and determining whether the group as a whole has altered its performance. In order to detect a deficit the mean of the group has to drop significantly. This makes the assumption that most patients will experience a deficit, but in fact only a minority of patients are likely to. Also it takes no account of the potential learning effect of the tests. The patients who improve through learning may mask the patients who deteriorate. This type of analysis therefore has flaws. Probably superior for single group studies are individual comparisons which examine individual changes with each patient acting as his own control. The main problem that then arises is how to define what extent of neuropsychological deterioration constitutes a deficit. There is still no consensus as to what constitutes a deficit. However most researchers accept that deterioration on

at least two tests is required as a poor performance on only one test may simply reflect a lapse in performance. (Although it may also mean that only one cognitive domain has been affected). A deficit has usually been defined in standard deviation (SD) terms with most investigators classifying a deficit as a drop in one or more SDs from the pre- operative to post-operative levels. The SD may be a population SD when standardised tests are used or it may be calculated from the pre-operative performance in an attempt to establish a notional standard score for the group being examined. However this method may become insensitive because it applies a fixed amount of deterioration to all individuals regardless of their absolute levels. Another similar approach is to take a drop in 20% in a test performance to reflect a significant deterioration. This therefore overcomes the problem by taking a proportional drop in performance to define a deficit.

When interventional studies are analysed, there are again a number of approaches. In these studies, there are two or more groups having cardiac surgery with or without a particular intervention. There are now several ways in which group comparisons can be performed in interventional studies. As with the descriptive studies, the use of group means is best avoided. One alternative method, the individual incidence approach, used by Pugsley[8], and described by Newman[39] enables a comparison to be made between the groups in terms of the number of individuals showing a deficit. A standard deviation (SD) unit is first calculated from all the patients' pre-operative scores. One then calculates the change in score for each patient for each test. In an identical manner to the methods of descriptive studies, a deficit in a test is said to occur when the patient's individual score in a test decreases by more than one SD from their preoperative score. A patient has a significant deficit if this occurs in 2 or more tests. One then compares the incidence of deficits between two or more groups. This conventional definition was widely applied in research until a few years ago when some drawbacks became apparent. In addition to the problem of arbitrarily defining a deficit, as explained above, it has also become

less sensitive. This method classifies a patient in a binary fashion as either having a deficit or not having a deficit with no intervening scale. Sensitivity is therefore reduced when analysing by incidence of deficits. As the incidence of NP deficits has declined with improvements in surgical and perfusion and anaesthesia practice, the lack of sensitivity has become more apparent. Another criticism of the incidence of deficits method is the phenomenon of "regression towards the mean". Regression towards the mean is the statistical observation of a biological phenomenon whereby extreme baseline scores tend to become less extreme on repeated testing. In the case of NP testing before and after cardiac surgery it has been argued that those with higher preoperative scores are more likely to regress towards the mean and show an apparent, although not real, deterioration[40]. Conversely, those with low scores pre-operatively would improve in a similarly spurious way if they regressed towards the mean. However this theoretical argument can be refuted by the facts observed after cardiac surgery which are the exact opposite: those patients with better pre-operative NP scores tend to deteriorate less[41].

More recently Arrowsmith et al[29;42-44] have introduced "Z", or "change" scores and these are now becoming an additional accepted way of analysing NP data with greater sensitivity. To calculate a Z score each patients' test score on each occasion is converted into a standardised score by dividing by the SD of the pre-operative group performance of all patients in a study. A change score is then calculated for each patient by subtracting the post-operative standardised score from the pre-operative standardised score. One can then calculate a mean of the change scores for each group. This method is more sensitive because it is a continuous change score without absolute cut-offs. It also allows group comparisons which take into account potential learning effects. Since Z scores have only recently been introduced, it is a more sensible approach to analyse NP results using deficit scores as well as Z scores. This has the advantage of making it possible to compare incidence of deficits to previous studies.

In summary, this chapter has shown how NP testing has been adapted from a single clinical assessment to repeated testing to measure change in NP performance from before to after cardiac surgery. Analysis has evolved from comparing pre-operative and post-operative means, via measuring incidence of individual deficits, to the use of Z change scores. The wide range of incidence of NP deficits reported in the literature can be accounted for by the variation in the way the tests are conducted, patient variables such as age, atrial fibrillation, endotoxin immunity and aortic atherosclerosis. Study design may also impact on the incidence of NP deficits found.

Chapter 2

Interventions which affect neuropsychological outcome after cardiac surgery

2.1 Types of Intervention

Having already reviewed the inherent patient risk factors such as age and genetic susceptibility, this section will review studies which have investigated various interventions to reduce NP deterioration after cardiac surgery. These interventions include filters, drugs (anti-inflammatory or neuroprotective), pH control and temperature control during bypass, surgery with and without CPB and other surgical techniques. Filtration will be discussed in most detail in Chapter 3. Only studies that have directly measured NP outcome will be discussed in this section. Other studies have used various interventions and then measured microemboli or surrogate markers of NP outcome such as S100b. Since the relationship of S100b or microemboli to NP outcome is complicated, these studies are discussed separately in later sections on S100b (Chapter 6) and microemboli (Chapter 3.3).

One can separate interventions into pharmacological and physical but there is an important theoretical consideration which distinguishes the two main types of intervention used to reduce cerebral injury. Agents such as arterial line filters or anti-inflammatory drugs act on specific postulated mechanisms of damage (i.e. microemboli and the inflammatory response). The other group includes agents such as hypothermia and neuroprotective drugs. They do not attempt to prevent or attenuate the agents and mechanisms of damage but they are designed to protect the brain against any type of injury.

There are good data from randomised controlled trials (RCTs) suggesting that conventional 40μm arterial line filtration and the use of alpha stat rather than pH stat acid base control reduces the incidence of cognitive decline, and the more widespread use of these interventions may have already contributed to the general reduction in decline seen in recent years. The data regarding other interventions such as temperature control, anti-inflammatory

agents, reducing aortic manipulations or avoiding CPB altogether are less clear and there are as yet no results from randomised trials to guide practice regarding these issues. However, this lack of data has not prevented strong recommendations regarding certain surgical practices. For example recent reviews have advocated avoiding repeated aortic cross clamping[45] or using aprotinin[46] when the evidence supporting such practices is not conclusive. Apart from remacemide[42] (an NMDA [N-methyl-D-aspartate] receptor antagonist), no drugs have been shown to have significant beneficial effects although some such as aprotinin have shown promise. This part of the review examines the current evidence for three main types of intervention to reduce the neuropsychological deficits seen after cardiac surgery. These are equipment (surgical and perfusion), drugs, and techniques (surgical and perfusion).

2.2 Filters

Arterial line filters have gained in popularity, especially in the USA, due to their ability to remove microemboli and also large air emboli in the event of a perfusion accident[47]. Their use has not been as great in the UK. Filters however have an important role in neuroprotection and are discussed more fully in Chapter 3.

2.3 Oxygenators and Type of Bypass Pump

Because membrane oxygenators produce less microemboli detected by transcranial Doppler (TCD) than bubble oxygenators[48], their almost universal use in current practice is likely to have a beneficial effect on neuropsychological outcome. There is one study which suggests that there is less neurocognitive decline when membrane oxygenators are compared to bubble oxygenators[49]. A randomised controlled trial of 103 patients has also found no difference in early (5 day) NP outcome when roller or centrifugal bypass pumps are used[50].

2.4 Pharmacological

There is an ongoing search for an effective neuroprotective drug for use in cardiac surgery. Surgery may be the ideal time to test such a drug because the injury is anticipated and treatment can be prophylactic. Two commonly used anaesthetic drugs, propofol and thiopentone have been studied the most, and some anti-inflammatory drugs may have a neuroprotective effect.

Nussmeir et al[51] first reported neuroprotective effects of thiopentone. They studied 182 patients having true open-heart surgery and randomised patients to receive either a thiopentone infusion during bypass or fentanyl. The dose of thiopentone was sufficient to maintain electroencephalographic silence throughout bypass. It was found that the thiopentone group had less "neuropsychiatric" (actual tests were neurological, psychiatric and the trailmaking tests) abnormalities at day 1 and day 10 post surgery. Unfortunately, longer-term effects were not studied. A later randomised trial[52] of 300 coronary artery bypass surgery patients found no neuroprotective effect but delayed awakening and greater inotrope dependence in the thiopentone group. A recent retrospective review by Pascoe et al also found no benefit of thiopentone in open-chamber cardiac surgery[53]. The evidence is therefore on the whole not supportive of thiopentone having a useful neuroprotective effect. The evidence that propofol has a neuroprotective effect is equally unconvincing. Although propofol has physiological effects on the brain similar to thiopentone, reducing cerebral metabolic rate for oxygen (CMRO2) and cerebral blood flow and inducing electroencephalographic (EEG) burst suppression[54], this does not seem to lead to clinically improved neurological or neuropsychological outcomes[55]. In a trial of 225 patients having valve surgery randomised to receive propofol or no propofol, NP outcome was the same at 5 to 7 days and 3 months post operation in both test and control groups[56]. Interestingly, it has

been suggested that propofol may reduce microembolic delivery to the brain by reducing cerebral blood flow and that any cerebroprotective effect may be due to this mechanism rather than reduction in CMRO2.

Calcium channel antagonists such as nimodipine have been shown to reduce ischaemic cerebral injury in animals by limiting the amount of ischaemia-induced calcium entry which precedes cell death. A small randomised study from 1990 of 35 patients having CABG or valve surgery suggested an improvement in certain neuropsychological tests at 6 months post surgery in the group given peri-operative nimodipine[57]. A later, larger, randomised study was halted prematurely when increased mortality and bleeding rates were found in the nimodipine group[58]. In the 150 patients randomised, no difference in neuropsychological outcome was found.

Remacemide is an NMDA (N-methyl-D-aspartate) receptor antagonist which has been shown to reduce cerebral injury in animal models of focal ischaemia. Arrowsmith et al[42] tested its clinical effects in a RCT of 171 patients receiving CABG surgery using a battery of 9 neuropsychological tests before and then 8 weeks after surgery. The group of patients who received remacemide had a significantly better global change (Z) score of neuropsychological function compared to the control group. This probably reflects an increased capacity for learning in the remacemide group who have received less cerebral injury and therefore provides evidence that remacemide is protective during cardiac surgery. Although, in contrast to previous studies of nimodipine or thiopentone, there were no serious adverse effects associated with remacemide, it should be noted that its use was associated with an increased frequency of dizziness, nausea and ataxia in the short term.

It has been known for about 20 years that cardiac surgery with CPB is associated with an abnormal systemic inflammatory response (see later). It is also likely that local inflammatory processes contribute to brain injury but it is extremely difficult to study this, at least in humans. Aprotinin is a non-specific serine protease inhibitor, used mainly to reduce peri-operative bleeding due to its anti-fibrinolytic effects. However, aprotinin's lack of specificity at inhibiting enzymes has also resulted in a number of other anti-inflammatory properties being discovered. As yet, there are no randomised trials assessing the effect of aprotinin on neuropsychological outcome. Retrospective analysis of data from trials investigating aprotinin's effects on other outcomes suggests that aprotinin may reduce stroke rate[46]. Therefore while there is potential for aprotinin to be neuroprotective, this concept remains untested.

Clomethiazole (also known as Heminevrin®) is the most recently tested potentially neuroprotective drug. It is more commonly used as a sedative to treat alcohol abusers. In a randomised controlled trial of 245 patients using a thorough pre-operative and post-operative battery of neuropsychological tests Kong et al[43] found no improvement in neuropsychological outcome using clomethiazole.

2.5 Acid base management

The solubility of a gas in blood increases as the temperature falls. Therefore during hypothermic bypass, when blood gas samples are corrected for temperature the patient appears to have a respiratory alkalosis - a low $PaCO_2$ and a high pH. It is possible to correct the pH to the normal range by increasing the $PaCO_2$ and this method is known as pH stat control. Alternatively it can be left uncorrected and this is known as alpha stat control. There is evidence that alpha stat management leads to a better neurological and neuropsychological outcome. Bashein[59] in 1990 found no difference in neuropsychological

outcome when either pH stat or alpha stat was used, but Stephan et al[60] found in 65 patients that hypothermia with pH stat management was associated with marked cerebral hyperaemia and an increase in neurological events by day seven post surgery. This finding was supported by a later study of 316 patients by Murkin et al[61] in 1995 which found that there was improved neuropsychological outcome at two months post surgery in a group randomised to receive alpha stat rather than pH stat.

Patel et al[62] (1996) randomised 70 patients to receive pH stat or alpha stat control with both groups cooled to 28 °C. A significantly greater proportion of the pH stat patients showed deterioration in NP function at six weeks post surgery. Interestingly, Patel also measured cerebral blood flow and found that it was greater in the pH stat group as was cerebral artery blood flow velocity. Although there was hyperaemia, as measured by cerebral extraction ratio for oxygen, in both groups it was more pronounced in the pH stat group. This study therefore suggested that the pH stat control disrupted the normal cerebral autoregulation and that the increased blood flow was harmful. It is not known why the increased cerebral blood flow associated with pH stat control increases the risk of diffuse cerebral injury. It has been suggested that an increase in flow leads to an increase in the number of microemboli delivered to the brain. Patel et al did not measure microemboli in their study, but there is evidence from experimental animal studies that an increase in PaCO2 leads to both increased cerebral blood flow and microembolic delivery[63]. Studies in humans having valve replacement surgery do not however show this relationship[64]. An alternative explanation for increased cerebral blood flow being harmful is if the blood brain barrier becomes more leaky during CPB. An increased cerebral blood flow could then lead to more oedema.

2.6 Temperature

The two main issues regarding temperature management and subsequent cognitive outcome are the degree of hypothermia at which bypass is conducted and then the rate and extent to which subsequent rewarming occurs.

Hypothermia has been used as both a cardioprotective and a neuroprotective strategy throughout the history of cardiac surgery. More recently, normothermia and normothermic cardioplegia have been investigated as a means of myocardial protection, leading to concern regarding possible compromise of cerebral protection using this technique. The following studies that have investigated the effect of normothermic compared to hypothermic bypass on neurological and neuropsychological outcome reveal conflicting results.

Singh et al[65] and also Mclean et al[66] found no difference in neurological outcome when normothermic or hypothermic bypass was used. In contrast Martin et al[67] found a marked increase in neurological events when normothermia was used. A criticism of all three of these studies is that the actual brain temperature was not measured. Depending on the bypass strategies used the actual brain temperature may be little different whether or not hypothermia is used. Also, it is possible that diffuse and focal cerebral injury (producing NP and neurological deficits respectively) may be differently affected by hypothermia. This is supported by a study of Mora et al[68] in which 138 patients were randomised to hypothermic or normothermic bypass and both neurological and neuropsychological outcomes were measured. Normothermic bypass increased the risk of stroke but not NP deficit. Regragui[69] randomised 96 adults to receive bypass at 37, 32 and 28°C. There was a lower incidence of cognitive deficit in the 32°C group compared to the 37°C group but cooling to 28°C conferred no additional benefit. More recently a study by Grimm et al[70] suggested that normothermia may be less harmful than hypothermia. However this study is seriously limited by a number of flaws. Grimm et al measured P300 auditory evoked potentials, the

Mini Mental State exam and Trailmaking A tests to assess neuropsychological outcome in patients randomised to normothermia (37°C) and hypothermia (32°C). There was no difference in Mini Mental State exam and Trailmaking A tests but a prolongation of P300 potentials in the hypothermia group four months after surgery. They failed to use a battery of tests as is recommended, and the relationship of P300 potentials to cognitive function in the setting of cardiac surgery is unknown. Also, the paper does not explain the important issue of exactly how temperature was managed and it is possible that the hypothermia group received damagingly rapid or excessive rewarming.

Nathan et al[71] studied the effects of two different rewarming strategies at the end of CPB. In a randomised controlled clinical trial all patients were cooled to 32°C on CPB and then rewarmed to either 37°C or 34°C with no further rewarming. Although it was found that the 34°C group had less cognitive deficit at 1 week and 3 months post-operatively, the method of analysing cognitive change was unusual. A battery of 11 tests was used but these were then grouped into three domains and patients were classified as having a deficit if there was a deterioration in 0.5 of a standard deviation or greater in a domain. At 3 months a difference in only one of the 11 tests was seen. This suggests that the difference between groups at one week was a temporary effect which had worn off by 3 months.
Grigore et al[72] randomised patients to slow or more rapid rewarming from hypothermia and found that those with slower rewarming had a better neuropsychological outcome sex weeks after surgery.

In summary, although hypothermia itself may be neuroprotective, the process of rewarming to normothermia and beyond may itself be harmful. Rewarming to 38°C either deliberately or inadvertently may directly injure neurones. It has also been suggested that rapid rewarming may force anaesthetic and other gases out of solution and so produce

microemboli. It should also be remembered that nasopharyngeal temperature is usually measured as a close approximation of "core temperature". However this may lag behind the actual temperature of the brain.

2.7 Blood Flow Management

Cardiopulmonary bypass may be performed with either pulsatile or non-pulsatile flow of blood. Neither have a clear advantage but pulsatile flow has been recommended for being, at least theoretically, more physiological. A single study[61] which compared the effect of pulsatile and non-pulsatile flow on neuropsychological outcome found no advantage of either method, however.

2.8 Surgical Techniques and "Off-pump" Surgery

It is possible that surgical techniques may have a profound effect on both neurological and NP outcome following surgery. Although strong opinions tend to be expressed with regard to surgical technique there has actually been very little scientific study of this issue. The common and logical view is that manipulating a calcific or atheromatous aorta is harmful. Such manipulation may dislodge macro- and micro-emboli with the subsequent adverse effects. There are three main manipulations to the aorta during CABG surgery which need to be considered. The first is the cannulation of the aorta necessary to initiate CPB. The second is the cross clamping of the aorta which precedes either cross clamp fibrillation or cardioplegic arrest. The third manipulation is the application of a side-biting clamp to proximal aorto-coronary anastamoses. Off-pump CABG avoids cannulation and cross clamping, but unless an exclusive Y-graft technique is used to avoid aorto-coronary anastamoses, side biting clamps remain necessary.

Performing coronary artery bypass surgery without the use of cardiopulmonary bypass has a number of theoretical advantages and elimination of cerebral injury is potentially one of the most significant of these. There is some evidence for a reduction in both microemboli production and the inflammatory response, thought to have a major role in causing cerebral injury on bypass, in patients who have CABG without CPB. However most of the studies carried out so far do not show any difference in neuropsychological outcome in on bypass and off bypass patients.

Malheiros et al[73] (1995) studied 81 patients who had CABG either with or without CPB according to surgical technical selection criteria. The CPB group had a mean of 2.9 grafts and a mean operative time of 325 minutes whereas the non-CPB group had a mean of 1.7 grafts and a mean operative time of 251 minutes. The groups were not therefore matched in terms of surgical trauma. No differences were found in early (5 to 7 days post surgery) tests of neurological or neuropsychological outcome. Although the neuropsychological tests were not conventional they were reasonably comprehensive. However at such an early post-operative stage many factors cloud neuropsychological performance and tests at this stage without later testing cannot be relied upon.

Bhasker-Rao et al[74] (1998) used ASEM (anti- saccadic eye movement) as a test of pre- and post-operative neurocognitive function in 322 CABG patients, 17 of whom had no CPB. Unfortunately, although ASEM is a good test of frontal lobe dysfunction in patients with dementia it has not been validated in cardiac surgery patients. Therefore the fact that the off-pump cases had fewer ASEM deficits post-operatively is of extremely doubtful significance. Interestingly they also used a conventional means of measuring intra-operative microemboli (transcranial Doppler) and were able to show that the off-pump cases had significantly less

microemboli produced during surgery. This is particularly significant because the off-pump cases in this study were selected on the basis of a history of stroke or TIA or the presence of a calcified aorta. All of these are factors which one would expect to lead to a greater number of intra-operative microemboli. If the elimination of CPB is able to reduce the number of microemboli then this could have an effect on neuropsychological outcome. It is logical to expect the elimination of CPB to reduce microemboli since there will be no cannulation, cross clamping or other surgical manipulations which can produce microemboli[75]. A short report by Malheiros et al in 1999[76] reports no microembolic signals detected in a "series" of 6 patients. They did however note fairly marked disturbances in middle cerebral artery blood velocity during manipulation of the heart. Off-pump surgery thus has the potential for a deleterious effect on cognitive function by compromising cerebral blood flow despite the relative lack of microemboli. Watters et al[77] showed a definite reduction in microemboli counts in off pump compared to CPB treated patients in a group of 20. However Watters et al's study was an unrandomised prospective comparative study and the results should be interpreted cautiously.

More recently, a number of studies have continued to give conflicting results regarding cognitive outcome after off-pump surgery. Diegler et al[78] in 2000 randomised 40 patients to off-pump or conventional CABG with CPB surgery. Unfortunately the tests they used to assess cognitive outcome (a "standardised *psychiatric* assessment" and the Syndrom Kurz Test) are un-validated in cardiac surgery and were performed only early after surgery (at seven days). Diegler et al's finding that there was a marked increase in cognitive impairment in the conventional group and no impairment at all in the off-pump group must therefore also be interpreted cautiously. However their positive correlation of conventionally measured microemboli with cognitive decline is supportive of microemboli causing deficits on bypass. In contrast Taggart et al[7] in a study using a conventional neuropsychological test battery pre-

surgery, at discharge and at 3 months post surgery were unable to find any difference in medium term cognitive outcome between either on-pump or off-pump cases. Taggart's study however was not randomised but studied 25 off pump patients compared to 50 matched on-pump controls. The fact that Taggart used a validated battery of neuropsychological tests lends that study more overall weight.

The most significant recent study is a large randomised trial comparing on-pump and off-pump surgery. Van Dijk et al[44] randomised 281 patients and measured cognitive outcome using a recognised battery of ten tests at 3 and 12 months after surgery. They found no difference in the two groups at 3 months post surgery using incidence analysis (21% in the off-pump group and 29% in the on-pump group) but there was a significant difference in the overall standardised change score at 3 months (0.19 vs. 0.13, $p = 0.03$). There was no difference at 12 months using either method of analysis. Van Dijk et al's follow up rate was good overall but the follow up rate was 90% in the on-pump group and 98% in the off-pump group at 3 months. It is therefore possible that a greater difference between the groups was missed because those patients with the greater cognitive impairment are more likely to drop out[79]. The latest study to be published does suggest that surgery without cardiopulmonary bypass causes less cognitive impairment than with bypass. Zamvar et al[80] (2002) randomised 60 patients to on- or off-pump CABG and performed pre-operative and 1 and 10 week post-operative NP testing with a battery of nine standard tests. Although the number of patients studied was relatively low, an impressive 100% follow up rate at 10 weeks was achieved. In a relatively low risk group (patients with previous TIAs and CVAs were excluded) incidence of decline (defined as deterioration by more than 1 SD in two or more tests) was 40% in the on-pump group and 10 % in the off- pump group (a highly significant difference). Such a high incidence is difficult to explain and unfortunately Zamvar et al give very little detail of operative procedures in either of their groups, thus limiting the applicability of their study.

In summary, there is convincing evidence that the use of both arterial line filters and alpha stat acid base control can reduce the incidence of cognitive decline after cardiac surgery. There is no evidence that hypothermia is of benefit and the only pharmacological agent to have a neuroprotective effect, remacemide, has not been used beyond the experimental stage. The evidence regarding on-pump and off-pump surgery is conflicting. The present study, in keeping with current best practice, uses alpha stat acid base control and has a strict temperature management protocol.

Chapter 3

Mechanisms of cerebral injury during cardiac surgery which impact on neuropsychological outcome

3.1 Inter-relation of Aetiological Factors

As the preceding review of interventions and their effect on NP outcome implies, the pathophysiology of cerebral damage secondary to CPB is complex and incompletely understood. The likely differing aetiologies for diffuse cerebral injury and stroke are again emphasised. The three main aetiological factors to be discussed in this chapter are cerebral blood flow, micro-embolism, and inflammatory mediators. It is likely that these factors are inter-related. One speculated inter-relation suggested is increased inflammation or cerebral blood flow leading to an increased microembolic load. When there is a greater inflammatory response there will be greater activation of neutrophils and platelets which when they aggregate may produce more particulate microemboli. Also an increased cerebral blood flow will potentially increase the microembolic load for a set microembolic concentration in the blood and so high cerebral blood flow rates may not always be beneficial. Microemboli and inflammation are potentially affected most by leucocyte depleting filtration and therefore will be discussed in greater detail. Most studies to date have concentrated on microemboli and their preponderance is reflected in this chapter. Little study has been made of the aetiological role of the inflammatory response and so this area is far more theoretical and a separate chapter (four) is devoted to the inflammatory response.

3.2 Cerebral Blood Flow

As cerebral blood flow (CBF) may be a factor, attempts are made to keep it constant in the present study. Cerebral blood flow is auto regulated across a range of blood pressure although this needs to be higher in hypertensive patients. Cerebral blood flow is normally 40-60mls/g/min and during bypass is 20-60mls/g/min. Astrup et al[81] have shown that CBF must fall to below 20mls/g/min before ischaemic cell death occurs at normothermia. Recent studies have shown that even with pump flow rates reduced to 1.75l/min/m^2 the lowest CBF was 24mls/g/min[62]. It can therefore be seen that although a global reduction in CBF may

cause ischaemia it is unlikely in itself to be the main cause of the cerebral injury occurring in 20% of cases. There is, however, some evidence that hypoperfusion can be a significant contributory factor to cardiac surgery related ischaemic cerebral injury. Tufo et al[82] found that in 100 patients having CABG (in 1970) that the incidence of post operative neurological signs was higher (78 vs. 27%) in those with a mean perfusion pressure of 40mmHg compared to 60mmHg. In a much more recent randomised study Gold et al[83] found that the incidence of strokes was higher (7.2 vs. 2.4%) at 6 months post surgery in patients maintained on CPB at a perfusion pressure of 50 to 60mmHg compared to 80 to 100mmHg. Gold et al however found no difference in cognitive outcomes between the two groups.

However, studies by Caplan et al[84] suggest that cerebral blood flow may have a significant effect upon neuropsychological and neurological outcome via effects on microemboli. Caplan et al hypothesise that the effect of emboli is worse when the perfusion pressure to the brain is less because there is less "washout" of emboli. However there is no direct evidence to support this. Conversely a higher cerebral blood flow (not necessarily resulting in a higher perfusion pressure) could theoretically result in more microemboli being delivered to the brain. These are the reasons why it was decided to measure cerebral blood flow velocity as well as microemboli in the present study.

Hypoxia is a common complication of coronary artery bypass surgery and a post hoc multiple regression analysis by Browne et al[85] in 115 patients having CABG surgery showed that hypoxia at 5 days was a weak but significant ($r = 0.24$, $p<0.03$) predictor of the Z score at 5 days. Although pain, hypoxia, effects of analgesia and anaesthesia are a reason for not performing tests five days after surgery early post-operative cognitive impairment has been shown to predict later deterioration[5]. It is therefore possible that early post-operative hypoxia is a contributing cause of long-term impairment.

3.3 Microembolism

Microemboli have received most attention as possible mediators of cerebral injury. This section will define microemboli, discuss their consistency, explain the various ways of measuring them with emphasis on transcranial Doppler (TCD), examine the evidence supporting their aetiological role in the production of neuropsychological deficits and, finally, outline theories as to how they could produce cerebral injury.

A microembolus has been arbitrarily defined as an embolus which measures no more than 200μm in diameter[86]. A larger embolus is then said to be a macroembolus. Microemboli may be gaseous, particulate or lipid with further subdivisions into organic or inorganic. Gaseous microemboli have a number of sources, which may be related either to the cardiopulmonary bypass circuit or to surgical techniques. Air inevitably enters the left side of the heart when it is opened for valve operations and there is evidence that even vigorous de-airing techniques do not completely remove the air[87]. Cannulation of the aorta and right atrium can theoretically entrain air. Although studies have shown that there is an increase in the number of microemboli at the time of aortic cannulation these could well be atheromatous or calcific particles rather than air. Air may continue to be entrained into the right atrium via the venous cannula if the purse string holding it is inadequate and the pressure in the right atrium is sub atmospheric. Air has also been demonstrated inside the heart during "closed heart" coronary graft operations. Using M-mode echocardiography, Oka et al[88] were able to detect air in 2 of 18 (11%) of patients having CABG compared to 12 of 15 (79%) having valve surgery. "Trace" or "moderate" grades of air were seen in the left atrium or aorta for 1 to 5 minutes after termination of bypass. Air or oxygen may also enter via the bypass equipment. Bubble oxygenators used to be a major source of microemboli but

membrane oxygenators, which are now used almost exclusively, produce far less microemboli[48]. Anaesthetic gases have also been implicated in the production of gaseous microemboli[86]. Bubbles are more likely to form or grow during the rewarming phase of cardiopulmonary bypass when the partial pressure of the gas will increase and tend to force it out of solution. Rapid rewarming could thus have a detrimental effect on neuropsychological function via this mechanism but there have been no studies examining the rate of rewarming and microemboli production.

There is a long list of potential particulate microemboli. Thrombin, fibrin, platelets, leucocytes, fat globules, atheroma, calcium and even denatured proteins may form organic particles. It is these types of microemboli that arterial line filters were designed to trap. There is further discussion of particulate microemboli in the section on filters. Liu et al attempted to quantify particulate microemboli during cardiopulmonary bypass in in-vitro, animal and clinical studies[89]. They used a Coulter counter (an electronic particle-size analyser) to detect microemboli between 15 and 80μm in diameter and also electron microscopy to directly view the particles. Liu et al found that the three main sources of microemboli were transfused blood, cardiotomy reservoirs and bubble oxygenators. These in-vitro experiments showed that the degree of destruction of blood elements in the perfusion circuit was most significant during the first hour on bypass and that there was a linear correlation between time of perfusion and blood destruction. The number of microemboli detected steadily increased during the first hour on the perfusion circuit and then ceased to change thereafter. Particulate microemboli may therefore be produced by damage to the blood elements and their subsequent aggregation. Activated blood is allowed to collect in cardiotomy reservoirs and Liu found that the number of microemboli increased 5 fold after passing through the reservoir. It is interesting that they also found that the number of particles produced by the bubble oxygenator was 2 to 3 times that of the membrane

oxygenator.

As well as the cellular blood element studied by Liu et al, lipid microemboli also seem to be produced by cardiotomy suction. Stump and Moody's group have devoted much attention to cardiotomy-derived lipids and their subsequent microembolisation to the brain. In 1998 Brooker at al[90] found a significantly greater number of small capillary and arteriolar dilatations (SCADs) on cross sections of dog's brains after 60 minutes of CPB in which cardiotomy suction was used. These SCADs are presumed to be lipid microemboli which are dissolved by the alkaline phosphatase staining technique used in preparing the sections of brain. Later work by the same group[91] shows that processing the cardiotomy suction blood with a cell saver was able to reduce the number of SCADs found. Interestingly, this study also showed that arterial line filtration including leucocyte filters did not reduce SCAD density. Kaza et al[92] have found that patients randomised to have cardiotomy suction rather than a cell saving device have greater lipid microemboli at the end of CPB. A standard 21 micron filter removed all these lipid microemboli.

Particulate microemboli are more susceptible to capture by arterial line filters and also, in a study by Harringer et al[93], an intra aortic filter has been used to capture particles within the aorta distal to the arterial cannula. The filter was a 120μm diameter pore size polyester mesh and it was inserted prior to removing the cross clamp used to construct the distal aorto-coronary anastamoses. The filter was then removed just before de-cannulation and was fixed and examined with light microscopy. Sixty two % of filters contained fibrous atheroma, 52% contained fibrin/platelet strands and 22% contained thrombus or red blood cells.

A number of investigators maintain that the atheroma of patients is the most significant of the microemboli sources and that the atheromatous microemboli are detrimental to

neuropsychological outcome[46;94;95]. However when all the studies are considered it seems that microemboli are heterogeneous with many sources. The fact that microemboli are detectable in children[46;94;96] having surgery to correct congenital cardiac defects suggests that non-atheromatous sources are important in the production of microemboli.

Researchers have used a variety of methods to measure the microemboli produced during cardiac surgery and have also focussed on a number of different sites. It is possible to measure the microemboli in the aorta, carotid artery, middle cerebral artery or even the retinal arteries. The methods themselves can be direct or indirect. An early, indirect method was the "screen filtration pressure" technique of Swank[97]. This was initially developed to measure micro aggregates in stored blood and then adapted for cardiac surgical patients' blood. The technique relied on the fact that the pressure required to push blood through a screen filter of a given pore size was directly proportional to the number of micro aggregates in the blood. This has not been used recently. Another indirect method, the Coulter counter has, however, continued to be used[89]. It measures micro particles of 0.4 to 800μm in diameter, again in vitro. Although reported to be sensitive and accurate[89] it can be criticised for not measuring gaseous microemboli and because any manipulation required to measure the microemboli is liable to alter the emboli[86]. It also cannot continuously measure microemboli, but measures at a single time point. Other workers have used histological methods to examine the brains of animals and humans after cardiac surgery, looking for evidence of microemboli. Moody[98] was the first to report small capillary and arterial dilatations (SCADS) in autopsy brains of patients post cardiac surgery. It is thought that these SCADS are the "footprint" left behind by mainly lipid microemboli when the solvent used to prepare the specimens has removed the actual embolic material. However, although SCADS are exclusive to cardiac surgical patients, their actual cause remains unknown[90]. Workers such as Blauth et al have used the retinal circulation as a visible extension of the

cerebral microvasculature[86;99]. Blauth et al used the photographic technique of retinal fluorescein angiography to demonstrate retinal micro-vascular occlusions consistent with retinal microemboli. They found that retinal micro-vascular occlusions were more common in those patients with neuropsychological deficit. Compared to Doppler ultrasound, this technique is invasive, complicated and also does not allow continuous monitoring for microemboli. With retinal fluorescein angiography, one merely sees the effect of microemboli at discrete time points. Blauth et al developed a method to quantify the microemboli producing the occlusions but the technique has not gained popularity. In contrast, TCD is the most commonly used method for micro embolus detection. As well as allowing the continuous "live" detection of microemboli it also measures blood velocity and so gives an indication of flow.

To obtain a TCD trace, an ultrasonic beam from a probe placed against the skull is directed at blood flowing in the middle cerebral artery. There is a variable temporal window of thinner bone above the zygomatic arch in about 90% of people. In the remainder it is not possible to obtain a TCD trace. The acoustic properties of embolic material, gaseous or particulate, differ from blood so that the emboli reflect the ultrasound much more strongly than blood. An increase in reflection produces a characteristic "high intensity transient signal" (HITS). These can be recorded on videotape for off line analysis. Software has been developed for automatically counting emboli but is not as good as an experienced human analyser[100]. HITS detected by TCD have been shown to correspond to microemboli made of air, platelets, fibrinogen or atheroma in laboratory in vivo experiments[101;102]. HITS may therefore be equated with microembolic events (MEE). TCD is able to detect both solid and gaseous microemboli, which is advantageous but cannot unfortunately distinguish between the two. Russell and Brucher has recently claimed to have developed technology that can automatically distinguish solid and gaseous emboli[103]. This has not yet been used clinically.

Blauth criticises TCD because the number of HITS may not exactly reflect the number of emboli, especially if automated counting is carried out. However, the same criticism applies to all the other methods previously mentioned which do not have TCD's advantage of continually detecting both types of emboli. In order to standardise their detection and make studies comparable a number of recommendations for their detection and recording have been made by an international consensus group[104]. In order to qualify as a HIT or MEE the Doppler signal must fulfil the following criteria:

1) Be transient – less than 300 millisecond
2) The amplitude should be at least 3 decibels higher than that of the background Doppler signal.
3) The signal should be unidirectional within the Doppler velocity spectrum (as the embolus is travelling in one direction in the bloodstream).

Padayachee et al[48] (1987) were the first to use TCD during cardiac surgery. They found that there were embolic signals in 22 out of 27 patients at the time of aortic cannulation. During CPB, they found that emboli were detected in all patients with a bubble oxygenator but in none with a membrane oxygenator. A few years later Pugsley[8], in a randomised controlled trial, found emboli in all patients with bubble oxygenators and that a 40μm filter reduced the number of emboli. The modern trend in perfusion technique of using membrane oxygenators and arterial line filters[105] has not led to the complete disappearance of microemboli and they continue to be detected by TCD.

An often discussed issue in the TCD technique of detecting cerebral emboli is whether bilateral or unilateral middle cerebral artery detection should be used. The majority of

studies have not shown a difference in right and left hemisphere counts although some have found more on the left. Some investigators argue that a combined bilateral count is more accurate as it gives the total number of microemboli. However even this is likely to only be a proportionate estimate unless all arteries supplying the brain are monitored. Moser et al[106] examined unilateral and bilateral monitoring in 29 patients and found only a 4% difference between sides. Therefore, which side is used in unilateral monitoring is probably not important. Wijman et al[107] have shown that cerebral microembolism occurs more frequently in the middle as compared to the anterior cerebral artery when TCD is used to detect microemboli. The number of microemboli detectable is probably related to the proportion of cerebral blood flow in each artery.

Mullges et al[108] have shown that microemboli counts during cardiopulmonary bypass also depend on the position of the aortic cannula. Mullges at al[108] randomised 60 patients to have the aortic cannula short so that it sat in the ascending aorta or long so that it sat in the descending aorta. The number of HITS detectable in the long cannula group was significantly lower (mean of 223 vs. 994).

The evidence relating microemboli to post-operative neuropsychological deficits is now fairly strong but not consistent. The earliest study to implicate microemboli was that of Aberg in 1974[109] in which 144 patients were given a battery of NP tests pre-op, eight days and then eight weeks post-op. Only six patients were given an arterial line filter but these patients had a better NP outcome. At that time no measure of microemboli was possible. Although there were a number of subsequent studies relating a variety of filters to NP outcome it was not until Pugsley's work, published in 1994, that the effect of arterial line filtration on microemboli was studied. Pugsley[8] studied the incidence of microemboli in the middle cerebral artery using TCD and NP outcome in a randomised controlled trial of 100

patients who received either a 40μm arterial line filter or no filter. It was found that patients with the 40μm filter had less microemboli during bypass than the non-filtered group and that the number of microemboli was related to the likelihood of a patient having a neuropsychological deficit at eight weeks post op. Significantly more patients in the non-filtered group had NP deficits at both 8 days and eight weeks post op.

Although Pugsley's was a well designed and appropriately controlled randomised trial, it may have limited applicability to modern practice because it was carried out at the time when bubble oxygenators and pH stat acid base balance were used during bypass. Bubble oxygenators are now known to produce more microemboli than membrane oxygenators and so membrane oxygenators are almost exclusively used. It is also known that cerebral blood flow auto regulation is better preserved with alpha stat pH balance during CPB and leads to a better NP outcome so most centres use this at present. An interesting study by Plochl[64], albeit in pigs, has shown that a reduction in $PaCO_2$ results in less embolisation of microspheres to the brain. It is therefore possible that as well as membrane oxygenators producing less microemboli, alpha stat may reduce their delivery to the brain.

Despite these caveats, the evidence linking microemboli and NP outcome from Pugsley et al's study[8] remains strong and is supported by later studies. Clark et al[110] showed that even when using alpha stat and membrane oxygenators the number of microemboli detected was related to the NP outcome. In a prospective study of 127 patients who had TCD monitoring during bypass, 41 of these patients had pre-operative and post-operative NP evaluation. It was shown that by dividing the patients into categories of microemboli count (<30, 30-60 and >60) that those with more microemboli had worse NP outcome. The major fault of this study is the timing of the postoperative tests at 5-10 days post op. As already discussed this is too early for results to be meaningful as so many factors influence NP deterioration at this

stage. Also, the exact battery of tests is not detailed and the number of patients studied (n=41) relatively low. Barbut et al[111] in 1997 used TCD to count middle cerebral artery emboli in 82 patients having coronary artery bypass grafting of whom 4 (4.9%) had peri-operative strokes. Patients with strokes had a mean microemboli count of 449 compared to a mean of 169 in those patients without strokes – this was a statistically significant difference. NP outcome was not assessed, but the finding regarding strokes was highly significant.

More recently, a number of investigators have studied the relationship between NP outcome and microemboli in more detail in an attempt to discover *how* they could be causally related. Sylviris et al[112] in Australia have investigated microemboli at different stages of CABG and both NP outcome and MRI changes. A relatively small population (n=41) was studied and of these only 32 had NP testing. Also, the NP tests were again performed too early at 5 or 6 days post surgery. Despite small numbers they were able to show a significant association between increased numbers of microemboli during bypass and increased NP deficits. In contrast to Barbut et al's study[111], an increase in microemboli during bypass was not associated with stroke (defined as cerebral infarction seen on post-operative MRI). However a strong association was found between pre-bypass microemboli and cerebral infarctions seen on MRI scans at 6 weeks post op. Only 28 patients in this study received both TCD and MRI and of the 5 of these who had MRI evidence of stroke 2 were also found to have significant carotid artery stenoses. Pre-bypass microemboli are detected before any surgical or bypass interventions have been made and are likely to be caused by atheroma and so this association is not surprising.

Jacobs et al[113] in Germany have examined the relationship between microemboli, NP outcome and cerebral glucose metabolism. This was also a small study of only 18 patients having coronary artery bypass grafting. Two patients also had valve replacements and thus

had much higher microemboli counts. They found that in those patients whose regional glucose metabolism was reduced post operation the regional reduction was correlated to the microemboli count. However there was no overall correlation between the number of HITS, the neuropsychological outcome or global cerebral glucose metabolism. The significance of all this is doubtful in such a small study.

Borger et al[114] have conducted several studies in which they have shown perfusionist interventions during which air is entrained into the venous side of the CPB circuit contribute to microemboli production. Borger et al's study was designed to test the hypothesis that an increase in air entrainment with these interventions would lead to impaired postoperative NP performance. They divided a non-randomised cohort of patients into two groups receiving either more or less than 10 interventions during CPB. They found that the group with less than 10 interventions had less NP impairment post surgery, however this group also had significantly shorter cross clamp and CPB times. A further flaw was the failure to perform actual microemboli counts such that they were not able to make a direct correlation between the microemboli count and NP outcome. More interventions could simply reflect longer more complicated surgery. Additionally Borger et al's hypothesis[114] that the majority of microemboli during CPB are gaseous secondary to perfusionist interventions is likely to be flawed. It certainly is not consistent with the majority of the microemboli literature which suggest that some microemboli are "bypass related" and some are aortic manipulation related.

Fearn et al[115] studied a group of 70 patients having CABG surgery with TCD of the middle cerebral artery to measure both microemboli and middle cerebral artery blood velocity and NP testing pre-operation then one week, two months and six months post operation. Fearn et al found a weak but significant ($r=0.3$, $p<0.02$) correlation between emboli and a reduction in

"overall memory reaction time" (a composite of several memory tests) at one week post surgery. Interestingly Fearn also found a correlation between measures of cerebral blood flow (perfusion pressure, middle cerebral artery blood velocity and cerebrovascular reactivity) and poor attention at one week. This therefore suggested that microemboli are a determinant of a specific NP outcome but that cerebral blood flow was also important. Neville at al[23], as has previously been discussed in chapter 1, seem to provide evidence to the contrary. That is, they suggest that the number of microemboli may not be a significant determinant of NP outcome, at least when comparing valve and CABG patients. Neville et al[23] confirmed previous findings that there were more microemboli detected during valve surgery but they were unable to find any difference in NP outcome. Although the two groups of valve and CABG patients in Neville's study[23] were well matched for age, other potentially significant characteristics such as diabetes prevalence were not well matched. Another possible explanation for the apparent disparity between Neville and Fearn's studies is that different types of microemboli may have been detected in the two studies. In Fearn's study all patients were having CABG surgery and so were likely to have had similar types, if not numbers, of microemboli (for example 40% particulate and 60% gaseous) thus allowing a correlation between microemboli and memory loss. In Neville's study there may have been more gaseous microemboli (possibly less harmful) in the valve group leading to a lack of correlation. Additionally, Fearn measured middle cerebral artery microemboli but Neville measured common carotid microemboli. It seems logical that middle cerebral artery microemboli are more likely to be associated with NP outcome than common carotid microemboli as many of the common carotid microemboli may not end up in the cerebral circulation. This comparison of Neville and Fearn's studies demonstrate how the differing methods and measures used to study microemboli lead to conflicting results regarding the relationship between microemboli and neuropsychological outcome.

Most recently, Stygall et al[37] investigated the relationship between intra-operative microemboli and outcome five years after surgery. In a longitudinal study of 107 patients having CABG surgery microemboli as well as post-operative change at 6 days and extent of recovery at 6 weeks were all found to predict NP performance at five years. No arterial line filters were used in this study and, as the authors point out, the number of microemboli detected may simply reflect the extent of the patients' cerebrovascular disease. Therefore as an alternative theory to microemboli causing the decline, microemboli could be a feature of those who will decline anyway.

The mechanism of damage arising from microemboli is unclear but unlikely to be merely a mechanical plugging effect. The small capillary and arterial dilatations that have been found in autopsy specimens post bypass and which seem to result from microemboli[116] suggest that microemboli may also have a locally destructive effect. Microemboli may induce a local inflammatory reaction as well as mechanically plugging capillaries.

Different types of microemboli are likely to produce different effects and although the evidence from valve versus CABG studies in which more microemboli (presumably air) in the valve groups does not lead to worse NP outcome suggest that air microemboli are less harmful, this is far from proven.

There is a consistent finding in the literature that when microemboli are compared in patients having surgery with and without CPB there are less microemboli in the patients not having CPB. This has been shown in both retrospective or non-randomised studies[74;77;78;117] and in a prospective randomised study[118]. Interestingly, in Lund's study[118] despite a significant reduction in the number of microemboli detected in the middle cerebral artery during off pump surgery, no difference in neuropsychological outcome was found. Cerebral blood flow

was not monitored by Lund and reduced cerebral blood flow in the off-pump group could have had an equally harmful effect as the microemboli in the on-pump group.

In summary at this stage, most work investigating mechanisms of the diffuse cerebral injury associated with cardiac surgery has concentrated on microemboli, that is solid, gaseous or lipid matter less than 200μm in diameter. Microemboli are most commonly and most accurately measured using transcranial Doppler, although even this technique has important limitations. A considerable number of investigators (eg Pugsley et al[8], Clark et al[110], Fearn et al[115] and Stygall et al[37]) have found a correlation between the numbers of microemboli detected by TCD and NP outcome, with more microemboli being associated with worse outcome. However, this has not been a consistent finding and others have found no relationship between microemboli and NP outcome (eg Neville et al[23], Arrowsmith et al[42], Mullges et al[108] and Lund et al[118]). It is likely that the limitations of TCD as well as methodological differences between studies have led to this inconsistency. The relative contribution of other potential causes of injury such as inflammation and altered cerebral blood flow could also interfere with a consistent relationship between microemboli and NP outcome. It is for this reason that cerebral blood flow is measured in the present study. The present study also uses TCD as the most accurate measure of cerebral microemboli available. The next chapter, chapter four, will therefore outline the inflammatory response to bypass with emphasis on the physiology of neutrophils, which the present study aims to filter, and then chapter five examines what investigation has already been made with various types of arterial line filter.

Chapter 4

The role of neutrophils in the inflammatory response to cardiopulmonary bypass and potential role in diffuse cerebral injury

A vast amount of attention has been devoted to demonstrating that cardiac surgery with cardiopulmonary bypass produces a systemic inflammatory response. As with neuropsychological injury, it has always been assumed that the CPB circuit was the main culprit in producing this frequently harmful response. However more recent studies performed on patients having cardiac surgery without CPB, show that there is still a marked inflammatory response. This suggests that the cardiac surgery and anaesthesia themselves are responsible for a considerable component of the inflammatory response. The purpose of this section of the literature review is to outline this systemic inflammatory response concentrating on the central role of the leucocytes as these cells are the focus of the present study. This section will explain how leucocytes, and in particular neutrophils, become activated and how, once activated they interact with other blood components and the vascular endothelium to produce tissue injury and possibly microemboli. The evidence in this chapter thus provides the rational for attempting to filter leucocytes from the circulation. As has already been mentioned, little study has been made of the role of the inflammatory response in causing cognitive decline, but the work that currently exists is examined.

Several factors have been implicated in initiating the inflammatory response. The factors include the exposure of blood to foreign surfaces, ischaemia-reperfusion of organs, surgical trauma, non-pulsatile flow and endotoxin release. These triggering factors then activate complement, coagulation, fibrinolysis and kallikrein-kinin pathways to cause leucocyte activation.

When blood is exposed to the foreign surface of the CPB circuit, the complement protein cascade is the first to be activated[119-121] and this leads to formation of anaphylatoxins C3a and C5a, mainly via the alternative complement pathway. Activation of complement via the classic pathway also occurs when blood meets air and when heparin is reversed with

protamine to form heparin-protamine complexes[122;123]. Although the surgical procedure can itself activate complement, it is seen that levels of C3a only rise markedly at the beginning of CPB and then peak at the end of CPB. Levels of C3a also correlate positively with the length of CPB[121]. Endotoxin which is released into the circulation (probably from the bowel) during CPB can also activate both the classic and the alternative complement pathways.

The C5a produced by complement activation then leads to both the margination and aggregation of neutrophils[124;125] and also the adherence of neutrophils to the vascular endothelial cells[126]. It is thought that both complement activation leading to this margination, aggregation and adherence of neutrophils is responsible for the initial fall in circulating neutrophils which is seen at the beginning of CPB[120].

The coagulation and fibrinolysis cascades are both activated by the contact of blood with the extracorporeal circuit. It is relatively far down this cascade that it is eventually inhibited by heparin to prevent coagulation. The negatively charged surfaces on the CPB circuit and also exposed collagen from tissue injury cause activation of factor XII. Factor XIIa then stimulates neutrophil aggregation and also the release of elastase[127]. Factor XIIa also generates kalekrein which causes neutrophil chemotaxis[128], aggregation[129] and degranulation[130].

At the beginning of CPB there is an immediate fall in the number of circulating leucocytes with a particular reduction in mature neutrophils and granulocytes. This is thought to be due to haemodilution of blood by the bypass prime, simple adhesion of neutrophils to the adsorbed protein layer of the CPB circuit and the complement mediated activation of neutrophils leading to their margination. There is a later rise in the leucocyte count towards the end of bypass which is presumably due to a release of new leucocytes from the bone

marrow or a reversal of their marginated state. Hypothermia reduces this response[131] and the rise in leucocyte count is often seen during rewarming following systemic hypothermia.

Neutrophils are activated by C3a, C4a, C5a, PAF (platelet activating factor), leukotriene B4 and other mediators. Within 20 seconds of being exposed to activated complement, Craddock et al[132] were able to photograph the morphological changes in neutrophils as they became rigid and grew pseudopods. When neutrophils become activated, they express specific adhesion molecules on their surface such as CD11b/CD18. Gu et al[133](1992) studied 16 patients having CPB and showed that CD11b/CD18 expression on neutrophils increased immediately after starting CPB at the same time as the rise in C3a. There was then a second peak in CD11b/CD18 expression after release of the aortic cross clamp and only this second peak was followed by a marked rise in serum elastase. Therefore, in order to cause tissue damage in vivo, neutrophils do not only themselves need to become activated, but also need to adhere to activated endothelial cells.

Endothelial cells in their resting condition are normally relatively inert. In response to a host of inflammatory signals such as cytokines, endotoxin, activated complement and others they are converted to an activated state and express their own adhesion molecules such as E-selectin (also known as Endothelial Leukocyte Adhesion Molecule 1) and ICAM (Intercellular Adhesion Molecule). There are two phases of endothelial cell activation which occur during cardiopulmonary bypass. In the first immediate phase circulating complement activation products cause an immediate but short-lived neutrophil adhesive response. The second phase occurs after several hours as the endothelial cells, in response to circulating cytokines and endotoxin, manufacture and express surface activation proteins such as ICAM and E-selectin.

Foreman et al[133;134] has shown in vitro that C5a is a potent agonist of endothelial cell P-selectin (also known as Platelet Activation-Dependent Granule) expression. It is therefore thought that the immediate expression of P-selectin resulting from endothelial C5a exposure could cause the sudden leucosequestration which follows commencement of CPB. P-selectin is the most readily available of all the endothelial cell activation molecules and is stored in cytoplasmic vacuoles and can be rapidly transported to the cell membrane for expression without having to be manufactured. Once neutrophils have adhered to the complement activated endothelium they release proteases such as elastase and oxygen derived free radicals. These harmful substances then cause endothelial cell dysfunction and subsequent leakage of fluid through the capillaries into the extravascular spaces. Although rapid, both in vivo[135] and in vitro[136] studies show that the response is short lived and as it wears of it is replaced by a more prolonged response.

During CPB cytokine and endotoxin levels rise in proportion to the length of the procedure and bind to the endothelial cells. In the first 4 to 8 hours after CPB the E-selectin and ICAM are expressed. The delay results from these proteins requiring synthesis. E-selectin cause the initial rolling phase of neutrophil-endothelial cell interaction through low affinity binding. ICAM then forms the form bond with the neutrophils. This phase is more prolonged and last up to 24 to 48 hours after CPB. Chello et al[137] have shown that following CPB apoptosis (programmed cell death) of leucocytes is delayed. That is the leucocytes live for longer. This effect was abolished by depleting the surgical plasma of IL-6 and IL-8. Leucocyte depleting arterial line filters can only reduce activated leucocytes during CPB and so if they have any effect it would be on the initial short lived neutrophil-endothelial cell interaction. Whether leucocyte filtration can inhibit the leucocyte platelet adhesion which occurs in bypass[138]has yet to be studied.

There are a considerable number of studies which report an increase in serum elastase during CPB. As an alternative to measuring the proportion of neutrophils expressing CD11b/CD18 molecules it has been used as a measure of neutrophil activation. Connective tissue in vivo is protected against elastase injury by an anti-protease called α-1-antitrypsin which binds irreversibly to elastase in the plasma and inactivates it. When elastase levels are measured what are actually being measured are levels of the inactivated elastase-α-1-antitrypsin complex.

All investigators have found that levels of serum elastase rise progressively during CPB and peak at the end of CPB. Levels then return to pre-operative levels by about 24 hours. Ascione et al[139] investigated the effect of CPB itself on the inflammatory response. They measured elastase, IL6, C3a and C5a peri-operatively in 60 patients randomised to receive CABG either with or without CPB. As with other studies, elastase was found to peak just after bypass in patients having CPB. In those not having CPB, elastase rose slowly and to a much less extent. C3a and C5a levels were similarly attenuated. Therefore this study suggested that avoiding CPB reduced but did not abolish the inflammatory response.

A significant study has recently suggested that neutrophils become primed and sensitised before CPB begins. In a relatively small study of 20 patients undergoing CPB for a variety of procedures, Gu et al[140] (2002) found that the primeability and release of elastase by neutrophils on stimulation in vitro by cytochalasin B increased and actually peaked before the beginning of CPB. In contrast the primeability of neutrophils was relatively low in the subsequent period during and after CPB. This suggested that neutrophils become preactivated by surgical trauma or anaesthetic treatment rather than contact of blood with the CPB circuit as most other studies have demonstrated. The exact relative roles of surgical

trauma, anaesthesia and CPB in initiating and maintaining the inflammatory response have yet to be elucidated.

This chapter has shown that neutrophils have a pivotal role in the inflammatory process which causes tissue injury. However, there has been little study so far of the link between the inflammatory response and NP outcome. A potential relationship between the two was first proposed by Smith in 1996[141]. There is only one study to date which has directly investigated any link between inflammatory mediators and post-operative NP functioning. Westaby et al[142] prospectively measured a variety of inflammatory mediators (beta-thromboglobulin, thromboxane, interleukin-6, prothrombin fragment 1 and 2, D-dimer, complement C4 split product and terminal complement complex) and NP performance pre-operatively and 5 days and 3 months post-operatively in 100 patients undergoing elective CABG surgery. Westaby et al[142] found no significant correlation between levels of inflammatory mediators and NP outcome. All the markers measured are ones which become raised when there is blood and foreign surface interaction. There is therefore, currently no evidence that the inflammatory reaction has any role in the cerebral injury that produces NP deterioration after cardiac surgery.

Chapter 5

The use of conventional and leucocyte depleting arterial line filters to reduce CPB induced injury and the inflammatory response

5.1 Development of Filters

Arterial line filters are an optional addition to the cardiopulmonary bypass circuit which have been clinically available in evolving forms since the early 1970s. These filters are designed to remove bubbles, particles and also, in the case of the LG-6 filter, activated leucocytes which may pass from the CPB circuit into the patient with potentially damaging results. Filters were originally developed in the 1960s by two American pioneers. Swank, in Oregon, developed a Dacron wool depth filter to first remove microaggregates from stored blood[97] and then for use during CPB[143]. Simultaneously, Patterson in New York City designed a screen micropore filter which was able to reduce the number of microemboli in animals and improve their neurological outcome following CPB[144]. This was a 25μm stainless steel filter and caused haemolysis to a degree unacceptable for clinical CPB. In the 1970s, the Dacron-wool depth filter made by Pioneer-Swank and the polyester-mesh screen filter made by Pall were the earliest commercially available filters suitable for placement in the arterial line. Variants of both were subsequently made by other companies but the screen filter became dominant, so that by the early 1980s, 99% of arterial line filters used were screen filters[145;146].

The depth filter became obsolete and the two types of filter available today are the conventional "stand alone" screen filter, of which there are several with different pore sizes from a variety of manufacturers, and the screen filter with a leucocyte depleting element (the LG-6 filter). Pall introduced leucocyte depleting arterial line filters in the early 1990s as a result of a number of animal studies which clearly showed that leucocyte filtration was able to reduce the inflammatory mediated damage to a number of organs.

In 1981, 64% of American perfusionists surveyed used an arterial line filter[146]. By 1995, this figure had increased to 92%[105]. Although filters have been available for 30 years, they

have not been universally accepted and, of note, there is a marked disparity in the prevalence of use between the USA and the UK. Despite Professor Treasure, working in a UK centre, stating in 1989 that "we should recommend their use in all cases"[147] a survey in the UK nine years later in 1998 revealed that they are used routinely by only 57% of surgeons[148]. This contrasts with a 98.5% use in the USA recorded in 2000[47].

This section of the review will analyse the clinical evidence relating to their use and determine, if possible, what benefit they may have. Although there is a great deal of published work showing a benefit from arterial line filters in animal models, this literature review is restricted to in-vivo studies on humans. Studies were selected if they used an arterial line filter on humans. Investigations into other aspects of filtration such as cardiotomy suction filtration, cardioplegia filtration, venous line leucodepletion and filtration of blood retransfused post CPB were excluded. A range of different outcomes have been studied including the broad (length of hospital stay)[149] to the more precise (biological assays such as the marker of neurological damage S100b)[150]. The two main types of filter, the conventional (25-40μm) filter and the leucocyte depleting (LD) filter, will be considered.

Table 3.21 (following page) summarises the design and sample characteristics of the studies reviewed. Most of the studies (23/28 = 82%) were randomised controlled trials (RCTs), but there were also some observational, historical and retrospective studies. Results of the latter types of study must be treated with caution. The RCTs fall into two main groups: those comparing a conventionally filtered group (screen or depth) to a

Name	Year	Type of Study	n	Population	Definite In/Exclusion criteria
Baksaas[151]	1999	RCT	40	elective 1st time CABG	age>70, infection, liver disease, DM, steroidal/renal or cerebral insufficiency
Mair[152]	1999	RCT	40	elective CABG patients	EF<50, raised WCC, pre-op pO2 < 60mmHg
Baksaas[153]	1998	RCT	40	elective 1st time CABG	infection,hepato-renal disease, malignancy,steroidal/nsaid medication
Gott[149]	1998	Observational	400	elective 1st/redo CABG/valve/aortic cases	None - was deliberately designed to include a wide range of cases
Hurst[154]	1997	RCT	24	elective valve cases	emergency or no informed consent
Taggart[150]	1997	RCT	40	elective first CABG	Hx of TIA/CVA or marked renal impairment
Di Salvo[155]	1996	RCT	20	urgent CABG	triple CAD + LMS>70% or Unstable angina or failed PTCA or EF<40%
Lust[156]	1996	RCT	50	open ht cases	None mentioned (not even pre-op lung function - an outcome measure)
Thurlow[157]	1996	RCT	14	elective first CABG	age>80, EF<30%, est. CPB time>60mins
Hachida[158]	1995	RCT	28	open heart valvular surgery	None mentioned
Henrikson[159]	1995	RCT	39	unselected open ht surgery(valve/CABG)	history of cerebrovascular disease
Johnson[160]	1995	RCT	32	elective CABG cases	None mentioned
Mihaljevic[161]	1995	RCT	32	elective CABG cases, males	female, abnormal pre-op lung function, age>75
Allen[162]	1994	RCT	50	not stated(letter report)	not stated
Coleman[163]	1994	Observational	189	CABG	None mentioned
Palanzo[164]	1994	Retrospective	36	CABG or Ao valve cases	abnormal pre-op lung function
Pugsley[8]	1994	RCT	100	elective CABG	age>70 Hx of TIA/strokes,uraemia,diabetes excluded
Sellman[165]	1993	RCT	54	elective male CABG patients age < 70	Hx of cerebrovascular,carotid,neurological or coagulation disorder
Borowiec[166]	1993	RCT	31	elective CABG	coagulopathy,DM,renal failure,redo,ASA/warfarin within 2 weeks
Palanzo[167]	1993	RCT	350	open ht cases	None mentioned
Sellman[168]	1992	RCT	29	elective CABG	age>70, cerebrovasc/neuro/carotid/coagulation disorders, alcoholism
Padayachee[169]	1988	RCT	18	elective CABG /AVRcases	None mentioned
Aris[170]	1986	RCT	100	elective cardiac cases	age>70 (N.B. left heart opened in 30% of cases)
Garvey[171]	1983	RCT	46	open ht "surg patients	Included if available and agreed to participate
Loop[172]	1976	Observational	60	CABG, CABG/valve, ventric. aneurysmect.	patients selected mainly by the magnitude of the operation"
Dutton[173]	1974	Historical	30	cardiac cases	None mentioned
Page[174]	1974	RCT	20	open ht cases	None mentioned
Connell[175]	1973	RCT	37	open ht "surg patients	None mentioned

Table 3.1 - Design and sample characteristics

Coleman et al[163], Connell[175] and Lust[156] have no exclusion criteria whereas Mihaljevic[161] and Palanzo[164;167] appropriately exclude patients with abnormal pre-operative lung function.

Another problem relates to the definition of "open heart surgery". Strictly, it should mean surgery in which chambers of the heart are opened, however in some studies it is not clear whether chambers of the heart have or have not been opened. Henrikson et al's[159] "open heart surgery" patients do include both CABG and valve surgery whereas one cannot be clear from Garvey et al's[171] , Page et al's[174] or Palanzo et al's[164;167] papers whether this is the case. This is likely to be of crucial importance in studies of microemboli or neuropsychological function because these outcome measures may be affected by whether or not the heart chambers are opened.

It is unclear in some papers whether the population used was high or low risk, especially as relative risk will have changed greatly over the time period from which these studies are drawn. Most studies seem to have used a low or mixed risk population. Di Salvo et al's[155] is the only randomised controlled trial to have selected a high risk population. There is a theoretical argument suggesting that filters may be of more benefit in high risk cases, but there is little evidence to support this. In fact, in the only study comparing the filter

non-filtered control group (which all investigated microemboli and/or cerebral outcomes) and those comparing a leucocyte-depleting filter to a conventional filter all of which measured inflammatory and related clinical outcome.

The sample size in these studies varies considerably from 14 to 400. One might expect investigators to perform a power calculation before deciding on a sample size but Hurst[154] was the only one to document such a calculation in the published paper. The power of a study is especially important when the outcome is negative but none of the studies with negative outcomes discuss power.

The characteristics of a study sample are important in relation to the generalisation of the findings. The details of the characteristics of the samples inevitably vary considerably between studies which span 30 years. However, only 15 out of 28 (54%) studies defined their population. In some cases the population was clearly defined, e.g. Taggart et al[150] used only elective first time coronary artery bypass graft (CABG) patients and excluded patients with a history of strokes, transient ischaemic attacks or renal impairment. This contrasts with studies such as Dutton et al's[173], which refers to "cardiac cases" with no definite inclusion or exclusion criteria. In those studies which examine the mechanical characteristics of filters or those relating to reduction in microemboli, the type of surgery or the risk stratification of the population may not have relevance. However, those studies whose outcomes are clinical should ideally have a more strictly defined selection criteria. For example, Henrikson[159], Sellman[168] and Taggart[150] investigated cerebral blood flow, magnetic resonance imaging (MRI) brain changes and S100b levels respectively. Since they were all measuring outcomes related to the brain and brain injury they all excluded from their study population patients with a history of cerebrovascular disease. In this respect these studies were well designed. This contrasts with a mix of design in the studies looking at post surgery lung function.

Name	Test Group/s	Control Group	Test/Control Similarity	Surgery	Anaesthesia	Perfusion
Allen[162]	Pall LG-6	Pall EC Plus	not mentioned	standardised	standardised	standardised
Aris[170]	Shiley 20 um	No filter	demonstrated	standard MP (CCC)	not mentioned	not mentioned
Baksaas[151]	Pall LG-6	Pall AV6	demonstrated	standardised	standardised	standardised
Baksaas[153]	Pall LG-6	Pall AV6	demonstrated	standardised	standardised	standardised
Borowiec[166]	heparin coated CPB/	standard CPB/	demonstrated	not mentioned	standardised	standardised
	<heparin dose*	heparin dose				
Coleman[163]	Pall LG-6	("standard art line filter")	---	not mentioned	not mentioned	not mentioned
Connell[175]	Dacron wool filtration*	No filter	not mentioned	not mentioned	not mentioned	standardised
Di Salvo[155]	Pall LG-6	Pall Autovent	demonstrated	standardised	not mentioned	not mentioned
Dutton[173]	Bentley 27um + 1)bubble or	400um stainless steel filter	not mentioned	not mentioned	not mentioned	standardised
	2)membrane oxygenator	and bubble oxygenator				
Garvey[171]	Pall 40um and	(No filter)	---	not mentioned	not mentioned	not mentioned
	Bentley AF-10 (25um)					
Gott[149]	4 test groups: steroid, aprotinin,		stated	standardised	not mentioned	not mentioned
	leucocyte depletion and					
	heparin bonded circuitry. No controls					
Hachida[158]	Pall LG-6	Pall Autovent SV	stated	standardised	not mentioned	standardised
Henrikson[159]	2: Johnson 20um and Pall 40um	No filter	demonstrated	not mentioned	standardised	standardised
Hurst[154]	Pall LG-6	Pall Autovent SP (40um)	demonstrated	standardised	standardised	not mentioned
Johnson[160]	Pall LG-6	Pall Autovent SP (40um)	demonstrated	not standardised	not mentioned	not mentioned
Loop[172]	4: Pall 40um,Bentley 27um,	None	---	not standardised	not mentioned	standardised
	JJ 20um,Pioneer Dacron wool					
Lust[156]	Pall LG-6	Pall Statprime	stated	not mentioned	not mentioned	not mentioned
Mair[152]	Pall LG-6	Pall AV6	demonstrated	standardised	standardised	standardised
Mihaljevic[161]	Pall LG-6	standard filter (not named)	demonstrated	standardised	standardised	standardised
Padayachee[169]	2: Bentley 40um and	No filter	not mentioned	standardised	standardised	standardised
	Bentley 25um					
Page[174]	Pioneer Dacron wool	No filter	not mentioned	not mentioned	not mentioned	not standardised
	(Swank)					
Palanzo[164]	Pall LG-6	Electromedics 40um filter	demonstrated	not mentioned	not mentioned	not mentioned
Palanzo[167]	Pall LG-6	Pall EC Plus	demonstrated	not mentioned	not mentioned	not mentioned
Pugsley[8]	Pall EC Plus	No filter	demonstrated	standardised	standardised	standardised
Sellman[165]	Swank high flow 6000	No filter	demonstrated	standardised	standardised	standardised
Sellman[168]	Swank high flow 6000	No filter	demonstrated	standardised	standardised	standardised
Taggart[150]	Cobe Sentry	No filter	demonstrated	standardised	standardised	standardised
	(43um heparin coated)					
Thurlow[157]	Pall LG-6	Pall AV6	demonstrated	not mentioned	not mentioned	not mentioned

Table 3.2 - Details of Study Design

between different risk groups, Gott[149] found LD filters to be more effective in the lower risk group of patients.

Table 3.22 (preceding page) shows details of study design

In RCTs it is conventional to demonstrate the similarity of the test and control populations. Most (19 out of 28 = 68%) of the studies in this review either demonstrated or stated the similarity.

Only 6/28 (21%) studies stated if and how they had controlled and standardised all the operative variables of surgery, anaesthesia and perfusion. Since these factors have a potential confounding effect upon outcome one would wish these to be standardised as much as possible.

Six separate outcomes have been identified and are discussed in turn. These are summarised in table 3.23 (following page)

	Primary Outcome Measure	Difference	Sig
Emboli			
Dutton[173]	platelet aggregate emboli	yes, filter removed microemboli	yes
Page[174]	screen filtration resistance (microemboli)	yes, lower SFR/microemboli after filtration	yes
Loop[172]	ultrasound detected microemboli pre- and post-filter	yes, Dacron wool filter most effective - removed 99%	---
Padayachee[169]	cerebral microemboli	yes, 25um<40um<no filter	yes
Borowiec[166]	scanning electron microscopy of art line filters	yes, <adhesion with heparin coated filter	yes
Cerebral			
Garvey[171]	neuropsychological (CLAT)	yes, decreased impairment with Pall	yes
Aris[170]	neuropsychological (battery)	no difference	---
Pugsley[8]	neuropsychological function and microemboli	yes,less microemboli,improved neuropsych function in filter gp	yes
Sellman[168]	magnetic resonance image brain changes	no difference	yes
Sellman[165]	neuropsychological (battery)	membrane oxygenator better than bubble oxygenator without filter in select tests	
Henrikson[159]	cerebral blood flow	yes, filters reduced cerebral hyperperfusion	yes
Taggart[150]	S100 (marker of brain injury)	yes, decreased S100 in filter group at 5 and 24 hrs post op	yes
Inflammatory			
Thurlow[157]	neutrophil activation markers	yes, decreased neutrophil activation across LG-6 filter	yes
Hurst[154]	1)yes, at 1, 4, and 24 hours post op 2)no		yes
Baksaas[153]	leucocytes,myeloperoxidase,C3a,IL-6,TNF-a	no difference	---
Baksaas[151]	1)inflammatory mediators 2)Leucocytes	1)no difference 2)decreased total WCC until 2hrs post op	yes
Mair[152]	1)inflammatory mediators 2)Lung function	1)increased elastase in LG-6 group 2)no difference	yes
Cardiac			
Hachida[158]	cardiac and lung function	yes,reduced CK-MB/Adr dose.increased pulm index	yes
Johnson[160]	cardiac and lung function	increased BP/decreased WBCs and intrapulm shunt 4 hrs post op	yes
Di Salvo[155]	myocardial damage(biochemical markers)	yes	yes
Pulmonary			
Connell[175]	histological damage to lung tissue	yes,decreased with Dacron filtration	
Coleman[163]	post operative oxygen requirement	yes,decreased with LG-6	
Lust[156]	Lung function	no difference	---
Mihaljevic[161]	Lung function	no difference	---
Palanzo[167]	lung function and post op complications	yes, only decreased ventilator hours in LG-6 group	yes
Palanzo[164]	lung function and length of hospital stay	yes, less vent.hours and post op days in LG-6 group	yes
Allen[162]	1)Lung function 2)White cell count	1)Trend towards improved oxygenation 2)Reduced post op leucocytosis	
Other			
Gott[149]	length of hospital stay and cost saving	reduced in low risk patients by LD	yes

Table 3.23 Outcomes

5.2 Microembolism

Of the three main aetiological factors thought to cause diffuse cerebral injury during cardiac surgery, namely microemboli, the inflammatory response and altered cerebral blood flow/pressure, microemboli have received most attention[4]. In fact, filters have been instrumental in establishing the causal link between microemboli and neuropsychological deterioration after cardiac surgery.

The first clinical controlled trial to show that filtration can reduce microemboli was that of Dutton et al[173] in 1974 which compared a 27μm polyurethane stratified filter (Bentley) with either a bubble or membrane oxygenator to bubble oxygenation with a 400μm stainless steel filter (effectively no filtration). The 400μm filter removed no microemboli (defined as emboli less than 200μm diameter) whereas the Bentley filter removed 90% of microemboli >150μm and 60% of microemboli 50 - 150μm. Dutton[173] examined a very narrow aspect of filtration as he was able to detect platelet aggregate microemboli only, whereas it is now known that microemboli may be gaseous or particulate and that the particulate emboli can consist of a wide range of substances - lipid, calcium, platelets, fibrin, spalated tubing to name a few. In the same year, Page et al[174] used the Pioneer Dacron wool filter in a small randomised controlled trial. They used an indirect measure of microemboli - the "screen filtration resistance" (SFR) which only reflects particulate microemboli - to show that filtration reduced the SFR and also, presumably, microemboli. Loop et al[172], two years later, evaluated four of the then available arterial line filters by recording pre- and post- filter ultrasound of the arterial line when bubble oxygenators were used. Their ultrasound transducer detected bubbles or particles (without distinction) down to 80μm in diameter, although microemboli may be smaller than this. The efficiency of the filter was then determined by subtracting from 100% the number of counts occurring downstream of the

filter divided by the number of counts occurring upstream. The Dacron wool depth absorption filter had the highest efficiency (99%) followed by the 20μm woven nylon mesh filter (92%), the 40μm polyester mesh filter (79%) and a 27μm stratified polyurethane filter (75%). Although the Dacron wool filter was the most efficient, Loop[172] notes that one of these filters became obstructed- a potentially dangerous complication of all filters. Also microemboli may be smaller than the 80μm limit of the transducer used and the filters may not be as efficient as they appear. The filters may be letting through microemboli less than 80μm diameter.

With previous studies showing filtration to remove microemboli from sampled blood, Padayachee et al[169] was subsequently able to show that filtration can reduce the number of microemboli that actually reach the cerebral circulation. Using bubble oxygenators, 18 patients were randomised either to no filter, a 40μm screen filter or a 25μm screen filter groups. Transcranial Doppler (TCD) was used to measure the number of microemboli reaching the middle cerebral artery and there was a significant reduction in microemboli counts in both the 40 and 25μm groups. The authors report this as a reduction in "gaseous microemboli" although their TCD could not distinguish between particles and bubbles. The fact that membrane oxygenators produce less microemboli than bubble oxygenators suggests that the excess produced by bubble oxygenators may be bubbles, however, as was mentioned in the previous chapter, bubble oxygenators also produce more particulate microemboli than membrane oxygenators so this is not certain.

Borowicz[166] showed that heparin coating of arterial line filters reduces the adhesion of cellular material to such filters. The biochemical or clinical consequences of this were not, however, studied.

5.3 Cerebral Outcomes

There are four published studies examining the effect of filtration on neuropsychological outcome. Garvey[171], in 1983, compared 46 unselected open heart surgery patients who received either a Pall 40μm or Bentley AF-10 (25μm) filter with a historical group from nine years previously which had received no filtration. Neither anaesthesia, surgical or perfusion protocols are mentioned and the fact that the controls are historical makes any comparison very problematic. Additionally, intellectual brain function was assessed using the CLAT (conceptual level analogy test) which is a single global measure of cognitive function. This type of assessment is unlikely to have the sensitivity required to detect changes following cardiac surgery. The decreased impairment found with the filter group could be due to many changes which could have occurred in the nine years between sampling the different groups.

In his RCT of 100 cases, Aris[170] used a more comprehensive battery of neuropsychological tests. This was therefore a much more rigorous study than that of Garvey. Aris compared patients who had a Shiley 20μm nylon screen filter with a non-filter control group but was unable to show any difference in outcome between the two. A negative result in a study of this size is unlikely to be the result of a type II error, but could be due to a number of other factors. The filter could have no effect or the filter could have a detrimental effect due to its small pore size or unusual form (nylon). More relevant is the possibility that the neuropsychological tests were performed at too early a stage post-operatively. Aris performed these tests at ten days post-operatively when many peri-operative factors such as anaesthesia, analgesia, pain or anxiety may continue to cloud the patients' neuropsychological function. The 1995 consensus suggests that the ideal time to perform post-operative testing is three months post surgery[14]. Many studies use four to twelve weeks.

A better NP assessment technique was used by Sellman[165] who tested three groups with a battery of NP tests before surgery and at one and six months post surgery. The post-operative testing was therefore likely to be far more sensitive than Aris'. Sellman did not find a significant difference between the bubble oxygenator group with 10μm in-depth adsorption filter and the bubble oxygenator group without a filter. However, the membrane oxygenator group (without filter) was found to perform better than the bubble oxygenator group without filter at one month post surgery on certain NP tests. Unfortunately the value of this study is limited by the small numbers (17 to 20 in each group).

It was not until the study of Pugsley et al[8] a year later which used a greater number of subjects and a neuropsychological battery at appropriate times that more convincing evidence of a neuroprotective effect of filtration was produced. One hundred patients receiving bubble oxygenated CPB were randomised to receive either the Pall 40μm (Polyester screen) arterial line filter or no filter. Perfusion, anaesthesia and surgical technique were all well controlled. NP function was assessed pre-operatively, at 8 days and 8 weeks post operatively. TCD was used to assess the number of microemboli occurring in the middle cerebral artery intra-operatively. Confirming the results of Padayachee et al's study[169], Pugsley showed that the 40μm filter significantly reduced the number of microemboli. Most importantly, Pugsley demonstrated a correlation between the number of microemboli detected and the incidence of a NP deficit post operatively. Those with a microemboli count < 200, 5/58 = 8.6% had a deficit whereas those with counts > 1000, 3/7 = 43% had deficits. Examining the effects of the filter, filtered patients at eight weeks post surgery showed less deterioration on eight out of ten NP tests compared to the non-filtered group. A deficit in performance (defined in Pugsley's study as a reduction in one SD of test score in two or more tests) occurred in 4 of 49 filtered patients and 12 of 45 non-filtered patients ($p<0.03$). Although Pugsley used bubble oxygenators and pH stat pH control, both

of which are little used today, the study provides good support for the value of arterial line filtration.

Three studies have investigated the effect of conventional filtration on cerebral outcomes other than neuropsychological function. Henrickson[159] (1995) investigated the effect of two different types of filter on cerebral blood flow, Sellman[168] (1992) examined the effect of a single filter type on post-operative magnetic resonance imaging (MRI) of the brain and Taggart[150] (1996) studied the effect of filtration on serum S100b. All three studies used suitable, although not identical populations. Sellman used elective CABG patients, Taggart used elective first time CABG patients but Henrikson used both valve and CABG patients. It has been shown that there are increased serum S100b levels after valve surgery compared to CABG and so there may be a higher potential for brain injury in valve patients. All three were RCTs and those of Taggart and Sellman were the more rigorously constructed as they standardised anaesthetic, surgical and perfusion protocols. Henrikson was unable to standardise surgical protocol.

Henrikson found that there was brain hyperperfusion on CPB in the control group and that both the 20 and 40 μm filters reduced this hyperperfusion but did not bring it to or below physiological levels. It would therefore seem unlikely that these filters are having a detrimental effect upon brain perfusion and could even protect the brain from hyperperfusion and the increased "embolic load" that this could entail. However, the relationship between cerebral blood flow and NP or neurological outcome is complicated and so one should not extrapolate too far beyond Henrikson's actual finding. Sellman was unable to find any MRI changes whether or not filters were used. This is not surprising as MRI has been found to be relatively insensitive at detecting brain injury at the diffuse cellular level (although some changes can be found post cardiac surgery[176]). Taggart found a significantly decreased

S100b level at 5 and 24 hours post surgery when 43μm heparin coated filters were used indicating that they may be reducing brain injury. Taggart's study was particularly interesting because although the previous studies discussed showed that filters could reduce microembolisation and improve neuropsychological function, these studies all used bubble oxygenators. Nearly everyone uses the superior membrane oxygenators now and Taggart's study provided evidence that filtration may reduce brain injury even when membrane oxygenators are used if S100b is a good marker of cerebral injury.

It therefore appears that the evidence regarding non-NP cerebral outcomes and filtration is inconclusive. The evidence regarding emboli and neuropsychological function is far more convincing. Neuropsychological outcome remains the gold standard measure of brain injury and S100b levels are a controversial measure in cardiac surgery.

5.4 Inflammatory Indices

Cardiac surgery with cardiopulmonary bypass produces a systemic inflammatory response. Without detailing the complex process which is well reviewed elsewhere[176;177] it is relevant to outline the pivotal role of leucocytes in this inflammatory response. Via the activation of complement, coagulation, fibrinolysis and the Kallikrein-kinin system leucocytes are themselves activated. When leucocytes become activated they express cell surface activation markers such as CD11b and CD18. They can then aggregate with each other, adhere to endothelial surfaces and degranulate to release superoxides, elastase and other proteases. These released substances destroy extracellular structures and thus increase tissue permeability causing inflammation and oedema. Activated leucocytes are therefore important mediators of tissue damage.

Leucocyte depletion for example by plasmapharesis was used as an experimental model in the 1980s to reduce the inflammatory response[162]. Depletion of leucocytes in animal models of cardiac surgery and bypass led to definite cardiopulmonary protective effects. The Leukoguard 6 (LG-6) filter was then developed to remove leucocytes from the arterial line in human CPB. The LG-6 filter has a conventional woven polyester 40μm screen component but it also contains a non woven polyester fibre mesh element which specifically traps leucocytes. Initial in vitro studies with this filter were encouraging. Using bovine blood the filter was able to remove 70% of neutrophils during 90 minutes of perfusion. Further studies with human blood showed the selective removal of activated neutrophils[178]. To date there have been five published clinical studies investigating the effects of the LG-6 filter on inflammatory markers.

Thurlow[157] randomised seven patients to LG-6 and seven to a conventional filter (AV-6) and measured peri-operative white cell count and differential, platelets, haemoglobin and in three patients only (two from the AV6 group and one from the LG-6 group) leucocyte cell surface activation markers and superoxide production. There was no difference in peri-bypass WCC, platelets or superoxide production. However, in the one patient studied there was a gradient in CD45RO and CD11a expression on leucocytes across the LG-6 filter that was not present in the two AV-6 patients similarly studied. This suggests that the LG-6 filter could selectively remove activated leucocytes. However as this was only demonstrated in one patient it might have been more appropriate to write it up as a case report.

Hurst[154] used a slightly larger sample size (n=24) to also investigate haematological measures including the cytokines IL-6 and IL-8 as well as CD11b and CD18 markers. Although they had used elective valve surgery patients in order to have cases with longer bypass times than CABG cases, the only significant difference found was a decrease in

CD18 on neutrophils in the LG-6 group. This difference persisted for up to 24 hours post surgery. The study therefore added some weight to the theory that the filter could selectively reduce the number of activated leucocytes. Hurst was unable to find any clinical (cardiac and lung function as measured by cardiac index, ejection fraction, blood pressure, inotrope use, FVC/FEV1, Pa O2 and length of hospital stay) in this relatively small group of patients. This result differed from a previous study by the same group[160] which had used CABG patients with shorter bypass times and showed a significant reduction in WCC at four hours post surgery. They suggest that the capacity of the LG-6 filter to filter leucocytes may have been exceeded by the longer bypass times in the valve study.

Baksaas[153] measured a raft of inflammatory markers in 40 patients who were randomised to receive LG-6 or a conventional filter. Leucocyte count, myeloperoxidase, cytokines, complement or platelets were not affected by the filter and, of note, bypass times were not particularly long in this study (about 80 minutes). This lack of a result led Baksaas et al to employ an alternative strategy of LG-6 filter use. In a study published a year later[151] the LG-6 filter was only used during the reperfusion phase of bypass. That is, only after cross clamp release until the end of bypass. The only significant finding was a reduced WCC at 2 hours post surgery. All other measures were unchanged. This strategy was therefore barely more successful than the usual method of using the filter throughout the whole period of bypass. Although there is a specific part of the inflammatory response which occurs during reperfusion, white cell activation occurs throughout bypass. Therefore if filtration is to be useful it seems logical to use it throughout bypass.

A recent study by Mair[152] was also unable to find any benefit from leucocyte depleting filtration in a prospective randomised clinical controlled trial. Forty patients were randomised to receive either a LG-6 or AV-6 filter. Surprisingly for a study investigating

inflammatory markers, Mair used low dose aprotinin in the pump prime. Aprotinin is a serine protease inhibitor with known anti-inflammatory effects which could thus potentially interfere with any filtration effect. They did not find a significant difference in WCC, differential, malondialdehyde and C-reactive protein. However, they found an increase in plasma elastase in the LG-6 filtered group during and immediately after bypass. Elastase is a proteolytic enzyme released by activated neutrophils. It is thought to have a locally destructive effect and becomes bound and inactivated in the bloodstream. Although it can logically be used as a marker of neutrophil activation, in the case of leucocyte filtration it crucially gives no indication as to *where* the leucocytes have become activated. A raised elastase could therefore reflect an increased release by those neutrophils trapped together in the filter rather than an increased activation of neutrophils in the tissues. One has to question whether trapping activated leucocytes in a filter can actually prevent them from doing harm as they are not totally removed from the circulation. They are clearly prevented from having a local destructive effect which is of potential benefit, but they could continue to release biologically active cytokines into the circulation which might then have a distant adverse effect.

In a more recent randomised study of 24 patients, Chen et al[179] in 2002 support the finding of leucocyte depletion being able to reduce expression of adhesion molecules. Chen et al showed that use of the LG-6 filter not only significantly reduced the total number of circulating neutrophils, but also reduced both CD11b and L-selectin expression during CPB, thus adding evidence that the LG-6 filter can selectively filter out activated leucocytes with these surface adhesion molecules.

In conclusion, it appears that the LG-6 filter has not been found to consistently reduce the actual number of circulating leucocytes during bypass. The bone marrow seems able to

supply more during bypass. Although three studies suggest that the filter may be able to selectively remove activated leucocytes this is not convincing and is not accompanied by a decrease in inflammatory mediators. One must conclude that even if filters can remove activated leucocytes these leucocytes are not totally removed from the circulation and are thus not prevented from releasing enzymes and possibly cytokines.

5.5 Cardiac Outcomes

The three studies that have investigated the effect of filters on measures of cardiac function all show a positive beneficial result despite being on different populations of cardiac surgery patients. Hachida et al[158] used 28 valve patients, Johnson et al[160] used elective CABG patients and Di Salvo et al[155] used 20 high risk CABG patients. All were RCTs and used LD filters tested against Pall Autovent 40μm filters. Di Salvo et al and Hachida et al were able to standardise surgery but not anaesthesia or perfusion techniques.

Di Salvo et al[155] found that the use of the LG-6 filter resulted in a decrease in serum troponin-T and an increased glutathione providing indirect evidence for a decrease in myocardial damage. Johnson et al's only positive clinical finding was increased blood pressure at 4 hours post surgery[160]. Hachida et al[158] showed that filtered patients had lower serum CK-MB levels and required less inotropic support. These studies therefore show either indirect evidence of decreased myocardial damage (by three different methods) or an only temporary clinical benefit. There is nothing to suggest a lasting clinical benefit from these outcomes and the long term effects on patients are unlikely to be as great as reducing neuropsychological deficits which may persist.

5.6 Pulmonary Outcomes

The greatest number of studies (eight) have addressed the effect of both conventional and leucocyte depleting filters on various pulmonary outcomes. A study by Connell[175] in 1973 showed that in a RCT of 37 unselected patients a Dacron wool filter was able to reduce the post bypass histopathological lung changes. Interestingly in relation to later studies using LD filters they found that the leucocyte aggregates found in the lungs of the unfiltered group were reduced by the Dacron filters. This was, however a descriptive decrease not supported by numerical data. The other seven studies are all recent and use the LG-6 filter. Palanzo's two studies from 1993 and 1994 using both valve and CABG patients indicated that leucocyte depleted patients spend less time ventilated and stay a shorter time in hospital[164;167]. However, both studies have flaws. The first, an RCT of 350 patients did not determine the characteristics of the patient population with regard to pre-operative lung function. The second study, which did exclude patients with abnormal pre-operative lung function is a retrospective study of only 36 patients. Coleman[163] conducted an observational study on 189 CABG patients. He found a decreased oxygen requirement in the filter group which, although statistically significant in those aged > 70, is a fairly soft outcome measure and must be treated with caution, especially in an observational study. Johnson[160] and Hachida[158] reported RCTs from 1995 in which they found a decrease pulmonary shunt and increased pulmonary index respectively. Lust[156] and Mihaljevic[161] found no difference in post-op lung function when LD filtration was compared to conventional filtration. The overall picture is therefore mixed but it does appear that LD filtration can have a beneficial effect on lung function and there is histological evidence to support and explain this.

5.7 Length of Stay and Cost Implications

Gott et al's large four arm study of 400 patients[149] was deliberately designed to incorporate a large range of risk and therefore selected all patients having CABG surgery. They used four anti-inflammatory measures – steroids, aprotinin, leucocyte depletion and heparin bonded circuitry. It was found that the LG-6 filter significantly reduced the length of hospital stay by one day in "low risk" (less than 5% predicted mortality) patients. It is unclear from this study exactly what specific clinical benefits were allowing this earlier discharge home. Sahlman et al[180] randomised 60 patients to either receive an LG-6 filter or no filter and measured clinical patient recovery. In this relatively small study no differences in length of ICU or hospital stay, use of inotropy or oxygenation index were found between the two groups.

5.8 Conclusions

Despite the wide ranging nature of the studies reviewed, a few conclusions are possible. The work of Pugsley[8] and the Middlesex Hospital group convincingly shows that conventional filters reduce middle cerebral artery microemboli and also the incidence of neuropsychological deficit. However, these studies were performed using bubble oxygenators and pH stat acid base management on bypass. Most centres now use membrane oxygenators and alpha stat acid base management and the neuroprotective effect of filters has not been assessed in this situation. Indeed it is possible that this is why some surgeons and perfusionists believe that arterial line filters are not necessary in this modern environment and this could account for their lower usage in the UK. Logically, conventional filters should filter out emboli produced by either type of oxygenator and there is no doubt that microemboli occur even with membrane oxygenators, if not in such great quantities as bubble oxygenators. Taggart et al's study[150] suggests that filters may reduce brain injury when membrane oxygenators and alpha stat are employed. Clark et al's study of 1995 has

shown that even when using alpha stat and membrane oxygenators the number of microemboli detected was related to the NP outcome[110].

The non-NP cerebral outcome studies are interesting, but even the potentially most significant, that of Taggart et al, awaits further validation of S100b as a marker of clinically relevant brain injury in cardiac surgery. Indeed all work involving S100b in which cardiotomy suction was used may now, in fact have little relevance because cardiotomy suction itself may make a major contribution to serum S100b levels[181]. Further work is required to relate these findings to those involving microemboli and neuropsychological change.

LD filtration has been the focus of recent studies showing a cardiopulmonary benefit. In high risk patients[155] there is certainly biochemical evidence for a cardioprotective benefit from LD filtration. Clinical benefit appears to be transient. As discussed, the effect of LD filtration on clinical lung function seems greater but there are some flawed studies as well as negative results and overall the studies are not conclusive.

Interestingly, there is a clear split between the outcome groups. All the cerebral studies compared a filtered to a non-filtered group. The more recently studied cardiac, pulmonary and inflammatory groups have compared the LG-6 filter to a conventional filter. This is obviously based on the reasoning that the LG-6 filter, by reducing the inflammatory response could attenuate inflammatory mediated damage to the heart and lungs. Since the studies of Pugsley, several potentially neuroprotective drugs have been tested in cardiac surgery with little or no benefit as far as the author is aware. To date there is no published data investigating the effect of the LG-6 filter on cerebral outcomes. The basis of this thesis is

therefore the investigation of the effect of leucocyte filtration on inflammatory response, microemboli production and neuropsychological outcome.

In conclusion there is enough evidence to justify the routine use of at least conventional filtration, if only for its neuroprotective effect. Leucocyte depleting filters are approximately twice the price of conventional filters and it is more difficult to justify their routine use as yet. They may benefit cardiac patients with co-morbid pulmonary disease, or they may benefit lower risk cases as demonstrated by Gott et al[149]. An increase in the use of both types of filter should be accompanied by further investigation into their clinical benefit using larger RCTs of sufficient power.

Chapter 6

The role of S100b as a marker of cerebral injury in cardiac surgery

Chapter one detailed the relatively laborious process of neuropsychological testing and also discussed the limitations of such testing. A simple biochemical marker of brain injury in the peri-operative period would therefore be an attractively convenient alternative to NP testing. It is for this reason that the role of the neuroprotein S100b as a marker for cerebral injury in cardiac surgery has been investigated quite intensively over the last ten years. This chapter will discuss what is already known about S100b and its relationship to cardiac surgery, cardiopulmonary bypass, microemboli and neuropsychological outcomes.

S100b is only one of a number of potential markers of brain injury. Other substances shown to be released from neurons, neuroglia, endothelium, platelets and leucocytes following cerebral hypoxia, ischaemia or other injury include Myelin Basic Protein (MBP) and Glial Fibrillary Acidic Protein (GFAP) from glia and Neuron Specific Enolase (NSE) and Adenylate Kinase (AK) from neurones. Such markers may be useful in indicating the severity or size of a cerebral lesion but they will all have certain inherent limitations. At the most they are likely to be useful as a guide rather than a substitute for clinical tests. A biochemical marker cannot give any anatomical localisation of a lesion nor may the degree of release reflect the clinical effect of a lesion. For example, a large infarct in the frontal lobe could produce a large release of marker with minimal clinical effect whereas a small infarct in the internal capsule might result in minimal marker release and yet a dense hemiplegia. However, these drawbacks of correlating marker release with neurological outcome may not apply to the global cerebral injury thought to produce neuropsychological deficits. As will be discussed in this chapter, there is some evidence that the degree of marker release does correlate with the degree of global brain injury as reflected by NP impairment. However, there still remains a lack of consensus in the literature on the relationship between S100b and neuropsychological outcome. It is not clear whether an increased S100b in the context of cardiac surgery reflects increased cerebral injury or is

actually an artefact. At present the evidence is mainly against S100b having a useful role. Nevertheless, at the time of planning this study there was intense interest in S100b and other assays as potential markers of brain injury after cardiac surgery. S100b was chosen as a marker for this study because an assay for S100b was available at a reasonable cost in our institution. S100b has no obvious superiority over other potential markers but as this study has utilised S100b as a marker of cerebral injury, discussion here is limited to this marker.

S100b is one of a family of 16 calcium binding proteins found in a variety of tissues. S100b is present in high concentrations in astroglial, microglial and Schwann cells and is highly specific to the brain. Its exact function is not known, but it has a role in the control of cell growth, cell structure and in signal transduction[182]. It was first identified in 1965 by BW Moore and called S100b due to its solubility in 100% ammonium sulphate. It is eliminated by the kidney and has a biological half-life of 113 minutes[183].

The nomenclature in the literature has changed as more members have been discovered[182] and the relevant nomenclature is as follows. There are three main dimeric isoforms S100a0, S100b and S100a made up from two monomeric subunits, A1 and B. S100a0 consists of A1-A1, S100b consists of B-B and S100a consists of A1-B. This is a crucial point because the initial studies which used S100b in the diagnosis of CNS pathology were describing the S100b dimer which is specific to the CNS. Later studies have, however, shown that the S100B subunit is present in extra CNS tissues such as the heart and aorta[184]. The terms S100b and S100B should not be used interchangeably, however the nomenclature in the literature is often confused and some researchers still refer to S100β[185].

To date and as far as the author is aware, studies evaluating cardiac surgical patients have exclusively used various versions of the Sangtec S100b assay which is commercially

available as a kit. The validity of the Sangtec S100b assay has never been published[184] and there are concerns that it may not be specific enough for cerebral S100b to make its use in cardiac surgery useful. As S100B is contained in the heart, aorta and other mediastinal tissues[182] which are disrupted by cardiac surgery there is therefore the potential for contamination from these tissues. The Sangtec assay detects S100B subunit and so may detect S100a. The fact that differing profiles of S100b release have been found after cardiac surgery depending on whether or not cardiotomy suction is used suggests that the Sangtec assay may be detecting mediastinally released S100B[181]. When cardiotomy suction is avoided the amount of S100b detected is far lower. Also a much higher S100b concentration has been detected in the cardiotomy suction blood compared to the systemic blood samples. This would suggest that the majority of the S100b being detected is mediastinal rather than cerebral in origin.

S100b was first used as a diagnostic and prognostic marker for central nervous system tumours. Increases in serum levels of S100b were then found after strokes, subarachnoid haemorrhage and cerebral trauma[186;187]. Since then, it has been found that S100b serum levels are raised after adult cardiac surgery. Johnsson et al[188] (1995) measured S100b levels in 38 patients undergoing various types of cardiac surgery with CPB. Eight patients had neurological complications - either stroke or delayed awakening after surgery. Seven of the patients with neurological complications had raised S100b. A raised S100b was found in only 4 of the remaining 30 who had no neurological complication. Westaby[189] in 1996 measured serial serum S100b levels in 34 patients undergoing CABG with CPB and compared them to 9 patients undergoing CABG without CPB. He found that in the non-CPB group none had a raised S100b apart from one patient who suffered a stroke. In the CPB group S100b was significantly raised after CPB, decreasing to normal levels within 24 hours. Of significance, none of the CPB group patients had a neurological event, despite raised

S100b levels. Also, S100b levels were strongly correlated ($r= 0.89$, $p<0.001$) with duration of CPB. These two facts raised the question of whether a raised S100b in the context of cardiac surgery with CPB reflected a sub clinical cerebral injury or was merely a bypass related epiphenomenon perhaps due to increased permeability of the blood brain barrier. Although it has been suggested that the inflammatory response to CPB may cause the blood brain barrier to become more leaky, a study in piglets has shown no increase in blood brain barrier permeability after 2 hours of bypass[190]. Also, the concentrations of S100b found in the serum after cardiac surgery are far greater than the normal CSF S100b concentration. It would thus seem unlikely that permeability changes alone could account for raised S100b after cardiac surgery.

Aberg[190] used a mock CPB circuit and compared S100b release in patients undergoing cardiac and non-cardiac operations. Peak S100b levels were found at the end of CPB and were not due to surgery, anaesthesia or blood trauma implying that they were due to cerebral injury. Westaby[191] was also able to show that S100b was not elevated in patients who receive CABG without CPB (supported by a more recent study from Bristol[192]). In the same study, Westaby also showed that there was a strong and significant correlation between S100b levels and the length of bypass but this was not repeatable in subsequent studies[150;193].

Taggart[193] has shown in a prospective study that patients in whom the heart is opened for valve replacement or atrial septal defect closure have a significantly greater elevation in S100b than CABG patients. S100b was more frequently elevated, elevated to a significantly greater magnitude, and also remained significantly elevated for longer. The interpretation of these results as showing that the brain suffers greater global injury when the heart is opened is consistent with those studies showing greater neuropsychological deficit following valve surgery compared to CABG surgery[24].

As has been discussed, there is strong evidence that microemboli are involved in the pathogenesis of global cerebral injury. Grocott showed a weak but highly significant correlation between the number of microemboli detected during aortic cannulation and the S100b elevation in a population of patients undergoing elective CABG[194].

Also, as has already been discussed at length, a reduction of microemboli by arterial line filtration may reduce NP deficit[8]. Taggart et al's prospective randomised controlled trial which demonstrates a reduced S100b in patients who receive arterial line filtration[150] therefore provides further indirect evidence that an elevated S100b does reflect a global cerebral injury. However neither of these two studies shows cause and effect, that is that microemboli cause cerebral injury which then leads to S100b release. A reduction in S100b, as in Taggart's filter study would not then imply a reduction in NP deficits. Also, the fact that more S100b is released after valve replacements may be directly due to the cut heart releasing more S100b rather than a consequence of increased cerebral injury even if there is an actual increased NP deficit.

There are now several studies in which S100b levels and neuropsychological changes have been directly compared. Overall findings remain inconclusive. Kilminster et al[195] (1999) studied 130 unselected cardiac surgical patients and evaluated S100b release and neuropsychological change. They used well-described and validated tests pre-operatively and at six to eight weeks post-operatively. Kilminster et al found that lower S100b was correlated with better neuropsychological outcome. Area under the curve of S100b release correlated with age and bypass time and the peak S100b level also correlated with bypass time. Kilminster et al used multiple regression models to show that neuropsychological performance accounted for 23% of the variance associated with S100b release after partialing out the effects of age and bypass time. This suggests that S100b release is not just an epi-phenomenon related to cardiotomy suction or bypass associated leakage of S100b via the

blood brain barrier but does have a true association with neuropsychological outcome. Hermann et al[196] also looked at S100b release in a smaller group of patients (n=36) undergoing CABG (N=18) or valve replacement (n=18). They found a weak correlation between S100b release and degree of post-operative decline in neuropsychological function in the valve replacement group only. Although Hermann et al found an increase in NP decline in the valve replacement group compared to the CABG group, there was no difference in S100b release between the groups. In addition to conflicting with Kilminster et al's results[195] this also contrasts with Taggarts study[193] which found a greater release of S100b in valve replacement and other open heart procedures compared to CABG patients.

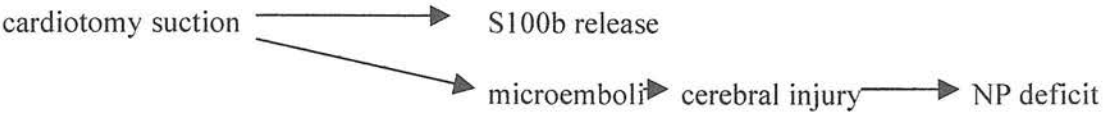
A more recent study by Westaby et al (2000) found no significant correlation between S100b release and neuropsychological outcome at 5 days or 6 months post operation[189]. In this study a comprehensive battery of neuropsychological tests were used as well as a new and supposedly more sensitive immunoluminometric S100b assay. However they were very careful to minimize surgical bleeding and there was no use of cardiotomy suction or the re-transfusion of mediastinal shed blood. This was done to avoid the detection of non-cerebral sources of S100b, as identified by Andersen[181], and may have led to the lower peaks of S100b found in this study. Westaby's study suggests that when non-cerebral sources of S100b are eliminated, there is no correlation of early S100b release with neurological outcome.

Although there is evidence that cardiotomy suction blood is a significant source of mainly lipid microemboli[90], Anderson has shown in two studies that the S100b detected by the Sangtec assay in cardiac surgical patients may well derive from the high concentrations of S100b in cardiotomy suction blood returned to patients. In his first study Andersen[181]

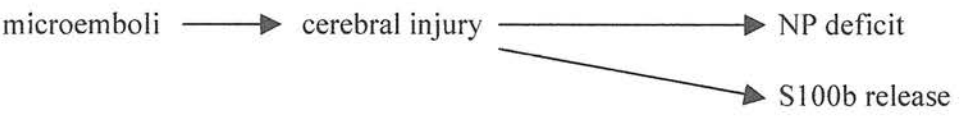
randomly allocated 20 patients having CABG surgery to either cardiotomy suction or a cell saving device and measured serial serum S100b (he labels it as S100β in this study) as well as S100b levels in the blood from the wound and the cell saving device. A significantly lower S100b level was found at the end of CPB in the cell saver group (0.44μg/L) compared to the cardiotomy suction group (2.47μg/L). The level in the cell saver group was comparable levels in patients having off-pump surgery[197]. Andersen also found that levels of S100b were 42μg/L in the cell saver reservoir and 33μg/L in the blood from the mediastinal wound. This study therefore showed that, at least when the Sangtec assay is used, it is the cardiotomy suction rather than CPB itself causing the rise in S100b levels. Jonsson[198] has found almost identical results to Anderson. Jonsson found that a group of patients treated with a cell saver had S100b levels of 0.53μg/L at the end of bypass compared to S100b levels of 2.40μg/L at the end of bypass in patients who had cardiotomy suction. In a further study[199]Anderson specifically measured S100 A1-B and S100 BB dimers (i.e. S100a and S100b) using specific antibodies. (In this paper he states " S100B is not a unique protein, but rather denotes collectively all S100 dimers that contain one β subunit." This statement is potentially confusing because by S100B he means not he monomer, which using the correct nomenclature it is, but what is detected by the Sangtec assay which detects any dimer with the S100B monomer. He calls the S100 monomer the "β subunit" but β is also not a correct term in the nomenclature. This confusion in the literature may well have arisen because the Sangtec assay detects the S100B monomer and is detecting S100a and S100b.) Anderson found that both S100b and S100a were released in approximately equal quantities during CPB with cardiotomy suction and in lower but equal quantities during CPB with a cell saver. Interestingly, the sum of the S100a and S100b did not quite account for the detected "S100B" suggesting that the Sangtec assay may even be detecting S100a0. This suggests that S100b may not be as specific to cerebral neurones as thought and that the detected release from the mediastinum may not be purely artefactual due to cross reactivity of the

Sangtec assay but that S100b may actually be released from mediastinal tissues.

Cardiotomy suction blood-containing mediastinally released S100b could therefore be a direct cause of both microemboli and increased S100b levels. This would then explain the relationship between S100b and neuropsychological outcome.



rather than



In conclusion one has to be very circumspect about interpreting papers which have found a positive correlation between S100b levels and neuropsychological outcome. These studies must be interpreted in the light of the later studies which have shown that most of the rise in S100b levels can be accounted for by the release of S100 from the mediastinum. Studies which have eliminated this mediastinal source by not using cardiotomy suction have not been able to find any correlation between S100b and neuropsychological outcome.

Chapter 7

Methods and Materials

7.1 Design

This study was a prospective, randomised, double blind, controlled clinical trial.

This single centre study was a collaboration between the University College of London Unit of Health Psychology and University College London Hospitals Department of Cardiothoracic Surgery.

7.2 Ethical Approval

Approval was obtained from the University College London Hospitals ethics committee (see **appendix 1**). The trial protocol was also approved by the hospital Research and Development committee.

7.3 Statistical Planning

Sample size calculation was based on the Z change scores in the previous study conducted at the institution of the present study by Arrowsmith et al[42]. The sample size was formulated on a two-sided hypothesis with alpha significance set at 0.05 and power set at 0.80. Mean difference and standard deviation data from the Arrowsmith et al data were 1.25 and 2.7 respectively. It was assumed that there would be equal size and equal variance for the control and treatment groups. Using the formula below:

Sample per group = $2*[Z_{(1-\alpha)} + Z_{(1-\beta)}]^2/ \Delta^2$

Where Δ^2 represents the standardised difference (i.e. the treatment difference divided by its standard deviation).

The sample size required was 74 per group, i.e. a total sample of 148. It was estimated that a 25% loss to follow up was possible and so 1.25 x148 = 197 patients in total were calculated

to be required.

7.4 Study Population

Patients scheduled for non emergency coronary artery bypass surgery in the department of cardiothoracic surgery at The Middlesex Hospital (University College Hospitals NHS Trust) were invited by the author to participate. A letter of invitation and a patient information sheet was sent to all patients together with the letter informing them of their date of surgery about 4 to 6 weeks prior to surgery. Both inpatients and patients from the "at home" waiting lists were recruited. Inpatients were patients who were kept in for surgery following coronary angiography. The decision to keep patients in was based on their coronary anatomy. Demographic and other differences between inpatients and "at home" patients were not analysed.

7.5 Recruitment and Consent of Patients

The following patients were excluded from recruitment: -

Patients taking oral or intravenous corticosteroid medication. Patients taking non-steroidal anti-inflammatory medication were allowed to participate.

Patients aged over 80 years. Although these patients are at most risk of NP injury, they also have the highest co-morbidity and mortality, which may make them less likely to attend for follow up.

Patients considered or found unable to perform the pre-operative NP testing for whatever reason. Examples were inability to speak or read English, blindness or physical disability.

Patients who had previously had cardiac surgery. Patients having such "re-do" surgery may have suffered a previous NP insult. They are also likely to be given aprotinin, an anti-inflammatory agent which is used to reduce bleeding but which might also confound the leucocyte depleting effects of the filters.

Patients with a history of transient ischaemic attacks or strokes or a recent history of cardiac arrest (within 1 month). A history of previous cerebral injury could also confound results.

Patients with carotid artery stenosis. (Patients were screened with auscultation of the neck. If a bruit was audible the patient then had carotid duplex scanning.) It is accepted that this is an imperfect method for screening for carotid stenoses. However this was the departmental policy and facilities were not available for screening all study patients with carotid Doppler.

Patients with insulin dependent diabetes mellitus. It is not known whether these patients are more at risk of NP deficits but there is evidence that the way in which serum glucose is managed on bypass can affect NP outcome[113].

Patients with renal failure (defined as creatinine greater than 150mmol/l pre – operatively). S100b is secreted predominantly by the kidney and so assays of this marker would be invalidated in patients with renal failure.

Patients with a pre- operative white cell count outside the normal range of $3\text{-}11 \times 10^6/mm^3$.

Patients fitting the criteria were then invited to participate. In accordance with ethics committee guidance, fully informed consent was obtained from all patients. The trial was

explained verbally to patients who were then given a written information sheet to read (see **appendix 2**). Patients were requested to sign a consent form if they agreed to participate (the consent form was part of the case report file, see **appendix 3**).

7.6 Randomisation

Full randomisation to test or control groups was used. 200 sealed envelopes containing 100 test and 100 control group slips were given to the perfusion department. A perfusionist drew an envelope at random when informed the day before surgery that a patient was in the trial. The perfusionist then set up the bypass circuit using the appropriate filter. Only the perfusion department kept a record of the filter used for each patient, so that all other investigators were blinded to the patient groups. If a patient was cancelled on the day of surgery then their allocation was kept for the date when they did have surgery. If a patient had to be removed from the trial due to breaking of the protocol (e.g. surgery done off-pump or propofol used) then their allocated envelope was not re-used.

7.7 Type of Filter

The original plan for the study was to compare two filters only. The test filter was the Pall Leukoguard-6 (LG-6) and the control was to have been the routine arterial line filter used in our unit, the Avecor affinity. Both filters have a 40μm screen polyester element but the LG-6 has an additional non-woven polyester mesh downstream of the screen, which gives it its leucocyte depleting properties. However the LG-6 is also an auto-venting filter. Auto venting allows the LG-6 to vent air filtered by the screen directly to the atmosphere. In

contrast, the Avecor filter vents any air back to the oxygenator through a closed vent line. This potential effect on microemboli production or elimination of this difference in venting properties was not appreciated until mid way through the trial. The effect of venting on microemboli has not been previously studied and its potential effect on microemboli is unknown. The AV-6 is auto-venting in an identical manner to the test filter but has no leucocyte-depleting element. It is arguably a more suitable control filter. It was therefore decided at this midway stage, to use a second control filter, the Pall Autovent-6 (AV-6). There was, however, a time delay between this decision and being able to implement the decision. This was because the second control filter had to be ordered and made up in pre-made bypass circuits. A second batch of envelopes was given to the perfusion department when the AV-6 filter began to be used. This led to an uneven number of patients eventually randomised to the three filter groups and a greater overall number of patients in the control group compared to the test group (see Table 8.3). The use of these two control filters also enabled a comparison to be made between an auto-venting and non-auto-venting filter, although the study was not originally designed or powered to do this.

7.8 Theatre Protocol

Anaesthesia, surgical technique, cardio pulmonary bypass, control of blood pressure and perfusion pressure, control of pH and p_aC0_2 all followed a set protocol developed in previous trials assessing neuropsychological outcome after cardiac surgery.

7.9 Anaesthesia

Premedication consisted of morphine (5-10mg) and hyoscine (0.3-0.4mg). Diazepam (5-10mg) or temazepam (10-20mg) was given according to the anaesthetist's preference. Supplemental oxygen was administered via a face mask at a rate of 4l/min from the time of

medication until induction of anaesthesia. Intravenous midazolam was used during insertion of peripheral and central cannulae, as necessary. Induction was carried out with midazolam (2-10mg), fentanyl (0.5–2.0mg), pancuronium and or suxamethonium. The trachea was intubated with a cuffed endotracheal tube and mechanical ventilation was used to achieve an end tidal CO_2 of 5.3 kPa. Maintenance was with nitrous oxide and oxygen and then isoflurane.

Propofol, etomidate and thiopentane were specifically avoided as these have potential but unproven neuroprotective effects but are used by only some anaesthetists. Post-operatively patients were kept sedated with intravenous midazolam.

7.10 Surgical Technique

Patients of all six consultants in the unit were used. These were Mr Pugsley, Mr Walesby, Mr Pattison, Mr Kallis, Mr Kolvekar and Mr Hayward. All consultants used the same technique of intermittent cross clamp fibrillation as myocardial protection. The left internal mammary artery and/or appropriate lengths of long saphenous vein were first harvested. After the commencement of CPB distal aorto-coronary anastamoses were fashioned on the surface of the heart with the proximal aorta cross-clamped and the heart in electrically induced ventricular fibrillation. Proximal aorto-coronary anastamoses were made after each distal anastamoses with the aortic cross clamp removed and the heart beating normally.

7.11 Cardiopulmonary Bypass Technique

Cardiopulmonary bypass was established between a cannula placed in the right atrium and a cannula in the ascending aorta. The bypass circuit was primed with 1.6 litres of Hartmanns solution heparinised with 5000iu of sodium heparin. Full anticoagulation was achieved prior to aortic cannulation with 300iu/kg heparin. Further doses of heparin were given throughout CPB to maintain the activated whole blood clotting time (ACT) greater than 400 seconds. Heparin concentration was not measured, as this assay was not available, although this may be a superior method of controlling anticoagulation[200]. A flat bed membrane oxygenator (5400, Bard, UK) and a roller pump (Stockard, USA) and tubing (Bard, UK) was used to give non-pulsatile flow. Flow was initially 2.4L/m^2/min at 37° C reducing to 1.8L/m^2/min at 32°C. Patients were actively cooled to 32°C (nasopharyngeal) and then re-warmed to 37°C (nasopharyngeal) during late CPB. During both cooling and re-warming the gradient between the arterial line blood and the nasopharyngeal temperature was always less than 10°C. Perfusion pressure was maintained between 50 and 60 mmHg using the pressor phenylephrine and vasodilator phentolamine, which have no direct effect on the cerebral circulation. $PaCO_2$ control was alpha stat; that is maintained at 5.3 kPa temperature uncorrected. Cardiotomy suction was via a Harvey cardiotomy reservoir connected to two or three low-pressure cardiotomy suckers. At the end of CPB the anticoagulant effect of heparin was reversed with protamine sulphate to correct the ACT to pre-CPB levels.

7.12 Monitoring

Apart from the transcranial Doppler and extra blood samples, theatre monitoring was routine. Patients were monitored with a right radial arterial line, central venous pressure via the right internal jugular vein, pulse oximetry, catheterisation of the bladder and end tidal CO_2. Temperature was monitored with a nasopharyngeal probe. This was left in for 24 hours to measure temperature for this period.

In addition to the recording of standard intra-operative variables such as bypass and cross clamp times, five specific intra-operative variables which have a potential influence on microemboli production and neuropsychological outcome were also recorded. These were: -

1) Clinical presence of aortic atheroma or calcification – either visible on CXR or palpable intra-operatively by the surgeon.
2) Whether or not a side-biting clamp was used for aortic cannulation.
3) Minimum temperature on CPB.
4) Maximum temperature reached during rewarming.
5) Time taken to rewarm from start of rewarming to reach 37°C.

Some surgeons preferred to use a side-biting clamp for cannulation whereas others did not and it was not possible to standardise this. Although there was a set protocol for cooling and rewarming as stated earlier there was some inevitable variation in the temperatures actually reached, and the speed at which rewarming occurred.

7.13 Tests

7.13.1 Neuropsychological

The following battery of nine neuropsychological tests was chosen to cover a range of domains as detailed below. The battery had been developed at UCLH and includes the core battery as recommended by the consensus meeting[14]. A trained psychologist who was blinded to the patients group administered these tests. A single psychologist saw all the patients pre-operatively. Patients were only recruited when this psychologist was available. There were six occasions (6/162 = 3.7%) post-operatively when this psychologist was not available and, because patients were returning on this one occasion only, the tests were performed by one of two other psychologists. All patients were brought from their ward to the same room where tests were carried out under relaxed conditions. The following battery of nine widely used tests was conducted:

7.13.1.1 The Rey Auditory Verbal Learning Test[201]

This is a test of immediate memory span, serial learning and also retention following distraction. A list of 15 words is read to the patient who then attempts to recall them immediately under free recall conditions. After the first recall, all 15 words are presented again and the patient is again asked to recall as many as possible. This is repeated on three occasions. A second list of 15 different words is then presented to the patient and they are again asked to recall as many as possible from this new list. Finally the patient is asked to recall as many as possible from the first list without the first list being re-read to the patient. This final test measures recall following distraction. The score is the total number of correct answers from list 1-5 and 7.

7.13.1.2 Trailmaking Tests A and B

This test has been taken from the Army Individual Test Battery and it assesses both attention and motor speed. It has been found to be very sensitive to brain injury. For trailmaking A the patient has to draw lines through consecutively numbered circles. For trailmaking B, which is more difficult, the patient has to join a sequence of alternating letters and numbers. The result of each test is a time to completion score.

7.13.1.3 Grooved Pegboard Test

This test requires the patient to put pegs into a board containing grooved slots. Each slot has a groove at various angles and each peg has a corresponding ridge along each side. The patient therefore has to rotate each peg to the correct orientation before it can be inserted. The grooved pegboard test therefore measures manual dexterity and fine motor co-ordination. The test is performed using the dominant and then the non-dominant hand so that both right and left cerebral hemisphere function is assessed. Again the result of each test is a time to completion score.

7.13.1.4 Symbol Digit Replacement Test[202]

This is a pen and paper test adapted for use on a computer. A key appears on the screen with nine different symbols each with its own corresponding digit from 1 to 9 shown underneath the symbol. Below the key on the screen there is a row of symbols with the digits missing. The patient is required to put the digit beneath each symbol. The number of correct responses and the time taken is recorded. The result of the test is a time to completion score.

7.13.1.5 Non-verbal Memory Test[8]

The patient is required to look at a chequered board design on the computer screen which is presented for 10 seconds and then disappears. Three smaller designs then appear on the

screen and the patient is required to identify which of these three is the one identical to the original design shown. This is repeated 20 times and the number correct and the response times are recorded. The result of the test is a time to completion score.

7.13.1.6 Letter Cancellation Test[203]

The patient is asked to scan an A4 sheet of paper containing rows of a list of randomly interspersed letters and cross out a designated target letter. Performance is scored on number correct and time to completion.

7.13.1.7 Choice Reaction Time Test [8]

In this test the patients are asked to discriminate between and respond, as quickly as possible, to two letters displayed on a computer screen. Response time and accuracy are recorded.

In addition to the above neuropsychological tests, measures of anxiety and depression were taken pre-operatively and post-operatively. Subjective Cognition, that is, the patients own perception of any change in cognitive function was assessed post-operatively with a simple nine question questionnaire. The CESD (Centre for Epidemiologic Studies Depression scale) was used to assess depression. It is a 20 item self-report scale which measures the presence and severity of depressive symptoms. The STAI (Spielberger state and trait anxiety inventory) was used to measure trait anxiety pre-operatively and state anxiety pre-operatively and post-operatively. Trait anxiety measures how anxious a personality a patient possesses, whereas state provides an immediate measure of anxiety in the patients current situation.

All tests were performed during the week pre-operatively (usually on the day preceding surgery) and then 6-8 weeks post surgery. The pre-operative test was usually done the afternoon prior to surgery and the post-operative test on the day that the patient returned to

the routine surgical follow-up clinic.

There is no ideal time for performing pre-operative neuropsychological tests. It has been conventional at the institution of the present study to perform the tests the day before surgery. In fact, as has previously been discussed in chapter 1, patients have been found to be less anxious a day before surgery compared to a week before surgery[38]. This difference in anxiety had no effect on NP performance[38].

7.13.2 Transcranial Doppler

Intra-operative transcranial Doppler was used to measure both a) cerebral artery blood velocity and b) embolic load. It therefore functioned both as a control measurement (as blood velocity should be the same in both groups), and also a measure of a potential end point variable (i.e. embolic load).

Cerebral artery blood velocity was assessed at the following discrete points:

1) 10 minutes pre bypass

2) 1 minute pre bypass

3) 15 minutes into CPB

4) 30 minutes into CPB

5) 60 minutes into CPB

6) 90 minutes into CPB

7) 10 minutes after discontinuing CPB

Monitoring for microemboli took place for two continuous periods:

1) During CPB

2) For the 10 minutes following bypass.

The Doppler machine used was a Nicolet EME Pioneer 4040 Transcranial Doppler system. The right middle cerebral artery was isonated using a 2 MHz pulsed wave transducer and the probe was secured to the skull using an elasticated headset, which allowed for prolonged monitoring. Microembolic events and cerebral blood flow velocity were recorded onto videotapes for subsequent analysis. A plot of mean velocity against time and spectra of detected microemboli were separately taped for each patient so values of blood velocity for the times detailed were then obtained. The micro emboli were counted manually "off-line" (i.e. after the operation) using their unique auditory and visual characteristics. International consensus criteria were used for defining microemboli[104]. The same investigator blind to the patient group performed microemboli counting. A second investigator (Jan Stygall) reviewing 10% of the tapes assessed inter-observer reliability. These data were unfortunately lost.

7.13.3 Biochemical

Haemoglobin, white cell count with differential, platelets, creatinine, S100b, and neutrophil elastase were measured. Neutrophil count was used to check the efficacy of the LD filter, although the removal of activated neutrophils by this filter tends to lead to their replacement from the bone marrow, and platelet count was used to assess the effect of the filter on this. Neutrophil elastase measured neutrophil activation and degranulation. Creatinine checked renal function, which may affect S100b clearance. We planned to be able to correlate the effect of leucocyte depleting filtration on neutrophil activation with the overall inflammatory status and neuropsychological changes. S100b was measured as a potential marker of cerebral injury. The following table summarises when each of these samples were taken:

Table 7.1 Timing of peri-operative blood tests

	Pre op (day before)	Pre Bypass (at cannulation)	On Bypass (30 mins)	Off Bypass (10 mins post CPB)	6hrs	24hrs	48hrs
Full Blood Count	+	+	+	+		+	+
Urea+ Electrolytes	+					+	+
S100b	+			+	+	+	+
Elastase	+	+	+	+		+	

S100b and elastase samples were taken from the first 80 patients in the trial. This was because funds were not available to test all patients. Full blood count and differential white cell count were taken from all 192 patients.

S100b

S100b was measured using a previously described sandwich enzyme linked immunoabsorbant assay (ELISA)[204;205]. For this assay, monoclonal anti-S100b antibody was bound to the wall of a microtitre plate. This is called the "capture" antibody. The capture antibody was then incubated with the patient's plasma sample.

The microtitre plates were first coated overnight with monoclonal anti-S100b at a concentration of 9.3μg/ml in 0.05 carbonate buffer (pH 9.5). The plates were then washed with 0.1% bovine serum albumin in phosphate buffer solution. 50μl of the patient's plasma sample was added in duplicate to the plate and 50μl of 0.06M barbitone buffer (pH 8.6) containing 1mM calcium lactate were then added to each well (this was the incubation buffer). Plates were then incubated at 37°C for three hours. After washing, rabbit anti-S100b antibody conjugated with horseradish peroxidase diluted 1/1000 with incubation buffer was

added. The microtitre plate was then incubated at room temperature for one hour. The plates were again washed and then 100μl of enzyme substrate (1mg/ml of phenylenediamine in 0.05 M acetate buffer containing 0.01% hydrogen peroxide) was added. After incubation in the dark for a further 30 minutes the reaction was stopped by adding 1 M HCl. Absorbance was then read at 492nm with 405 nm as the reference wavelength using an Anthos 2001 Plate Reader (Denley Instruments, Sussex, UK). An ELISA standard curve had been constructed using S100b standard samples and the S100b concentration was then read from this curve.

Elastase

Neutrophil elastase was assayed as a complex with alpha-1-antitrypsin, in citrated plasma (0.105M citrate). The complex was measured using an enzyme linked immunoabsorbant assay (ELISA), as previously described[206]. Sheep antihuman neutrophil elastase was used as a coating antibody, and a peroxidase conjugated sheep anti-human alpha-1-antitrypsin antibody was used as a detector (Serotec, Kidlington, UK). A range of dilutions of test and standard plasmas were applied. The assay was standardised using neutrophil elastase: alpha-1-antitrypsin complexes generated according to Brower & Harpel[207] using human neutrophil elastase isolated according to Baugh & Travis[208].

All results were written by the author in each patient's individual case report file as the data was collected. Data were then transferred to a database (SPSS version 6.1) on a personal computer for subsequent statistical analysis.

As well as the specific tests detailed above other descriptive data were prospectively collected for each patient as follows:-

7.14 Demographic and other pre-operative data

Age, sex, race, body mass index, grades of angina and dyspnoea, history of myocardial infarction, hypertension, peripheral vascular disease and smoking, ejection fraction, Parsonnet score, and pre-operative medication.

7.15 Intra-operative data

Operation time, intra-operative systolic diastolic and mean arterial pressures, time on CPB, cross clamp time, number of grafts and whether or not a left internal mammary artery was used, presence of aortic atheroma or calcification (this was a subjective decision made by the operating surgeon – yes or no), whether a side clamp was used at cannulation of the aorta, minimum temperature, maximum temperature, and time taken to rewarm from minimum temperature to 37 °C.

7.16 Post-operative data

Pulse, SBP, DBP, respiratory rate, oxygen saturation, arterial oxygen tension, inspired oxygen concentration and temperature at 1,2,3,4,8,12,16,20,24 hrs post op, use of inotropes, time to extubation, cumulative blood loss at 6,12,18,24 hrs, record of complications, and time in days to discharge.

7.17 Neuropsychological and other Test Statistical Analysis

The χ^2 test was used for all categorical data. The Kolmagorov- Smirnoff test was used to test whether all continuous data was normally or non-normally distributed. Normally distributed data was analysed using the t test to test for significant differences in means between groups at a single time point. Normally distributed data was analysed using repeated measures analysis of variance (ANOVA) to test for significant differences in means between groups at several time points. Non-normally distributed data was analysed using the Mann Whitney U

test. Correlation between normally and non-normally distributed data was analysed using Spearman's correlation coefficient. Significance was set at the 95% level. That is: $P<0.05$ was taken as significant. A summary of all statistical tests used for all tables is given at the end of the results chapter.

The primary outcome measure of the study was the standardised change (Z) score. Z scores were calculated for individual tests and to give a total Z score for each group using the standard deviation of the pre-operative group performance. Z score was calculated as $Z = (X_2-X_1)/ \mu SD_1)$, where X_1 is the pre-operative score, X_2 is the post-operative score and μSD_1 is the standard deviation of the pre-operative group scores. A higher postoperative score gives a positive Z score and a lower post-operative score gives a negative Z score. However, some of the tests are timed tests and a better performance is reflected in a lower time score. Therefore X_2 and X_1 are swapped for timed tests to ensure that positive Z scores consistently indicate improved performance. The t test was used to test for significant differences in Z score between groups. The previous conventional definition of a neuropsychological deficit is that a patient's postoperative score has dropped by one standard deviation or greater from their preoperative score in two or more tests. As a secondary neuropsychological outcome the incidence of deficits in each group using this definition measure were calculated. Comparison of the proportion of patients with deficit in the test and control groups was performed using the χ^2 test.

7.18 Time plan of methods

Time	Action
6-8 weeks before surgery	Write to patient with information sheet
Day before surgery	Recruit Randomise Blood tests Neuropsychological tests
Day of surgery	Blood tests Transcranial Doppler
1 day post surgery	Blood tests
2 days post surgery	Blood tests
6 weeks post surgery	Return to clinic Neuropsychological tests

Chapter 8

Results

8.1 Recruitment

One hundred and ninety eight patients were recruited over 30 months between April 1999 and September 2001. All 198 patients received pre-operative neuropsychological testing and were randomised to test or control filter groups. A summary of the number of patients receiving the main tests is given at the end of this chapter (Fig 8.26).

8.2 Removal from study before or during surgery

One patient was found to have carotid artery stenosis and was removed from the study before surgery. A further five patients were removed from the study during surgery either because their surgery was performed without cardiopulmonary bypass or because the anaesthetic protocol was broken by using Propofol.

Table 8.1 Summary of reasons for removal from study after recruitment

Reason for Removal	No. of Patients
CPB not used	4
Propofol used	1
Carotid artery stenosis discovered	1

Therefore 192 patients were randomised, completed pre-operative neuropsychological assessment and successfully completed theatre protocol.

8.3 Outcome after surgery

162 out of 192 patients (84%) returned for repeat neuropsychological assessment. Thirty patients failed to return for follow up for reasons summarised in Table 8.2

Table 8.2 Summary of reasons for not returning for follow up neuropsychological assessment.

Reason for Not Returning for Follow-Up	No. of Patients
Death	9
CVA	2
Medically unfit to return for testing (No CVA)	11
Unwilling to return for testing	8

There were no significant differences in final outcomes between the filter groups (Table 8.3)

Table 8.3 Outcomes for all patients and the three filter groups.

Final Outcome	All	%	LD	%	Av	%	AV-6	%
	n =192		n=82		n=73		n=37	
Dead	9	4.6	4	4.9	4	5.5	1	2.7
CVA	2	1.1	0	0	2	2.7	0	0
Medically unable to FU	11	5.7	5	6.1	4	5.5	2	5.4
Unwilling to attend FU	8	4.2	2	2.4	4	5.5	2	5.4
Completed	162	84.4	71	86.6	59	80.8	32	86.5
Total	**192**	**100**	**82**	**100**	**73**	**100**	**37**	**100**

LD = LG-6 leucocyte depleting filter group, **Av** = Avecor conventional filter group and **AV-6** = AV-6 conventional filter group. There were no statistical differences found between groups using the χ^2 test.

As has been explained in the methods section, and will be discussed in the final chapter, the uneven distribution between the three filter groups arose because the control filters were changed about three quarters of the way through the trial.

Of the 4 deaths in the LD group 3 died of multi-organ failure on ITU and one had a fatal MI on post-operative day 6. The 4 deaths in the Av group consisted of 1 patient with intra-operative myocardial infarction who could not be weaned from bypass, 1 patient with sepsis and 2 patients with multi-organ failure. The one death in the AV-6 group was caused by post-operative cardiac failure on post-operative day 17.

8.4 Patient Characteristics

As table 8.4 shows, there were no differences in the demographic and clinical characteristics between the three filter groups.

Table 8.4 Pre-operative and demographic data for all patients and separate groups.

	ALL (192)	LD (82)	Avecor (73)	AV-6 (37)
Age (years)	64.1 (8.7)	63.6 (8.7)	65.5* (8.3)	62.3* (9.1)
Sex (n m:f)	165:27	69:13	65:8	31:6
Sex (% m:f)	86:14	84:16	89:11	84:16
Body mass index (Kg/m^2)	27.8 (4.5)	27.9 (4.9)	27.6 (4.1)	28.1 (4.2)
Grade of angina (CCS)	2.16 (0.8)	2.20 (0.8)	2.14 (0.8)	2.14 (0.9)
Grade of dyspnoea (NYHA)	1.68 (0.8)	1.67 (0.8)	1.68 (0.9)	1.74 (0.8)
History of MI (n yes)	74	28	31	15
History of MI (%yes)	38	34	42	40
Hypertension (n yes)	102	44	42	16
Hypertension (%yes)	53	54	57	43
Peripheral vascular disease (n yes)	13	6	6	1
Peripheral vascular disease (%yes)	7	7	8	3
Ejection fraction (n good/mod/poor)	27/115/50	13/43/26	6/48/19	8/24/5
Ejection fraction (% good/mod/poor)	14/60/26	16/52/32	9/66/25	21/66/13
Parsonnet score	6.13 (5.6)	6.21 (5.9)	6.46 (5.5)	5.26 (4.9)
Smoker (n ex/yes/never)	142/42/8	57/22/3	55/16/2	30/4/3
Smoker (% ex/yes/never)	74/22/4	69/27/4	75/22/3	80/14/6
Recruitment (n at-home/in-patient)	170/22	71/11	66/7	33/4
Recruitment (% at-home/in-patient)	89/11	87/13	91/9	89/11

Numeric data are expressed as means with standard deviation in brackets. There were no

statistical differences found between groups using the χ^2 test for categorical data and the t test for continuous data. Despite an apparent difference in age between the Avecor and AV-6 groups, this was not statistically significant (*p = 0.07) using the 2 tailed t test.

The average age of the patients and the predominance of male patients are both typical of the UK population of patients having CABG surgery (UK National Adult Cardiac Surgical Database Report 2000-2001).

This study has used the original Parsonnet risk stratification system[209]. This was the first to become popular of various models used to predict mortality from pre-operative patient variables. Although it is still used it has more recently been superseded by the Euroscore model. This issue is discussed further in chapter 9.

An average Parsonnet risk stratification score of 5 – 6 places patients into the "elevated risk" group with a predicted operative mortality of 5% (Table 8.3 shows that overall actual operative mortality was 4.6%).

8.5 Pre-operative Medication

The pre-operative medication regime was dependent partly on the discretion of the patients' attending physicians. However as it may also reflect the severity of disease and may potentially affect outcome variables such as microemboli production or the inflammatory response, details of the percentage of patients in each group taking the major groups of medication used to control ischaemic heart disease is shown in Table 8.5.

Although aspirin was stopped seven days prior to surgery in most patients Table 8.5 includes the numbers who were on aspirin prior to this time. A small percentage of patients were taking warfarin for AF. A record of each patient's rhythm was not taken.

Table 8.5 Pre-operative medication rates in each group.

	ALL (192)	LD (82)	Avecor (73)	AV-6 (37)
Statins (% yes)	76	79	66	89
B Blockers (% yes)	77	76	77	83
Aspirin (% yes)*	92	94	92	89
Nitrates (% yes)	56	44	63	71
Calcium channel blockers (% yes)	43	46	45	34
ACE inhibitors (% yes)	30	33	26	28
K channel antagonists (% yes)	25	20	30	23
Warfarin (% yes)	1.6	2.5	1.4	0

* This included patients who stopped asprin 7 days prior to surgery.
All figures are expressed as percentages. There were no statistical differences found using the χ^2 test in the pre-operative medication use between groups.

8.6 Intra-operative data

No statistically significant differences were found between the groups. It can be seen in Table 8.6 that for all groups, mean operation time is under 3 hours, mean bypass time is 66 – 68 minutes and mean cross clamp time is 30 – 32 minutes. The mean number of coronary bypass grafts performed per patient was approximately 3. This relative speed of surgery compared to other, published studies is most likely due to the use of cross clamp fibrillation for myocardial protection. Most studies in the literature have used cardioplegic myocardial protection which is associated with longer cross clamp and bypass times.

Table 8.6 Intra-operative data.

	ALL (192)	LD (82)	Avecor (73)	AV-6 (37)
Operation time (mins)	169 (30.5)	166 (33.9)	172 (29.1)	171 (25.0)
CPB time (mins)	67.7 (19.1)	66.5 (22.1)	68.7 (17.0)	68.4 (15.5)
cross clamp time (mins)	30.9 (9.2)	30.6 (10.1)	31.9 (8.9)	29.5 (7.0)
number of grafts	2.88 (0.7)	2.86 (0.7)	2.95 (0.7)	2.78 (0.6)
LIMA (n / % yes)	157/82	68/83	58/79	31/84
Aortic atheroma or calcification (n / % yes)	13/7	3/3	7/10	3/10
Side clamp (n / % yes)	25/13	9/11	11/15	5/13
Min temperature (°C)	31.9 (0.8)	32.1 (0.8)	31.7 (0.8)	31.8 (0.5)
Max temperature (°C)	37.7 (0.3)	37.7 (0.3)	37.7 (0.4)	37.6 (0.3)
Time taken to rewarm (mins)	12.7 (4.4)	12.0 (3.8)	12.7 (4.8)	14.7 (4.1)

For numeric data, figures are expressed as means with standard deviation in brackets. The remainder are expressed as percentages. Numeric data are expressed as means with standard deviation in brackets. There were no statistical differences found between groups using the χ^2 test for categorical data and the t test for continuous data.

Factors which potentially affect the degree of aortic manipulation and potential subsequent microemboli production – i.e. number of grafts, use of the LIMA, side clamp use for cannulation and aortic disease – were equal in all groups. Although the aim of rewarming was to reach 37 °C, this target temperature was overshot, with the maximum temperature reached in all groups being 37.7 °C.

8.7 Neuropsychological Tests

8.7.1 Pre-operative

All patients received baseline pre-operative neuropsychological testing. Table 8.7 details these results.

Table 8.7 Pre-operative Neuropsychological Test Scores.

	ALL (192)	LD (82)	Avecor (73)	AV-6 (37)
Choice Reaction Time Test (secs)[¶]	0.64 (0.18)	0.63 (0.14)	0.67 (0.23)	0.59 (0.13)
Grooved Pegboard Test D (secs)*	79.16 (14.49)	77.30 (12.81)	80.42 (13.68)	80.86 (18.95)
Grooved Pegboard Test ND (secs)*	86.52 (18.28)	86.78 (18.05)	87.08 (19.05)	84.83 (17.62)
Letter Cancellation Test (secs)*	92.56 (25.04)	90.81 (22.49)	95.68 (29.16)	90.46 (21.46)
Non-verbal Memory Test (secs)[#]	77.72 (21.18)	76.13 (21.35)	77.53 (21.26)	81.45 (20.79)
Rey Auditory Verbal Learning Test[µ]	55.32 (11.84)	55.78 (12.26)	53.44 (11.44)	58.09 (11.29)
Symbol Digit Replacement Test (secs)*	182.66 (53.94)	177.51 (51.22)	187.57 (54.70)	184.11 (58.48)
Trail Making Test A (secs)*	39.99 (14.09)	39.25 (12.85)	41.04 (15.50)	39.57 (14.09)
Trail Making Test B (secs)*	92.06 (36.96)	90.63 (31.71)	94.01 (39.33)	91.46 (43.34)
Speilberger Anxiety State[α]	36.68 (9.75)	36.38 (10.01)	37.42 (9.98)	35.61 (8.50)
Speilberger Anxiety Trait[α]	35.01 (9.48)	33.54 (9.09)	37.28 (10.03)	33.32 (8.41)
CESD Depression Score[α]	11.81 (8.65)	11.73 (9.11)	12.89 (8.58)	9.25 (7.08)

*time to completion [#]total answer time [¶]mean response time [α]score on questionnaire [µ]total number of words recalled in 7 trials (max 105). ND non-dominant, D dominant.

There were no significant differences using the t test in pre-operative neuropsychological scores between the three filter groups. The three groups were therefore well matched in terms of pre-operative neuropsychological performance.

8.7.2 Six Week Post-operative Mean Z Change Scores

There was no significant differences between mean Z score changes for the Avecor and AV-6 filter groups (Table 8.8) and so the data from these two control groups were combined to give "standard" filter data. The purpose of this was to increase power of analysis.

Table 8.8 Mean Z Change Scores for neuropsychological tests at six weeks post surgery in Avecor and AV-6 filter groups.

	Av (n=58)	AV-6 (n=33)	p value
Choice Reaction Time Test	-0.13	-0.04	0.56
Grooved Pegboard Test D	0.18	-0.01	0.35
Grooved Pegboard Test ND	0.02	-0.01	0.82
Letter Cancellation Test	0.04	-0.17	0.18
Non-verbal Memory Test	0.03	0.33	0.09
Rey Auditory Verbal Learning Test	0.36	0.35	0.93
Symbol Digit Replacement Test	0.24	0.15	0.51
Trail Making Test A	0.13	0.12	0.93
Trail Making Test B	0.16	-0.14	0.22
Total Z score	0.98	0.58	0.57

Differences between groups were analysed with the independent 2 tailed t test

Table 8.9 Mean Z Change Scores for all neuropsychological tests at six weeks post surgery in leucocyte depleting (LD) and standard filter groups.

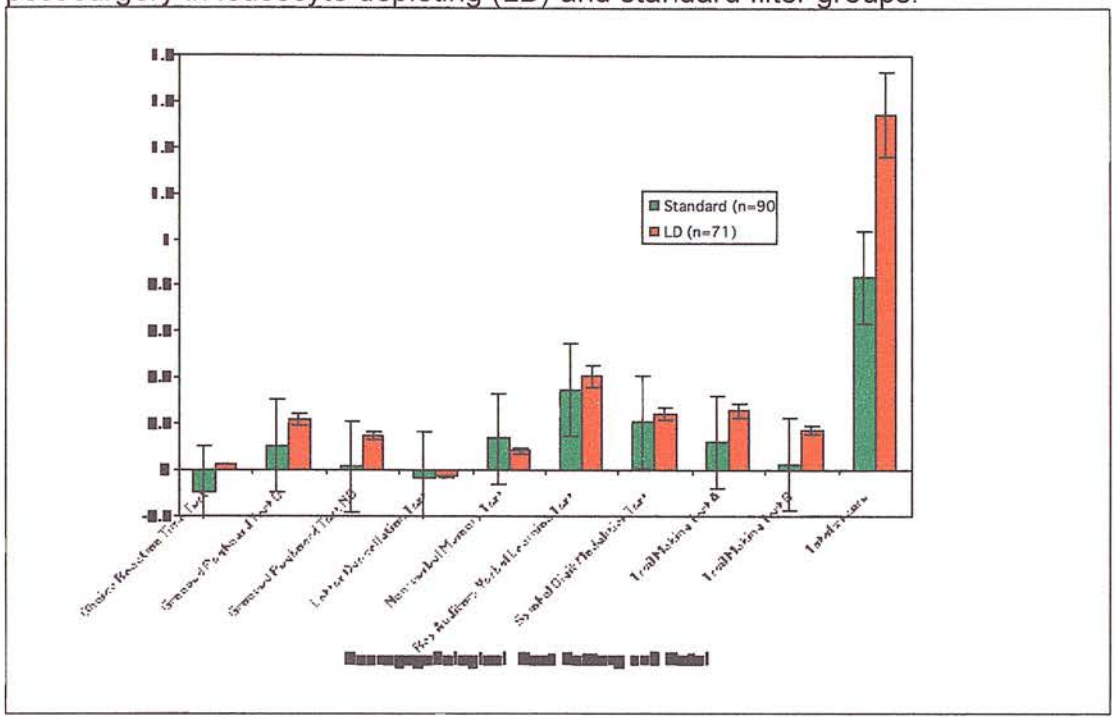
	LD (n=71)	**Standard** (n=91)	**p value**
Choice Reaction Time Test	0.03	-0.10	0.13
Grooved Pegboard Test D	0.22	0.11	0.19
Grooved Pegboard Test ND	0.15	0.01	0.06
Letter Cancellation Test	-0.03	-0.04	0.48
Non-verbal Memory Test	0.08	0.14	0.33
Rey Auditory Verbal Learning Test	0.41	0.35	0.31
Symbol Digit Replacement Test	0.25	0.21	0.34
Trail Making Test A	0.26	0.13	0.13
Trail Making Test B	0.18	0.02	0.16
Total Z score	**1.54**	**0.84**	**0.07**

Differences between groups were analysed with the independent 1 tailed t test

There was no difference in the Speilberger Anxiety Trait between LD (28.88 +/- 8.83) and standard (29.28 +/- 8.27), p=0.54, groups at the 6 week follow up visit. Also, there was no difference in the CESD Depression Score with scores of 9.19 +/- 7.63 in the LD group and 10.74 +/- 8.95 in the standard group, p=0.40.

It can therefore be seen that there was no statistically significant difference between the leucocyte depleting and standard filter groups for post-operative Z scores either for individual tests or for total Z change score. However, as is better illustrated in Figure 8.1, there was an overall trend towards greater improvement (that is: a greater positive change) in Z scores for all tests, apart from the non-verbal memory test, in the leucocyte depletion group.

Figure 8.1 Mean Z Change Scores for all neuropsychological tests at six weeks post surgery in leucocyte depleting (LD) and standard filter groups.



8.7.3 Six Week Deficit Scores

Deficit is defined as a decline by more than one standard deviation in two or more tests. In the standard filter group the incidence of deficit was 7/91 (7.8%) and in the LD group 4/71 (5.6%).

Table 8.10 Incidence of deficits in standard and leucocyte depleting (LD) filter groups.

		Filter	
		Standard (n=91)	LD (n=71)
Deficit	No	84	67
	Yes	7	4

Figures are absolute numbers of patients

This difference did not reach statistical significance using the Chi squared test. (χ^2 =0.30, p>0.50)

8.7.4 Comparison of characteristics of patients followed up and withdrawn

As shown in Table 8.3, there was an overall follow up rate of 84% for the 6 week repeat NP testing. In order to see if there was any selective attrition of patients, pre-operative and intra-operative characteristics were compared between those who did and did not return for follow up. The aim was to examine all patients who failed to return for whatever reason and so the patients who had died were included in the 30 patients withdrawn.

Table 8.11 Peri-operative characteristics of patients followed up or withdawn.

	Returned (n=162)	**Withdrawn** (n=30)	**p value**
Age	63.9 (8.3)	63.8 (8.3)	0.9
Yrs of Education	10.7 (1.7)	10.8 (1.6)	0.6
Parsonnet Score	5.9 (5.4)	6.3 (4.3)	0.8
Speilberger State (anxiety)	36.4 (9.3)	38.3 (11.7)	0.3
CESD (depression)	11.4 (8.3)	14.0 (10.1)	0.1
Bypass time	67.2 (19.1)	70.7 (19.2)	0.4
Number of Grafts	2.9 (0.7)	2.8 (0.5)	0.6
Microemboli on CPB	**61.6 (101.7)**	**125.2 (193.2)**	**0.04**
Days to Discharge	6.9 (2.3)	7.1 (2.5)	0.2

Data was analysed using the two-tailed t test for all characteristics which were normally distributed. Only microemboli on CPB was not normally distributed and this was analysed using the Mann Whitney U test.

It can be seen that those who did not return to follow up had a significantly greater number of microemboli on CPB.

Table 8.12 Pre-operative NP tests of patients followed up or withdawn.

	Returned (n=162)	**Withdrawn** (n=30)	**p value on two tailed t test**
Choice Reaction Time Test[¶]	0.64 (0.18)	0.66 (0.22)	0.80
Grooved Pegboard Test D (secs)*	78.50 (14.86)	83.00 (11.66)	0.10
Grooved Pegboard Test ND (secs)*	**85.50 (18.72)**	**92.32 (14.47)**	**0.03**
Letter Cancellation Test (secs)*	91.45 (22.16)	98.93 (37.09)	0.23
Non-verbal Memory Test[#]	76.92 (18.40)	82.11 (32.54)	0.41
Rey Auditory Verbal Learning Test[µ]	55.16 (12.04)	56.22 (10.76)	0.67
Symbol Digit Replacement Test (secs)*	178.46 (44.85)	205.69 (86.20)	0.13
Trail Making Test A (secs)*	**38.98 (13.03)**	**45.62 (18.20)**	**0.02**
Trail Making Test B (secs)*	89.99 (33.74)	103.52 (50.44)	0.12

*time to completion [#]total answer time [¶]mean response time [µ]total number of words recalled in 7 trials (max 105). ND = non-dominant, D = dominant.

Those patients who withdrew from the study had a significantly poorer pre-operative performance in the Grooved Pegboard Test (non-dominant) and Trail Making Test A tests. It is therefore possible that those patients who returned had deteriorated less than those who withdrew and that the degree of post-operative neuropsychological impairment in the study population was thus underestimated.

8.8 Transcranial Doppler Data

127/192 patients received successful intra-operative TCD monitoring of the right middle cerebral artery and video recording. Data from the first 35 patients were lost due to malfunction of the video recorder. The video recorder failed to record pictures onto tape and required servicing.14 patients had no suitable transcranial window or the probe was irretrievably dislodged and for the remaining 16 the TCD machine was unavailable for monitoring because another patient was simultaneously being studied.

127 video tapes were then reviewed off line. Velocity of middle cerebral artery blood was recorded and microembolic events were counted as described in Methods and Materials.

8.8.1 Middle cerebral artery blood velocity

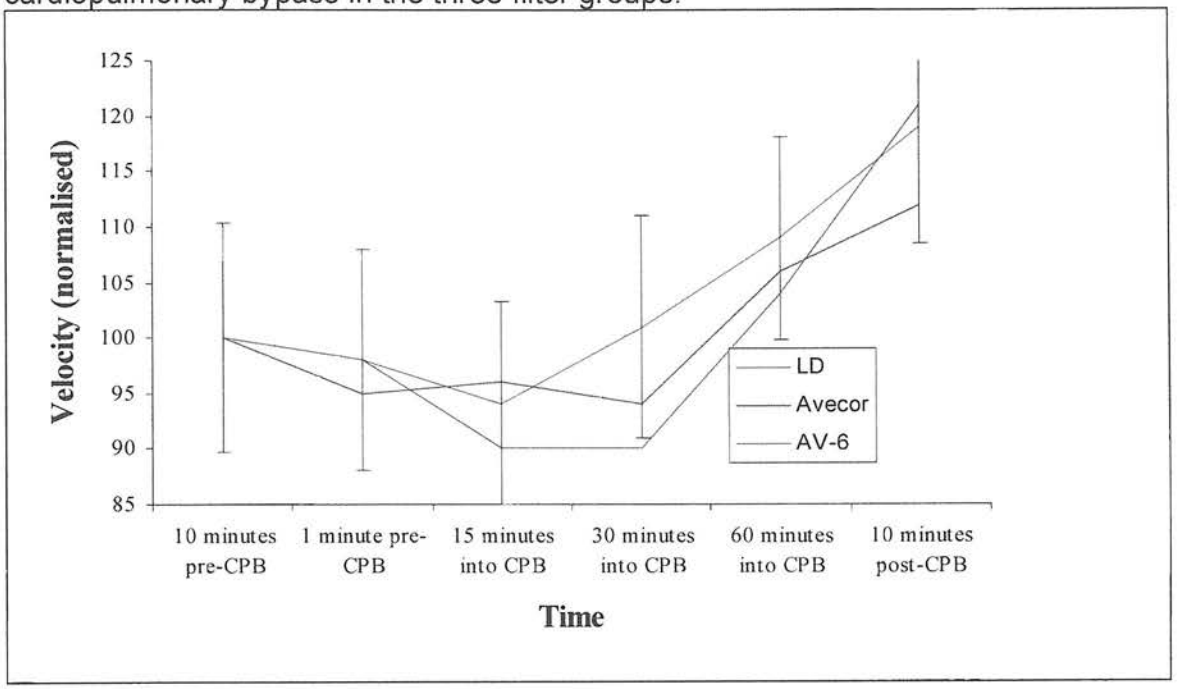
In all three filter groups there was a slight reduction in mean cerebral artery blood velocity at 15 and 30 minutes into cardiopulmonary bypass compared to pre-CPB velocity followed by an increase in velocity during rewarming and at 10 minutes post. Using repeated measures ANOVA there were no significant differences over time or between groups.

Table 8.13 Mean middle cerebral artery blood velocity before during and after cardiopulmonary bypass in the three filter groups.

	Mean Velocity (SD) (cm/s) (normalised velocity)		
Time	**LD (n=56)**	**Avecor (n=42)**	**AV-6 (n=26)**
1 10 minutes pre-CPB	28.8 (10.3) (100)	31.6 (13.7) (100)	30.3 (12.4) (100)
2 1 minute pre-CPB	28.3 (9.9) (98)	30.1 (13.2) (95)	26.9 (10.8) (98)
3 15 minutes into CPB	27.0 (9.3) (94)	30.4 (11.5) (96)	27.4 (9.0) (90)
4 30 minutes into CPB	29.1 (10.0) (101)	29.7 (11.3) (94)	27.3 (11.1) (90)
5 60 minutes into CPB	31.4 (9.2) (109)	33.8 (14.6) (106)	31.5 (15.3) (104)
6 10 minutes post-CPB	34.3 (10.5) (119)	35.3 (11.5) (112)	36.7 (12.1) (121)

The data in Table 8.13 is also shown in the following Figure 8.2 Values were normalised to a baseline of 100% for all three groups in order to compare the groups graphically.

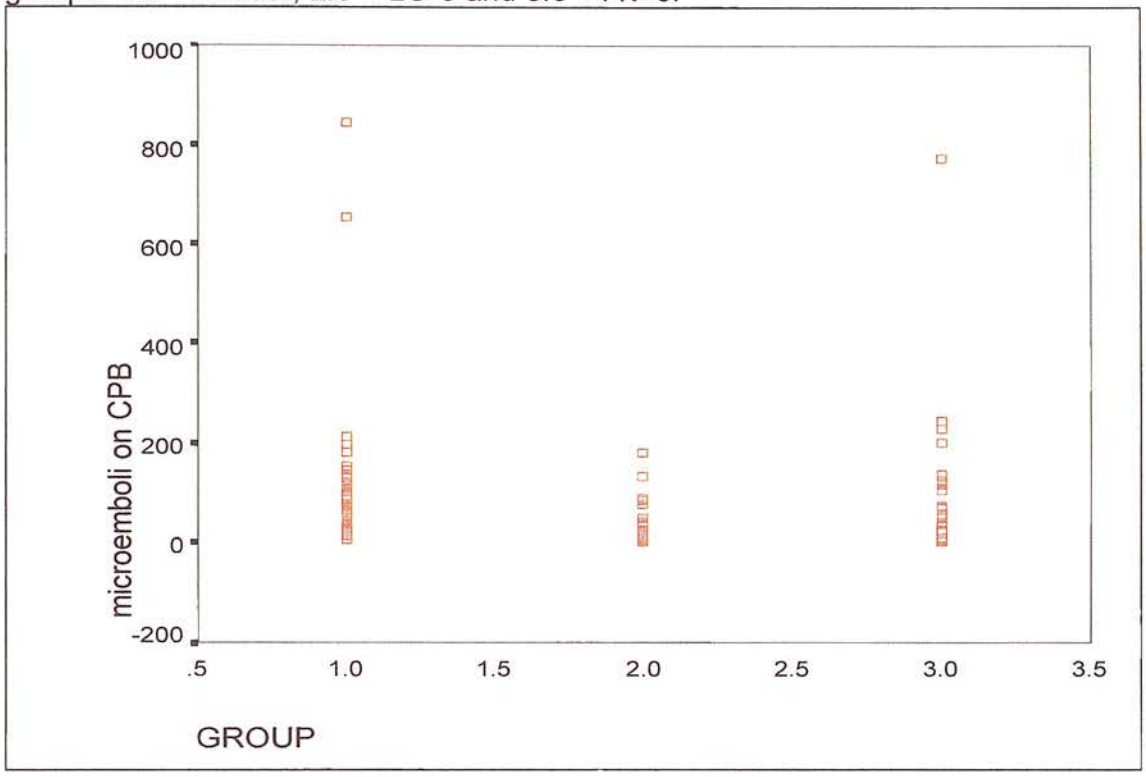
Figure 8.2 Mean middle cerebral artery blood velocity before during and after cardiopulmonary bypass in the three filter groups.



8.8.2 Microemboli Counts During CPB

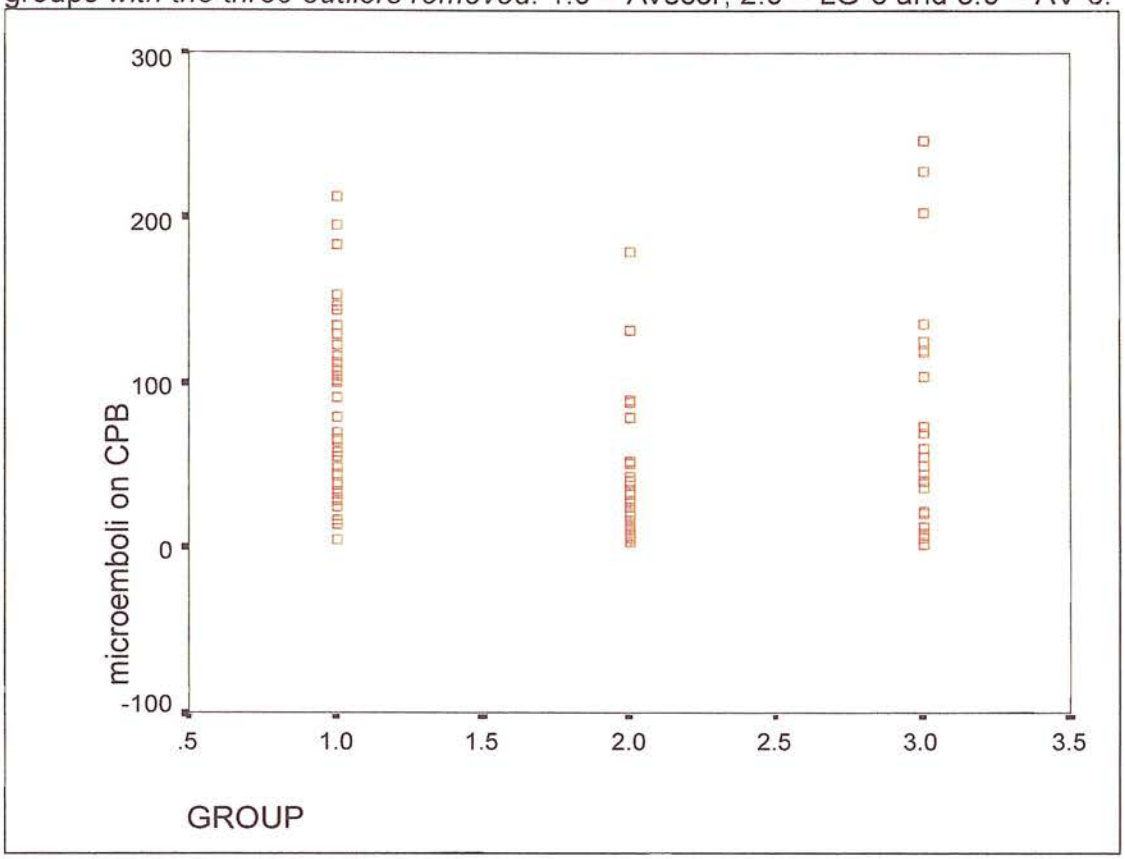
The following scattergram, Figure 8.3, shows that the number of microemboli detected were not normally distributed. Results for microemboli are therefore expressed as medians.

Figure 8.3 Scattergram showing range of microemboli count for the three filter groups. 1.0 = Avecor, 2.0 = LG-6 and 3.0 = AV-6.



There were three distinct outliers. Two in the Avecor group and one in the AV-6 group. Although careful analysis of the conduct of these cases and their characteristics did not reveal any cause for the increased number of microemboli, these patients were thought unlikely to be representative of the patient population. Data from these three patients was therefore removed from further microemboli data analysis. The scattergram in Figure 8.4 show the range of microemboli counts with the outliers removed.

Figure 8.4 Scattergram showing range of microemboli count for the three filter groups *with the three outliers removed.* 1.0 = Avecor, 2.0 = LG-6 and 3.0 = AV-6.



There were less microemboli detected on CPB in the LG-6 group with a median of 15 compared to 66 and 55 in the Avecor and AV-6 groups respectively. There was no statistically significant difference between the two control filters on CPB ($p = 0.46$).

Very few microemboli were detected in the ten minute period after CPB except in a few cases (Table 8.14)

Table 8.14 Median microemboli counts on CPB and for the ten minutes post CPB for the three filter groups (outliers removed).

Microemboli	LD (n=56)	Avecor (n=42)	AV-6 (n=26)
On CPB	15 (3-177)	66 (5-212)	55 (2-245)
10 minutes post bypass	0 (0-26)	1 (0-29)	0 (0-1)

Table 8.15 Mann Whitney U test comparing median numbers of microemboli on CPB both with and without outliers

	With Outliers		Without Outliers	
	U	p	U	p
LG-6 vs. Avecor	325	< 0.001	325	< 0.001
LG-6 vs. AV-6	349	< 0.001	349	< 0.001
Avecor vs. AV-6	521	0.47	479	0.49

It can therefore be seen that inclusion or exclusion of the outliers makes no difference to the significance of the results.

8.8.3 Relationship of Cerebral Microemboli on CPB to Cerebral Blood Velocity

Although there was no difference in middle cerebral artery blood velocity between the three filter groups, we wished to see if there was any effect of blood velocity per se on microemboli counts. Controlling for filter group, there was no correlation found between cerebral blood velocity at any time point and microemboli count on CPB (see Table 8.16).

Table 8.16 Partial correlation coefficients for microemboli counts and cerebral blood velocity controlling for filter group.

	V1	V2	V3	V4	V5	V6
Microemboli on CPB	R = -0.12 p = 0.37	R = -0.16 p = 0.19	R = -0.02 p = 0.86	R = -0.06 p = 0.62	R = -0.12 p = 0.33	R = 0.01 p = 0.93

V1=10 mins pre-CPB, V2=1 min pre-CPB, V3=15 mins into CPB, V4=30 mins into CPB, V5=60 mins into CPB and V6=10 mins post-CPB.

8.8.4 Relationship between patient and intra-operative variables and microemboli count

There was no correlation between the age of the patient, the bypass and cross clamp times or number of grafts performed and microemboli count.

Table 8.17 Correlation coefficients (Spearmans) for microemboli counts patient and intra-operative variables.

	Microemboli count	
	R	p
Age	-0.15	0.87
Bypass time	0.09	0.29
Cross clamp time	0.15	0.10
Number of grafts	0.09	0.36

Examining whether the presence of symptomatic peripheral vascular disease, clinically detectable atheroma or side-biting clamp usage influenced microemboli count revealed that none of these factors led to a statistically significant difference in microemboli count. This can be seen in Table 8.18. In order to enable comparison of these findings, only those patients who had data on all these variables are included in Table 8.18. In 6 of the patients with a TCD record, the surgeon was unable to say whether there was digital palpation evidence of atheroma of the aorta. These 6 are excluded.

Table 8.18 Influence of variables potentially affecting microemboli count on CPB.

Factor		Microemboli on CPB		P (Mann Whitney U test)
		Mean	Standard deviation	
PVD	Yes (n = 7) No (n = 111)	58.7 71.1	52.8 126.6	0.81
Atheroma	Yes (n = 10) No (n = 108)	72.6 70.2	58.4 128.6	0.95

Side-clamp use	Yes (n = 11) No (n = 107)	135.5 63.7	223.3 108.6	 0.07

Table 8.18 indicates that there was a trend towards an increase in microemboli count when the side-biting clamp was used to cannulate.

8.8.5 Relationship of Microemboli to NP Outcome

A total of 107 patients received intra-operative TCD and then completed NP follow up at six weeks.

Despite the significant decrease in microemboli with the leucocyte-depleting filter this did not lead to a significant difference in NP outcome using deficit and Z score analysis. It was therefore decided to look at the relationship between microemboli and NP outcome more closely.

No Spearmans correlation was found between microemboli count on CPB and Z scores for each of the tests and the total Z scores. This held true for the whole study population and also for each of the filter groups (Table 8.19).

Table 8.19 Spearmans correlation for microemboli count and total Z scores

	R	P
For all cases	-0.06	0.57
For LD filter group	-0.16	0.25
For Avecor filter group	-0.15	0.39
For AV-6 filter group	0.06	0.77

8.8.6 Relationship of Microemboli to Other Outcomes (all cases)

Table 8.20 Correlation coefficients (Spearmans) for microemboli counts and post-operative variables.

	Microemboli count	
	R	P
Time to extubation	0.05	0.64
Days to discharge	0.14	0.23

Table 8.21 Spearmans correlation coefficients for microemboli counts and neuropsychological Z change scores.

	Microemboli count on CPB			
	All cases	**Avecor (36)**	**LD (50)**	**Av-6 (21)**
Choice Reaction Time Test	-0.06 (0.51)	-0.12 (0.47)	-0.16 (0.26)	0.06 (0.79)
Grooved Pegboard Test D	0.09 (0.36)	0.18 (0.31)	-0.10 (0.17)	0.16 (0.47)
Grooved Pegboard Test ND	0.06 (0.55)	0.08 (0.65)	0.09 (0.52)	0.11 (0.62)
Letter Cancellation Test	-0.07 (0.47)	-0.01 (0.98)	-0.11 (0.47)	-0.26 (0.22)
Non-verbal Memory Test	-0.05 (0.64)	-0.17 (0.33)	0.09 (0.50)	-0.05 (0.84)
Rey Auditory Verbal Learning Test	-0.07 (0.47)	-0.06 (0.74)	-0.05 (0.71)	-0.13 (0.57)
Symbol Digit Replacement Test	-0.07 (0.49)	-0.18 (0.31)	-0.22 (0.12)	0.09 (0.66)
Trail Making Test A	0.08 (0.44)	0.17 (0.34)	0.07 (0.63)	-0.07 (0.74)
Trail Making Test B	0.01 (0.90)	-0.02 (0.90)	-0.22 (0.12)	0.16 (0.47)
Total z score	-0.06 (0.57)	-0.15 (0.39)	-0.16 (0.25)	0.06 (0.77)

P values given in brackets

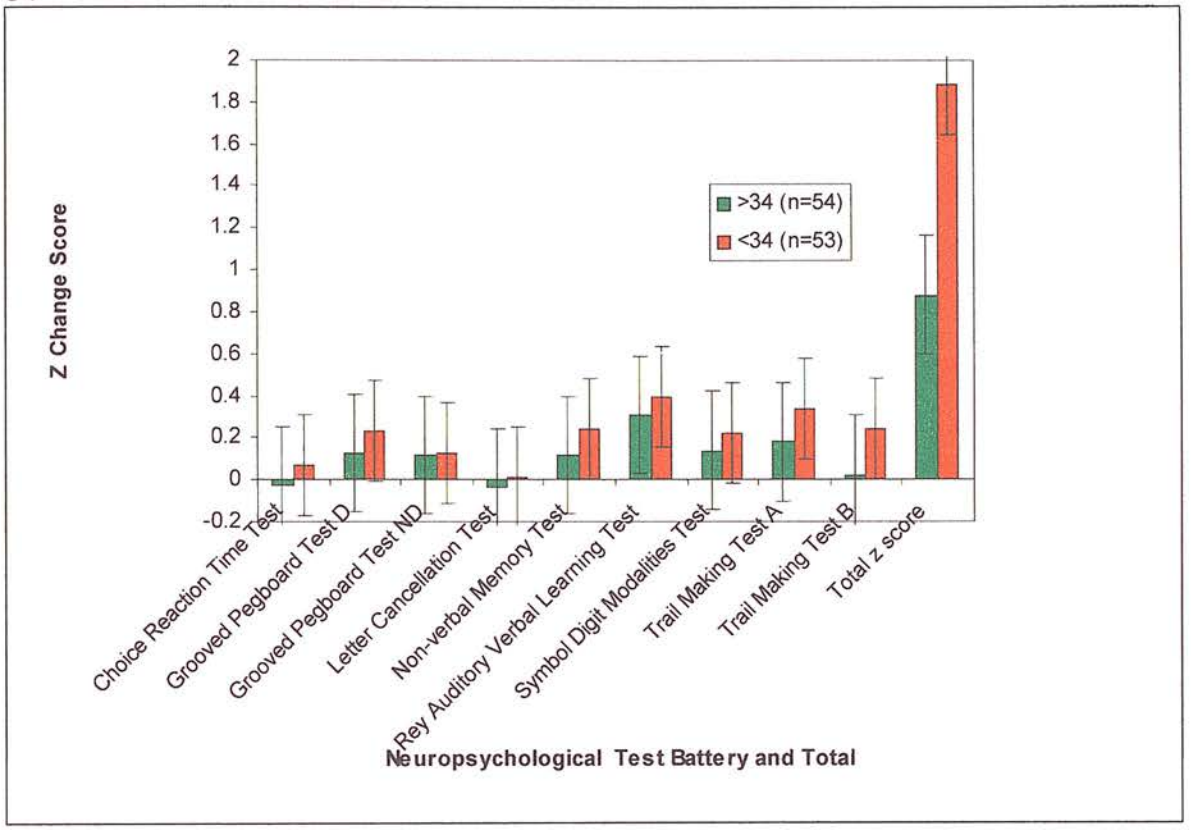
However dividing the whole cohort into two equal groups using a median split with greater or less than 34 microemboli on CPB showed that those with fewer microemboli had greater Z scores.

Table 8.22 Mean Z scores according to microemboli count using a median split of 34

	< 34 (n=53)	**>34** (n=54)	**p** (t test,1 tailed)
Choice Reaction Time Test	0.07	- 0.03	0.20
Grooved Pegboard Test D	0.23	0.13	0.23
Grooved Pegboard Test ND	0.13	0.12	0.45
Letter Cancellation Test	0.01	- 0.06	0.30
Non-verbal Memory Test	0.24	0.12	0.21
Rey Auditory Verbal Learning Test	0.40	0.31	0.25
Symbol Digit Replacement Test	0.22	0.14	0.20
Trail Making Test A	0.34	0.18	0.11
Trail Making Test B	0.24	- 0.03	0.08
Total Z score	**1.88**	**0.88**	**0.02**

This difference reached statistical significance for the total Z scores only. However, as with the leucocyte depleting filter group, there was an overall trend towards greater improvement (i.e. a greater positive Z score) in the <34 microemboli filter group. This is illustrated graphically in Figure 8.5

Figure 8.5 Mean Z scores according to microemboli count using a median split of 34



8.9 Clinical Data

8.9.1 Intra-operative clinical data

8.91.1 Peri-operative blood pressure

Table 8.23 Mean peri-operative systolic blood pressure for the three filter groups.

	Systolic Blood Pressure (SD) (mmHg)		
Time	**LD**	**Avecor**	**AV-6**
Pre-induction	132 (21)	136 (19)	135 (17)
Post-induction	98 (19)	103(16)	100 (23)
Pre-sternotomy	116 (19)	113 (18)	116 (14)
Post sternotomy	112 (20)	112 (18)	121 (19)
Pre-CPB	91 (17)	94 (17)	88 (17)
Post-CPB	94 (17)	91 (16)	98 (18)
Post skin closure	99 (16)	100 (17)	103 (13)

Using repeated measures ANOVA there were no significant differences over time or between groups.

There were no significant differences at any time point. The same data are shown in Figure 8.6

Figure 8.6 Mean peri-operative systolic blood pressure for the three filter groups.

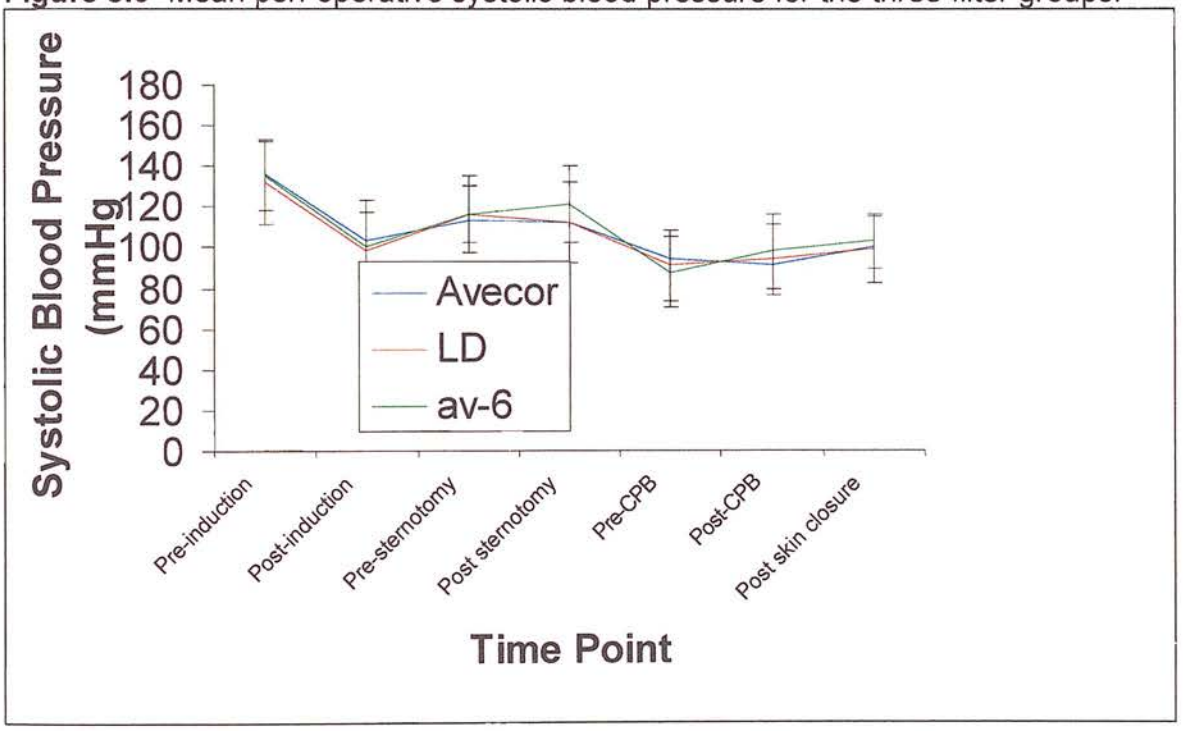


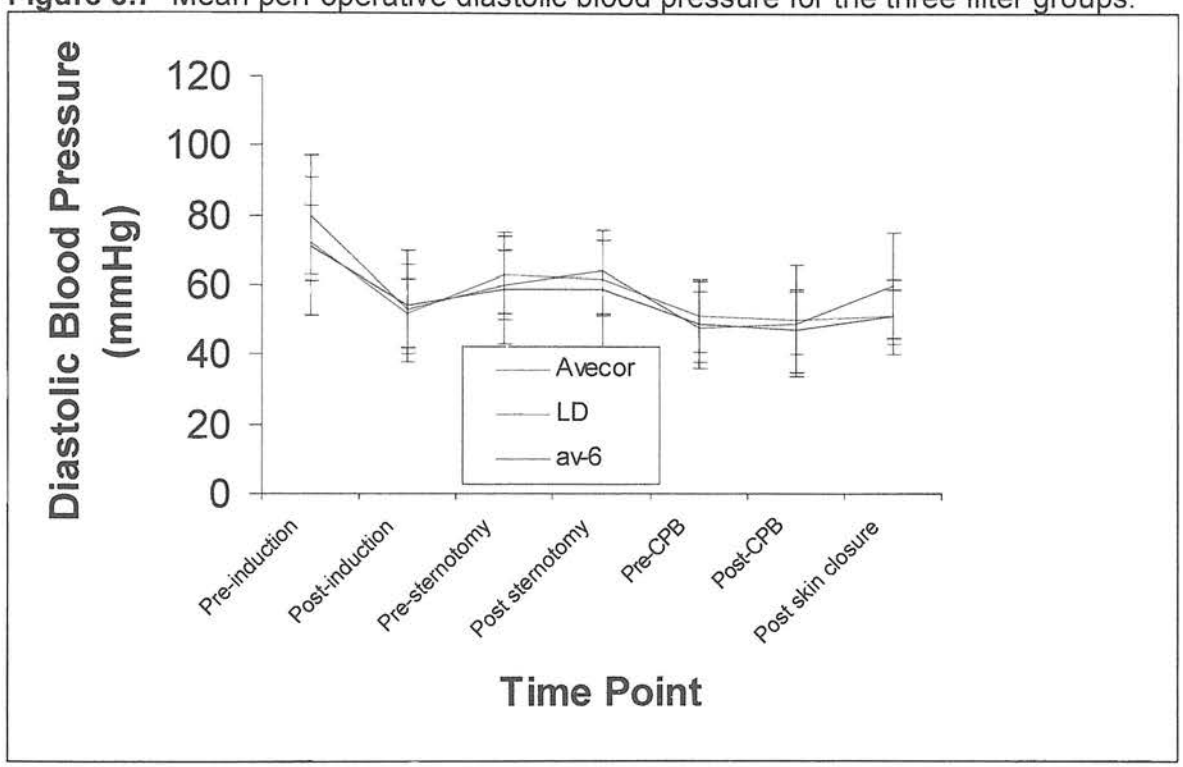
Table 8.24 Mean peri-operative diastolic blood pressure for the three filter groups.

	Diastolic Blood Pressure (SD) (mmHg)		
Time	**LD**	**Avecor**	**AV-6**
Pre-induction	71 (11)	72 (20)	80 (17)
Post-induction	54 (10)	52 (16)	53 (13)
Pre-sternotomy	59 (11)	63 (16)	60 (10)
Post sternotomy	59 (11)	62 (17)	64 (12)
Pre-CPB	49 (10)	51 (13)	48 (10)
Post-CPB	47 (16)	50 (12)	49(9)
Post skin closure	51 (8)	51 (11)	60 (15)

Using repeated measures ANOVA there were no significant differences over time or between groups.

There were no significant differences at any time point. The same data are shown in Figure 8.7

Figure 8.7 Mean peri-operative diastolic blood pressure for the three filter groups.



Although there was a decline in peri-operative systolic and diastolic blood pressures there was no significant differences between the three filter groups.

8.9.1.2 Peri-operative Oxygenation

Table 8.25 Peri-operative pO2 for all three filter groups

	pO2 (kPa) (SD)		
Time	**LD**	**Avecor**	**AV-6**
Pre-op	12.1 (1.6)	11.4 (1.9)	-
1 hour	21.6 (8.8)	19.2 (7.1)	25.6 (8.7)
2 hrs	17.4 (7.3)	17.3 (6.2)	20.8 (7.1)
3 hrs	16.4 (4.9)	16.5 (4.7)	19.3 (7.9)
4 hrs	16.5 (5)	16.5 (3.9)	16.9 (4.6)
8 hrs	15.2 (3.3)	14.4 (3.4)	15.8 (3.9)
12 hrs	14.6 (3.8)	14.3 (3.4)	14.1 (3)
16 hrs	13.5 (2.9)	13.5 (3.3)	13.7 (4.6)
20 hrs	14.2 (7.1)	13.2 (2.7)	14.2 (4.1)
24 hrs	11.7 (3.3)	12.1 (2.5)	10.7 (1.4)
5 days	9.9 (1.3)	9.5 (1.1)	-

Using repeated measures ANOVA there were significant differences over time but no difference between groups.

Figure 8.8 Peri-operative pO2

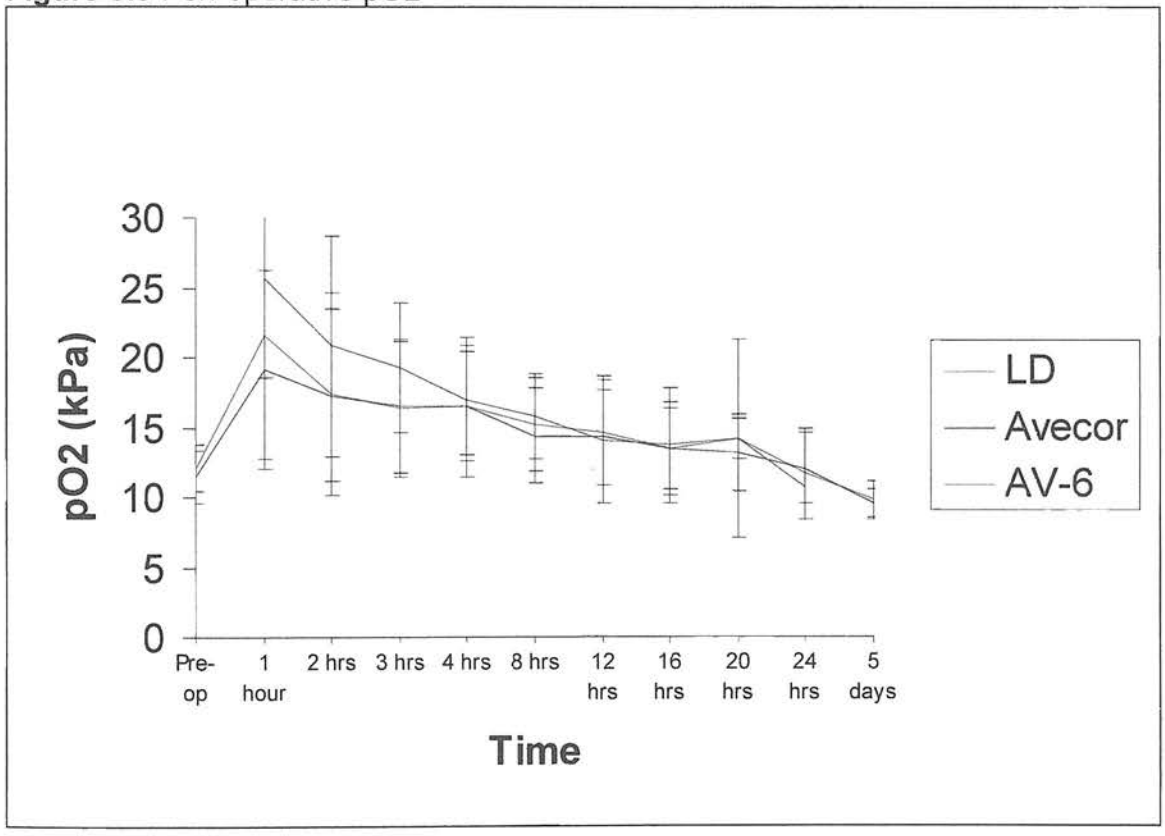


Table 8.26 Peri-operative Oxygen saturation for all three filter groups

	Oxygen Saturation (SD) (%)		
Time	**LD**	**Avecor**	**AV-6**
Pre-op	96.6 (1.1)	96.5 (1.3)	-
1 hour	98 (1.6)	98 (1.5)	98.1 (1.1)
2 hrs	97.5 (1.9)	97.6 (1.9)	97.7 (1.1)
3 hrs	97.3 (1.5)	97.7 (1.4)	97.4 (1.6)
4 hrs	97.5 (1.5)	97.7 (1.7)	97.2 (1.3)
8 hrs	97.3 (1.2)	97.2 (1.5)	96.9 (1.3)
12 hrs	96.9 (1.7)	97 (1.7)	96.7 (1.1)
16 hrs	96.8 (1.3)	96.9 (1.6)	96.3 (1.4)
20 hrs	96.5 (1.3)	96.8 (1.7)	96.3 (1.7)
24 hrs	95.4 (1.8)	96.4 (1.7)	94.8 (1.6)
5 days	94.8 (2.1)	94.3 (1.7)	-

Using repeated measures ANOVA there were no significant differences over time or between groups.

Figure 8.9 Peri-operative Oxygen Saturation

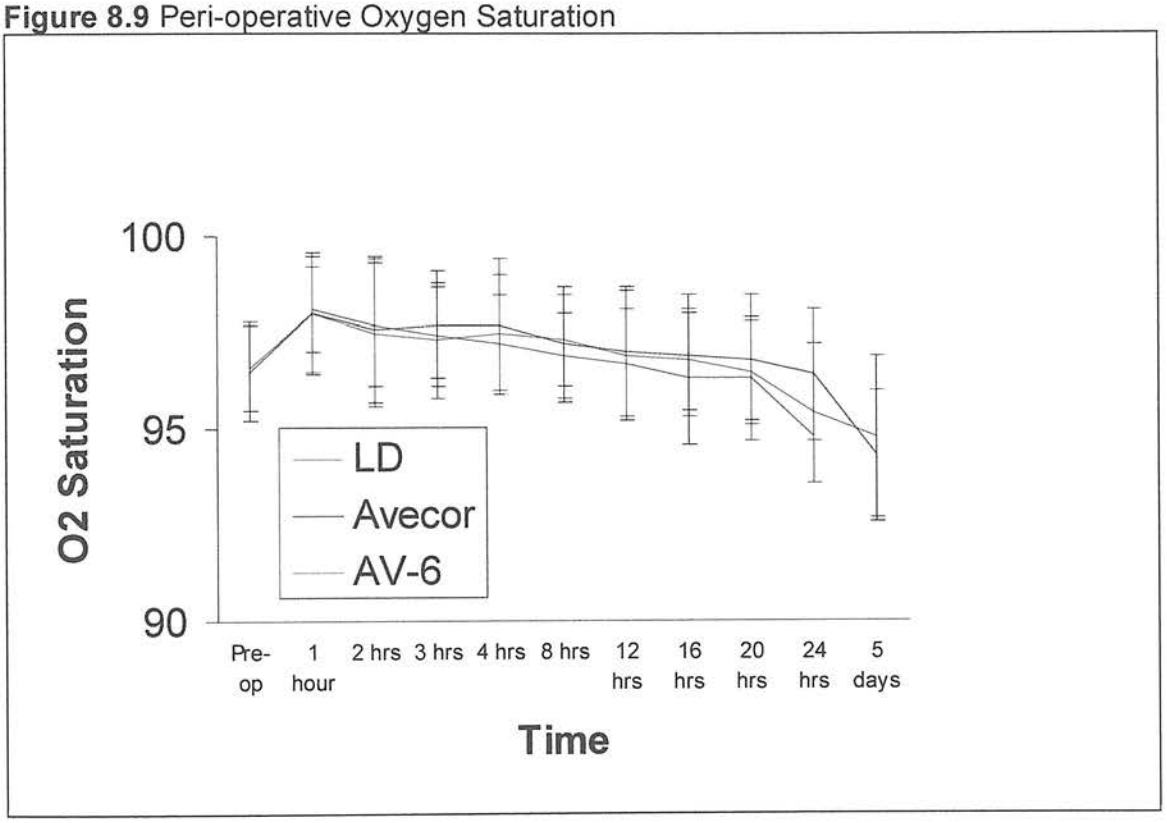
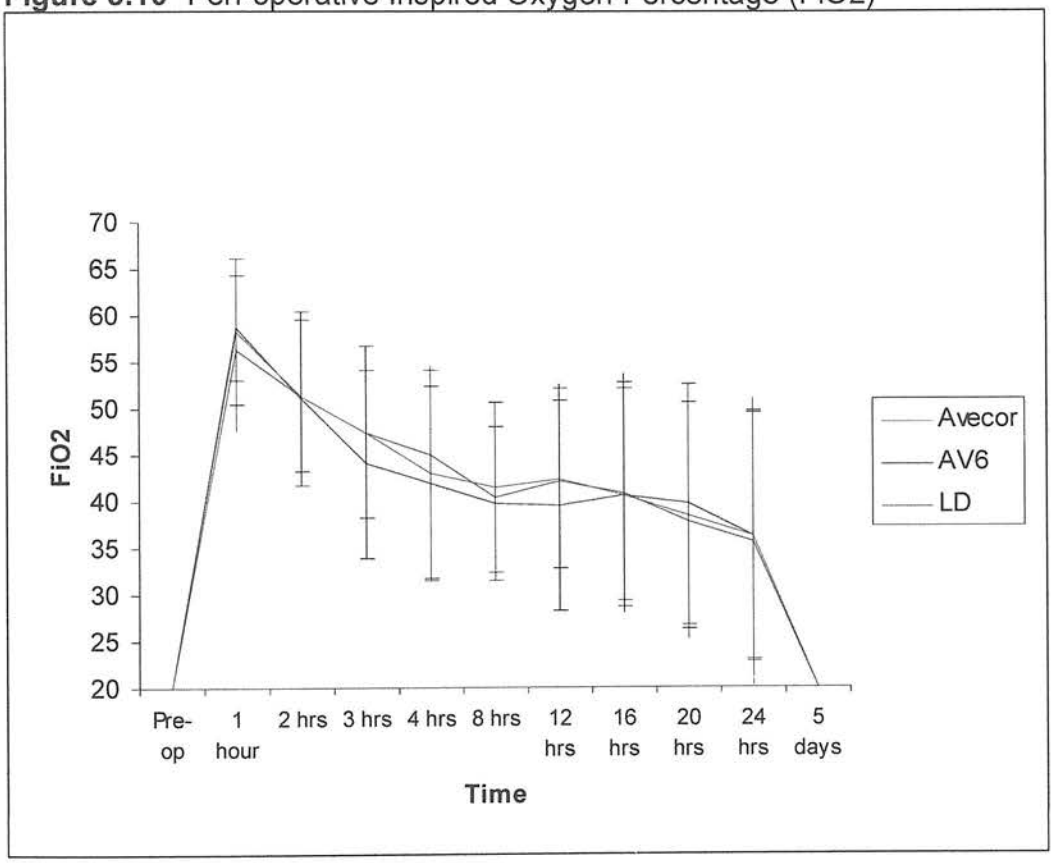


Table 8.27 Peri-operative FiO2 for all three filter groups

	FiO2 (SD) (kPa)		
Time	**LD**	**Avecor**	**AV-6**
Pre-op	20 (0)	20 (0)	20 (0)
1 hour	58.2 (7.8)	56.3 (8.6)	58.7 (58.7)
2 hrs	51.4 (8.1)	51.3 (8.2)	51.1 (51.1)
3 hrs	47.5 (9.2)	47.3 (8.8)	44.1 (44.1)
4 hrs	43 (11.2)	44.9 (9.7)	41.9 (41.9)
8 hrs	41.5 (9.2)	40.5 (9)	39.8 (39.8)
12 hrs	42.5 (9.6)	42.1 (10.6)	39.6 (39.6)
16 hrs	40.7 (11.4)	40.9 (12.9)	40.7 (40.7)
20 hrs	38.5 (12.1)	37.9 (12.6)	39.7 (39.7)
24 hrs	36.3 (13.3)	35.7 (15.4)	36.3 (36.3)
5 days	20 (0)	20 (0)	20 (0)

Using repeated measures ANOVA there were no significant differences over time or between groups.

Figure 8.10 Peri-operative Inspired Oxygen Percentage (FiO2)



8.9.1.3 Peri-operative Temperature

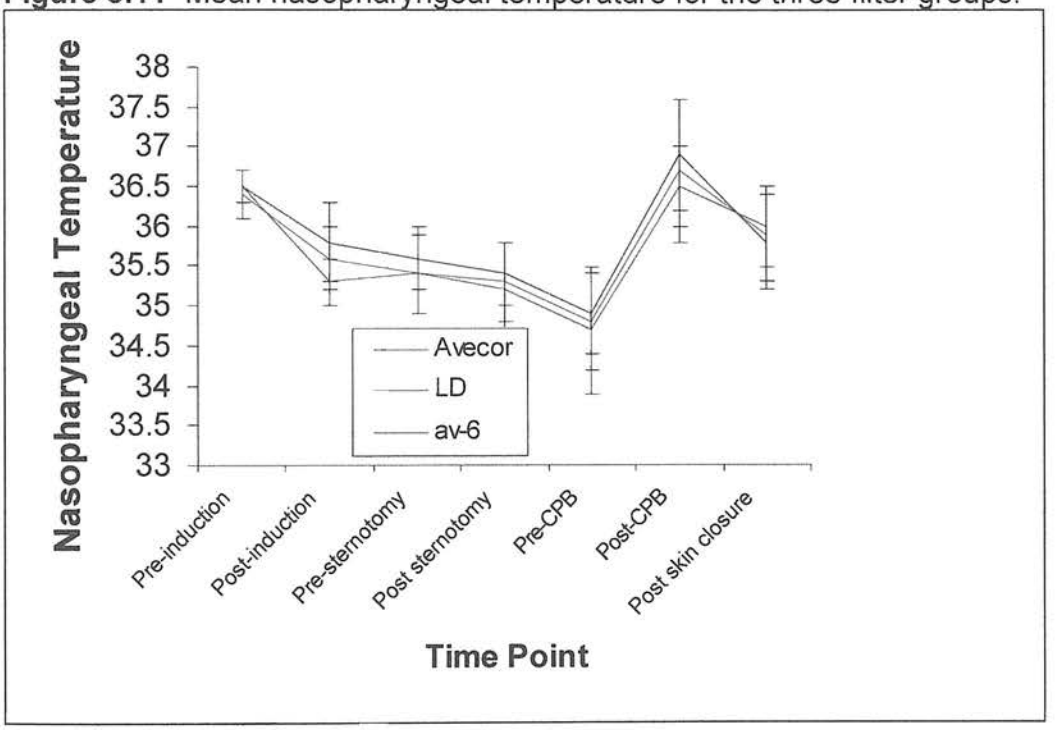
Table 8.28 Mean peri-operative nasopharyngeal temperature for the three filter groups.

	Nasopharyngeal Temperature (SD) (°C)		
Time	**LD**	**Avecor**	**AV-6**
Pre-induction	36.4 (0.3)	36.5 (0.2)	36.5 (0.2)
Post-induction	35.6 (0.4)	35.8 (0.5)	35.3 (0.3)
Pre-sternotomy	35.4 (0.5)	35.6 (0.4)	35.4 (0.5)
Post sternotomy	35.3 (0.5)	35.4 (0.4)	35.2 (0.6)
Pre-CPB	34.8 (0.6)	34.9 (0.5)	34.7 (0.8)
Post-CPB	36.7 (0.9)	36.9 (0.7)	36.5 (0.5)
Post skin closure	35.9 (0.6)	35.8 (0.6)	36.0 (0.5)

Using repeated measures ANOVA there were significant differences over time but no differences between groups at any time point.

There were no significant differences at any time point. The same data are shown in Figure 8.11

Figure 8.11 Mean nasopharyngeal temperature for the three filter groups.



In all groups there was a decline in temperature after induction to the time of beginning cardiopulmonary bypass. After rewarming to 37 ° C at the end of CPB there was then a second decrease in temperature to the time of skin closure. Figure 8.11 omits to show the cooling to 32 ° C which occurred during CPB.

8.9.2 Post-operative blood pressure

Table 8.29 Mean systolic blood pressure in the first 24 hours post surgery for the three filter groups.

	Systolic Blood Pressure (SD) (mmHg)		
Time (hrs post op)	**LD**	**Avecor**	**AV-6**
1	115 (21)	117 (19)	116 (20)
2	124 (18)	122 (17)	125 (20)
3	118 (15)	120 (19)	118 (15)
4	117 (14)	117 (19)	114 (17)
8	113 (15)	114 (16)	110 (17)
12	116 (14)	116 (15)	117 (13)
16	118 (16)	116 (19)	112 (12)
20	118 (18)	118 (18)	116 (14)
24	118 (16)	118 (16)	116 (18)

Using repeated measures ANOVA there were no significant differences over time or between groups.

The same data are shown in Figure 8.12

Figure 8.12 Mean post-operative systolic blood pressure for the three filter groups.

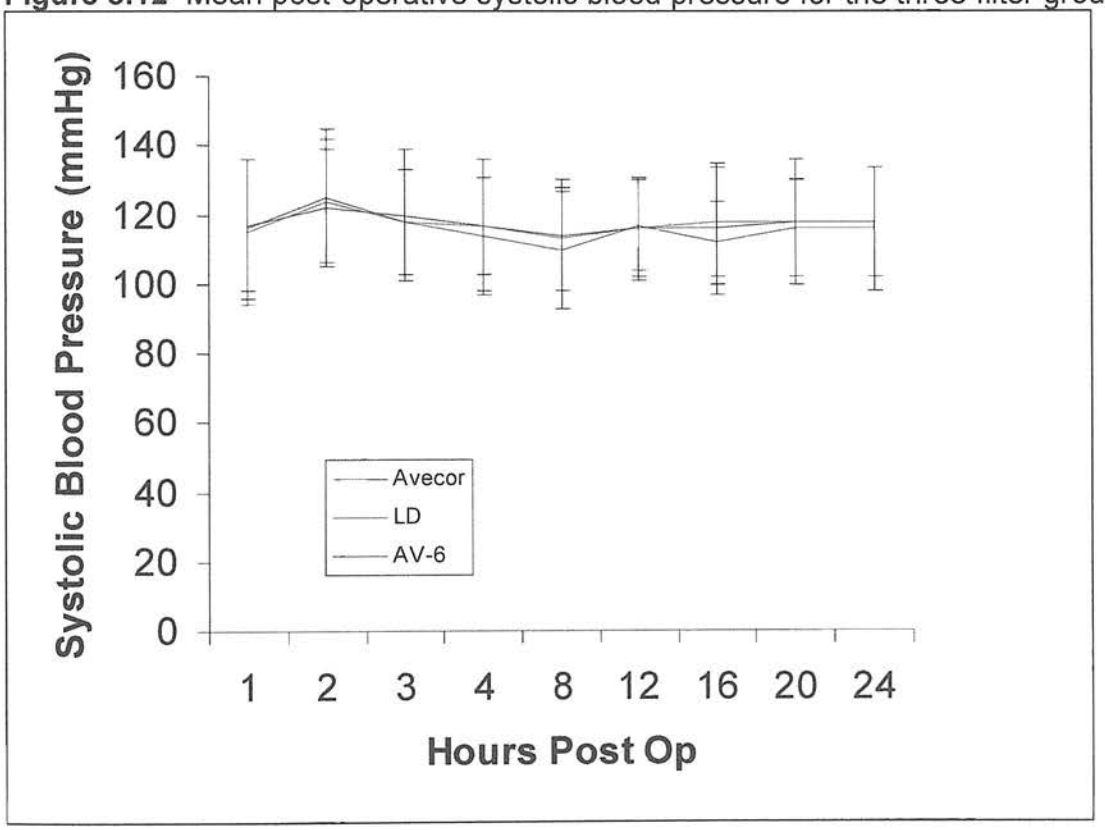
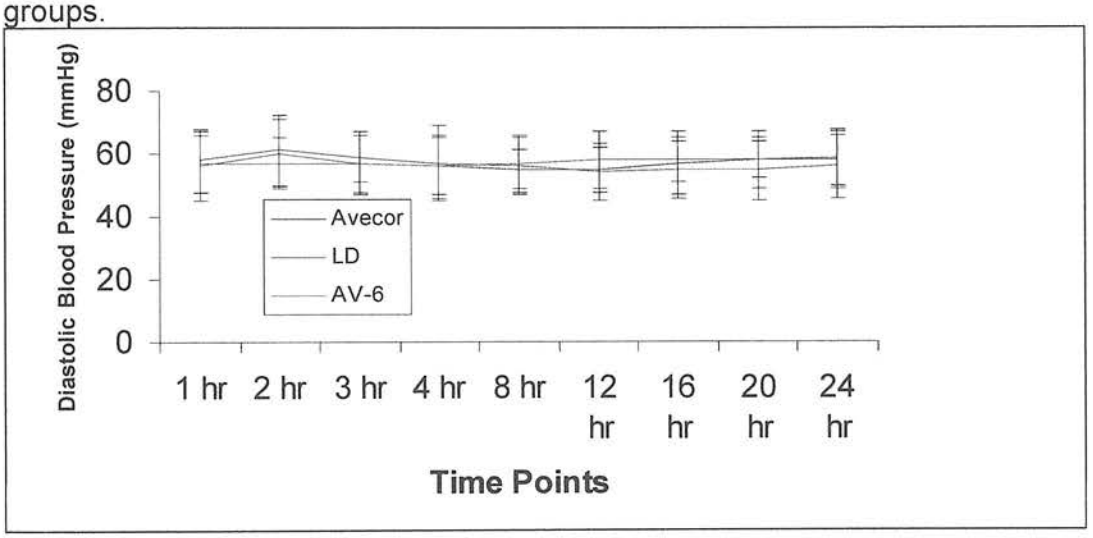


Table 8.30 Mean diastolic blood pressure in the first 24 hours post surgery for the three filter groups.

	Diastolic Blood Pressure (SD) (mmHg)		
Time (hrs post op)	LD	Avecor	AV-6
1	56 (11)	57 (9)	58 (10)
2	60 (11)	57 (8)	61 (11)
3	57 (9)	57 (10)	59 (8)
4	56 (10)	56 (9)	57 (12)
8	57 (9)	55 (6)	56 (9)
12	58 (9)	55 (7)	54 (9)
16	58 (7)	57 (10)	55 (9)
20	58 (9)	58 (6)	55 (10)
24	59 (9)	58 (9)	56 (10)

Using repeated measures ANOVA there were no significant differences over time or between groups.
The same data are shown in Figure 8.13

Figure 8.13 Mean post-operative diastolic blood pressure for the three filter groups.



8.9.3 Post-operative Temperature

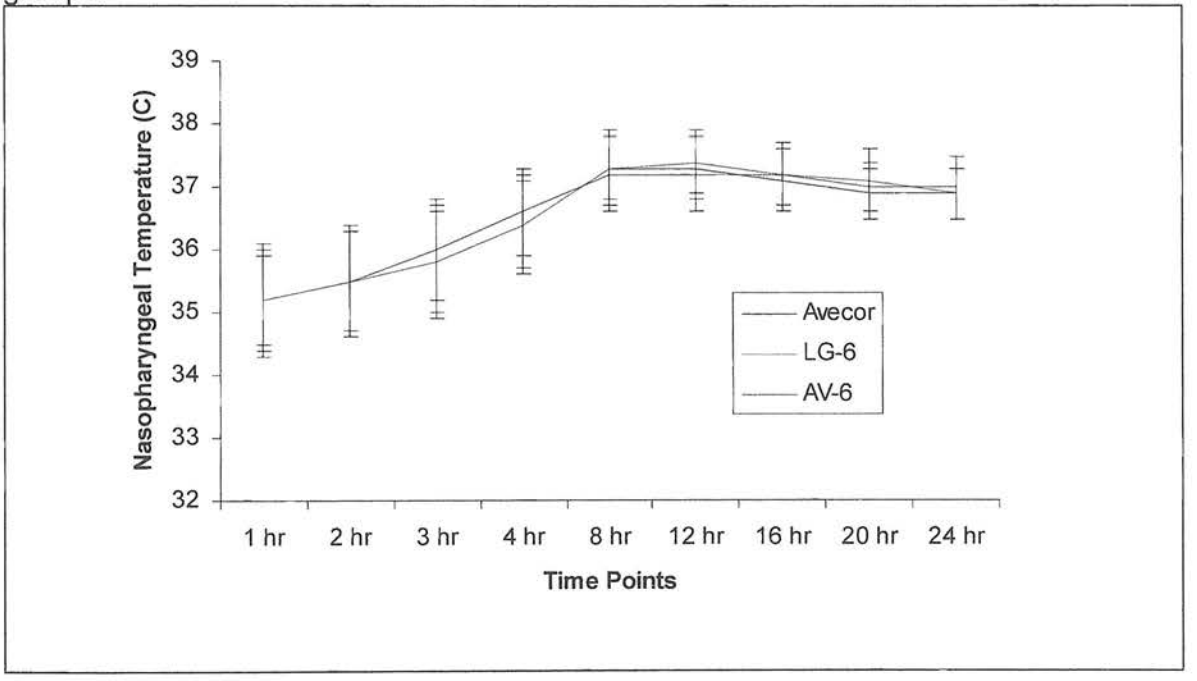
Table 8.31 Mean nasopharyngeal temperature in the first 24 hours post surgery for the three filter groups.

	Nasopharyngeal Temperature (SD) (°C)		
Time (hrs post op)	LD	Avecor	AV-6
1	35.2 (0.8)	35.2 (0.9)	35.2 (0.7)
2	35.5 (0.8)	35.5 (0.9)	35.5 (0.8)
3	35.8 (0.8)	35.8 (0.9)	36.0 (0.8)
4	36.4 (0.7)	36.4 (0.8)	36.6 (0.7)
8	37.3 (0.5)	37.3 (0.6)	37.2 (0.6)
12	37.4 (0.5)	37.3 (0.5)	37.2 (0.6)
16	37.2 (0.5)	37.1 (0.5)	37.2 (0.5)
20	37.1 (0.5)	36.9 (0.4)	37.0 (0.4)
24	36.9 (0.4)	36.9 (0.4)	37.0 (0.5)

Using repeated measures ANOVA there were no significant differences over time or between groups.

The same data are shown in Figure 8.14

Figure 8.14 Mean post-operative nasopharyngeal temperature for the three filter groups.



8.9.4 Time to extubation

Figure 8.15 Scattergram of time to extubation for all three filter groups. 1.0 = Avecor, 2.0 = LD and 3.0 = AV-6.

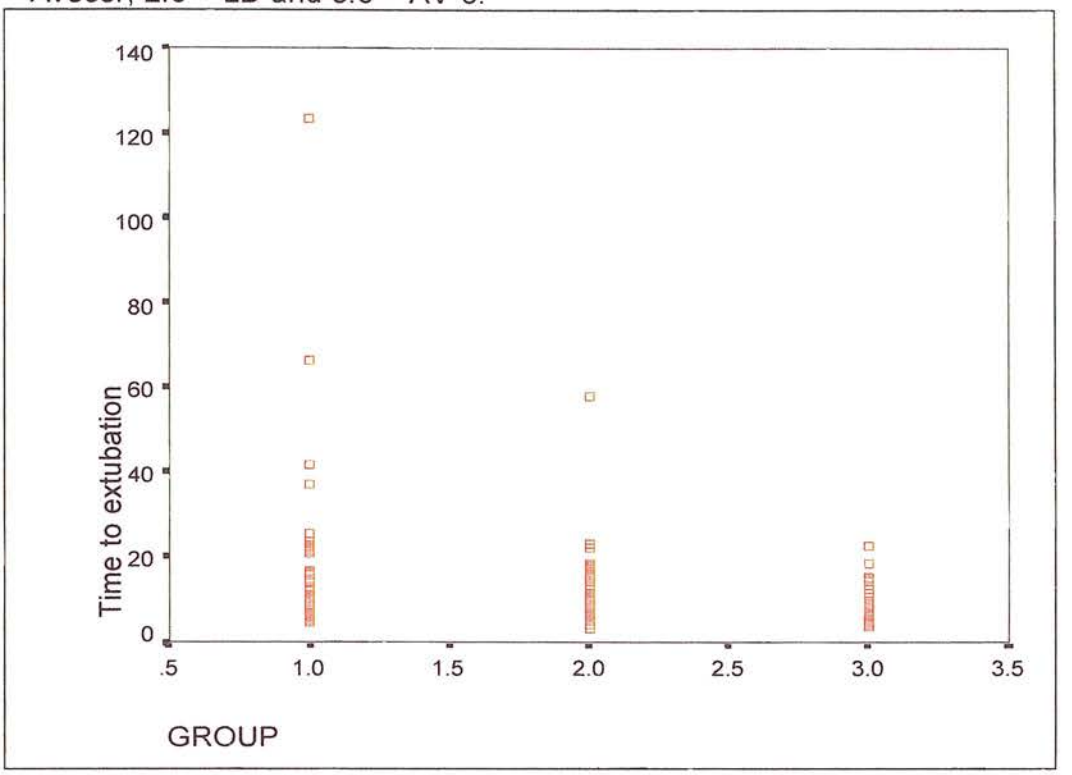


Table 8.32 Median time to extubation for all three filter groups. The range is given in brackets.

	Avecor (n=69)	LD (n=78)	AV-6 (n=36)
Extubation Time (Hrs)	**10.8** (5 - 124)	**9.8** (3-58)	**7.8** (4-23)

Table 8.33 Mann Whitney U test to test for significant differences between extubation times.

LG-6 vs. Avecor	p = 0.08
LG-6 vs. AV-6	p = 0.03
Avecor vs. AV-6	p = 0.02

There were significant differences between extubation times between the groups. As this difference may still be due to the outliers the analyses were repeated with the removal of extreme values (3 standard deviations) and outliers (2 standard deviations). The same pattern of significant differences was found in both these analyses as in Table 8.33.

8.9.5 Days to Discharge

Figure 8.16 Scattergram of days to discharge for all three filter groups. 1.0 = Avecor, 2.0 = LG-6 and 3.0 = AV-6.

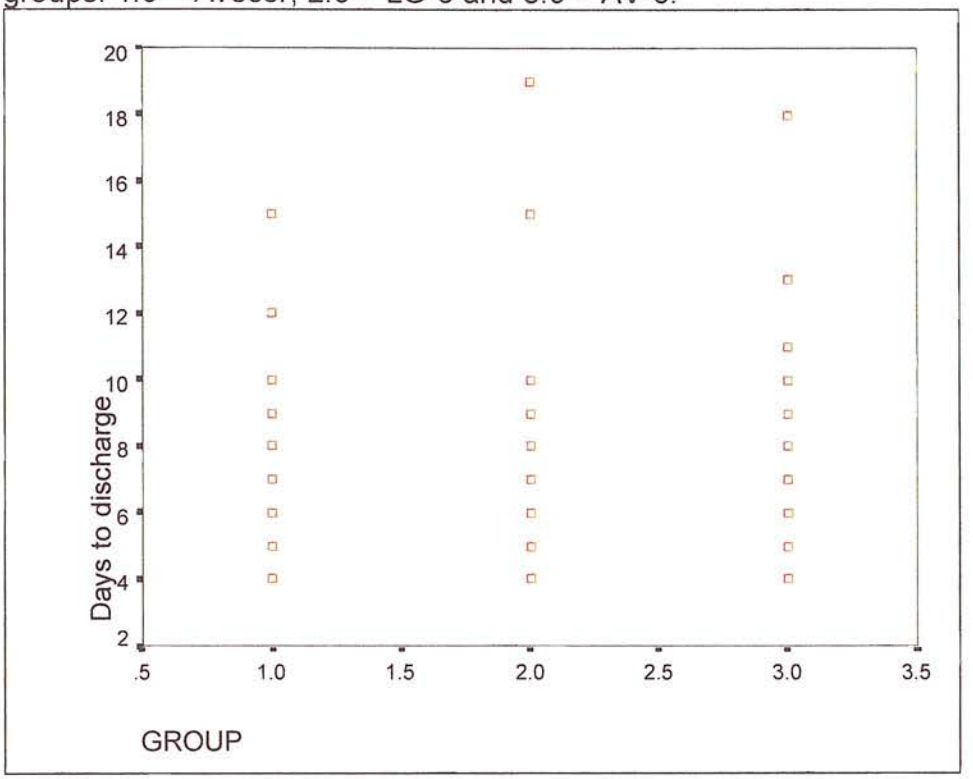


Table 8.34 Median number of days to discharge for all three filter groups.

	Avecor (n=69)	LG-6 (n=78)	AV-6 (n=36)
Days to discharge	**6** (4-15)	**6** (4-19)	7 (4-18)

The range is given in brackets.

Table 8.35 Mann Whitney U test to test for significant differences in days to discharge between all three filter groups.

LG-6 vs. Avecor	p = 0.78
LG-6 vs. AV-6	p = 0.09
Avecor vs. AV-6	p = 0.15

8.10 Haematological Results

8.10.1 Total White Cell Count

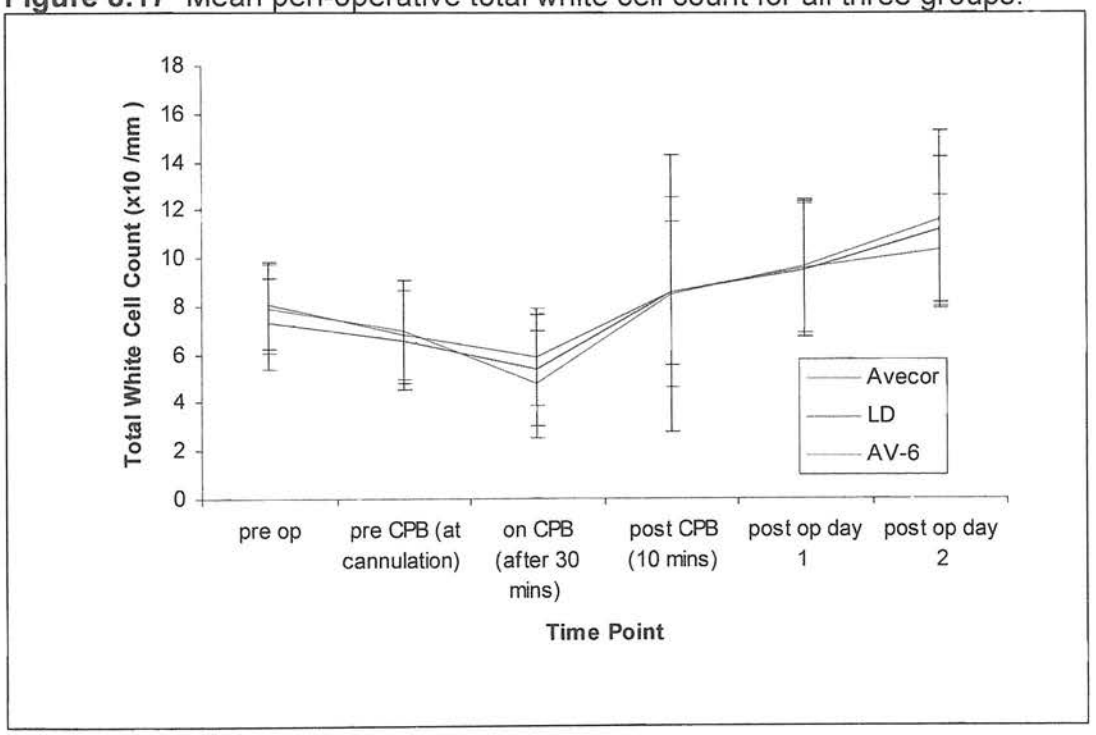
One hundred and seventy patients had serial FBC testing. There was a characteristic fall in total white cell count in all groups during cardiopulmonary bypass. This was then followed by a rise in total white cell count post-operatively.

Table 8.36 Mean peri-operative total white cell count for all three groups.

	Mean WCC (SD) (x 10^3 /mm^3)		
Time	**LD (n=78)**	**Avecor (n=63)**	**AV-6 (n=29)**
1 pre op	7.89 (1.85)	7.31 (1.89)	8.05 (1.79)
2 pre CPB (at cannulation)	6.95 (2.15)	6.59 (2.08)	6.80 (1.84)
3 on CPB (after 30 mins)	4.76 (2.26)	5.35 (2.30)	5.88 (2.02)
4 post CPB (10 mins)	8.53 (5.74)	8.57 (3.94)	8.56 (2.99)
5 post op day 1	9.69 (2.79)	9.50 (2.74)	9.62 (2.74)
6 post op day 2	11.62 (3.71)	11.22 (3.02)	10.31 (2.34)

Using repeated measures ANOVA there were significant differences over time but not between groups at any time points.

Figure 8.17 Mean peri-operative total white cell count for all three groups.



8.10.1.1 Correlation of WCC with Microemboli

Of the one hundred and seventy patients who had serial FBC testing, 120 also had intra-operative TCD monitoring of microemboli. The following Table 8.37 shows that there was no correlation between white cell count at any time point measured and the intra-operative microemboli count.

Table 8.37 Spearman correlation coefficients between WCC and microemboli.

	WCC1	WCC2	WCC3	WCC4	WCC5	WCC6
Microemboli	R = -0.2 P = 0.82	R = 0.03 P = 0.79	R = 0.17 P = 0.16	R = 0.16 P = 0.07	R = 0.07 P = 0.44	R = 0.08 P = 0.41

8.10.1.2 Correlation of WCC with Z scores

Of the one hundred and seventy patients who had serial FBC testing, 120 also returned for post-operative NP testing and thus had Z scores. The following Table 8.38 shows that there were weak but significant inverse correlations between both pre-operative and post-operative measures of white cell count and total Z scores as well as various individual test Z scores.

Table 8.38 Spearman correlation coefficients between WCC and Z scores.

	WCC1 Pre-op	WCC2 Pre-CPB	WCC3 On-CPB	WCC4 Post-CPB	WCC5 Day 1	WCC6 Day 2
CRTT	R = -0.13 P = 0.12	R = -0.08 P = 0.36	R = -0.06 P = 0.49	R = -0.06 P = 0.49	R = -0.11 P = 0.20	R = -0.09 P = 0.29
GPD	R = -0.08 P = 0.31	R = -0.02 P = 0.79	R = -0.07 P = 0.41	R = -0.07 P = 0.39	R = -0.06 P = 0.15	R = -0.03 P = 0.77
GPND	**R = -0.19** **P = 0.02**	R = -0.12 P = 0.16	R = -0.11 P = 0.21	**R = -0.23** **P < 0.01**	R = -0.10 P = 0.22	R = -0.06 P = 0.49
LCT	**R = -0.19** **P = 0.02**	R = -0.02 P = 0.87	R = 0.08 P = 0.34	R = 0.03 P = 0.69	R = -0.06 P = 0.46	R = -0.02 P = 0.82
NVMT	R = -0.12 P = 0.14	R = -0.11 P = 0.20	R = -0.03 P = 0.77	**R = -0.17** **P = 0.04**	R = -0.04 P = 0.64	R = -0.13 P = 0.12
REY	R = 0.08 P = 0.33	R = 0.00 P = 0.98	R = 0.08 P = 0.37	R = -0.03 P = 0.77	**R = -0.17** **P = 0.04**	R = -0.07 P = 0.42
SDRT	R = -0.13 P = 0.11	R = -0.13 P = 0.12	R = -0.05 P = 0.58	R = -0.15 P = 0.17	R = -0.13 P = 0.12	R = 0.04 P = 0.68
TMTA	R = -0.09 P = 0.28	R = -0.02 P = 0.81	R = -0.10 P = 0.23	**R = -0.20** **P = 0.02**	R = -0.05 P = 0.56	R = 0.00 P = 0.96
TMTB	R = -0.03 P = 0.76	R = -0.12 P = 0.16	R = 0.05 P = 0.59	R = -0.08 P = 0.38	R = -0.05 P = 0.59	R = 0.04 P = 0.64
TOTAL	**R = -0.19** **P = 0.02**	R = -0.12 P = 0.15	R = -0.05 P = 0.15	**R = -0.21** **P < 0.01**	R = -0.15 P = 0.28	R = -0.06 P = 0.18

8.10.2 Neutrophil Count

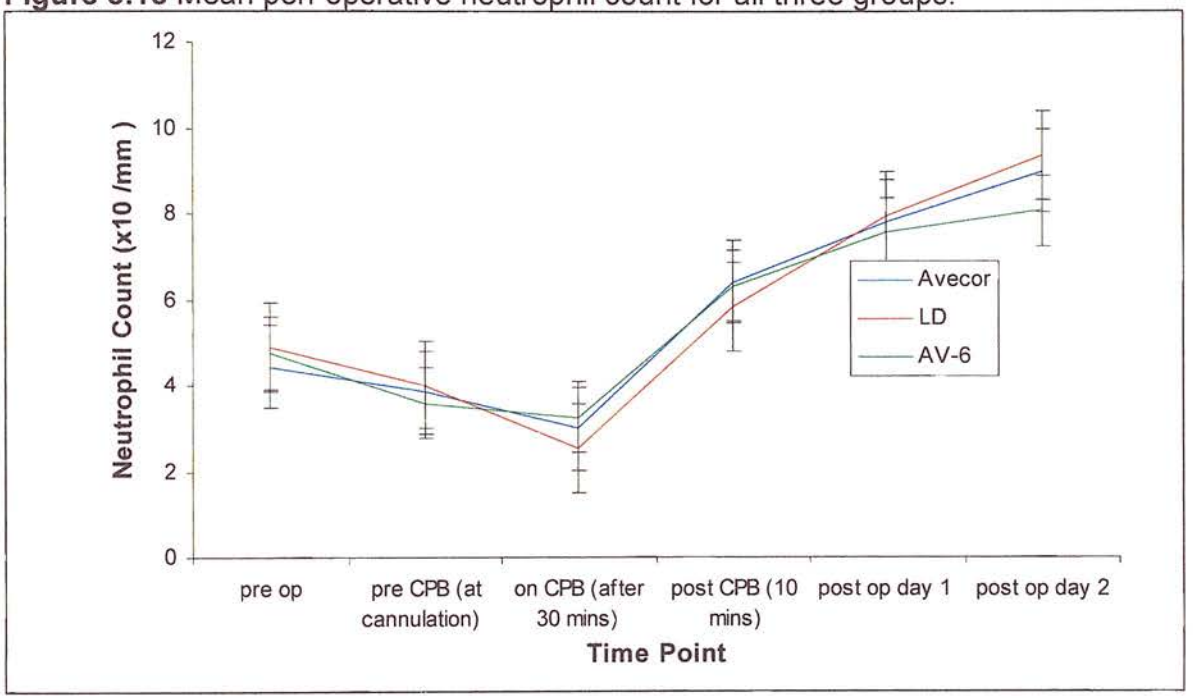
The pattern of a fall and then rise in total white cell count is mainly attributable to the similar fall and rise in neutrophil count (Table 8.39)

Table 8.39 Mean peri-operative neutrophil count for all three groups.

	Mean Neutrophil count (SD) (x 10^3 /mm^3)		
Time	**LD (n=78)**	**Avecor (n=63)**	**AV-6 (n=29)**
1 pre op	4.89 (1.44)	4.43 (1.49)	4.75 (1.19)
2 pre CPB (at cannulation)	4.02 (1.38)	3.85 (1.60)	3.60 (1.07)
3 on CPB (after 30 mins)	2.54 (1.46)	2.99 (1.62)	3.26 (1.39)
4 post CPB (10 mins)	5.82 (2.62)	6.42 (3.48)	6.32 (2.51)
5 post op day 1	7.95 (2.47)	7.81 (2.49)	7.56 (1.82)
6 post op day 2	9.35 (3.27)	9.01 (2.78)	8.08 (2.14)

Using repeated measures ANOVA there were significant differences over time but not between groups at any time points.

Figure 8.18 Mean peri-operative neutrophil count for all three groups.



8.10.2.1 Correlation of Neutrophil Count with Microemboli

Of the one hundred and seventy patients who had serial FBC testing, 120 also had intra-operative TCD monitoring of microemboli. The following Table 8.40 shows that there was a weak but significant correlation between the neutrophil count 10 minutes after CPB and the intra-operative microemboli count.

Table 8.40 Spearman correlation coefficients between neutrophil count and microemboli.

	N1 Pre-op	N2 Pre-CPB	N3 On-CPB	N4 Post-CPB	N5 Day 1	N6 Day 2
Microemboli	R = -0.08 P = 0.31	R = -0.10 P = 0.29	R = 0.13 P = 0.16	**R = 0.23** **P = 0.01**	R = 0.01 P = 0.89	R = 0.05 P = 0.61

8.10.2.2 Correlation of Neutrophil Count with Neuropsychological Z scores

Of the one hundred and seventy patients who had serial FBC testing, 120 also returned for post-operative NP testing and thus had Z scores. The following Table 8.41 shows that there were weak but significant inverse correlations between the neutrophil count 10 minutes after CPB and the total and grooved pegboard (non dominant), non verbal memory test, and the trailmaking test A Z scores.

Table 8.41 Spearman correlation coefficients between neutrophil count and Z scores.

	N1 Pre-op	N2 Pre-CPB	N3 On-CPB	N4 Post-CPB	N5 Day 1	N6 Day 2
CRTT	R = -0.07 P = 0.42	R = -0.09 P = 0.33	R = -0.04 P = 0.68	R = -0.02 P = 0.84	R = -0.09 P = 0.29	R = -0.07 P = 0.37
GPD	R = -0.07 P = 0.38	R = 0.06 P = 0.48	R = -0.03 P = 0.76	R = -0.07 P = 0.42	R = -0.04 P = 0.62	R = -0.01 P = 0.91
GPND	**R = -0.23** **P = <0.01**	R = -0.11 P = 0.23	R = -0.11 P = 0.23	**R = -0.20** **P = 0.02**	R = -0.11 P = 0.18	R = -0.08 P = 0.36
LCT	R = -0.09 P = 0.26	R = 0.03 P = 0.78	R = 0.12 P = 0.15	R = 0.04 P = 0.64	R = -0.06 P = 0.46	R = -0.02 P = 0.65
NVMT	R = -0.09 P = 0.26	R = -0.09 P = 0.28	R = -0.02 P = 0.79	**R = -0.18** **P = 0.04**	R = -0.04 P = 0.63	R = -0.13 P = 0.14
REY	R = 0.11 P = 0.19	R = 0.03 P = 0.72	R = 0.05 P = 0.54	R = -0.01 P = 0.17	R = -0.12 P = 0.19	R = 0.05 P = 0.14
SDRT	R = -0.09 P = 0.31	R = -0.04 P = 0.34	R = -0.02 P = 0.32	R = -0.16 P = 0.55	R = -0.13 P = 0.13	R = 0.05 P = 0.54
TMTA	R = -0.09 P = 0.27	R = -0.01 P = 0.87	R = -0.13 P = 0.13	**R = -0.17** **P = 0.04**	R = -0.05 P = 0.52	R = 0.00 P = 0.99
TMTB	R = 0.09 P = 0.23	R = -0.01 P = 0.91	R = 0.11 P = 0.19	R = -0.05 P = 0.54	R = -0.01 P = 0.88	R = 0.07 P = 0.39
TOTAL	R = -0.12 P = 0.15	R = -0.03 P = 0.73	R = -0.03 P = 0.75	**R = -0.18** **P = 0.04**	R = -0.12 P = 0.15	R = -0.03 P = 0.70

8.10.3 Lymphocyte Count

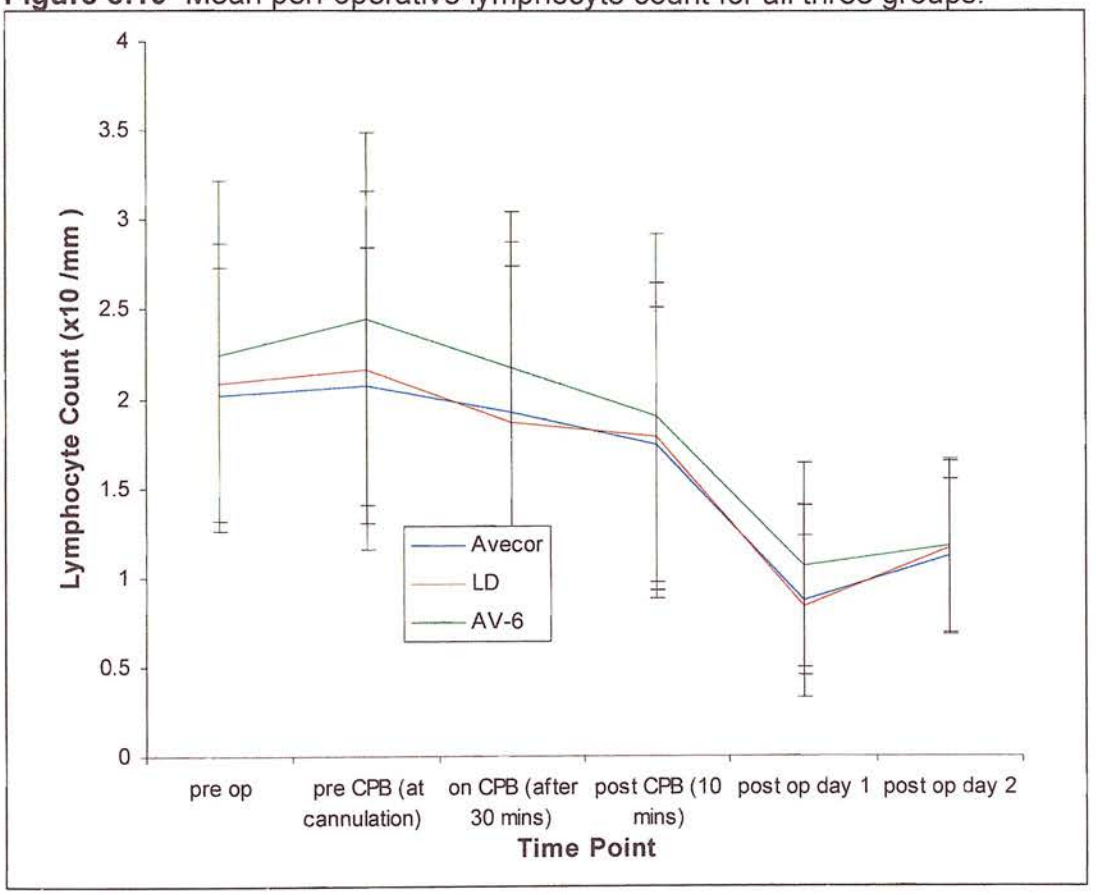
Lymphocytes in all three groups began to fall during CPB and reached a low on post operative day one. There were no significant differences between the groups at any time point.

Table 8.42 Mean peri-operative lymphocyte count for all three groups.

	Mean Lymphocyte count (SD) (x 10^3 /mm^3)		
Time	**LD (n=78)**	**Avecor (n=63)**	**AV-6 (n=29)**
1 pre op	2.09 (0.78)	2.02 (0.71)	2.24 (0.98)
2 pre CPB (at cannulation)	2.16 (1.00)	2.07 (0.77)	2.45 (1.04)
3 on CPB (after 30 mins)	1.87(1.01)	1.93 (0.81)	2.17 (0.88)
4 post CPB (10 mins)	1.79 (0.86)	1.74 (0.77)	1.90 (1.02)
5 post op day 1	0.84 (0.39)	0.87 (0.54)	1.07 (0.57)
6 post op day 2	1.17 (0.49)	1.12 (0.43)	1.18 (0.49)

Using repeated measures ANOVA there were significant differences over time but not between groups at any time points.

Figure 8.19 Mean peri-operative lymphocyte count for all three groups.



8.10.4 Monocyte Count

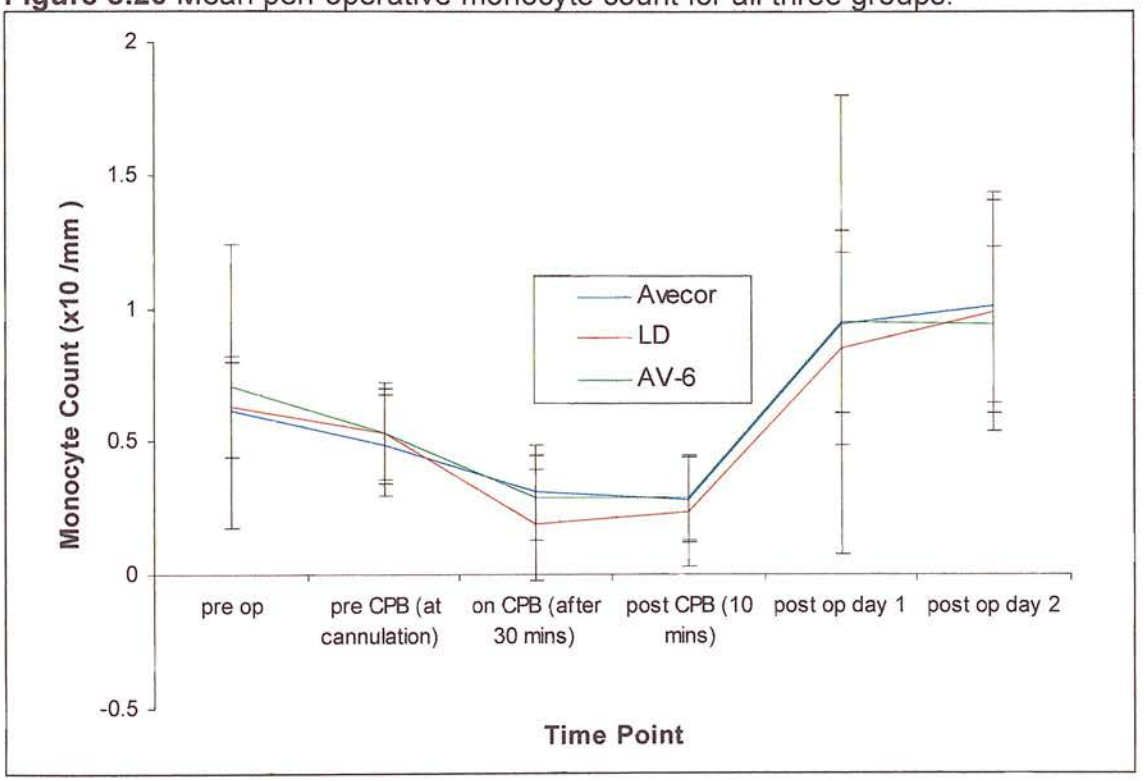
Monocyte count fell sharply in all groups while on cardiopulmonary bypass and then rose to higher than pre-operative levels by post-operative day one.

Table 8.43 Mean peri-operative monocyte count for all three groups.

	Mean Monocyte count (SD) (x 10^3 /mm^3)		
Time	**LD (n=78)**	**Avecor (n=63)**	**AV-6 (n=29)**
1 pre op	0.63 (0.19)	0.62 (0.18)	0.71 (0.53)
2 pre CPB (at cannulation)	0.53 (0.19)	0.49 (0.19)	0.53 (0.17)
3 on CPB (after 30 mins)	0.19 (0.21)	0.31 (0.18)	0.29 (0.16)
4 post CPB (10 mins)	0.24 (0.21)	0.28 (0.16)	0.29 (0.16)
5 post op day 1	0.85 (0.36)	0.94 (0.86)	0.95 (0.34)
6 post op day 2	0.99 (0.45)	1.01 (0.41)	0.94 (0.29)

Using repeated measures ANOVA there were no significant differences over time or between groups.

Figure 8.20 Mean peri-operative monocyte count for all three groups.



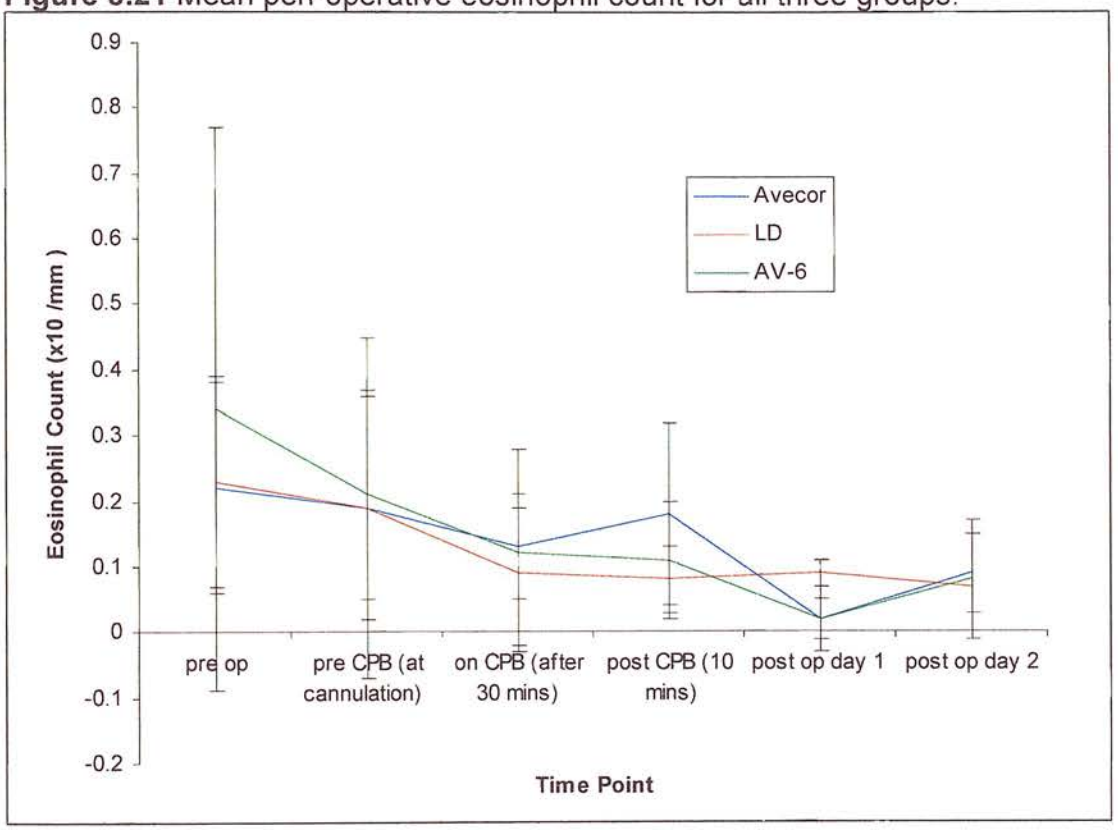
8.10.5 Eosinophil Count

Table 8.44 Mean peri-operative eosinophil count for all three groups.

	Mean Eosinophil count (SD) (x 10^3 /mm^3)		
Time	**LD (n=78)**	**Avecor (n=63)**	**AV-6 (n=29)**
1 pre op	0.23 (0.16)	0.22 (0.16)	0.34 (0.43)
2 pre CPB (at cannulation)	0.19 (0.17)	0.19 (0.26)	0.21 (0.16)
3 on CPB (after 30 mins)	0.09 (0.12)	0.13 (0.15)	0.12 (0.07)
4 post CPB (10 mins)	0.08 (0.05)	0.18 (0.14)	0.11 (0.09)
5 post op day 1	0.09 (0.02)	0.02 (0.03)	0.02 (0.05)
6 post op day 2	0.07 (0.08)	0.09 (0.06)	0.08 (0.09)

Using repeated measures ANOVA there were no significant differences over time or between groups.

Figure 8.21 Mean peri-operative eosinophil count for all three groups.



8.10.6 Platelet Count

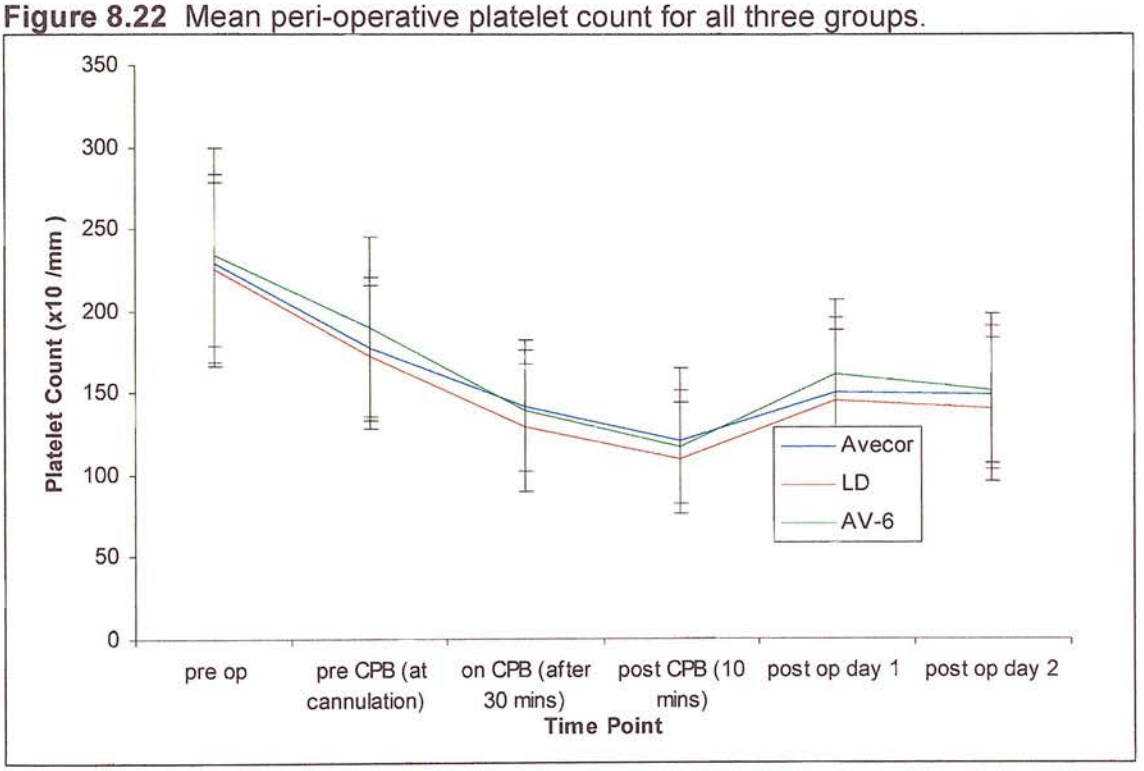
The platelet count decreased steadily from pre-operative levels to a trough after CPB in all three filter groups. There was then a slight increase post-operatively but this did not reach pre-operative levels.

Table 8.45 Mean peri-operative platelet count for all three groups.

	Mean Platelet count (SD) (x 10^3 /mm^3)		
Time	**LD (n=78)**	**Avecor (n=63)**	**AV-6 (n=29)**
1 pre op	225 (59)	229 (50)	234(65)
2 pre CPB (at cannulation)	172 (44)	177 (44)	190 (55)
3 on CPB (after 30 mins)	129 (39)	142 (40)	139 (37)
4 post CPB (10 mins)	110 (34)	121 (44)	117 (34)
5 post op day 1	145 (44)	150 (46)	162 (45)
6 post op day 2	140 (44)	149 (42)	151 (48)

Using repeated measures ANOVA there were significant differences over time but not between groups at any time points.

Figure 8.22 Mean peri-operative platelet count for all three groups.



8.10.7 Haemoglobin

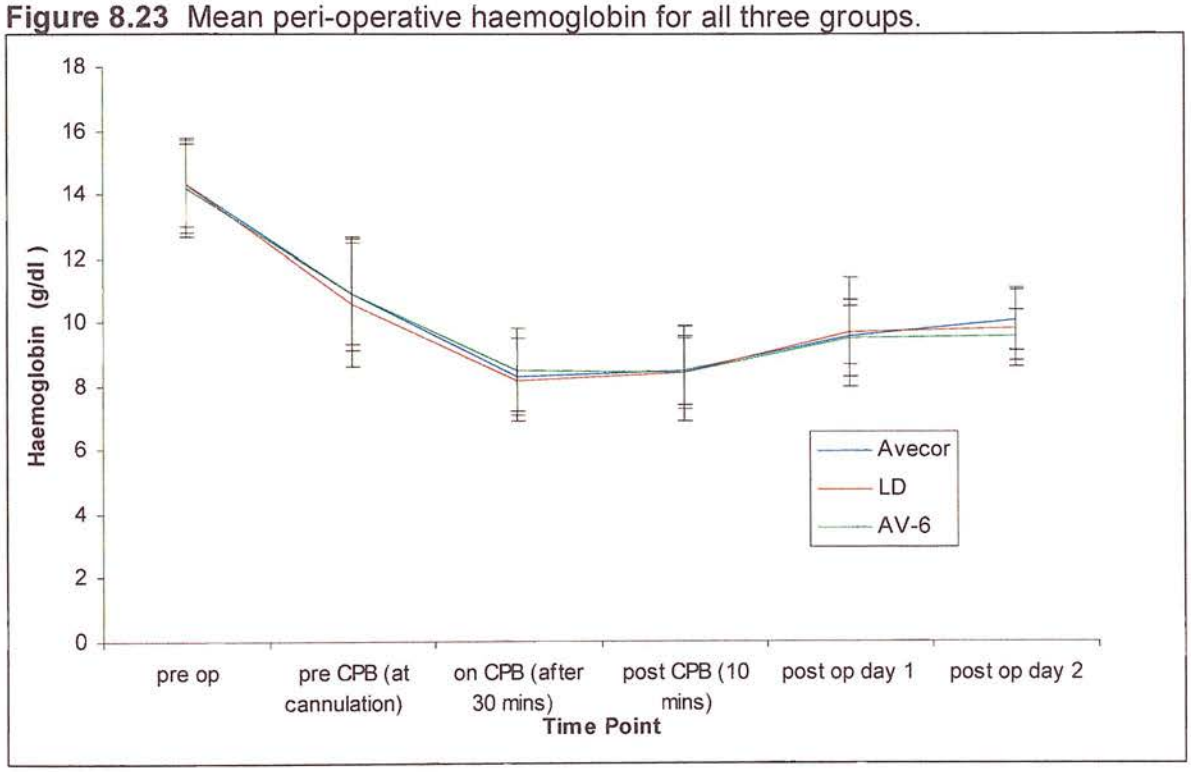
Haemoglobin decreased steadily from pre-operative levels to a trough after CPB in all three filter groups. There was then a slight increase post-operatively but this did not reach pre-operative levels. There was no statistical differences between groups at any time point.

Table 8.46 Mean peri-operative haemoglobin levels for all three groups.

	Mean Haemoglobin (SD) (g/dl)		
Time	**LD (n=78)**	**Avecor (n=63)**	**AV-6 (n=29)**
1 pre op	14.3 (1.5)	14.3 (1.3)	14.2 (1.5)
2 pre CPB (at cannulation)	10.6 (2.0)	10.9 (1.8)	10.9 (1.6)
3 on CPB (after 30 mins)	8.2 (1.3)	8.3 (1.2)	8.5 (1.3)
4 post CPB (10 mins)	8.4 (1.1)	8.5 (1.1)	8.4 (1.5)
5 post op day 1	9.7 (1.7)	9.6 (0.9)	9.5 (1.2)
6 post op day 2	9.8 (1.2)	10.1 (1.0)	9.6 (0.8)

Using repeated measures ANOVA there were significant differences over time but not between groups at any time points.

Figure 8.23 Mean peri-operative haemoglobin for all three groups.



8.10.8 Serum Elastase

Serum elastase was measured in the first 60 patients of the study and thus for the LD and Avecor groups only. Elastase rose in both groups on CPB and 10 minutes post CPB. However the rise was significantly greater in the LD group as shown in Table 8.47 and Figure 8.24. Elastase levels fell by post-operative day one but remained significantly elevated from pre-operative levels. There was no difference between the two groups by post-operative day one.

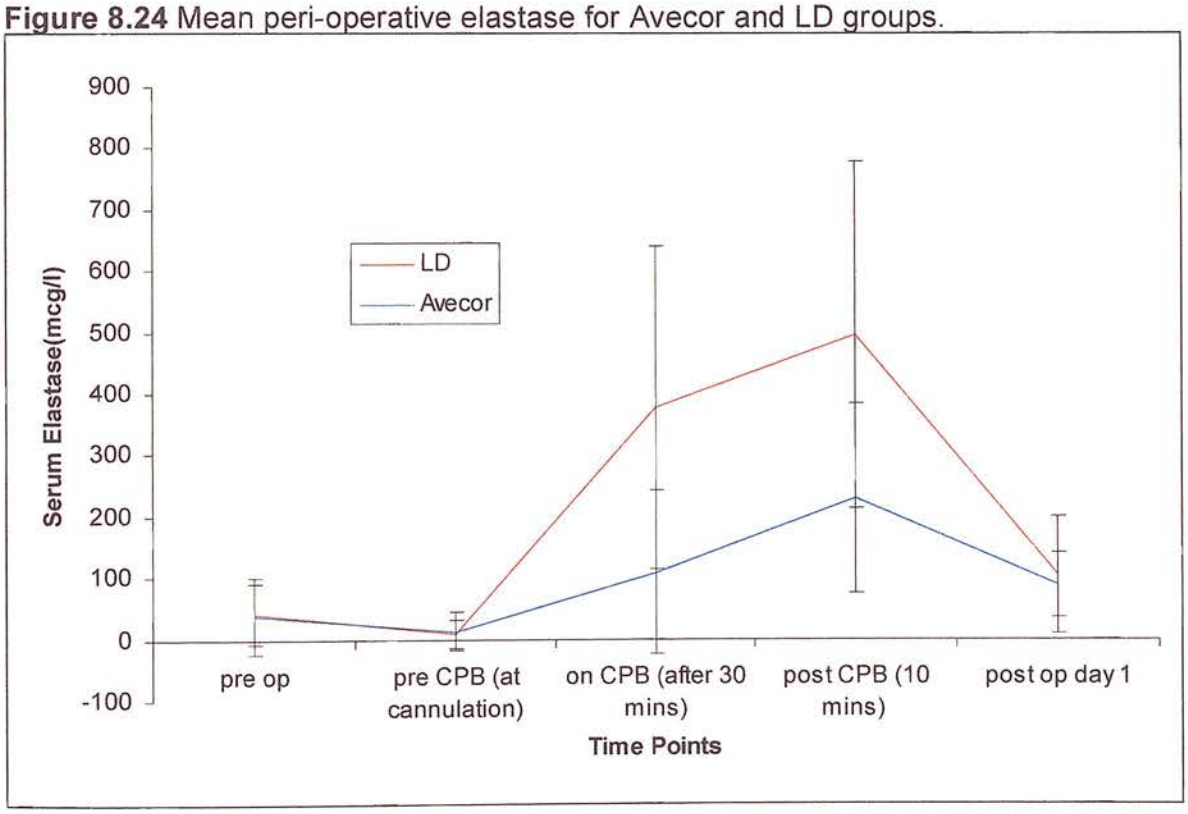
Table 8.47 Mean peri-operative serum elastase levels.

	Mean Elastase (SD) (mcg/l)		
Time	**LD (n=26)**	**Avecor (n=34)**	**p (ANOVA)**
1 pre op	40.5 (49.4)	37.4 (62.6)	0.57
2 pre CPB (at cannulation)	9.1 (23.6)	12.6 (31.1)	0.75
3 on CPB (after 30 mins)	376.8 (263.3)	108.3 (133.1)	0.002
4 post CPB (10 mins)	496.7 (283.2)	228.5 (154.8)	0.002
5 post op day 1	103.6 (95.6)	87.9 (53.8)	0.62

Using repeated measures ANOVA there were significant differences over time and between groups on CPB and 10 minutes after CPB.

There appears to be a difference between the pre-operative and pre-bypass serum elastase levels. This was not statistically significant ($p = 0.45$ for the LD group and $p = 0.62$ for the Avecor group).

Figure 8.24 Mean peri-operative elastase for Avecor and LD groups.



8.10.10 Serum S100b

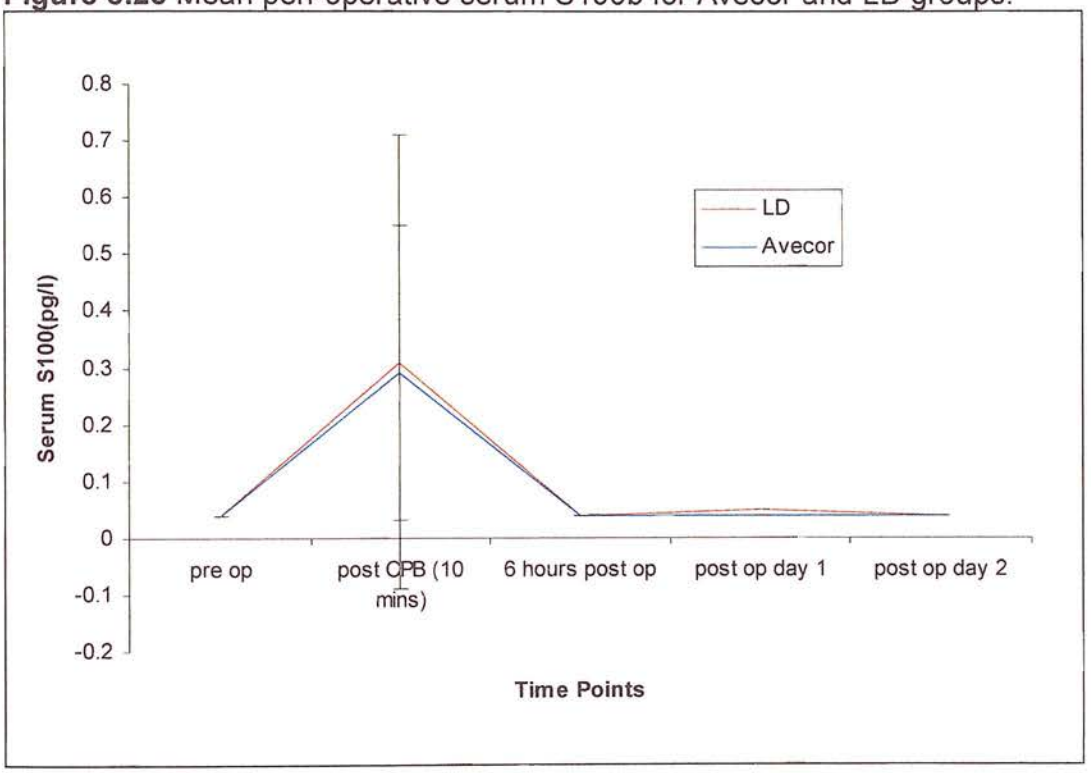
Serum S100b was measured in the first 60 patients of the study and thus for the LD and Avecor groups only. Serum S100b was elevated only at 10 minutes post bypass in both groups. There was no difference between LD and Avecor groups in the rise seen.

Table 8.48 Mean peri-operative serum S100b levels for Avecor and LD groups.

	Mean S100 (SD) (pg/l)	
Time	**LD (n=29)**	**Avecor (n=31)**
1 pre op	0.04 (0)	0.04 (0)
2 post CPB (10 mins)	0.31 (0.4)	0.29 (0.26)
3 6 hours post op	0.04 (0)	0.04 (0)
4 post op day 1	0.05 (0.01)	0.04 (0)
5 post op day 2	0.04 (0)	0.04 (0)

Using repeated measures ANOVA there were significant differences over time but not between groups at any time points.

Figure 8.25 Mean peri-operative serum S100b for Avecor and LD groups.



8.10.9.1 Correlation between Serum S100b and microemboli on bypass and NP outcome

Of the 60 patients who received S100b sampling, 43 also had intra-operative transcranial Doppler monitoring.

No correlation between serum S100b and microemboli on bypass was seen:

Table 8.49 Correlation (Spearman's) between Serum S100b and microemboli on bypass

	S100b (post CPB)
Microemboli	r = 0.08 p = 0.69

Of the 60 patients who received S100b sampling, 51 also returned for post-operative NP testing and thus had 6 week Z scores.

No correlation between Serum S100b and Z scores was seen:

Table 8.50 Correlation (Spearman's) between Serum S100b and Z scores at 6 weeks post surgery

	S100b (post CPB)
CRTT	r = 0.00 p = 0.99
GPD	r = 0.23 p = 0.14
GPND	r = 0.15 p = 0.34
LCT	r = -0.16 p = 0.30
NVMT	r = -0.12 p = 0.32
REY	r = 0.27 p = 0.08
SDRT	r = -0.22 p = 0.16
TMTA	r = 0.05 p = 0.75
TMTB	r = -0.05 p = 0.73
TOTAL	r = 0.12 p = 0.43

8.11 Summary of statistical tests used

The χ^2 test was used for all categorical data:
Tables 8.3, 8.4, 8.5, 8.6, 8.10

The Kolmagorov- Smirnoff test was used to test whether all continuous data was normally or not normally distributed.

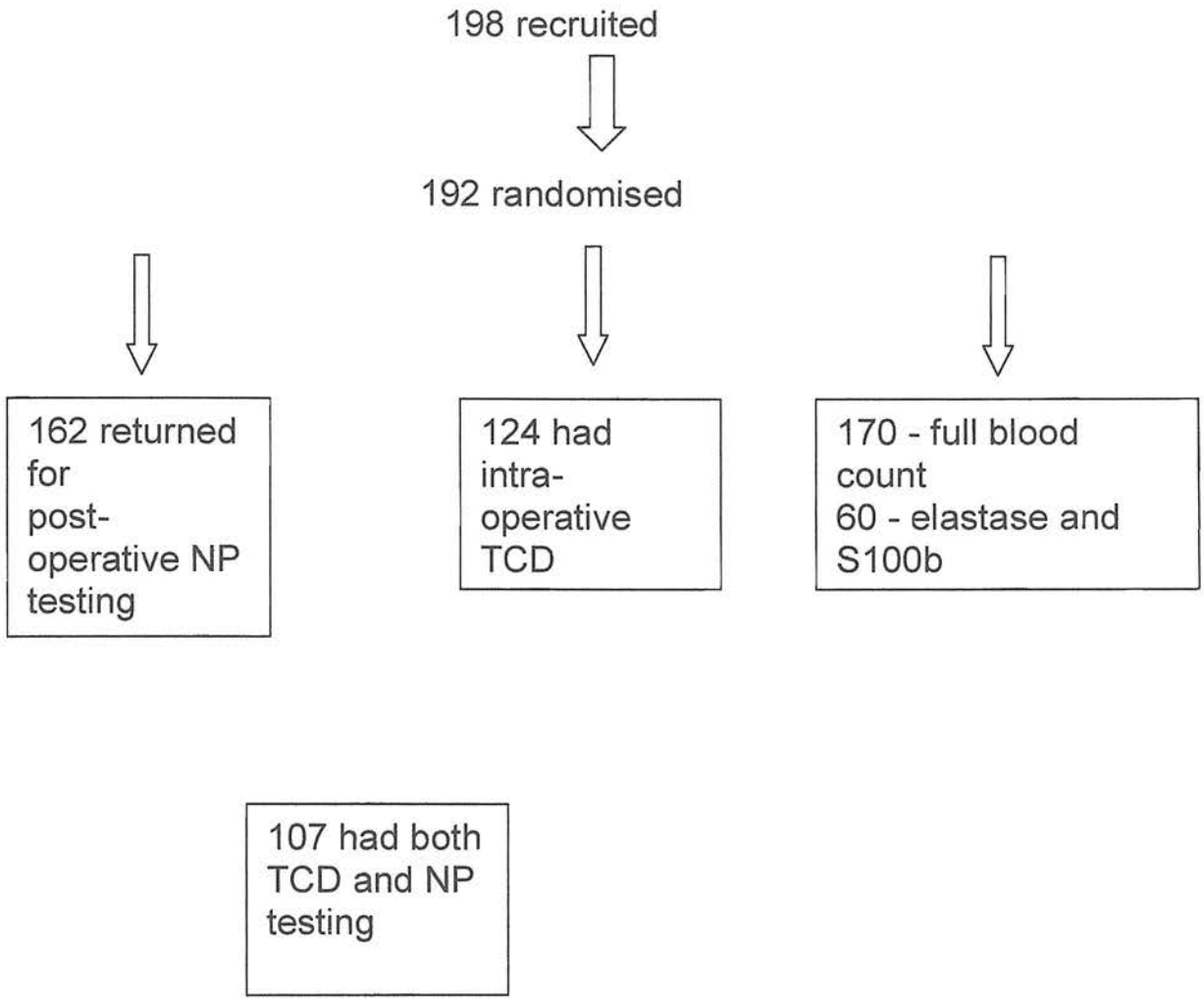
Normally distributed data was analysed using the t test to test for significant differences in means between groups at a single time point:
Tables 8.4, 8.6, 8.7, 8.8, 8.9, 8.11, 8.12, 8.22

Normally distributed data was analysed using repeated measures analysis of variance (ANOVA) to test for significant differences in means between groups at several time points:
Tables 8.13, 8.23, 8.24, 8.25, 8.26, 8.27, 8.28, 8.29, 8.30, 8.31, 8.36, 8.39, 8.42, 8.43, 8.44, 8.45, 8.46, 8.47, 8.48

Non-normally distributed data was analysed using the Mann Whitney U test:
Tables 8.11, 8.15, 8.18, 8.33, 8.35

Correlation between normally and non-normally distributed data was analysed using Spearman's correlation coefficient:
Tables 8.16, 8.17, 8.19, 8.20, 8.21, 8.37, 8.38, 8.40, 8.41, 8.49, 8.50

Fig 8.26 Summary of Number of Patients Receiving the Main Tests



Chapter 9

Discussion of Results

This study has shown that leucocyte depleting filters, when compared to standard arterial line filters, further reduce the number of microembolic events recorded by transcranial Doppler during CABG, and that neuropsychological test results suggest an accompanying degree of non statistically significant neuroprotection. The primary outcome measure of this study was neuropsychological performance at six weeks after surgery and this will be discussed first. The secondary outcomes such as the incidence of microemboli, serum S100b levels and their relationship to the neuropsychology results are then discussed individually in turn. Finally all the results are summarised and some conclusions are drawn.

9.1 Neuropsychological Outcome

It was decided at the outset of this study to analyse the neuropsychological data in two distinct ways: firstly to have a Z change score analysis as the primary NP outcome and to have conventional analysis of the incidence of deficit as the secondary outcome. Deficit was defined as a greater than 1 standard deviation deterioration in 2 or more tests.

The most important finding of this study is that both methods of analysis show a consistent trend towards improved post-operative NP outcome with the use of the LD filter (Fig 8.1). The difference however was not statistically significant ($p = 0.07$). Therefore, although standard arterial line filters have previously been shown by Pugsley et al[8] to reduce the incidence of neuropsychological deficit compared to using no filter at all, the use of a leucocyte depleting filter does not appear to offer significant additional benefit.

This study used two methods of NP analysis because deficit analysis, although previously widely used, is arbitrary and has a binary cut-off and is therefore less sensitive analysis than

Z change score[8;42;210]. Z change score analysis is now an accepted method of analysis[29;36;37;42;211] used by researchers in both Europe and the United States. Some recent studies have continued to use only incidence of deficit analysis[34;80;212-214]. By using both methods in this study it is possible to demonstrate the difference in sensitivity between the two methods as well as using the deficit incidence to compare the results of this study to others.

The incidence method showed minimal difference between the test and control groups in this study. However, when the Z change score was used further insight into the potential difference between test and control groups becomes apparent. In seven of the nine tests used there was a better improvement in the Z change score in the leucocyte depletion group (Fig 8.1). Also the difference in the total Z change score only just failed to reach statistical significance using the 1 tailed t test (Table 8.9). Therefore, although there was no statistically significant difference, the Z change score analysis suggests that there is a trend towards an improvement in NP outcome with the LD filters compared to controls. The fact that this trend extends across almost all the tests in the battery suggests that this is a real effect that has not reached significance due to statistical under powering, rather than a chance finding. Use of the Z change scores therefore reveals that most patients learn and improve between repeat assessments. The difference between groups can be interpreted as better preservation of learning in the LD group. Van Dijk et al[44] in their randomised controlled trial comparing NP outcome in patients having on-pump and off pump CABG also used both deficit and Z score analysis. Using a 20% decline in 20% (three of fifteen) of the NP tests used to define a deficit, Van Dijk et al found an incidence of deficit of 21% in the off-pump group and 29% in the on-pump group three months after surgery. This was not statistically significant using Fisher exact test. However the mean total Z change score was 0.19 in the off-pump group and 0.12 in the on-pump group, which was statistically significant using the Wilcoxon test.

The data of the present study therefore concur with the findings of Van Dijk et al in terms of comparing methods of analysis, as both studies show Z change score analysis to be more sensitive than deficit analysis.

The incidence of deficits was low in this study compared to most other studies. As discussed in chapter one there are many real (patient) factors and also artefactual (differences in study design and analysis) factors which influence measured NP outcome and could explain the difference between this and other studies. Also the standard interventions in place in this study (such as alpha stat acid base balance) in addition to the test intervention may have contributed to the low incidence. This is especially relevant when comparing this study to older studies such as that of Pugsley et al[8]. There are a number of possible explanations for the difference in result between this study and Pugsley et al's. Pugsley et al found that four of 49 filtered patients had deficits and 12 of 45 non-filtered patients had deficits. However the overall numbers of microemboli in Pugsley et al's study were much greater, probably as a result of the use of bubble oxygenators which produce more microemboli[48] and pH stat acid base control which may increase cerebral blood flow and so microemboli delivery[215]. Additionally, Pugsley et al's study was fundamentally different as it compared the use of filters to no filters rather than comparing different filters as the present study has done.

The incidences of 5.6% in the LD group and 7.8% in the control group six weeks post surgery in the present study (Table 8.10) were much lower than most recently reported incidences for example by Lund et al[118], Nathan et al[71], Zamvar et al[80] and Van Dijk et al[44] but similar to those reported by Kadoi et al[213]. It is of note that the study of Kadoi et al is the most recently published and so the most contemporaneous to the present study.

In a randomised controlled trial comparing off-pump and on-pump CABG, Lund et al[118]

found incidences of cognitive decline of 29% in the off pump group and 35% in the on-pump group 3 months after surgery. In terms of the demographics of the sample in Lund et al's study the mean ages in their two groups were 62 and 64, similar to the mean age of the sample in this study (64.1). The male to female ratio and operation times were also similar even though the mean number of grafts per patient was lower (2.3-2.5 in Lund et al vs. 2.8-2.9 in the present study). However, Lund et al included patients with diabetes or who had a history of previous stokes whereas such patients were excluded from the present study. Lund et al's study sample was therefore at a higher risk of poor neuropsychological outcome[216] and this could explain the higher incidence of deficits. There were a few minor differences in study design and analysis between the present study and Lund et al's. He used a comprehensive battery of 12 tests but followed up patients slightly later at 3 months rather than six weeks. Also deficit was defined by Lund et al as a reduction of more than 20% in two or more tests rather than one standard deviation, although this would not be expected to give rise to any major difference. Lund et al do not state in their methods whether they used arterial line filters, the type of acid base balance employed or the levels of intra-operative blood pressure. All three of these factors can affect NP outcome[8;62;83]. A lack of arterial line filters, pH stat balance and low intra-operative blood pressure could possibly all have contributed to the higher incidence of cognitive decline reported by Lund et al[118].

Nathan et al[71] found incidences of deficit of 62% and 48% at one week post surgery in patients randomly allocate to normothermia or hypothermia at the time of rewarming on CPB. As previously discussed, the consensus[14] recommends testing at three months once peri-operative issues have resolved. Patients in Nathan et al's study were slightly older, diabetics were included, and bypass and aortic cross clamp times were longer which might account for the higher incidences found. Also the method of NP analysis in which patients were defined as having a deficit if they had a decline from pre-operative testing of more than

0.5 standard deviation in one of three domain scores is likely to have classified a higher proportion of patients as having a deficit. Incidence of deficit is higher at one week after surgery with some recovery by 6 to 12 weeks[5;37]. Indeed, Nathan et al found some recovery by three months although details are not given[71].

Zamvar et al[80] found incidences of deficit of 27% in the off-pump group and 63% in the on-pump group one week after surgery and 10% in the off-pump group and 40% in the on-pump group at 10 weeks after surgery. Zamvar et al's sample had similar demographic features to the sample in the present study and patients with previous strokes were excluded. No mention is made of diabetes. Also, the definition of deficit in Zamvar et al's study is the same as the present study and so it is difficult to explain the high incidence (40%) of deficit at 10 weeks. Zamvar et al[80], like Lund et al[118] make no mention of neuroprotective techniques such as alpha stat acid base control and arterial line filters leaving their absence as a possible explanation. However, one would expect alpha stat and the use of arterial line filters to be standard. Interestingly, both Lund et al and Zamvar et al were investigating off-pump surgery and therefore were likely to be off-pump enthusiasts, which may explain their lack of reporting or actual use of CPB neuroprotective techniques. Conversely, most controlled trials in which both groups receive cardiopulmonary bypass are more likely to report the use of such techniques[43;213;217]. A positive point, which could partially explain Zamvar et al's relatively high incidence of deficit, is the 100% follow up rate achieved in his study. There would have been no selective attrition of those with worse outcome in Zamvar et al's study.

Although Van Dijk et al[44] did use neuroprotective techniques and a method of defining incidence of deficit comparable to the present study, they found incidences of NP deficit of 21% and 29%. The inclusion of patients with diabetes and a history of strokes may explain

the higher incidence in Van Dijk et al's study.

Comparing the present study to that of Kadoi et al[213] (also only recently completed) in which incidences of 6% and 7% were found it is apparent that the design and conduct of the two studies was very similar. Kadoi et al were examining the effect of two different anaesthetic agents used during CPB and so both study groups had CABG carried out using normothermic CPB. Arterial line filters and alpha stat pH regulation were used and perfusion pressure was maintained between 50 and 80mmHg. Patients with diabetes or a history of stroke were excluded. A battery of six NP tests was used and a deficit was defined as deterioration by more than one SD at six months. Therefore taking Kadoi et al's results in addition to the results of this study it would seem that a low incidence of deficits using the method of the present study could be expected when the study sample is relatively fit and healthy and neuroprotective regimes employed.

The incidence of NP deficits found post-operatively may be influenced by the follow-up rate of the study in question. It has previously been found that those patients who perform less well on the pre-operative tests are less likely to attend for post-operative follow-up[3]. Therefore unless 100% NP follow-up is achieved there remains the possibility that selective attrition leads to an underestimate of the deficit rate in the study sample. Relative to the range of follow up rates reported in the literature the follow up rate of 84% found in this study is unexceptional. Table 9.1 gives some examples of follow up rates reported from prospective randomised interventional studies similar to the present study carried out in the last 10 years. Many of the patients who took part in this study (approximately 50%) were not local residents and had to travel distances of 50 to 80 miles from their home to attend the follow-up clinic. Although bringing patients back to their tertiary referral centre for follow-up was the normal practice of the study institution, the issue of travel may have influenced

the decision regarding those patients who were unwilling to return or were deemed too unwell to return.

Table 9.1 Follow up rates.

Author	Year	n (total)	Age	Weeks post-op	Follow up rate
Pugsley et al[8]	1994	105	55	8	**95**
Murkin et al[61]	1995	316	61	8	**85**
Patel et al[62]	1996	70	57	6	**100***
Mora et al[68]	1996	99	63	6	**87**
Regragui et al[69]	1996	96	59	6	**83**
Heyer et al[218]	1997	99	64	6	**26**
Arrowsmith et al[42]	1998	171	59	8	**93**
Roach et al[56]	1999	225	63	8	**NR**
Grimm et al[70]	2000	144	62	12	**NR**
Nathan et al[71]	2001	221	68	12	**NR**
Van Dijk et al[44]	2002	281	61	12	**88**
Kong et al[43]	2002	245	64	6	**89**
Zamvar et al[80]	2002	60	63	10	**100**
Lund et al[118]	2003	52	63	12	**NR**
Taggart et al[217]	2003	150	62	12	**93**
Kadoi et al[213]	2003	180	65	24	**84**

* 2 patients died peri-operatively and 2 extra patients were recruited to replace them, NR = not reported

Although not all studies have reported follow up rates those which do, do not analyse for peri-operative differences between those patients who did or did not return for follow up. In order to determine whether there might be some selective difference the peri-operative variables of these two groups of patients were analysed in this study. It was found that there were no differences in demographic data between the patients who did and did not return but that those who did not return had a greater number of microemboli on bypass (Table 8.11). However Table 8.12 shows that those who did not return had also performed significantly less well on two out of the nine pre-operative NP tests. There is evidence to suggest that poor pre-operative NP performance predicts a poor post-operative outcome[41]. The pre-operative differences in this study may mean that those who did not return were suffering more neuropsychological impairment than those who did. The incidences of 5.6% and 7.8% found in this study may therefore reflect less than the true incidence of deficit.

Initial power calculations for the present study were based on Z change scores from the previous study conducted at this institution. Therefore despite conservative statistical planning, it is possible that the trial ended up being underpowered. A power calculation based on the present study's findings and those of Kadoi et al for detecting a 20% reduction in incidence of 8% with 90% power at the 5% level, shows that approximately 3000 subjects would be needed. This would be a considerable undertaking. Kadoi et al recruited 180 patients – a similar number to the present study.

In summary the present study, using the two methods of NP analysis discussed, has found a low incidence of NP deficits in comparison with most reported studies. Although the follow up rate at six weeks in this study was in accordance with other studies, the loss to NP follow up of 16% of patients may through selective attrition have led to an underestimation of the true incidence of deficit. The follow up rate was the same in test and control groups (Tble 8.3). The low incidence of deficits found may have led to under powering of the present study. The Z change score analysis did

not show a statistically significant difference between the leucocyte-depleted and control groups but suggests a real difference between the groups.

9.2 Transcranial Doppler Data – Microemboli and Middle Cerebral Artery Blood Velocity

This study has shown that leucocyte-depleting filters reduce the number of microembolic events recorded by transcranial Doppler during CABG compared to standard arterial line filters. This remains true whether the control arterial line filter is auto-venting or has a vent line (Table 8.14). Although this study was originally designed to compare only two different types of arterial line filter (the leucocyte depleting and auto-venting LG-6 and the conventional 40μm Avecor), the inclusion of the Pall AV-6, which is conventional and also auto-venting as an additional control, allows comparison between the differential effects of auto-venting and leucocyte depletion. There is no difference in microemboli count between the two conventional filter groups (Table 8.14) indicating that auto-venting itself has no direct effect on microemboli count. This indicates that the reduction in microemboli in the LD group is due to the leucocyte-depleting element of the LG-6 filter rather than its auto-venting properties. Therefore, although no definite neuropsychological benefit has been proven in this study, LD filters do seem to be better than standard filters in terms of reducing "microembolic load".

Henrikson et al have shown that 20 and 40 μm arterial line filters reduce cerebral blood flow measured using xenon 133[159]. In the present study the cerebral artery blood velocity has been shown to be the same in all three groups (Figure 8.2) suggesting that a reduction in cerebral blood flow is not a mechanism by which these filters influence microemboli delivery.

This study has not been able to determine how the leucocyte-depleting element achieves a reduction in the number of microemboli. Speculation on the mechanism of microemboli reduction is hampered because current technology cannot determine whether the LD filters

are reducing gaseous or particulate microemboli or both. It is unlikely that the LD filter is able to remove more gaseous microemboli as it is the screen filter, identical in both types of filter, which is designed to remove bubbles (as well as particles). The unwoven mesh of the LD filters is designed to adhere to leucocytes but it is possible that bubbles passing through the screen are somehow trapped downstream by the leucocyte depleting mesh.

It would not be possible for any type of arterial line filter to reduce microemboli of atheromatous origin (although an intra-aortic filter could). These microemboli would arise downstream of the filter from the arterial walls and pass directly to the brain. If the LD filter is removing more particulate microemboli it may be simple physical adherence of leucocyte and other cellular aggregates to the non-woven mesh of the filter that is occurring. Alternatively, by filtering out the activated leucocytes from the circulation the number of leucocyte, platelet and fibrin aggregates, which form elsewhere[138], may actually be reduced. This is speculative and further study would be needed to investigate these potential mechanisms.

Lipid microemboli which derive mainly from cardiotomy suction blood[90] have been found in autopsy specimens of the brain[219]. However, Kincaid et al[91] in a study performed on dogs found that although using a cell saver rather than cardiotomy suction reduced lipid microemboli, the use of standard arterial line filter (25μm or 40μm) or a leucocyte depleting filter (40μm) did not reduce lipid microemboli as determined by the number of small capillary and arterial dilatations (SCADs) found on cross section of the dogs' brains. More recently a study in humans by Kaza et al[92] has found that a 21μm filter was able to remove all lipid emboli detectable by red O stain in the blood. The conflicting results may be explained by the different methods of detecting microemboli both of which are different again from the method used in the present study. It remains possible that the LD filters in this study are somehow reducing lipid microemboli. Any role of the LD filters in reducing the inflammatory response to surgery will be discussed later.

This study has found a low overall number of microemboli detected in all groups (Figures 8.3 and 8.4, Tables 8.14 and 8.15). Although there are three outliers in the Avecor and AV-6 groups which had between 600 and 900, microemboli the majority (124/127 = 98%) of patients had less than 250 microemboli and the median microemboli counts ranged from 15 (LG-6 group) to 67 (AV6 group). To put the microemboli counts in this study in context of other recent studies Fearn et al[115] have found 57% of patients had more than 200 microemboli during CPB, Taylor et al[220] found a median of 132 and Bowles et al[117] reported means of 1,766 +/- 2,455. The following Table 9.2 shows some recent studies which have measured microemboli.

Table 9.2 Studies measuring microemboli

Author	Year	N	Age	CPB Time	Arterial Filters	PH Control	Doppler Equipment	Total Microemboli Count	NP Correlation
Arrowsmith[42]	1998	171	59	81	No	alpha	2 MHz	**146** (med) **198** (med)	No
Taylor[220]	1999	18	64	85	32μm	?	2MHz (DWL)	**132**	ND
Fearn[221]	2001	70	60	73	40μm	alpha	2MHz	**>200 in 57%**	Yes
Neville[23]	2001	193 73	61 59	116 145	? ?	? ?	5MHz	**105** (med) **479** (med)	No
Bowles[117]	2001	20 20	65 66	-	- 40μm	- ?	Nicolet (? Freq)	**27** **1766**	ND
Mullges[108]	2003	30 30	59 61	138 114	40μm 40μm	alpha alpha	2MHz 2MHz	**413** (med) **175** (med)	No
Plochl[64]	2001	30 31	61 63	117 110	40μm 40μm	alpha alpha	2MHz 2MHz	**80*** (med) **96*** (med)	ND
Lund[118]	2003	29 23	62 64	-	- ?	- ?	DWL (? freq)	**16.3** (med) **90** (med)	No

ND = not analysed, ? = not stated, - = not relevant, * these counts were for 15 minutes after cross clamp release only, med = median

It is virtually impossible to compare microemboli counts between different studies in a meaningful way because of the various techniques used to measure them. As Table 9.2 shows, the probe frequency of Doppler used varies and is not always reported. It is rarer still for the more precise settings of the Doppler probe to be reported. Although there are

consensus criteria for defining a microembolus on the Doppler trace there may be inter-observer variability in identifying microemboli between studies.

The use of filters in all the patients of the present study may have contributed to the low microemboli counts. However the studies of Mullges et al[108], Taylor et al[220], Fearn et al[221] and Bowles et al[117] shown in the above table also all used 32μm to 44μm filters yet found far higher microemboli counts. Additional factors other than filters must therefore be contributing to the low microemboli counts in this study. The relatively short bypass and cross clamp times in the present study (Table 8.6) may have decreased microemboli counts. Other studies often report far longer bypass and cross clamp times[64;108]. The use of cross clamp fibrillation (CCF) is a relatively unusual feature of this study. Although CCF is a common practice in the UK, most studies in the literature have used cardioplegic myocardial protection techniques which tend to be associated with longer bypass and cross clamp times. Although repeated cross clamping can theoretically increase microemboli production there is no reliable evidence that intermittent cross clamp fibrillation produces more microemboli than cardioplegic techniques. Indeed, it is possible that since intermittent cross clamping tends to result in shorter cross clamp and bypass times, total microemboli production could be less than with cardioplegia. The two methods have only ever been compared in one randomised study in terms of microemboli production or neuropsychological outcome. This study reported no difference but was underpowered[64;222]. An interesting future study would be to compare the effects of CCF and cardioplegia on microemboli production and NP outcome, and this is planned.

Whatever the cause of the low overall numbers of microemboli, the effects of individual filters on microemboli counts in our study must be seen in the context of a low baseline count. In this study no correlation was found between age, cross clamp or bypass times and

microemboli counts (Table 8.17). Other investigators have found such correlations[19;64;223] and it is possible that these correlations only become apparent when bypass and cross clamp times are longer.

Most investigators using TCD report microemboli data but not cerebral blood velocity. One exception was Benaroia et al[224] who found no effect of cerebral blood velocity or type of arterial cannula used on the number of microemboli detected. This finding was confirmed in the present study with no correlation between cerebral blood velocity at any time point and microemboli count (Table 8.16).

Although TCD is the best current method for assessing intra-operative microemboli it also has a number of limitations. Only about 90% of people have an adequate window in the temporal bone through which to isonate the middle cerebral artery. This anatomical fact and technical problems (as explained in the results chapter, the video recorder failed to record a picture for the first 35 patients) with trace recording meant that not all (127/192 = 66%) patients in the present study received TCD monitoring. The right middle cerebral artery is only one of six arteries supplying the circle of Willis leading to the question of whether bilateral or unilateral middle cerebral artery detection should be used. Some investigators argue that a combined bilateral count is more accurate as it is closer to the total number of microemboli. However even this is likely to only be a proportionate estimate unless all arteries supplying the brain are monitored (not a realistic proposition). Moser et al[106] examined unilateral and bilateral monitoring in 29 patients and found only a 4% error between sides. This suggests that it does not matter whether the right or left middle cerebral artery is used for monitoring. Mullges et al[225], on the other hand, found a significant difference in microemboli counts between sides with either right or left having more microemboli varying for each individual patient. Wijman et al[107] have shown that cerebral

microembolism occurs more frequently in the middle as compared to the anterior cerebral artery when TCD is used to detect microemboli. Other investigators use Doppler of the common carotid artery[23] but this may detect microemboli which are destined for the extra cerebral circulation. Another limitation of TCD is its current inability to distinguish between gaseous and particulate microemboli, let alone the many potential types of particulate microemboli. Additionally the size of each embolus detected by TCD is not known. Size and substance of microemboli are likely to have considerable impact on their pathological effect and so a simple "microemboli count" as is currently available must be accepted as a fairly crude measure. New technology offers the possibility of identifying size and nature of microemboli and a future study should have the potential to determine which factors carry pathogenic influence.

As previously discussed, the relationship between microemboli and neuropsychological outcome is complex. In this study the highly significant difference in microemboli counts between LD and non-LD filter groups did not lead to a significant difference in neuropsychological outcome. If a causative link between microemboli and cognitive deficits does exist, the low numbers of microemboli in all groups in our study may have masked this link (as well as leading to the low incidence of neuropsychological deficits in both groups). It is possible that the difference in microemboli explains the trend towards improved cognitive function in the LD group but it is equally possible that other factors such as inflammatory changes or alterations of brain perfusion are also important. In this study there was no correlation between microemboli count on bypass and Z change scores (Table 8.19). However when the study population was divided using a median split into those with more or less than 34 microemboli on bypass, there was a significantly higher ("better") total Z change score in the group with less than 34 microemboli (Figure 8.5). Although such a mean split was made arbitrarily the finding would suggest that microemboli do have an involvement in

cognitive decline. A recent study of 107 patients followed up at UCLH to five years after surgery has shown that the number of microemboli recorded during surgery predicts NP outcome at five years[37]. This study therefore may add weight to the theory that microemboli cause cerebral injury. However, as the authors of that study point out[37], the number of microemboli may be a simple reflection of the cardiovascular disease process which itself contributes to NP decline over the study period. It is interesting to note that in the present study the patients who did not return for follow up testing (including those who had died) had a significantly higher number of intra-operative microemboli (Table 8.11). It is therefore possible that those who were unable to return had a worse NP outcome due to microembolism but because they did not return the relationship between NP outcome and microemboli was not revealed.

Given the limitations of microemboli detection it is unsurprising to find no consensus in the literature regarding an association between microemboli and NP outcome. The crude microemboli counts used may not be representative of the true embolic load to the brain and the potential variation in size and substance and therefore pathological effect of the microemboli in each study probably accounts for the inconsistency of the relationship found between microemboli and NP outcome.

9.3 Haematological Measures

9.3.1 White Cells

In the present study leucocyte-depleting filters were not found to reduce the total white cell count (WCC) (Table 8.36) and the neutrophil count (Table 8.39) after 30 minutes on bypass or at subsequent time points. As far as the author is aware, this study is the largest to have measured these cells serially in a comparison of an LD filter to a standard arterial line filter. There has previously been conflicting data in the literature as to whether LD filters are able to cause a significant reduction in circulating white cells during cardiopulmonary bypass.

Thurlow in 1995 used a simulated *in vitro* cardiopulmonary bypass circuit and showed a 45% reduction in total white cells and a 70% reduction in neutrophils using a LG-6 filter compared to an AV6[178]. However a subsequent randomised study in 14 patients having CABG surgery again using a LG-6 filter and an AV6 found no intra-operative reduction in neutrophils[157]. Palanzo, in a randomised trial of 36 patients compared the LG-6 to a standard filter and found that the LG-6 significantly reduced the WCC immediately after, and at four and 24 hours post CPB[167]. Palanzo did not sample intra-operatively or report neutrophil counts. Mihaljevic[161], Lust[156] and Hurst[154] in clinical studies of 32, 50 and 24 patients respectively found no difference in intra-operative or post-operative WCC or neutrophil count when the LG-6 was compared to a standard filter. These findings were confirmed by subsequent studies[152;153;180]. However two recent studies appear to contradict these studies. Ohto et al[226] in 2000 randomised 26 patients to an LG-6 filter or a standard filter and found a significant reduction in neutrophils five minutes after commencing cardiopulmonary bypass. However, there was no difference at subsequent time points, which is when the previous studies had made measurements. The timing of sampling may therefore account for Ohto's unusual finding. Most recently Chen et al[179]

randomised 24 patients to an LG-6 filter or a control and they actually found a significant reduction in both WCC and neutrophils at 30 and 60 minutes into CPB. This suggests that the LG-6 may be effective for certain patients. Ohto et al's study was conducted in Japan and Chenet al's study was from Taiwan and it is possible that the filter is more effective in these populations although neither Ohto et al, Chenet et al or the author know of a theoretical mechanism to explain such a difference.

The results of the present study, conducted on a predominantly Caucasian population with a larger sample size than any of the studies discussed (170 patients having serial WCC sampling), suggest that the LG-6 filter does not significantly reduce the WCC and neutrophil count 30 minutes into CPB. Unlike Palanzo[167] and Chen[179] but in accordance with most other studies there were no differences found at subsequent time points. The results of the present study therefore confirm previous findings that any effect of the filter on white cells in temporary. The common finding that the LG-6 does not actually reduce the total white cell count has been explained by the bone marrow rapidly replacing the activated neutrophils which had been filtered out[157]. This study suggests that at 30 minutes into CPB the LG-6 continues to be able to filter out the neutrophils released by the bone marrow.

There is evidence[179] that the LG-6 can reduce the CD11b count on neutrophils suggesting that it is the activated neutrophils which are being removed. Funds were not available for the present study to use the flow cytometry necessary to measure white cell markers, which would have shown whether activated neutrophils were being filtered preferentially.

This study has found a direct correlation between neutrophil count at ten minutes after bypass and microemboli count on CPB (Table 8.41). A higher neutrophil count was

associated with more microemboli. This suggests that the microemboli may be causing an inflammatory reaction and so raising the neutrophils count. However as the LG-6 filters have been found to simultaneously reduce WCC, neutrophils and microemboli and there may not be a direct causal link between the microemboli and neutrophil counts. It is possible therefore that the reduction in neutrophils and microemboli are two unrelated effects of the LG-6 filter.

It is interesting that there was also a correlation between WCC and NP outcome (Table 8.38). A high white cell count both pre-operatively and at ten minutes after bypass predict a poorer NP outcome. No studies, as far as the author is aware have previously found a relationship between inflammatory markers and NP outcome[142]. However, a significant positive correlation between stroke and raised WCC in cardiac surgery has been described[227].

9.3.2 Elastase

A statistically significant elevation of elastase in the LG-6 group during and immediately after CPB compared to the Avecor group has been found in this study (Fig 8.25). Elastase was measured as a marker of inflammation. Since elastase is a proteolytic enzyme released by activated neutrophils this rise in elastase levels is somewhat paradoxical because the LG-6 filters reduced the circulating neutrophils during CPB. However, some but not all other studies have found a similar elevation in elastase. Palanzo et al found no difference in elastase levels when LG-6 and standard filters were compared[167]. At the end of bypass Mihaljevic et al[161] found levels of elastase were 460 mcg/L in the LG-6 group compared to 230mcg/L in the control group. These figures are very similar to those in our study– 497mcg/L in the LG-6 group and 229mcg/L in the

control group (Table 8.48). Mair et al[152] also found a greater rise in elastase in the LG-6 group with similar figures. Although it can be used as a marker of neutrophil activation, in the case of leucocyte filtration it crucially gives no indication as to *where* the leucocytes have become activated. A raised elastase may reflect an increased release by those neutrophils trapped together in the filter rather than an increased activation of neutrophils in the patients' tissues. This hypothesis would require further study to test. It should also be pointed out that as elastase was only measured for the LG-6 and Avecor filters the auto-venting aspect of the LG-6 was not controlled. It is unlikely that venting rather than the LD aspect of the LG-6 is influencing elastase but this cannot be ruled out.

9.3.3 Platelets and Haemoglobin

This study confirms previous studies by showing that the LG-6 filter has no effect on haemoglobin or platelets peri-operatively. This study therefore provides no evidence that the LG-6 causes haemolysis or destruction of platelets.

9.4 S100b

In this study we measured S100b as a supplementary endpoint to further investigate is use as a marker of peri-operative cerebral injury. The evidence discussed in chapter 6 has no clear conclusion. There is conflicting evidence as to whether the rise in S100b seen using the Sangtec assay reflects a true cerebral injury or is an epiphenomenon resulting from the Sangtec S100b assay detecting mediastinally released S100a and S100a0. The Sangtec validation data has never been published and its potential cross reactivity with S100a and S100a0 is problematic.

In our study, using an ELISA assay not previously used in cardiac surgery, but which does have published validation data[204;205], we found a post-operative elevation of S100b at 10 minutes post bypass only (Table 8.49). There was no difference between the LD and conventional filter groups and there was no relationship between S100b and either microemboli detected during bypass or neuropsychological outcome. Despite using cardiotomy suction in our study, the levels of S100b detected were relatively low and comparable to levels found in studies in which no CPB or cell saving devices have been used. It is possible that our assay is more specific than the Sangtec assay and is only detecting cerebral S100b rather than mediastinally released S100b.

9.5 Strengths and weaknesses of the Study

Choice of neuropsychological tests

All the neuropsychological tests were delivered according to the clear standardised instructions detailed in the methods chapter. However any neuropsychological test battery used within the domain of cardiac surgery will always be a compromise given the limitations placed over time. The battery used in this study is one that is widely used by other centres and reported in a number of articles. However, the battery is limited in that it does not include all possible domains although the spread of domains is quite large in that it covers verbal and non-verbal memory, attention and concentration, speed of response and perceptuo-motor skills. The areas not covered in the battery are issues to do with executive function tests (e.g. the Wisconsin Card Sorting test) but these are limited in that they cannot be applied repeatedly in any domain as they centrally examine the ability to learn a rule. A further limitation of the battery is that two of the tests do not have a large battery of normative data from controls. These two tests, the choice reaction time test and the non-verbal memory test, have been specifically developed for this domain in order to capture certain types of responses that were not easily available on standardised and norm referenced tests.

There are other groups that have selected to use other tests in the limited time available for this kind of research but it must be borne in mind that out of the approximately 1,500 tests easily available it would not be surprising for certain neuropsychologists to choose different tests. The tests chosen in this study are perhaps the most widely used and certainly concur with the recommendations made by the consensus statement for assessing neuropsychological changes following cardiac surgery[14].

Statistical Analysis of Neuropsychological Data

There are a number of approaches to assessing cognitive change following cardiopulmonary bypass some of which have been discussed in the literature review. In particular the binary classification suffers from a number of inherent concerns regarding its sensitivity as well as the fact that it is conventionally defined. These two factors limit the ability of a binary classificatory system to be sensitive enough to the kinds of interventions performed in cardiac surgery in which neuropsychological outcomes are examined. The alternative is to use a continuous measure of performance. Here the standardised Z score used in this thesis has been widely applied in other studies[44]. The two measures have both been used in this thesis, albeit that it is recognised that the binary classificatory system is severely limited[42, 44].

There are however alternative approaches that people have used but it was felt that in the design of the study that these suffer from a number of limitations. Some researchers[5] have used a system in which they perform a principle component analysis of the scores that the tests produce in the particular sample that they are studying. Whilst this might seem a parsimonious approach to reduce the numbers of variables and provide factor scores it has its own limitations. It is unclear until the study has been conducted which tests and which scores will finally group together. The method of grouping them by means of principle component/factor analysis is a volatile technique and often the results and the weightings differ by sample. Because these factor groupings are not standardised and well recognised, comparisons to normative data are not easily made. Most of the studies that have been performed using this technique have used absolute performance preoperatively. It is clear that change scores and the sensitivity of change scores over time, could provide a radically different grouping to those that are using absolute scores prior to surgery. On balance it

was felt that in this study to use both the binary classification as a secondary outcome as this is widely used in the literature and the continuous measure which has been highly recommended by other researchers and now more commonly practiced.

Some studies, particularly in non-cardiac surgery have used control groups in order to make comparisons. The real difficulties in finding control groups for this sample given that these individuals have significant coronary artery disease and MRI analysis has suggested that at least 80% of elective coronary artery bypass graft surgery have abnormalities in the brain. They may be particularly sensitive to some of the factors that occur in cardiac surgery and this might not be well represented in any control group. Furthermore, the appropriateness of either a surgical control group or alternatively a normal control group outside of surgery would not necessarily be particularly helpful in looking at this subset. Most importantly however, this was a comparative study not attempting to establish the absolute decline in performance of individuals undergoing cardiac surgery but to see whether a difference could be detected within the context of a randomised control trial of the use of two types of filter.

Parsonnet

When the author was designing this study the Parsonnet score[209] was the most popular method of risk stratifying patients, that is: predicting their 30 day mortality from pre-operative characteristics. Although the Parsonnet score was developed in North America, it has been shown to be applicable to UK patients[228;229]. Bridgewater et al in 1998 stated "the Parsonnet score and preliminary version of the UK national scores seem to be the best available scores at present"[229]. However the Parsonnet score has also been shown to

overestimate mortality, especially in high risk patients[230]. The "Euroscore" was subsequently developed by Sam Nashef and better predicts mortality in the UK[231-233]. The Euroscore has become more popular due to its superiority and would therefore be the risk stratification method of choice in future studies. However the Parsonnet score was useful and available at the time of planning this study and was an acceptable method to use. Moreover, the purpose of using a risk stratification system in this study was to compare groups, not accurately predict mortality .

Carotid bruit screening

The method used in this study to screen for carotid artery stenoses was auscultation for carotid bruits. If a bruit was heard, the patient proceeded to have carotid artery Duplex scanning. Auscultation is a less sensitive method than Duplex scanning for detecting carotid stenoses and it is thus possible that patients with stenoses were missed and therefore included in the study. The method of the present study was the practice at the institution of the study and it was not possible to routinely screen all patients with Doppler scans of the carotid arteries before inclusion in the study. To have done so would, however, have been ideal. It should be stressed that the same criteria were applied to all three groups in this randomised controlled trial. Duplex scanning has a sensitivity of approximately 85% and a specificity of 90%[234] whereas others have found the sensitivity of auscultation was 56% and specificity was 91%[235].

Diabetics

The present study only excluded insulin dependent diabetics. The effect on neuropsychological outcome of diabetes and blood sugar management in the peri-operative period is unknown. This study could be criticised if all diabetics or no diabetics were

excluded. There is no perfect study.

Aspirin

In this study all the patients from home stopped aspirin seven days before surgery. It was not possible to stop aspirin so far in advance in all the inpatients included in the study. This could have had an effect on platelet function and microemboli generation.

AF/ warfarin

Patients with AF were not excluded from this study. The effect of AF on microemboli generation is not known but Stanley et al in 2002[27] found that patients with AF had worse NP outcome. This was published three years after the present study was designed but it would have been useful, if not to exclude patients with AF, to record the number of patients in each group with AF.

Neuropsychological testing

As stated in the methods section, all pre-operative NP testing was performed by the same trained psychologist. If this psychologist was not available no patients were recruited pre-operatively. At the six week return visit, the patients had to have the tests done on this set day. It was very rare (6/162 = 3.7%) for the post-operative testing to be done by a psychologist other than the one who had performed the pre-operative testing. When it did occur, it was unavoidable.

Switching of control filters

At the start of the study the potential difference between auto-venting and vent-line filters was not appreciated and so a leucocyte depleting auto vent filter was being compared with a conventional screen and vent-line filter. This is a potential flaw. Fortunately it was possible to include a more appropriate second control filter that was auto-venting as well as

having the conventional screen filter. The inclusion of this second control filter also had the positive aspect of enabling a comparison to be made between the effects of auto-venting versus vent-lines on microemboli. In fact, there was no difference between the vent-line and auto-venting filters in terms of microemboli count, although the study was not initially powered to test this hypothesis. Switching of the filters also led to an uneven distribution of recruitment such that 82 patients were recruited to the LD group, 73 to the AV group and 37 to the AV-6 group (Table 8.3).

Study population

Potential differences between the at home patients and the inpatients were not analysed.

Before drawing some conclusions it is worth commenting on the strengths and weaknesses of the study and discussing how this should limit interpretation. A prospective randomised trial is the most scientifically valid of the various types of clinical study. However such clinical trials have inherent limitations because, however rigorous the attempts are to standardise procedure, there will be events and variables beyond the investigators' control. For example, although we devised a standard protocol for anaesthesia it was necessary to leave some choices of anaesthetic drug usage to the anaesthetists' discretion. Approximately 10 anaesthetists were involved and they each had individual preferences for types and amounts of drugs used. It would not have been practical to have only one anaesthetist performing all the anaesthetics for the trial patients, although this would have been the ideal. Also there are limitations to the extent one can impose protocols on clinicians however willing they are to participate in trials. In this study, we believed it important to avoid the uncontrolled use of Propofol, which is a popular intravenous anaesthetic agent, because it may have neuroprotective effect. It was possible to incorporate this restriction into the trial protocol and achieve compliance from those anaesthetists who would ordinarily have used Propofol. Moreover, it is not known how

anaesthesia affects NP outcome and the only way the effect of surgery could be distinguished from the effect of anaesthesia would be to perform anaesthesia without surgery or surgery without anaesthesia, both of which would be unethical. Randomisation should deal with the uncontrollable variables if numbers are adequate and this has been the case in the present study.

Power calculations showed that the reduction in the Z scores after cardiac surgery required 197 patients, a relatively large sample size for a single centre prospective clinical study. In order to recruit these patients within a realistic time period it was necessary to recruit widely. We therefore recruited patients operated on by six different consultant surgeons whereas ideally this should have been kept to fewer. As with the anaesthetists, the surgeons had an agreed protocol and all used cross clamp fibrillation for myocardial protection. Other minor differences in operative procedure such as use of a side-biting clamp for cannulation could not be controlled, but were recorded and so available for analysis. At the beginning of the study there were three consultants working in the unit. By the end one had left, and a further three had joined.

There were therefore some unavoidable limitations to this study. In addition, some aspects could be criticised. In an attempt to reduce confounding variables we did not recruit patients who had had previous cerebral injury, including previous CABG and patients with insulin dependent diabetes. We also excluded particularly elderly patients over the age of eighty as it was thought these would have been more difficult to follow up. However, it could be argued that although we were attempting to reduce variables outside our control, we were actually excluding the very patients who could potentially derive more benefit from neuroprotection. Excluding both these types of patient will have reduced further the expected incidence of deficit and will have reduced the statistical power of the study. The strict selection criteria may also mean that the present study is not representative of the whole CABG surgery population

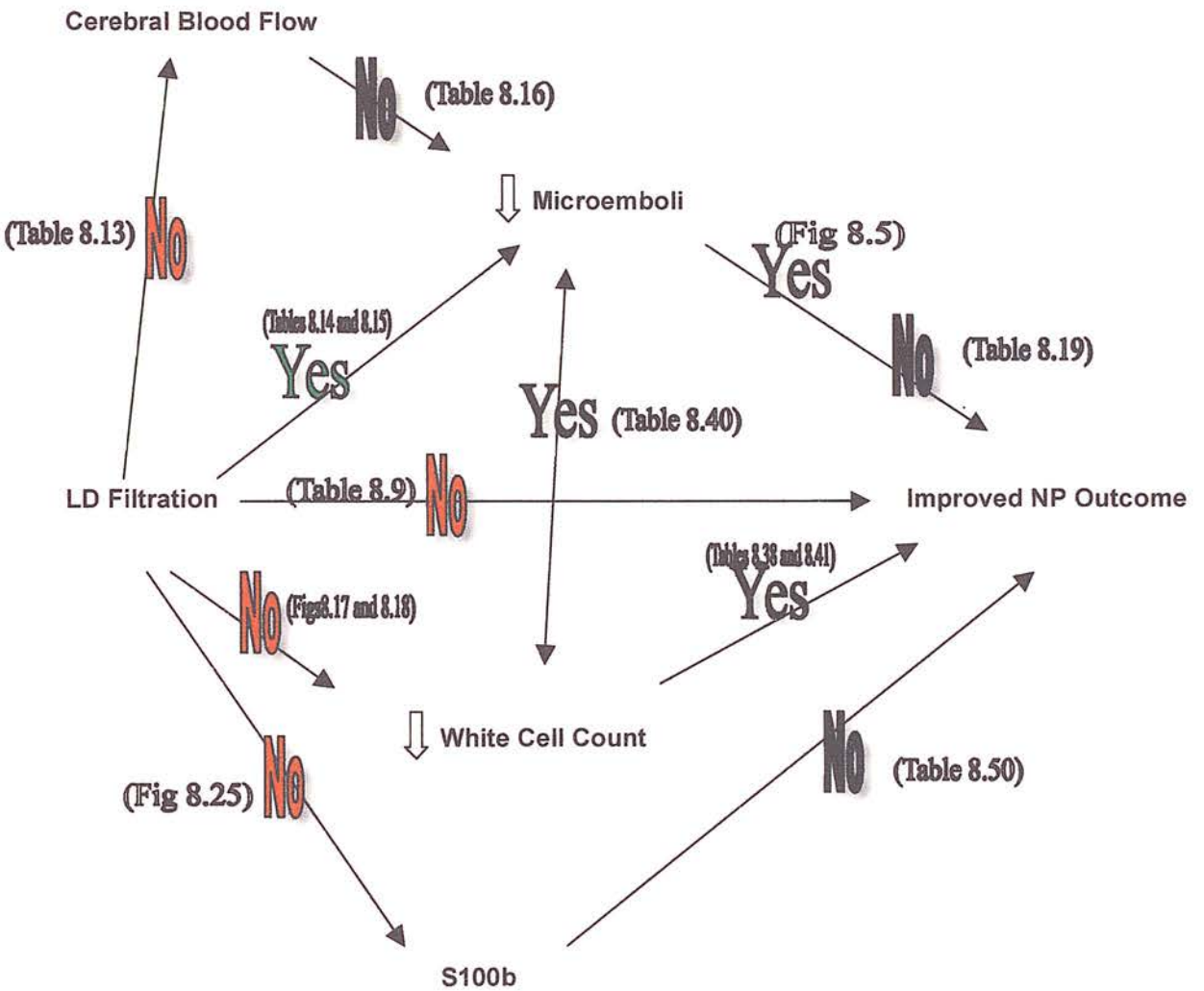
and thus limit the ability to generalise from its results. The UK society database has shown that 15-20% of UK patients having CABG have diabetes[10].

The aim of randomisation is to have similar variability between groups. In this randomised controlled study we are able to demonstrate the similarity of test and control groups' pre-operative patient characteristics (Table 8.4). There was a trend towards a slightly younger age in the AV-6 group compared to the Avecor group ($p = 0.07$), but there were no statistically significant differences in demographic and preoperative characteristics of the three groups. The age and sex ratio was representative of the UK population reported in the National Adult Cardiac Surgical Database Report 2000-2001[10] and mortality was as predicted using the Parsonnet score[236]. Intra-operatively, all groups had similar number of grafts performed and a corresponding similarity of cross clamp and bypass times. The proportion of patients who had left internal mammary artery grafts used was similar between groups meaning that all patients would have had a similar number of proximal aorto-coronary anastamoses (with attendant side biting clamp usage). The LG-6 group did have a lower incidence of aortic atheroma or calcification detectable by relatively crude methods (Table 8.6). However, analysis of both presence of atheroma/calcification and whether or not a side-biting clamp was used showed that neither had a statistically significant effect on microemboli production (Table 8.18). There was only a trend towards use of the side-biting clamp at cannulation leading to more microemboli. It is worth emphasising the benefits of full randomisation because many studies measuring microemboli have not been randomised, especially those testing the effect of a particular technique such as off pump surgery[117] or epiaortic scanning and exclusive Y-grafting[95].

9.6 Conclusions of Discussion

The main findings of the present study have now been discussed and are summarised in Fig 9.1 below:-

Figure 9.1 Summary of Results Discussed



Coloured results indicate results from randomised trial. Black results indicate secondary analysis.

The null hypotheses tested were:-

1) There will be no significant difference in the neuropsychological outcome when leucocyte-depleting filters are used.

2) There will be no significant difference in the microemboli count or inflammatory response when leucocyte-depleting filters are used.

This study has found the first null hypothesis to be true. No significant difference in NP outcome was found when the LD filter group were compared to the standard filter group (Table 8.9). Using both incidence of deficit and standardised Z change scores, this randomised controlled clinical trial of 192 patients has not found conclusive evidence of a neuroprotective effect of leucocyte depleting arterial line filters compared to standard arterial line filters. However in eight out of a battery of nine tests there was a non-significant trend towards improved neuropsychological outcome. The incidence of neuropsychological deficits was lower in the present study than in most studies previously reported. As discussed, this is probably the result of strict inclusion criteria in the present study. The study may thus have been underpowered despite adequate statistical planning.

The second null hypothesis has been disproved because differences were found between the LD and standard filter groups for both microemboli and for the inflammatory response.

This study has found that leucocyte-depleting filters reduce the number of microembolic events detected by transcranial Doppler in the right middle cerebral artery (Tables 8.14 and 8.15). There was no direct correlation of microemboli with NP outcome (Table 8.19), but using a median split patients with lower numbers of microemboli had a better neuropsychological outcome (Fig 8.5). This is therefore further indirect evidence that the LD filters, by reducing microemboli, can improve NP outcome. LD filtration had no effect upon cerebral blood flow velocity (Table 8.13) nor was there any correlation of cerebral blood flow velocity and

microemboli count (Table 8.16). Altering cerebral blood flow is not a mechanism by which the LD filters reduce microemboli.

In terms of the inflammatory response to surgery and cardiopulmonary bypass, it remains unclear exactly what effect the LD filters have. The LD filter does not significantly reduce total white cell count (Fig 8.17) or neutrophil count (Fig 8.18). The present study has, however, found a correlation between increased white cell count and worse NP outcome (Table 8.38). There is also a positive correlation between neutrophil count post bypass and microemboli count (Table 8.40). The finding of the present study that LD filtration increases serum elastase is, as has been discussed, paradoxical. If, by filtering out activated white cells, the LD filters are reducing the inflammatory response, the one would expect serum elastase to be lower. It is possible that elastase continues to be released by activated neutrophils trapped in the LD filter. Elastase is probably not the best inflammatory marker to use in the presence of the LD filter because it is not possible to interpret the difference in inflammatory response found. Although the LD filter has been shown to have had no quantitative effect on the white cell and neutrophil counts, without measuring white cell activation markers it is not possible to determine what qualitative effect the filters had on white cells (i.e. it is not known whether the LD filters are reducing the proportion of activated white cells).

This study has found no relationship between peri-operative S100b levels and microemboli count or neuropsychological outcome. This study therefore lends weight to the current consensus that S100b may not be as useful as a marker of cerebral injury as was once hoped.

9.7 Suggestions for Further Research

Further study would be necessary to determine how much of the mechanism by which the leucocyte depleting filters reduce microemboli is mechanical and how much is inflammatory mediated. The measurement of white cell activation markers such as CB11b and CD18, which was not possible in the present study, would determine the qualitative effect of LD filters on the white cell population. Measuring additional inflammatory markers such as the interleukins or tumour necrosis factor would also give more information on how the LD filters influence the overall inflammatory response.

Newer Doppler techniques may be able to determine whether LD filters differentially reduce gaseous or particulate microemboli or whether both are reduced in the same proportion. The relative pathogenicicity of particulate, gaseous and lipid microemboli is also yet to be determined.

The low incidence of neuropsychological deficits in this study suggests that future studies will need to either have less strict inclusion criteria or have greater numbers of participants. The latter suggestion is less practical. Alternatively refinement of existing neuropsychological tests may be necessary to make them more sensitive.

Reference List

1. Ahlgren E, Lundqvist A, Nordlund A, Aren C, Rutberg H. Neurocognitive impairment and driving performance after coronary artery bypass surgery. *Eur.J.Cardiothorac.Surg.* 2003;**23**:334-40.

2. McKhann GM, Goldsborough MA, Borowicz LM, Jr., Selnes OA, Mellits ED, Enger C *et al.* Cognitive outcome after coronary artery bypass: a one-year prospective study. *Ann.Thorac.Surg.* 1997;**63**:510-5.

3. Borowicz LM, Goldsborough MA, Selnes OA, McKhann GM. Neuropsychologic change after cardiac surgery: a critical review. *J.Cardiothorac.Vasc.Anesth.* 1996;**10**:105-11.

4. Taylor KM. Brain damage during cardiopulmonary bypass. *Ann.Thorac.Surg.* 1998;**65**:S20-S26.

5. Newman MF, Kirchner JL, Phillips-Bute B, Gaver V, Grocott H, Jones RH *et al.* Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery. *N.Engl.J.Med.* 2001;**344**:395-402.

6. Vingerhoets G, Van Nooten G, Vermassen F, De Soete G, Jannes C. Short-term and long-term neuropsychological consequences of cardiac surgery with extracorporeal circulation. *Eur.J.Cardiothorac.Surg.* 1997;**11**:424-31.

7. Taggart DP, Browne SM, Halligan PW, Wade DT. Is cardiopulmonary bypass still the cause of cognitive dysfunction after cardiac operations? *J.Thorac.Cardiovasc.Surg.* 1999;**118**:414-20.

8. Pugsley W, Klinger L, Paschalis C, Treasure T, Harrison M, Newman S. The impact of microemboli during cardiopulmonary bypass on neuropsychological functioning. *Stroke* 1994;**25**:1393-9.

9. Souttar, HS. The surgical treatment of mitral stenosis. BMJ 3, 603-606. 1925.

10. Keogh BE, Kinsman R. UK National Adult Cardiac Surgical Database Report 2000-2001. Henley on Thames: Dendrite Clinical Systems, 2002.

11. Lezak MD. Neuropsychological Assessment. New York: Oxford University Press, 1995.

12. Mills SA, Prough DS. Neuropsychiatric complications following cardiac surgery. *Semin.Thorac.Cardiovasc.Surg.* 1991;**3**:39-46.

13. McDowell I, Newell C. Measuring Health. New York: Oxford University Press, 1996.

14. Murkin JM, Newman SP, Stump DA, Blumenthal JA. Statement of consensus on assessment of neurobehavioral outcomes after cardiac surgery. *Ann.Thorac.Surg.* 1995;**59**:1289-95.

15. Newman MF, Croughwell ND, Blumenthal JA, Lowry E, White WD, Spillane W *et al.* Predictors of cognitive decline after cardiac operation. *Ann.Thorac.Surg.* 1995;**59**:1326-30.

16. O'Brien DJ, Bauer RM, Yarandi H, Knauf DG, Bramblett P, Alexander JA. Patient memory before and after cardiac operations. *J.Thorac.Cardiovasc.Surg.* 1992;**104**:1116-24.

17. Sotaniemi KA, Mononen H, Hokkanen TE. Long-term cerebral outcome after open-heart surgery. A five-year neuropsychological follow-up study. *Stroke* 1986;**17**:410-6.

18. Townes BD, Bashein G, Hornbein TF, Coppel DB, Goldstein DE, Davis KB *et al.* Neurobehavioral outcomes in cardiac operations. A prospective controlled study. *J.Thorac.Cardiovasc.Surg.* 1989;**98**:774-82.

19. Stump, D. A., Tegeler, C. H, Newman, S. P., Wallenhaupt, S., and Roy, C. Older patients have more emboli during coronary artery bypass surgery. Anesthesiology 77, A52. 1992.

20. Sotaniemi KA, Juolasmaa A, Hokkanen ET. Neuropsychologic outcome after open-heart surgery. *Arch.Neurol.* 1981;**38**:2-8.

21. Di Carlo A, Perna AM, Pantoni L, Basile AM, Bonacchi M, Pracucci G *et al.* Clinically relevant cognitive impairment after cardiac surgery: a 6-month follow-up study. *J.Neurol.Sci.* 2001;**188**:85-93.

22. Braekken SK, Reinvang I, Russell D, Brucher R, Svennevig JL. Association between intraoperative cerebral microembolic signals and postoperative neuropsychological deficit: comparison between patients with cardiac valve replacement and patients with coronary artery bypass grafting. *J.Neurol.Neurosurg.Psychiatry* 1998;**65**:573-6.

23. Neville MJ, Butterworth J, James RL, Hammon JW, Stump DA. Similar neurobehavioral outcome after valve or coronary artery operations despite differing carotid embolic counts. *J.Thorac.Cardiovasc.Surg.* 2001;**121**:125-36.

24. Zimpfer D, Czerny M, Kilo J, Kasimir MT, Madl C, Kramer L *et al.* Cognitive deficit after aortic valve replacement. *Ann.Thorac.Surg.* 2002;**74**:407-12.

25. Archbold RA. Predictors of neurological morbidity after coronary artery bypass surgery. *Eur.J.Cardiothorac.Surg.* 1999;**16**:397-8.

26. Stamou SC, Hill PC, Dangas G, Pfister AJ, Boyce SW, Dullum MK *et al.* Stroke after coronary artery bypass: incidence, predictors, and clinical outcome. *Stroke* 2001;**32**:1508-13.

27. Stanley TO, Mackensen GB, Grocott HP, White WD, Blumenthal JA, Laskowitz DT *et al.* The impact of postoperative atrial fibrillation on neurocognitive outcome after coronary artery bypass graft surgery. *Anesth.Analg.* 2002;**94**:290-5.

28. Tardiff BE, Newman MF, Saunders AM, Strittmatter WJ, Blumenthal JA, White WD *et al.* Preliminary report of a genetic basis for cognitive decline after cardiac operations. The Neurologic Outcome Research Group of the Duke Heart Center. *Ann.Thorac.Surg.* 1997;**64**:715-20.

29. Steed L, Kong R, Stygall J, Acharya J, Bolla M, Harrison MJ *et al.* The role of apolipoprotein E in cognitive decline after cardiac operation. *Ann.Thorac.Surg.* 2001;**71**:823-6.

30. Mathew JP, Rinder CS, Howe JG, Fontes M, Crouch J, Newman MF *et al.* Platelet PlA2 polymorphism enhances risk of neurocognitive decline after cardiopulmonary bypass. Multicenter Study of Perioperative Ischemia (McSPI) Research Group. *Ann.Thorac.Surg.* 2001;**71**:663-6.

31. Hammon JW, Jr., Stump DA, Kon ND, Cordell AR, Hudspeth AS, Oaks TE *et al.* Risk factors and solutions for the development of neurobehavioral changes after coronary artery bypass grafting. *Ann.Thorac.Surg.* 1997;**63**:1613-8.

32. Andersen LW, Baek L, Degn H, Lehd J, Krasnik M, Rasmussen JP. Presence of circulating endotoxins during cardiac operations. *J.Thorac.Cardiovasc.Surg.* 1987;**93**:115-9.

33. Bennett-Guerrero E, Ayuso L, Hamilton-Davies C, White WD, Barclay GR, Smith PK *et al.* Relationship of preoperative antiendotoxin core antibodies and adverse outcomes following cardiac surgery. *JAMA* 1997;**277**:646-50.

34. Mathew JP, Grocott HP, Phillips-Bute B, Stafford-Smith M, Laskowitz DT, Rossignol D *et al.* Lower endotoxin immunity predicts increased cognitive dysfunction in elderly patients after cardiac surgery. *Stroke* 2003;**34**:508-13.

35. Vingerhoets G, Van Nooten G, Jannes C. Neuropsychological impairment in candidates for cardiac surgery. *J.Int.Neuropsychol.Soc.* 1997;**3**:480-4.

36. Selnes OA, Grega MA, Borowicz LM, Jr., Royall RM, McKhann GM, Baumgartner WA. Cognitive changes with coronary artery disease: a prospective study of coronary artery bypass graft patients and nonsurgical controls. *Ann.Thorac.Surg.* 2003;**75**:1377-84.

37. Stygall J, Newman SP, Fitzgerald G, Steed L, Mulligan K, Arrowsmith JE *et al.* Cognitive change 5 years after coronary artery bypass surgery. *Health Psychol.* 2003;**22**:579-86.

38. Stygall J, Mulligan K, Steed L, Harrison M and Newman SP. The impact of anxiety on pre-operative cognitive functioning - a comparison of the week and the day before surgery. Presented at the 17th conference of the European Health Psychology Society September 2003. 2003.

39. Newman SP. Analysis and interpretation of neuropsychologic tests in cardiac surgery. *Ann.Thorac.Surg.* 1995;**59**:1351-5.

40. Browne SM, Halligan PW, Wade DT, Taggart DP. Cognitive performance after cardiac operation: implications of regression toward the mean. *J.Thorac.Cardiovasc.Surg.* 1999;**117**:481-5.

41. Selnes OA, Goldsborough MA, Borowicz LM, Jr., Enger C, Quaskey SA, McKhann GM. Determinants of cognitive change after coronary artery bypass surgery: a multifactorial problem. *Ann.Thorac.Surg.* 1999;**67**:1669-76.

42. Arrowsmith JE, Harrison MJ, Newman SP, Stygall J, Timberlake N, Pugsley WB. Neuroprotection of the brain during cardiopulmonary bypass: a randomized trial of remacemide during coronary artery bypass in 171 patients. *Stroke* 1998;**29**:2357-62.

43. Kong RS, Butterworth J, Aveling W, Stump DA, Harrison MJ, Hammon J *et al.* Clinical trial of the neuroprotectant clomethiazole in coronary artery bypass graft surgery: a randomized controlled trial. *Anesthesiology* 2002;**97**:585-91.

44. Van Dijk D, Jansen EW, Hijman R, Nierich AP, Diephuis JC, Moons KG *et al.* Cognitive outcome after off-pump and on-pump coronary artery bypass graft surgery: a randomized trial. *JAMA* 2002;**287**:1405-12.

45. Taggart DP,.Westaby S. Neurological and cognitive disorders after coronary artery bypass grafting. *Curr.Opin.Cardiol.* 2001;**16**:271-6.

46. Murkin JM. Attenuation of neurologic injury during cardiac surgery. *Ann.Thorac.Surg.* 2001;**72**:S1838-S1844.

47. Mejak BL, Stammers A, Rauch E, Vang S, Viessman T. A retrospective study on perfusion incidents and safety devices. *Perfusion* 2000;**15**:51-61.

48. Padayachee TS, Parsons S, Theobold R, Linley J, Gosling RG, Deverall PB. The detection of microemboli in the middle cerebral artery during cardiopulmonary bypass: a transcranial Doppler ultrasound investigation using membrane and bubble oxygenators. *Ann.Thorac.Surg.* 1987;**44**:298-302.

49. Smith PL, Blauth CI, Newman S. Cerebral microembolism and neuropsychological outcome following coronary artery bypass surgery (CABS) with either a membrane or a bubble oxygenator. In Willner A, Rodewald G, eds. *Impact of cardiac surgery on the quality of life.*, pp 337-42. New York: Plenum Press, 2005.

50. Scott DA, Silbert BS, Doyle TJ, Blyth C, Borton MC, O'brien JL *et al.* Centrifugal versus roller head pumps for cardiopulmonary bypass: effect on early neuropsychologic outcomes after coronary artery surgery. *J.Cardiothorac.Vasc.Anesth.* 2002;**16**:715-22.

51. Nussmeier NA, Arlund C, Slogoff S. Neuropsychiatric complications after cardiopulmonary bypass: cerebral protection by a barbiturate. *Anesthesiology* 1986;**64**:165-70.

52. Zaidan JR, Klochany A, Martin WM, Ziegler JS, Harless DM, Andrews RB. Effect of thiopental on neurologic outcome following coronary artery bypass grafting. *Anesthesiology* 1991;**74**:406-11.

53. Pascoe EA, Hudson RJ, Anderson BA, Kassum DA, Shanks A, Rosenbloom M *et al.* High-dose thiopentone for open-chamber cardiac surgery: a retrospective review. *Can.J.Anaesth.* 1996;**43**:575-9.

54. Newman MF, Murkin JM, Roach G, Croughwell ND, White WD, Clements FM *et al.* Cerebral physiologic effects of burst suppression doses of propofol during nonpulsatile cardiopulmonary bypass. CNS Subgroup of McSPI. *Anesth.Analg.* 1995;**81**:452-7.

55. Hindman BJ,.Todd MM. Improving neurologic outcome after cardiac surgery. *Anesthesiology* 1999;**90**:1243-7.

56. Roach GW, Newman MF, Murkin JM, Martzke J, Ruskin A, Li J *et al.* Ineffectiveness of burst suppression therapy in mitigating perioperative cerebrovascular dysfunction. Multicenter Study of Perioperative Ischemia (McSPI) Research Group. *Anesthesiology* 1999;**90**:1255-64.

57. Forsman M, Olsnes BT, Semb G, Steen PA. Effects of nimodipine on cerebral blood flow and neuropsychological outcome after cardiac surgery. *Br.J.Anaesth.* 1990;**65**:514-20.

58. Legault C, Furberg CD, Wagenknecht LE, Rogers AT, Stump DA, Coker L *et al.* Nimodipine neuroprotection in cardiac valve replacement: report of an early terminated trial. *Stroke* 1996;**27**:593-8.

59. Bashein G, Townes BD, Nessly ML, Bledsoe SW, Hornbein TF, Davis KB *et al.* A randomized study of carbon dioxide management during hypothermic cardiopulmonary bypass. *Anesthesiology* 1990;**72**:7-15.

60. Stephan H, Weyland A, Kazmaier S, Henze T, Menck S, Sonntag H. Acid-base management during hypothermic cardiopulmonary bypass does not affect cerebral metabolism but does affect blood flow and neurological outcome. *Br.J.Anaesth.* 1992;**69**:51-7.

61. Murkin JM, Martzke JS, Buchan AM, Bentley C, Wong CJ. A randomized study of the influence of perfusion technique and pH management strategy in 316 patients undergoing coronary artery bypass surgery. II. Neurologic and cognitive outcomes. *J.Thorac.Cardiovasc.Surg.* 1995;**110**:349-62.

62. Patel RL, Turtle MR, Chambers DJ, James DN, Newman S, Venn GE. Alpha-stat acid-base regulation during cardiopulmonary bypass improves neuropsychologic outcome in patients undergoing coronary artery bypass grafting. *J.Thorac.Cardiovasc.Surg.* 1996;**111**:1267-79.

63. Cook DJ, Plochl W, Orszulak TA. Effect of temperature and PaCO2 on cerebral embolization during cardiopulmonary bypass in swine. *Ann.Thorac.Surg.* 2000;**69**:415-20.

64. Plochl W, Krenn CG, Cook DJ, Gollob E, Pezawas T, Schima H *et al.* Can hypocapnia reduce cerebral embolization during cardiopulmonary bypass? *Ann.Thorac.Surg.* 2001;**72**:845-9.

65. Singh AK, Bert AA, Feng WC, Rotenberg FA. Stroke during coronary artery bypass grafting using hypothermic versus normothermic perfusion. *Ann.Thorac.Surg.* 1995;**59**:84-9.

66. McLean RF,.Wong BI. Normothermic versus hypothermic cardiopulmonary bypass: central nervous system outcomes. *J.Cardiothorac.Vasc.Anesth.* 1996;**10**:45-52.

67. Martin TD, Craver JM, Gott JP, Weintraub WS, Ramsay J, Mora CT *et al.* Prospective, randomized trial of retrograde warm blood cardioplegia: myocardial benefit and neurologic threat. *Ann.Thorac.Surg.* 1994;**57**:298-302.

68. Mora CT, Henson MB, Weintraub WS, Murkin JM, Martin TD, Craver JM *et al.* The effect of temperature management during cardiopulmonary bypass on neurologic and neuropsychologic outcomes in patients undergoing coronary revascularization. *J.Thorac.Cardiovasc.Surg.* 1996;**112**:514-22.

69. Regragui I, Birdi I, Izzat MB, Black AM, Lopatatzidis A, Day CJ *et al.* The effects of cardiopulmonary bypass temperature on neuropsychologic outcome after coronary artery operations: a prospective randomized trial. *J.Thorac.Cardiovasc.Surg.* 1996;**112**:1036-45.

70. Grimm M, Czerny M, Baumer H, Kilo J, Madl C, Kramer L *et al.* Normothermic cardiopulmonary bypass is beneficial for cognitive brain function after coronary artery bypass grafting--a prospective randomized trial. *Eur.J.Cardiothorac.Surg.* 2000;**18**:270-5.

71. Nathan HJ, Wells GA, Munson JL, Wozny D. Neuroprotective effect of mild hypothermia in patients undergoing coronary artery surgery with cardiopulmonary bypass: a randomized trial. *Circulation* 2001;**104**:I85-I91.

72. Grigore AM, Grocott HP, Mathew JP, Phillips-Bute B, Stanley TO, Butler A *et al.* The rewarming rate and increased peak temperature alter neurocognitive outcome after cardiac surgery. *Anesth.Analg.* 2002;**94**:4-10, table.

73. Malheiros SM, Brucki SM, Gabbai AA, Bertolucci PH, Juliano Y, Carvalho AC *et al.* Neurological outcome in coronary artery surgery with and without cardiopulmonary bypass. *Acta Neurol.Scand.* 1995;**92**:256-60.

74. BhaskerRao B, VanHimbergen D, Edmonds HL, Jr., Jaber S, Ali AT, Pagni S *et al.* Evidence for improved cerebral function after minimally invasive bypass surgery. *J.Card Surg.* 1998;**13**:27-31.

75. Braekken SK, Russell D, Brucher R, Abdelnoor M, Svennevig JL. Cerebral microembolic signals during cardiopulmonary bypass surgery. Frequency, time of occurrence, and association with patient and surgical characteristics. *Stroke* 1997;**28**:1988-92.

76. Malheiros SM, Massaro AR, Carvalho AC, Moises VA, Mussi A, Federico D *et al.* Transesophageal echocardiography and transcranial doppler monitoring in coronary surgery without cardiopulmonary bypass: preliminary results. *Cerebrovasc.Dis.*

1999;**9**:358-60.

77. Watters MP, Cohen AM, Monk CR, Angelini GD, Ryder IG. Reduced cerebral embolic signals in beating heart coronary surgery detected by transcranial Doppler ultrasound. *Br.J.Anaesth.* 2000;**84**:629-31.

78. Diegeler A, Hirsch R, Schneider F, Schilling LO, Falk V, Rauch T *et al.* Neuromonitoring and neurocognitive outcome in off-pump versus conventional coronary bypass operation. *Ann.Thorac.Surg.* 2000;**69**:1162-6.

79. Blumenthal JA, Mahanna EP, Madden DJ, White WD, Croughwell ND, Newman MF. Methodological issues in the assessment of neuropsychologic function after cardiac surgery. *Ann.Thorac.Surg.* 1995;**59**:1345-50.

80. Zamvar V, Williams D, Hall J, Payne N, Cann C, Young K *et al.* Assessment of neurocognitive impairment after off-pump and on-pump techniques for coronary artery bypass graft surgery: prospective randomised controlled trial. *BMJ* 2002;**325**:1268.

81. Astrup J, Symon L, Branston NM, Lassen NA. Cortical evoked potential and extracellular K+ and H+ at critical levels of brain ischemia. *Stroke* 1977;**8**:51-7.

82. Tufo HM, Ostfeld AM, Shekelle R. Central nervous system dysfunction following open-heart surgery. *JAMA* 1970;**212**:1333-40.

83. Gold JP, Charlson ME, Williams-Russo P, Szatrowski TP, Peterson JC, Pirraglia PA *et al.* Improvement of outcomes after coronary artery bypass. A randomized trial comparing intraoperative high versus low mean arterial pressure. *J.Thorac.Cardiovasc.Surg.* 1995;**110**:1302-11.

84. Caplan LR,.Hennerici M. Impaired clearance of emboli (washout) is an important link between hypoperfusion, embolism, and ischemic stroke. *Arch.Neurol.* 1998;**55**:1475-82.

85. Browne SM, Halligan PW, Wade DT, Taggart DP. Postoperative hypoxia is a contributory factor to cognitive impairment after cardiac surgery. *J.Thorac.Cardiovasc.Surg.* 2003;**126**:1061-4.

86. Blauth CI. Macroemboli and microemboli during cardiopulmonary bypass. *Ann.Thorac.Surg.* 1995;**59**:1300-3.

87. Orihashi K, Matsuura Y, Hamanaka Y, Sueda T, Shikata H, Hayashi S *et al.* Retained intracardiac air in open heart operations examined by transesophageal echocardiography. *Ann.Thorac.Surg.* 1993;**55**:1467-71.

88. Oka Y, Inoue T, Hong Y, Sisto DA, Strom JA, Frater RW. Retained intracardiac air. Transesophageal echocardiography for definition of incidence and monitoring removal by improved techniques. *J.Thorac.Cardiovasc.Surg.* 1986;**91**:329-38.

89. Liu JF, Su ZK, Ding WX. Quantitation of particulate microemboli during cardiopulmonary bypass: experimental and clinical studies. *Ann.Thorac.Surg.*

1992;**54**:1196-202.

90. Brooker RF, Brown WR, Moody DM, Hammon JW, Jr., Reboussin DM, Deal DD *et al.* Cardiotomy suction: a major source of brain lipid emboli during cardiopulmonary bypass. *Ann.Thorac.Surg.* 1998;**65**:1651-5.

91. Kincaid EH, Jones TJ, Stump DA, Brown WR, Moody DM, Deal DD *et al.* Processing scavenged blood with a cell saver reduces cerebral lipid microembolization. *Ann.Thorac.Surg.* 2000;**70**:1296-300.

92. Kaza AK, Cope JT, Fiser SM, Long SM, Kern JA, Kron IL *et al.* Elimination of fat microemboli during cardiopulmonary bypass. *Ann.Thorac.Surg.* 2003;**75**:555-9.

93. Harringer W. Capture of particulate emboli during cardiac procedures in which aortic cross-clamp is used. International Council of Emboli Management Study Group. *Ann.Thorac.Surg.* 2000;**70**:1119-23.

94. Barbut D, Hinton RB, Szatrowski TP, Hartman GS, Bruefach M, Williams-Russo P *et al.* Cerebral emboli detected during bypass surgery are associated with clamp removal. *Stroke* 1994;**25**:2398-402.

95. Royse AG, Royse CF, Ajani AE, Symes E, Maruff P, Karagiannis S *et al.* Reduced neuropsychological dysfunction using epiaortic echocardiography and the exclusive Y graft. *Ann.Thorac.Surg.* 2000;**69**:1431-8.

96. O'Brien JJ, Butterworth J, Hammon JW, Morris KJ, Phipps JM, Stump DA. Cerebral emboli during cardiac surgery in children. *Anesthesiology* 1997;**87**:1063-9.

97. Swank RL. Alteration of blood on storage: measurement of adhesiveness of "aging" platelets and leukocytes and their removal by filtration. *N.Engl.J.Med.* 1961;**265**:728-33.

98. Moody DM, Bell MA, Challa VR, Johnston WE, Prough DS. Brain microemboli during cardiac surgery or aortography. *Ann.Neurol.* 1990;**28**:477-86.

99. Blauth CI, Arnold JV, Schulenberg WE, McCartney AC, Taylor KM. Cerebral microembolism during cardiopulmonary bypass. Retinal microvascular studies in vivo with fluorescein angiography. *J.Thorac.Cardiovasc.Surg.* 1988;**95**:668-76.

100. Van Zuilen EV, Mess WH, Jansen C, Van dT, I, Van Gijn J, Ackerstaff GA. Automatic embolus detection compared with human experts. A Doppler ultrasound study. *Stroke* 1996;**27**:1840-3.

101. Markus HS and Brown MM. Differentiation between different pathological cerebral embolic materials using transcranial Doppler in an in vitro model. *Stroke* 1993;**24**:1-5.

102. Russell D, Madden KP, Clark WM, Sandset PM, Zivin JA. Detection of arterial emboli using Doppler ultrasound in rabbits. *Stroke* 1991;**22**:253-8.

103. Russell D and Brucher R. Online automatic discrimination between solid and

gaseous cerebral microemboli with the first multifrequency transcranial Doppler. *Stroke* 2002;**33**:1975-80.

104. Ringelstein EB, Droste DW, Babikian VL, Evans DH, Grosset DG, Kaps M *et al.* Consensus on microembolus detection by TCD. International Consensus Group on Microembolus Detection. *Stroke* 1998;**29**:725-9.

105. Silvay G, Ammar T, Reich DL, Vela-Cantos F, Joffe D, Ergin AM. Cardiopulmonary bypass for adult patients: a survey of equipment and techniques. *J.Cardiothorac.Vasc.Anesth.* 1995;**9**:420-4.

106. Moser DJ, Ferneyhough KC, Bauer RM, Arndt S, Schultz SK, Haynes WG *et al.* Unilateral vs. bilateral ultrasound in the monitoring of cerebral microemboli. *Ultrasound Med.Biol.* 2001;**27**:757-60.

107. Wijman CA, Babikian VL, Winter MR, Pochay VE. Distribution of cerebral microembolism in the anterior and middle cerebral arteries. *Acta Neurol.Scand.* 2000;**101**:122-7.

108. Mullges W, Franke D, Reents W, Babin-Ebell J, Toyka KV. Reduced rate of microembolism by optimized aortic cannula position does not influence early postoperative cognitive performance in CABG patients. *Cerebrovasc.Dis.* 2003;**15**:192-8.

109. Aberg T. Effect of open heart surgery on intellectual function. *Scand.J.Thorac.Cardiovasc.Surg.Suppl* 1974;1-63.

110. Clark RE, Brillman J, Davis DA, Lovell MR, Price TR, Magovern GJ. Microemboli during coronary artery bypass grafting. Genesis and effect on outcome. *J.Thorac.Cardiovasc.Surg.* 1995;**109**:249-57.

111. Barbut D, Lo YW, Gold JP, Trifiletti RR, Yao FS, Hager DN *et al.* Impact of embolization during coronary artery bypass grafting on outcome and length of stay. *Ann.Thorac.Surg.* 1997;**63**:998-1002.

112. Sylivris S, Levi C, Matalanis G, Rosalion A, Buxton BF, Mitchell A *et al.* Pattern and significance of cerebral microemboli during coronary artery bypass grafting. *Ann.Thorac.Surg.* 1998;**66**:1674-8.

113. Jacobs A, Neveling M, Horst M, Ghaemi M, Kessler J, Eichstaedt H *et al.* Alterations of neuropsychological function and cerebral glucose metabolism after cardiac surgery are not related only to intraoperative microembolic events. *Stroke* 1998;**29**:660-7.

114. Borger MA, Peniston CM, Weisel RD, Vasiliou M, Green RE, Feindel CM. Neuropsychologic impairment after coronary bypass surgery: effect of gaseous microemboli during perfusionist interventions. *J.Thorac.Cardiovasc.Surg.* 2001;**121**:743-9.

115. Fearn SJ, Pole R, Wesnes K, Faragher EB, Hooper TL, McCollum CN. Cerebral injury during cardiopulmonary bypass: emboli impair memory.

J.Thorac.Cardiovasc.Surg. 2001;**121**:1150-60.

116. Moody DM, Brown WR, Challa VR, Stump DA, Reboussin DM, Legault C. Brain microemboli associated with cardiopulmonary bypass: a histologic and magnetic resonance imaging study. *Ann.Thorac.Surg.* 1995;**59**:1304-7.

117. Bowles BJ, Lee JD, Dang CR, Taoka SN, Johnson EW, Lau EM *et al.* Coronary artery bypass performed without the use of cardiopulmonary bypass is associated with reduced cerebral microemboli and improved clinical results. *Chest* 2001;**119**:25-30.

118. Lund C, Hol PK, Lundblad R, Fosse E, Sundet K, Tennoe B *et al.* Comparison of cerebral embolization during off-pump and on-pump coronary artery bypass surgery. *Ann.Thorac.Surg.* 2003;**76**:765-70.

119. Chenoweth DE, Cooper SW, Hugli TE, Stewart RW, Blackstone EH, Kirklin JW. Complement activation during cardiopulmonary bypass: evidence for generation of C3a and C5a anaphylatoxins. *N.Engl.J.Med.* 1981;**304**:497-503.

120. Hammerschmidt DE, Stroncek DF, Bowers TK, Lammi-Keefe CJ, Kurth DM, Ozalins A *et al.* Complement activation and neutropenia occurring during cardiopulmonary bypass. *J.Thorac.Cardiovasc.Surg.* 1981;**81**:370-7.

121. Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenoweth DE, Pacifico AD. Complement and the damaging effects of cardiopulmonary bypass. *J.Thorac.Cardiovasc.Surg.* 1983;**86**:845-57.

122. Cavarocchi NC, Schaff HV, Orszulak TA, Homburger HA, Schnell WA, Jr., Pluth JR. Evidence for complement activation by protamine-heparin interaction after cardiopulmonary bypass. *Surgery* 1985;**98**:525-31.

123. Kirklin JK, Chenoweth DE, Naftel DC, Blackstone EH, Kirklin JW, Bitran DD *et al.* Effects of protamine administration after cardiopulmonary bypass on complement, blood elements, and the hemodynamic state. *Ann.Thorac.Surg.* 1986;**41**:193-9.

124. Chenoweth DE,.Hugli TE. Demonstration of specific C5a receptor on intact human polymorphonuclear leukocytes. *Proc.Natl.Acad.Sci.U.S.A* 1978;**75**:3943-7.

125. Henson PM, Zanolari B, Schwartzman NA, Hong SR. Intracellular control of human neutrophil secretion. I. C5a-induced stimulus-specific desensitization and the effects of cytochalasin B. *J.Immunol.* 1978;**121**:851-5.

126. Charo IF, Yuen C, Perez HD, Goldstein IM. Chemotactic peptides modulate adherence of human polymorphonuclear leukocytes to monolayers of cultured endothelial cells. *J.Immunol.* 1986;**136**:3412-9.

127. Wachtfogel YT, Kucich U, James HL, Scott CF, Schapira M, Zimmerman M *et al.* Human plasma kallikrein releases neutrophil elastase during blood coagulation. *J.Clin.Invest* 1983;**72**:1672-7.

128. Kaplan AP, Kay AB, Austen KF. A prealbumin activator of prekallikrein. 3. Appearance of chemotactic activity for human neutrophils by the conversion of human prekallikrein to kallikrein. *J.Exp.Med.* 1972;**135**:81-97.

129. Schapira M, Despland E, Scott CF, Boxer LA, Colman RW. Purified human plasma kallikrein aggregates human blood neutrophils. *J.Clin.Invest* 1982;**69**:1199-202.

130. Wachtfogel YT, Kucich U, Greenplate J, Gluszko P, Abrams W, Weinbaum G *et al.* Human neutrophil degranulation during extracorporeal circulation. *Blood* 1987;**69**:324-30.

131. Quiroga MM, Miyagishima R, Haendschen LC, Glovsky M, Martin BA, Hogg JC. The effect of body temperature on leukocyte kinetics during cardiopulmonary bypass. *J.Thorac.Cardiovasc.Surg.* 1985;**90**:91-6.

132. Craddock PR, Hammerschmidt D, White JG, Dalmosso AP, Jacob HS. Complement (C5-a)-induced granulocyte aggregation in vitro. A possible mechanism of complement-mediated leukostasis and leukopenia. *J.Clin.Invest* 1977;**60**:260-4.

133. Gu YJ, van Oeveren W, Boonstra PW, de Haan J, Wildevuur CR. Leukocyte activation with increased expression of CR3 receptors during cardiopulmonary bypass. *Ann.Thorac.Surg.* 1992;**53**:839-43.

134. Foreman KE, Vaporciyan AA, Bonish BK, Jones ML, Johnson KJ, Glovsky MM *et al.* C5a-induced expression of P-selectin in endothelial cells. *J.Clin.Invest* 1994;**94**:1147-55.

135. Burns SA, DeGuzman BJ, Newburger JW, Mayer JE, Jr., Neufeld EJ, Briscoe DM. P-selectin expression in myocardium of children undergoing cardiopulmonary bypass. *J.Thorac.Cardiovasc.Surg.* 1995;**110**:924-33.

136. Lorant DE, Topham MK, Whatley RE, McEver RP, McIntyre TM, Prescott SM *et al.* Inflammatory roles of P-selectin. *J.Clin.Invest* 1993;**92**:559-70.

137. Chello M, Mastroroberto P, Quirino A, Cuda G, Perticone F, Cirillo F *et al.* Inhibition of neutrophil apoptosis after coronary bypass operation with cardiopulmonary bypass. *Ann.Thorac.Surg.* 2002;**73**:123-9.

138. Rinder CS, Bonan JL, Rinder HM, Mathew J, Hines R, Smith BR. Cardiopulmonary bypass induces leukocyte-platelet adhesion. *Blood* 1992;**79**:1201-5.

139. Ascione R, Lloyd CT, Underwood MJ, Lotto AA, Pitsis AA, Angelini GD. Inflammatory response after coronary revascularization with or without cardiopulmonary bypass. *Ann.Thorac.Surg.* 2000;**69**:1198-204.

140. Gu YJ, Schoen P, Tigchelaar I, Loef BG, Ebels T, Rankin AJ *et al.* Increased neutrophil priming and sensitization before commencing cardiopulmonary bypass in cardiac surgical patients. *Ann.Thorac.Surg.* 2002;**74**:1173-9.

141. Smith PL. The systemic inflammatory response to cardiopulmonary bypass and the brain. *Perfusion* 1996;**11**:196-9.

142. Westaby S, Saatvedt K, White S, Katsumata T, van Oeveren W, Halligan PW. Is there a relationship between cognitive dysfunction and systemic inflammatory response after cardiopulmonary bypass? *Ann.Thorac.Surg.* 2001;**71**:667-72.

143. Hill JD, Osborn JJ, Swank RL, Aguilar MJ, Gerbode F, de Lanerolle P. Experience using a new dacron wool filter during extracorporeal circulation. *Arch.Surg.* 1970;**101**:649-52.

144. Patterson RH, Jr., Kessler J, Bergland RM. A filter to prevent cerebral damage during experimental cardiopulmonary bypass. *Surg.Gynecol.Obstet.* 1971;**132**:71-4.

145. Kurusz M, Schneider B, Brown, J and Conti V. Filtration during open heart surgery: devices, techniques, opinions and complications. Acad Cardiovasc Perf 4, 123-129. 1983.

146. Miller DW, Jr., Binford JM, Hessel EA. Results of a survey of the professional activities of 811 cardiopulmonary perfusionists. *J.Thorac.Cardiovasc.Surg.* 1982;**83**:385-9.

147. Treasure T. Interventions to reduce cerebral injury during cardiac surgery - the effect of arterial line filtration. Perfusion 4, 147-152. 1989.

148. Kong R, Stygall J, Aveling W, Pugsley W, Harrison M and Newman S. Protection and anaestheia for adult cardiac surgery in the UK and Ireland. Presented at the meeting of the Association of Cardiothoracic Anaesthetists. November 1998. 1998.

149. Gott JP, Cooper WA, Schmidt FE, Jr., Brown WM, III, Wright CE, Merlino JD *et al.* Modifying risk for extracorporeal circulation: trial of four antiinflammatory strategies. *Ann.Thorac.Surg.* 1998;**66**:747-53.

150. Taggart DP, Bhattacharya K, Meston N, Standing SJ, Kay JD, Pillai R *et al.* Serum S-100 protein concentration after cardiac surgery: a randomized trial of arterial line filtration. *Eur.J.Cardiothorac.Surg.* 1997;**11**:645-9.

151. Baksaas ST, Flom-Halvorsen HI, Ovrum E, Videm V, Mollnes TE, Brosstad F *et al.* Leucocyte filtration during cardiopulmonary reperfusion in coronary artery bypass surgery. *Perfusion* 1999;**14**:107-17.

152. Mair P, Hoermann C, Mair J, Margreiter J, Puschendorf B, Balogh D. Effects of a leucocyte depleting arterial line filter on perioperative proteolytic enzyme and oxygen free radical release in patients undergoing aortocoronary bypass surgery. *Acta Anaesthesiol.Scand.* 1999;**43**:452-7.

153. Baksaas ST, Videm V, Mollnes TE, Tollofsrud S, Hetland G, Pedersen T *et al.* Leucocyte filtration during cardiopulmonary bypass hardly changed leucocyte counts and did not influence myeloperoxidase, complement, cytokines or platelets.

Perfusion 1998;**13**:429-36.

154. Hurst T, Johnson D, Cujec B, Thomson D, Mycyk T, Burbridge B *et al.* Depletion of activated neutrophils by a filter during cardiac valve surgery. *Can.J.Anaesth.* 1997;**44**:131-9.

155. Di Salvo C, Louca LL, Pattichis K, Hooper J, Walesby RK. Does activated neutrophil depletion on bypass by leukocyte filtration reduce myocardial damage? A preliminary report. *J.Cardiovasc.Surg.(Torino)* 1996;**37**:93-100.

156. Lust RM, Bode AP, Yang L, Hodges W, Chitwood WR, Jr. In-line leukocyte filtration during bypass. Clinical results from a randomized prospective trial. *ASAIO J.* 1996;**42**:M819-M822.

157. Thurlow PJ, Doolan L, Sharp R, Sullivan M, Smith B, Andersen LW. Laboratory studies of the effect of Pall extracorporeal leucocyte filters LG6 and AV6 on patients undergoing coronary bypass grafts. *Perfusion* 1996;**11**:29-37.

158. Hachida M, Hanayama N, Okamura T, Akasawa T, Maeda T, Bonkohara Y *et al.* The role of leukocyte depletion in reducing injury to myocardium and lung during cardiopulmonary bypass. *ASAIO J.* 1995;**41**:M291-M294.

159. Henriksen L,.Hjelms E. Cerebral blood flow during cardiopulmonary bypass in man: effect of arterial filtration. *Thorax* 1986;**41**:386-95.

160. Johnson D, Thomson D, Mycyk T, Burbridge B, Mayers I. Depletion of neutrophils by filter during aortocoronary bypass surgery transiently improves postoperative cardiorespiratory status. *Chest* 1995;**107**:1253-9.

161. Mihaljevic T, Tonz M, von Segesser LK, Pasic M, Grob P, Fehr J *et al.* The influence of leukocyte filtration during cardiopulmonary bypass on postoperative lung function. A clinical study. *J.Thorac.Cardiovasc.Surg.* 1995;**109**:1138-45.

162. Allen S. The role of leucocytes in the systemic inflammatory response and the potential impact of leucocyte depletion. Cardiovascular Engineering 2, 34-54. 1997.

163. Coleman SM,.Demastrice L. Leukocyte depletion reduces postoperative oxygen requirements. *Ann.Thorac.Surg.* 1994;**58**:1567-8.

164. Palanzo DA and Manley NJ. Cost effectiveness of using a leucodepleting arterial line filter. Proc American Acad Cardiovasc Pefusion 15, 124-127. 1994.

165. Sellman M, Holm L, Ivert T, Semb BK. A randomized study of neuropsychological function in patients undergoing coronary bypass surgery. *Thorac.Cardiovasc.Surg.* 1993;**41**:349-54.

166. Borowiec JW, Bylock A, van der LJ, Thelin S. Heparin coating reduces blood cell adhesion to arterial filters during coronary bypass: a clinical study. *Ann.Thorac.Surg.* 1993;**55**:1540-5.

167. Palanzo DA, Manley NJ, Montesano RM, Yeisley GL and Gordon D. Clinical evaluation of the LG-6 arterial line filter for routine open heart surgery. Perfusion 8, 489-496. 1993.

168. Sellman M, Hindmarsh T, Ivert T, Semb BK. Magnetic resonance imaging of the brain before and after open heart operations. *Ann.Thorac.Surg.* 1992;**53**:807-12.

169. Padayachee TS, Parsons S, Theobold R, Gosling RG, Deverall PB. The effect of arterial filtration on reduction of gaseous microemboli in the middle cerebral artery during cardiopulmonary bypass. *Ann.Thorac.Surg.* 1988;**45**:647-9.

170. Aris A, Solanes H, Camara ML, Junque C, Escartin A, Caralps JM. Arterial line filtration during cardiopulmonary bypass. Neurologic, neuropsychologic, and hematologic studies. *J.Thorac.Cardiovasc.Surg.* 1986;**91**:526-33.

171. Garvey JW, Willner A, Wolpowitz A, Caramante L, Rabiner CJ, Weisz D *et al.* The effect of arterial filtration during open heart surgery on cerebral function. *Circulation* 1983;**68**:II125-II128.

172. Loop FD, Szabo J, Rowlinson RD, Urbanek K. Events related to microembolism during extracorporeal perfusion in man: effectiveness of in-line filtration recorded by ultrasound. *Ann.Thorac.Surg.* 1976;**21**:412-20.

173. Dutton RC, Edmunds LH, Jr., Hutchinson JC, Roe BB. Platelet aggregate emboli produced in patients during cardiopulmonary bypass with membrane and bubble oxygenators and blood filters. *J.Thorac.Cardiovasc.Surg.* 1974;**67**:258-65.

174. Page US, Bigelow JC, Carter CR, Swank RL. Emboli (debris) produced by bubble oxygenators. Removal by filtration. *Ann.Thorac.Surg.* 1974;**18**:164-71.

175. Connell RS, Page US, Bartley TD, Bigelow JC, Webb MC. The effect on pulmonary ultrastructure of dacron-wool filtration during cardiopulmonary bypass. *Ann.Thorac.Surg.* 1973;**15**:217-29.

176. Harris DN, Bailey SM, Smith PL, Taylor KM, Oatridge A, Bydder GM. Brain swelling in first hour after coronary artery bypass surgery. *Lancet* 1993;**342**:586-7.

177. Boyle EM, Jr., Pohlman TH, Johnson MC, Verrier ED. Endothelial cell injury in cardiovascular surgery: the systemic inflammatory response. *Ann.Thorac.Surg.* 1997;**63**:277-84.

178. Thurlow PJ, Doolan L, Sharp R, Sullivan M, Smith B, Andersen LW. Studies of the effect of Pall leucocyte filters LG6 and AV6 in an in vitro simulated extracorporeal circulatory system. *Perfusion* 1995;**10**:291-300.

179. Chen YF, Tsai WC, Lin CC, Lee CS, Huang CH, Pan PC *et al.* Leukocyte depletion attenuates expression of neutrophil adhesion molecules during cardiopulmonary bypass in human beings. *J.Thorac.Cardiovasc.Surg.* 2002;**123**:218-24.

180. Sahlman A, Ahonen J, Salo JA, Ramo OJ. No impact of a leucocyte depleting

arterial line filter on patient recovery after cardiopulmonary bypass. *Acta Anaesthesiol.Scand.* 2001;**45**:558-63.

181. Anderson RE, Hansson LO, Liska J, Settergren G, Vaage J. The effect of cardiotomy suction on the brain injury marker S100beta after cardiopulmonary bypass. *Ann.Thorac.Surg.* 2000;**69**:847-50.

182. Zimmer DB, Cornwall EH, Landar A, Song W. The S100 protein family: history, function, and expression. *Brain Res.Bull.* 1995;**37**:417-29.

183. Kligman D,.Hilt DC. The S100 protein family. *Trends Biochem.Sci.* 1988;**13**:437-43.

184. Pisa EK. Serum S-100 protein in stroke and cardiac surgery. *Stroke* 1999;**30**:1153-4.

185. Vaage J,.Anderson R. Biochemical markers of neurologic injury in cardiac surgery: the rise and fall of S100beta. *J.Thorac.Cardiovasc.Surg.* 2001;**122**:853-5.

186. Persson L, Hardemark HG, Gustafsson J, Rundstrom G, Mendel-Hartvig I, Esscher T *et al.* S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: markers of cell damage in human central nervous system. *Stroke* 1987;**18**:911-8.

187. Persson L, Hardemark H, Edner G, Ronne E, Mendel-Hartvig I, Pahlman S. S-100 protein in cerebrospinal fluid of patients with subarachnoid haemorrhage: a potential marker of brain damage. *Acta Neurochir.(Wien.)* 1988;**93**:116-22.

188. Johnsson P, Lundqvist C, Lindgren A, Ferencz I, Alling C, Stahl E. Cerebral complications after cardiac surgery assessed by S-100 and NSE levels in blood. *J.Cardiothorac.Vasc.Anesth.* 1995;**9**:694-9.

189. Westaby S, Saatvedt K, White S, Katsumata T, van Oeveren W, Bhatnagar NK *et al.* Is there a relationship between serum S-100beta protein and neuropsychologic dysfunction after cardiopulmonary bypass? *J.Thorac.Cardiovasc.Surg.* 2000;**119**:132-7.

190. Aberg T. Signs of brain cell injury during open heart operations: past and present. *Ann.Thorac.Surg.* 1995;**59**:1312-5.

191. Westaby S, Johnsson P, Parry AJ, Blomqvist S, Solem JO, Alling C *et al.* Serum S100 protein: a potential marker for cerebral events during cardiopulmonary bypass. *Ann.Thorac.Surg.* 1996;**61**:88-92.

192. Lloyd CT, Ascione R, Underwood MJ, Gardner F, Black A, Angelini GD. Serum S-100 protein release and neuropsychologic outcome during coronary revascularization on the beating heart: a prospective randomized study. *J.Thorac.Cardiovasc.Surg.* 2000;**119**:148-54.

193. Taggart DP, Mazel JW, Bhattacharya K, Meston N, Standing SJ, Kay JD *et al.* Comparison of serum S-100 beta levels during CABG and intracardiac operations. *Ann.Thorac.Surg.* 1997;**63**:492-6.

194. Grocott HP, Croughwell ND, Amory DW, White WD, Kirchner JL, Newman MF. Cerebral emboli and serum S100beta during cardiac operations. *Ann.Thorac.Surg.* 1998;**65**:1645-9.

195. Kilminster S, Treasure T, McMillan T, Holt DW. Neuropsychological change and S-100 protein release in 130 unselected patients undergoing cardiac surgery. *Stroke* 1999;**30**:1869-74.

196. Herrmann M, Ebert AD, Tober D, Hann J, Huth C. A contrastive analysis of release patterns of biochemical markers of brain damage after coronary artery bypass grafting and valve replacement and their association with the neurobehavioral outcome after cardiac surgery. *Eur.J.Cardiothorac.Surg.* 1999;**16**:513-8.

197. Anderson RE, Hansson LO, Vaage J. Release of S100B during coronary artery bypass grafting is reduced by off-pump surgery. *Ann.Thorac.Surg.* 1999;**67**:1721-5.

198. Jonsson H, Johnsson P, Alling C, Backstrom M, Bergh C, Blomquist S. S100beta after coronary artery surgery: release pattern, source of contamination, and relation to neuropsychological outcome. *Ann.Thorac.Surg.* 1999;**68**:2202-8.

199. Anderson RE, Hansson LO, Nilsson O, Liska J, Settergren G, Vaage J. Increase in serum S100A1-B and S100BB during cardiac surgery arises from extracerebral sources. *Ann.Thorac.Surg.* 2001;**71**:1512-7.

200. Hashimoto K, Sasaki T, Hachiya T, Takakura H, Onoguchi K, Nagahori R *et al.* Real time measurement of heparin concentration during cardiopulmonary bypass. *J.Cardiovasc Surg.(Torino)* 1999;**40**:645-51.

201. Rey A. L'examen clinique en psychologie. Paris: Presses Universitaires de France., 1964.

202. Smith A. The symbol digit modalities test: a neuropsychological test for economic screening of learning and other cerebral disorders. Learning Disorders 3, 83-89. 1968.

203. Talland GA and Schwab RS. Performance with multiple sets in Parkinson's disease. Neuropsychologia 2, 45-53. 1964.

204. Green AJ, Keir G, Thompson EJ. A specific and sensitive ELISA for measuring S-100b in cerebrospinal fluid. *J.Immunol.Methods* 1997;**205**:35-41.

205. Petzold A, Keir G, Lim D, Smith M, Thompson EJ. Cerebrospinal fluid (CSF) and serum S100B: release and wash-out pattern. *Brain Res.Bull.* 2003;**61**:281-5.

206. Galloway MJ, Mackie MJ, McVerry BA. Elastase-alpha 1 antitrypsin complexes in acute leukaemia. *Thromb.Res.* 1985;**38**:311-20.

207. Brower MS,.Harpel PC. Alpha-1-antitrypsin-human leukocyte elastase complexes in blood: quantification by an enzyme-linked differential antibody immunosorbent assay and comparison with alpha-2-plasmin inhibitor-plasmin complexes. *Blood*

1983;**61**:842-9.

208. Baugh RJ,.Travis J. Human leukocyte granule elastase: rapid isolation and characterization. *Biochemistry* 1976;**15**:836-41.

209. Parsonnet V, Dean D, Bernstein AD. A method of uniform stratification of risk for evaluating the results of surgery in acquired adult heart disease. *Circulation* 1989;**79**:I3-12.

210. Whitaker D. The use of Z scores in assessing neuropsychological change after cardiac operations. *Ann.Thorac.Surg.* 2003;**75**:1066-7.

211. Selnes OA, Royall RM, Grega MA, Borowicz LM, Jr., Quaskey S, McKhann GM. Cognitive changes 5 years after coronary artery bypass grafting: is there evidence of late decline? *Arch.Neurol.* 2001;**58**:598-604.

212. Grocott HP, Mackensen GB, Grigore AM, Mathew J, Reves JG, Phillips-Bute B *et al*. Postoperative hyperthermia is associated with cognitive dysfunction after coronary artery bypass graft surgery. *Stroke* 2002;**33**:537-41.

213. Kadoi Y, Saito S, Kunimoto F, Goto F, Fujita N. Comparative effects of propofol versus fentanyl on cerebral oxygenation state during normothermic cardiopulmonary bypass and postoperative cognitive dysfunction. *Ann.Thorac.Surg.* 2003;**75**:840-6.

214. Patel NC, Deodhar AP, Grayson AD, Pullan DM, Keenan DJ, Hasan R *et al*. Neurological outcomes in coronary surgery: independent effect of avoiding cardiopulmonary bypass. *Ann.Thorac.Surg.* 2002;**74**:400-5.

215. Plochl W,.Cook DJ. Quantification and distribution of cerebral emboli during cardiopulmonary bypass in the swine: the impact of PaCO2. *Anesthesiology* 1999;**90**:183-90.

216. Roach GW, Kanchuger M, Mangano CM, Newman M, Nussmeier N, Wolman R *et al*. Adverse cerebral outcomes after coronary bypass surgery. Multicenter Study of Perioperative Ischemia Research Group and the Ischemia Research and Education Foundation Investigators. *N.Engl.J.Med.* 1996;**335**:1857-63.

217. Taggart DP, Browne SM, Wade DT, Halligan PW. Neuroprotection during cardiac surgery: a randomised trial of a platelet activating factor antagonist. *Heart* 2003;**89**:897-900.

218. Heyer EJ, Adams DC, Delphin E, McMahon DJ, Steneck SD, Oz MC *et al*. Cerebral dysfunction after coronary artery bypass grafting done with mild or moderate hypothermia. *J.Thorac.Cardiovasc.Surg.* 1997;**114**:270-7.

219. Brown WR, Moody DM, Challa VR, Stump DA. Histologic Studies of Brain Microemboli in Humans and Dogs After Cardiopulmonary Bypass. *Echocardiography.* 1996;**13**:559-66.

220. Taylor RL, Borger MA, Weisel RD, Fedorko L, Feindel CM. Cerebral microemboli

during cardiopulmonary bypass: increased emboli during perfusionist interventions. *Ann.Thorac.Surg.* 1999;**68**:89-93.

221. Fearn SJ, Pole R, Burgess M, Ray SG, Hooper TL, McCollum CN. Cerebral embolisation during modern cardiopulmonary bypass. *Eur.J.Cardiothorac.Surg.* 2001;**20**:1163-7.

222. Musumeci F, Feccia M, MacCarthy PA, Ellis GR, Mammana L, Brinn F *et al.* Prospective randomized trial of single clamp technique versus intermittent ischaemic arrest: myocardial and neurological outcome. *Eur.J.Cardiothorac.Surg.* 1998;**13**:702-9.

223. Brown WR, Moody DM, Challa VR, Stump DA, Hammon JW. Longer duration of cardiopulmonary bypass is associated with greater numbers of cerebral microemboli. *Stroke* 2000;**31**:707-13.

224. Benaroia M, Baker AJ, Mazer CD, Errett L. Effect of aortic cannula characteristics and blood velocity on transcranial doppler-detected microemboli during cardiopulmonary bypass. *J.Cardiothorac.Vasc.Anesth.* 1998;**12**:266-9.

225. Mullges W, Berg D, Toyka KV. Bilateral cerebral emboli monitoring during extracorporeal circulation. *Ultrasound Med.Biol.* 1999;**25**:755-8.

226. Ohto T, Yamamoto F, Nakajima N. Evaluation of leukocyte-reducing arterial line filter (LG6) for postoperative lung function, using cardiopulmonary bypass. *Jpn.J.Thorac.Cardiovasc.Surg.* 2000;**48**:295-300.

227. Albert AA, Beller CJ, Walter JA, Arnrich B, Rosendahl UP, Priss H *et al.* Preoperative high leukocyte count: a novel risk factor for stroke after cardiac surgery. *Ann.Thorac.Surg.* 2003;**75**:1550-7.

228. Nashef SA, Carey F, Silcock MM, Oommen PK, Levy RD, Jones MT. Risk stratification for open heart surgery: trial of the Parsonnet system in a British hospital. *BMJ* 1992;**305**:1066-7.

229. Bridgewater B, Neve H, Moat N, Hooper T, Jones M. Predicting operative risk for coronary artery surgery in the United Kingdom: a comparison of various risk prediction algorithms. *Heart* 1998;**79**:350-5.

230. Wynne-Jones K, Jackson M, Grotte G, Bridgewater B. Limitations of the Parsonnet score for measuring risk stratified mortality in the north west of England. The North West Regional Cardiac Surgery Audit Steering Group. *Heart* 2000;**84**:71-8.

231. Roques F, Michel P, Goldstone AR, Nashef SA. The logistic EuroSCORE. *Eur.Heart J.* 2003;**24**:881-2.

232. Roques F, Nashef SA, Michel P, Pinna PP, David M, Baudet E *et al.* Does EuroSCORE work in individual European countries? *Eur.J.Cardiothorac.Surg.* 2000;**18**:27-30.

233. Nashef SA, Roques F, Michel P, Gauducheau E, Lemeshow S, Salamon R.

European system for cardiac operative risk evaluation (EuroSCORE). *Eur.J.Cardiothorac.Surg.* 1999;**16**:9-13.

234. Feussner JR,.Matchar DB. When and how to study the carotid arteries. *Ann.Intern.Med.* 1988;**109**:805-18.

235. Magyar MT, Nam EM, Csiba L, Ritter MA, Ringelstein EB, Droste DW. Carotid artery auscultation--anachronism or useful screening procedure? *Neurol.Res.* 2002;**24**:705-8.

236. Parsonnet V, Bernstein AD, Gera M. Clinical usefulness of risk-stratified outcome analysis in cardiac surgery in New Jersey. *Ann.Thorac.Surg.* 1996;**61**:S8-11.

Appendix 1 - Ethical Approval

UCL
HOSPITALS

The University College London Hospitals

The Joint UCL/UCLH Committees on the Ethics of Human Research

Committee A Chairman: Dr F D Thompson

Please address all correspondence to:
Mrs Iwona Nowicka
Research & Development Directorate
9th Floor, St Martin's House
140 Tottenham Court Road, LONDON W1P 9LN
Tel. 0171- 380 9579 Fax 0171-380 9937
e-mail: i.nowicka@academic.uclh.nthames.nhs.uk

Mr D Whitaker
Honorary Research Fellow
Department of Cardothoracic Surgery
The Middlesex Hospital

23 October 1998

By post and fax: 0171 436 1755

Dear Mr Whitaker

Study No: **98/0216**
Title: **Investigation into the use of Leucocyte depleting filters during cardiopulmonary bypass; Do they reduce neuropsychological deficit and the inflammatory response?**

The committee met and discuss your proposal, and were basically happy. One minor amendment might be included in that the information sheet seems to suggest that every patient is going to have some memory defect. If this is the case, it should be left in, but we wondered whether a phrase such as 'may have a memory defect' should be included. Apart from this, once your study has been approved by the Trust (via the R&D office) you may proceed with your research.

Please note that it is important that you notify the Committee of any adverse events or changes (name of investigator etc) relating to this project. You should also notify the Committee on completion of the project, or indeed if the project is abandoned. **Please remember to quote the above number in any correspondence.**

Yours sincerely

pp Dr F D Thompson
Chairman

Appendix 2 - Patient Information Sheet

The University College London Hospitals

HOLMES SELLORS CARDIOTHORACIC UNIT

The Middlesex Hospital
Mortimer Street
London W1N 8AA

LEUCOCYTE DEPLETION TRIAL INFORMATION SHEET

Principal Investigators

Mr WB Pugsley Professor SP Newman Professor MG Harrison Mrs J Stygall Mr DC Whitaker

Contact: Donald Whitaker, Holmes Sellors Dept of Cardiac Surgery, Middlesex Hospital - 0207 679 9419

You are soon to have coronary artery bypass surgery performed at the Middlesex Hospital. In addition you are invited to take part in the following research study.

Purpose of the Research

Coronary artery bypass surgery usually involves the use of the heart-lung bypass machine which takes over the function of the heart and lungs during the procedure. It is known that a small proportion of patients may have subtle changes in memory, concentration and attention after such cardiac surgery. The cause of this is unknown. However, previous studies conducted at this hospital have shown that arterial line filters which filter the blood as it leaves the bypass machine to return to the patient can reduce the incidence of changes in memory and so these filters are now used routinely. A newer filter is now available which may in theory be superior at filtering the blood. The purpose of this study is to evaluate this new filter which, in addition to filtering out bubbles and particles which may arise in the blood, also filters out some of the white blood cells ("leucocyte depleting filter").

What the Study Involves for You

If you agree to take part in this study, you will be randomly allocated to receive either the standard filter or the leucocyte depleting filter. The doctors looking after you nor you will be able too know which filter is used. If you agree to take part in this study, we will ask you to have some tests of memory and concentration before your surgery and these will then be repeated when you return to clinic about six weeks after surgery. The tests are conducted in the hospital by a friendly psychologist and they take about one to one and a half hours. During surgery blood flow to your brain will be monitored using a Doppler probe. This probe uses ultrasound similar to the probes which are used to scan

The University College London Hospitals

HOLMES SELLORS CARDIOTHORACIC UNIT

The Middlesex Hospital
Mortimer Street
London W1N 8AA

LEUCOCYTE DEPLETION TRIAL INFORMATION SHEET

pregnant women. The probe simply sits on the side of the head and is not harmful. Some extra blood tests will be performed before, during and after surgery at the same time that routine blood tests are taken. All other aspects of your medical care will be the same as if you were not in the study.

What if You Do Not Wish to Take Part in the Study

If you do not wish to take part in the study then you will be treated in the normal way and will receive the standard filter. You do not have to take part in the study if you do not wish to and you are free to withdraw from the study at any time. Although you will be asked to sign a consent form to give us permission to include you in the study this is not in any way binding. If you wish to withdraw from the study please inform one of the doctors looking after you.

Benefits of the Study

It is possible that there are benefits to the leucocyte filter however there is currently no information on this and this is why the study is being carried out.

Risks of the Study

We are not aware of any risks involved in taking part in this study. However they may exist and we will be monitoring any side effects.

Confidentiality of Records

Records of you details will be kept only by the study investigators and will be kept confidential. The results of the trial may be published but the identity of individuals will not be disclosed.

Further Questions

If you have any further questions after I have spoken to you, please contact me on the ward or on 0207 679 9122.

Appendix 3 - Patient Case Report File

INDIVIDUAL CASE REPORT FORM

LEUCOCYTE DEPLETION TRIAL

An investigation into the use of leucocyte depletion during cardiopulmonary bypass; Do they reduce the neuropsychological deficit and the inflammatory response?

Patient Initials	Trial No.

Hospital No.	Consultant

Principal Investigators

Mr WB Pugsley
Middlesex Hospital
Mortimer St
London
W1N 8AA

Professor SP Newman
Middlesex Hospital
Mortimer St
London
W1N 8AA

Professor MG Harrison
Middlesex Hospital
Mortimer St
London
W1N 8AA

Mrs J Stygall
Middlesex Hospital
Mortimer St
London
W1N 8AA

Mr DC Whitaker
Middlesex Hospital
Mortimer St
London
W1N 8AA

(If found, please return to Donald Whitaker, Holmes Sellors Dept of Cardiac Surgery, Middlesex Hospital - 0207 679 9419)

Index

INFORMED CONSENT FORM

An investigation into the use of leucocyte depletion during cardiopulmonary bypass; Do they reduce the neuropsychological deficit and the inflammatory response?

Investigators: Mr Pugsley (Consultant Cardiothoracic Surgeon)
Professor Newman (Professor of Psychiatry)
Professor Harrison (Professor of Neurology)
Mrs Stygall (Lecturer in Health Psychology)
Mr Whitaker (Research Fellow In Cardiac Surgery)

1) HAVE YOU READ THE INFORMATION SHEET ABOUT THIS STUDY?	YES/NO
2) HAVE YOU HAD AN OPPORTUNITY TO ASK QUESTIONS AND DISCUSS THIS STUDY?	YES/NO
3) HAVE YOUR QUESTIONS BEEN ANSWERED SATISFACTORILY?	YES/NO
4) HAVE YOU BEEN GIVEN ENOUGH INFORMATION ABOUT THIS STUDY?	YES/NO
5) I HAVE SPOKEN TO DR/MR..... ABOUT THIS STUDY	YES/NO
6) DO YOU UNDERSTAND THAT YOU ARE FREE TO WITHDRAW FROM THIS STUDY AT ANY TIME?	YES/NO
7) DO YOU AGREE TO TAKE PART IN THIS STUDY?	YES/NO

Doctors Declaration

I have explained the above study to a patient in the presence of a witness

Signed **Date** **Time**

Patients Declaration

The nature and purpose of the study has been explained to me and I agree to participate

Signed **Date** **Time**

UCL HOSPITALS, MORTIMER STREET, LONDON W1N 8AA

Assessment Dates

Assessment 1 **Outpatient or Inpatient**					
Prior to scheduled surgery date					
d	d	m	m	y	y
Assessment 2 **Inpatient**					
Intra-operative					
d	d	m	m	y	y
Assessment 3 **Inpatient**					
Post -operative					
d	d	m	m	y	y
Assessment 4 **Outpatient**					
Post -operative					
d	d	m	m	y	y

Assessment 1

Inpatient or Outpatient prior to surgery

- **Inclusion and Exclusion criteria**
- **Informed Consent**
- **Demographic Data**
- **General Medical History**
- **Cardiovascular History**
- **Parsonnet Score**
- **General Clinical Exam**
- **Neuropsychological Testing**
- **Blood tests** **FBC**
  U and Es
  S100
  Elastase

INCLUSION CRITERIA

	YES	NO
1. Able to speak and read English	☐	☐
2. Aged 80 or less	☐	☐
3. Elective 1st time CABG	☐	☐
4. No history of TIA or CVA	☐	☐
5. No history of renal failure	☐	☐
6. White cell count 3-11 X 10^6	☐	☐
7. Has signed the consent form	☐	☐
8. Is not enrolled in another study	☐	☐

NOTE

IF ANY "NO" BOX IS TICKED, THE PATIENT IS NOT ELIGIBLE FOR THE STUDY

	YES	NO
Is the patient eligible for the study?	☐	☐

If yes,

- **Complete details of scheduled date of surgery and assessment dates at front of file.**
- **Inform Perfusionists**
- **Inform Anaesthetists (theatre list)**
- **Continue with demographics and medical exam.**

DEMOGRAPHICS

Date of Birth ___/__/___.

Age _____.

Sex ☐ male

☐ female

Years of Education _____.
(5 onwards)

Race

c	caucasian
b	black
a	asian
o	oriental
os	other, specify .....

Height (m)		Weight (kg)		BMI	

GENERAL MEDICAL HISTORY

Enter any significant medical/surgical history

☐ Tick if none

DESCRIPTION	DATE OF ONSET	DATE CEASED

MEDICATION	DOSE

PARSONNET SCORE		
Female	1	
Obesity (BMI > 30)	3	
Diabetes	3	
Hypertension (Systolic BP > 140mmHg)	3	
Ejection Fraction Good (>50%)	0	
Mod (30-49)	2	
Poor (<30%)	4	
Age 70-74	7	
75-79	12	
80 +	20	
Re-operation First	5	0
Second	10	0
Pre-operative IABP	2	
Left Ventricular Aneurysm	5	
Emergency Surgery	10	0
Dialysis Dependency	10	0
Catastrophic States	10-50	0
Other Rare Circumstances (eg severe asthma)	2-10	
Valve Surgery	N/A	0

Total	

CARDIOVASCULAR HISTORY

EVENT	YES	NO	START DATE	FINISH DATE
Stable Angina	grade:		D D M M Y Y	D D M M Y Y
Unstable Angina	grade:		D D M M Y Y	D D M M Y Y
Myocardial Infarction			D D M M Y Y	D D M M Y Y
Dyspnoea	grade:		D D M M Y Y	D D M M Y Y
Angiogram			D D M M Y Y	D D M M Y Y
Angioplasty or Stenting			D D M M Y Y	D D M M Y Y
HYPERTENSION			D D M M Y Y	D D M M Y Y
LV Aneurysm			D D M M Y Y	D D M M Y Y
Peripheral Vascular Disease			D D M M Y Y	D D M M Y
Time of Angio	months ago :			
Time on waiting list	months			
? Working at present				

If Not working, due to 1)normal retirement pre-symptoms, 2)early retirement due to symptoms or 3)off sick due to symptoms

Smoker	Yes	No	Ex	PACK YEARS	D D M M Y Y	D D M M Y Y

EJECTION FRACTION	Good (>50%)	Moderate (30-49%)	Poor (<30%)

UCL HOSPITALS, MORTIMER STREET, LONDON W1N 8AA

GENERAL PHYSICAL EXAM

SYSTEM	NORMAL	ABNORMAL	NOT KNOWN
1.CARDIOVASCULAR			
? CAROTID BRUITS			
2.RESPIRATORY			
3.GI			
4.MUSCULOSKELETAL			
5.URINALYSIS			
6.ENDOCRINE			

DETAILS OF ABNORMALITIES FOUND	
SYSTEM NO.	FINDINGS

Neuropsychological Tests

NART	

Rey Auditory Verbal Learning Test							
Score	1:	2:	3:	4:	5:	6:	7:

Trail Making Test A		Trail Making Test B	
Time to Completion		**Time to Completion**	

Grooved Pegboard Test	
Time to Completion, dominant hand	
Time to Completion, on-dominant hand	

Symbol Digits Modalities Test			
Total time		**Number correct answers**	

Non-verbal Memory Test			
Number correct		**Time to completion**	

Letter Cancellation Test			
Number of Intrusion Errors Made			
Number of targets missed		**Time to completion**	

Choice Reaction Time Test			
A **Accuracy**		A **Time**	
B **Accuracy**		B **Time**	

Speilberger state		Speilberger trait	
CESD			

Assessment 2

Inpatient – intra-operative

- **Transcranial Doppler Data**
- **Blood tests**
- **Anaesthesia Protocol Check**
- **Intra-operative Vital Signs**
- **Record of Intra-operative Events**

START (KNIFE TO SKIN)		FINISH (SKIN CLOSURE)		OP TIME	
STERNOTOMY					

TRANSCRANIAL DOPPLER											
TRACE OBTAINED	RIGHT	Good mod poor		LEFT	Good Mod poor		NEITHER				
DEPTH		POWER			GAIN						

TIME	ACTUAL TIME	MCA BLOOD VELOCITY
Pre CPB (just before cannulation)		
Pre-CPB (immed prior to CPB)		
During CPB (15mins into CPB)		
Post-CPB (10mins after stopping)		

TIME	MICROEMBOLI COUNT
For 15mins after sternotomy	
During CPB	

INTRA-OP BLOOD TESTS			
TIME			
	Pre-Bypass (at cannulation)	On Bypass (30 mins)	Off Bypass (10 mins)
FBC (3mls)			
S100 (3mls) *			
Elastase (3mls) **			

* LITH HEP TUBE ** Na CITRATE TUBE

mls of blood needed	9	9	12

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INTRA-OP VITAL SIGNS					
TIME		MAP	SBP	DBP	TEMP
	Pre-induction				
	Post-induction				
	Pre-sternotomy				
	Post-sternotomy				
	Pre-CPB				
	Post-CPB				
	Post-skin closure				

ON CPB		OFF CPB		CPB TIME	
NO OF GRAFTS		TOTAL CROSS CLAMP TIME		? LIMA YES / NO	
INDIVIDUAL CROSS CLAMP TIMES	OFF TIME	OFF TIME	OFF TIME	OFF TIME	OFF TIME
CLINICAL SIGNS OF AORTIC ATHEROMA OR CALCIFICATION		NO			
METHOD OF CANNULATION SIDE CLAMP OR NOT?	YES	NO			
TIME OF CANNULATION	TIME OF DE-CANNULATION				

TEMPERATURE DATA	
MINIMUM TEMP	MAXIMUM TEMP
TIME OF START OF REWARM	
TIME TAKEN TO REACH 37°C FROM MINIMUM	

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Assessment 3

Inpatient – immediately post op until discharge

- **Cardiovascular and Respiratory Vital Signs**
- **Blood Loss**
- **Adverse Events and Complications**

Cardiovascular and Respiratory Vital Signs

Time(Post CPB)	Pulse	SBP	DBP	Resp Rate	pO2	O2 SATS	FiO2	Temp
1Hr								
2Hr								
3Hr								
4Hr								
8Hr								
12Hr								
16Hr								
20Hr								
24Hr								

NOTROPES	YES / NO
Adren / norad / dopamine / other	DURATION (HRS) :

TIME OF EXTUBATION	
TIME TO EXTUBATION (HRS)	

BLOOD LOSS	
TIME	**CUMMULATIVE LOSS**
6Hr	
12Hr	
18Hr	
24Hr	

TRANSFUSIONS (RCC) GIVEN		
DATE	TIME	DEPLETED OR NOT

Assessment 4

Out-patient at 6 weeks

- **Neuropsychological Tests**

Neuropsychological Tests

Rey Auditory Verbal Learning Test							
Score	1:	2:	3:	4:	5:	6:	7:

Trail Making Test A		Trail Making Test B	
Time to Completion		**Time to Completion**	

Grooved Pegboard Test	
Time to Completion, dominant hand	
Time to Completion, non-dominant hand	

Symbol Digits Modalities Test			
Total time		**Number correct answers**	

Non-verbal Memory Test			
Number correct		**Time to completion**	

Letter Cancellation Test			
Number of Intrusion Errors Made			
Number of targets missed		**Time to completion**	

Choice Reaction Time Test			
A **Accuracy**		A **Time**	
B **Accuracy**		B **Time**	

Speilberger state		CESD	

SUBJECTIVE COGNITION SCALE

Since your operation are you:	More	No Change	Less
More or less alert and thinking more or less clearly			
Forgetting things, for example, things that have happened recently, where you put things or keeping appointments			
Have more or less minor accidents, for example dropping things, tripping			
Reacting more or less quickly to things that are said or done			
Having more or less difficulty in solving problems and learning new things			
Having more or less difficulty in making decisions			
Able to keep your attention to a task for more or less time			
Making more or less mistakes			
Having more or less difficulty in doing things which involve thought and concentration			

Appendix 4 - Publication Arising from Thesis

ELSEVIER

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EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY

www.elsevier.com/locate/ejcts

The effect of leucocyte-depleting arterial line filters on cerebral microemboli and neuropsychological outcome following coronary artery bypass surgery☆

Donald C. Whitaker, Stanton P. Newman*, Jan Stygall, Chris Hope-Wynne, Michael J.G. Harrison, Robin K. Walesby

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Received 26 November 2002; received in revised form 31 July 2003; accepted 12 November 2003

Abstract

Objective: A randomised clinical trial sought evidence as to whether leucocyte-depleting (LD) arterial line filters added a further degree of neuroprotection in patients undergoing elective coronary artery bypass graft (CABG) surgery. **Methods**: One hundred and ninety-two patients were randomised to the use of a Pall Leukoguard-6 LD filter or either an Avecor Affinity or Pall Autovent-6 control filter. Cerebral microemboli during surgery were recorded by transcranial Doppler (TCD) monitor over the right middle cerebral artery. Evidence of cerebral impairment was obtained by comparing patients' performance in a neuropsychological (NP) test battery (nine tests) administered 6–8 weeks post-operatively with their pre-operative scores. **Results**: The groups proved well balanced in pre-operative variables. During cardiopulmonary bypass (CPB) the *median* number and range of microemboli was 15 (3–180) in the LD group compared to 67 (5–846) and 55 (2–773) for the Avecor and AV6 groups, respectively ($P < 0.0001$). One hundred and sixty-two patients completed all the NP tests. The LD group showed better post-operative performance in all but one of the nine tests although the difference in a total change score just failed to reach significance ($P = 0.07$ one-tailed t-test). **Conclusions**: LD filtration during CABG reduced the number of cerebral microemboli recorded by TCD and showed a strong trend towards improving NP performance post-operatively. These findings suggest that the use of such filters in CABG surgery may offer increased neuroprotection.

Keywords: Coronary artery bypass graft surgery; Neuropsychology; Leucocyte; Filters; Microemboli

1. Introduction

Diffuse cerebral injury leading to cognitive dysfunction remains a concern associated with coronary artery bypass graft (CABG) surgery. Defined by a reduction in test performance it affects approximately 20–40% of patients followed up 2–3 months after surgery [1]. Its cause is probably multi-factorial involving poor cerebral perfusion, inflammatory responses to surgery, and particularly cerebral microembolism, as detected by transcranial Doppler (TCD).

Pugsley et al. [2] have demonstrated a correlation between the number of microemboli recorded during surgery and the incidence of post-operative neuropsychological (NP) deficits. Clark [3] subsequently also found that patients with highest counts of emboli had the worst NP outcome. A study by Neville et al. [4] however found that more microemboli accompany valve surgery than CABG but without a comparable difference in cognitive outcome. The weight of evidence has been sufficient to lead to widespread use of arterial line filters especially as Pugsley's randomised study demonstrated both reduced microemboli, and improved NP performance when an arterial line filter was added to the cardiopulmonary bypass (CPB) circuit. More recently, the screen filter with an additional leucocyte-depleting (LD) element (the Pall Leukoguard-6 or LG-6 filter) has been introduced following experimental studies in animals showing protection from inflammatory mediated damage to a number of organs after CPB [5]. In the clinical

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situation there is some suggestive evidence of improved post-bypass pulmonary function in particular [6,7].

The purpose of this study was to test the effect of LD filters on intra-operative microembolic load to the brain and NP sequelae when compared with conventional arterial line filters. To this end we performed a randomised controlled clinical trial of patients undergoing elective CABG surgery.

2. Materials and methods

Between May 1999 and September 2001, 198 patients were prospectively randomised to three groups. The initial plan was to compare two filters namely the LG-6 as the test filter and our unit's routine arterial line filter, the Avecor Affinity as a control. Both have a 40 μm screen polyester element and the LG-6 has an additional non-woven polyester mesh downstream of the screen which gives it its LD properties. However, the LG-6 is an autoventing filter which allows the LG-6 to vent air filtered by the screen directly to the atmosphere. In contrast, the Avecor filter vents any air back to the oxygenator through a closed vent line. From the midpoint of the trial we therefore used a second control filter, the Pall Autovent-6 (AV-6), which is autoventing in an identical manner to the test filter but has no LD element.

Patients scheduled for elective CABG surgery in the department of cardiothoracic surgery at The Middlesex Hospital (University College London Hospitals NHS Trust) were invited to participate. We recruited patients of all six consultants from the elective waiting list and inpatients awaiting urgent surgery. Prior approval was obtained from the hospital ethics committee and all patients gave written informed consent before participation. The following patients were excluded from recruitment.

Patients over 80 years or having re-do procedures were excluded as were any with a history of transient ischaemic attacks or strokes or if they had a cardiac arrest in the preceding month. All patients with carotid bruits were excluded. Patients with insulin-dependent diabetes mellitus, pre-operative renal failure (defined as creatinine >150 mmol/l) or a pre-operative leucocyte count outside the normal range of $3-11 \times 10^6/mm^3$ were also excluded.

Randomisation was achieved with sealed envelopes given to the perfusion department, whose staff then set up the bypass circuit using the appropriate filter. The perfusionists kept a record of the filter used for each patient and all other investigators were blinded to the patients' assignment.

2.1. Surgical and anaesthetic technique

Anaesthesia, surgical technique, CPB, control of blood pressure and perfusion pressure, control of pH and p_aCO_2 all followed a set protocol.

Pre-medication consisted of morphine (5–10 mg) and hyoscine (0.3–0.4 mg). Diazepam (5–10 mg) or temazepam (10–20 mg) was given according to the anaesthetist's preference. Intravenous midazolam was used during insertion of peripheral and central cannulae, as necessary. Induction was carried out with midazolam (2–10 mg), fentanyl (0.5–2.0 mg), pancuronium and or suxamethonium. Maintenance was with nitrous oxide and oxygen and then isoflurane.

All surgeons used the technique of intermittent cross clamp fibrillation for myocardial protection.

The details of the CPB technique included crystalloid prime, non-pulsatile flow, using a flat membrane oxygenator, a flow rate of 2.4 l/m^2 per min at 37 °C reducing to 1.8 l/m^2 per min at 32 °C. Perfusion pressure was maintained between 50 and 70 mmHg using phenylephrine and phentolamine. p_aCO_2 was maintained at 5.3 kPa (alpha stat).

Intra-operative TCD was used to record cerebral blood flow and monitor microembolic events. Records were taken from the right middle cerebral artery through the temporal window using a Nicolet EME Pioneer 4040 system and a 2 MHz pulsed wave transducer secured by a headband. All subsequent measurements were made off-line, using international consensus criteria [8] and the second observer reviewed 10% of tapes to assess inter-rater reliability. Recordings of velocity were made 10 and 1 min before bypass, 15, 30 and 60 min into CPB and 10 min after discontinuing bypass. Microembolic events were counted throughout CPB and for 10 min after bypass.

2.2. Blood tests

EDTA blood samples for full blood count (haemoglobin, platelets, white cell count and differential) were taken (1) pre-operatively, (2) at cannulation, (3) after 30 min of CPB, (4) 10 min after coming off CPB, (5) first post-operative day and (6) second post-operative day.

2.3. Neuropsychological assessments

One psychologist administered the battery of nine NP tests. The assessments were conducted during the pre-operative week, usually on the day before, and 6–8 weeks later during a routine clinic. On each occasion the patient was tested in the same quiet room. The test battery consisted of the Rey Auditory Verbal Learning Test, Trailmaking A and B, Grooved Pegboard (dominant and non-dominant), Symbol Digit Replacement Test, Non-verbal Memory, Letter Cancellation and Choice Reaction Time as previously described [9].The Center for Epidemiologic Studies Depression Scale (CES-D) [10] was used to assess depression and the Spielberger state and trait anxiety inventory (STAI) [11] was used to measure state anxiety on each occasion of NP testing and trait anxiety pre-operatively.

Table 1
Clinical outcomes and attendance at follow-up

	All, $n = 192$	%	LD, $n = 82$	%	Av, $n = 73$	%	AV-6, $n = 37$	%
Dead	9	4.6	4	4.9	4	5.5	1	2.7
CVA	2	1.1	0	0	2	2.7	0	0
Medically unable to FU	11	5.7	5	6.1	4	5.5	2	5.4
Unwilling to attend FU	8	4.2	2	2.4	4	5.5	2	5.4
Completed	162	84.4	71	86.6	59	80.8	32	86.5
Total	192	100	82	100	73	100	37	100

LD, LG-6 leucocyte-depleting filter group; Av, Avecor conventional filter group; AV-6, AV-6 conventional filter group. There were no differences between groups (χ^2-test).

To make optimal use of the NP data each patients pre- and post-operative scores were compared to create a change score. Z scores were thus obtained for individual tests and for an overall score. (Z score was calculated as $(X_2 - X_1)/\mu SD1$). A positive Z score indicates an improved performance and the higher the Z score the better the improvement, whereas a negative score indicates a deterioration. Differences were analysed between groups using the one-tailed t-test.

In addition a secondary endpoint for neuropsychology was the proportion of patients showing a drop in score of ≥ 1 SD from their pre-operative performance in two or more tests were compared (χ^2). This is a conventional incidence analysis method but is relatively crude as it reduces a score to a binary outcome [1].

3. Results

3.1. Recruitment

One hundred and ninety-eight patients were recruited and randomised to one of the three groups. Six patients were removed from the study between the time of recruitment/randomisation and surgery either because their surgery was performed without CPB (4), because the protocol was broken by using Propofol (1) or because the patient was found to have carotid artery stenosis (1). Of those 192 who were randomised, completed pre-operative NP assessment and successfully completed theatre protocol 162 (84%) returned for repeat NP assessment at 6–8 weeks. One hundred and twenty-seven received intra-operative TCD monitoring. There were no significant differences in patients lost to follow-up between the filter groups (Table 1).

The demographic and operative details of all the randomised patients are shown in Table 2. The AV-6 group had the lowest Parsonnet scores, which measures surgical risk profile, but neither this or any other difference was significant, and the groups proved well balanced.

Fig. 1 shows the characteristic drop in neutrophil count during CPB and post-operative neutrophils in all three groups. The count was lowest 30 min into bypass. An ANOVA on the difference in count between the LG-6 group [2.54 (SD 1.46) $\times 10^9$/l] and the AV-6 [3.26 (SD 1.39) $\times 10^9$/l] was not significant.

The cerebral blood velocity data (Fig. 2) revealed the expected slight fall during bypass, and increase with rewarming and end of bypass. There were no significant differences between the different filter groups.

Significantly fewer microemboli were detected in patients randomised to LD filters (Fig. 3). The median number and range of microemboli was 15 (3–180) in the LD group compared to 67 (5–846) and 55 (2–773) for the Avecor and AV6 groups, respectively ($P < 0.0001$). There was no difference between the two control filters.

Table 2
Demographic and intra-operative data

	All (198)	LD (82)	Avecor (73)	AV-6 (37)
Age (years)	64.1 (8.7)	63.6 (8.7)	65.5 (8.3)	62.3 (9.1)
Sex (% m:f)	86:14	84:16	89:11	81:19
Body mass index (kg/m^2)	27.8 (4.5)	27.9 (4.9)	27.6 (4.1)	28.1 (4.2)
Parsonnet score	6.13 (5.6)	6.21 (5.9)	6.46 (5.5)	5.26 (4.9)
Operation time (min)	169 (30.5)	166 (33.9)	172 (29.1)	171 (25.0)
CPB time (min)	67.7 (19.1)	66.5 (22.1)	68.7 (17.0)	68.4 (15.5)
Cross clamp time (min)	30.9 (9.2)	30.6 (10.1)	31.9 (8.9)	29.5 (7.0)
Number of grafts	2.88 (0.7)	2.86 (0.7)	2.95 (0.7)	2.78 (0.6)

Figures are expressed as means with SD in brackets. There were no significant differences found in demographic data between groups. Mann–Whitney U-test.

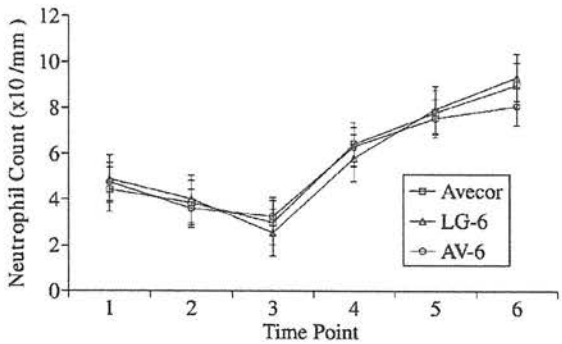

Fig. 1. Perioperative neutrophil count. Time points (1) pre-operatively, (2) at cannulation, (3) after 30 min of CPB, (4) 10 min after coming off CPB, (5) first post-operative day and (6) second post-operative day. ANOVA showed no statistically significant differences between groups at any time point.

3.2. Neuropsychological outcome

There were no differences in the pre-operative NP scores between the three groups. There were also no differences in CES-D or STAI scores pre- or post-operatively. Analysis of post-operative Z change scores showed no difference between the two control groups. These two groups were therefore merged to a single group to increase power of analysis. At 6 weeks post-surgery there was a trend towards greater improvement in NP performance expressed as Z scores in the LD group compared to the control group. The LD group showed better performance in all tests of the battery apart from the non-verbal memory test (see Fig. 4) The total Z score for the LD group was 1.55 (SD 2.71) compared to 0.84 (SD 2.70) in the control group ($P = 0.07$ one-tailed t-test).

Using the definition of a deficit as a decline by more than 1 SD in two or more tests, there was an incidence of 7.8% (7/90) in the standard filter group and 5.6% (4/71) in the LD group. This difference in incidence of 2.8% did not reach statistical significance ($\chi^2 = 0.18$).

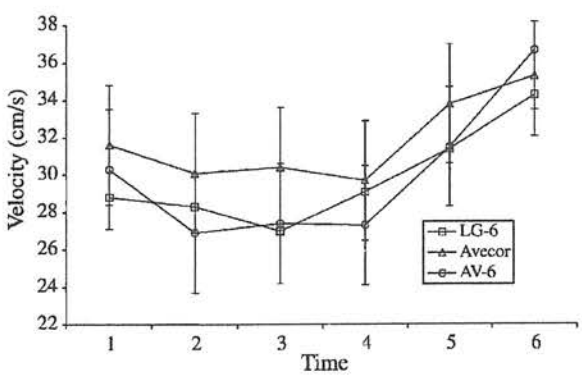

Fig. 2. Middle cerebral artery blood velocity. Time points (1) 10 min pre-bypass, (2) 1 min pre-bypass, (3) 15 min into CPB when the patient has cooled to 32 °C, (4) 30 min into CPB, (5) 60 min into CPB, and (6) 10 minutes after discontinuing CPB. ANOVA showed no statistically significant differences between groups at any time point.

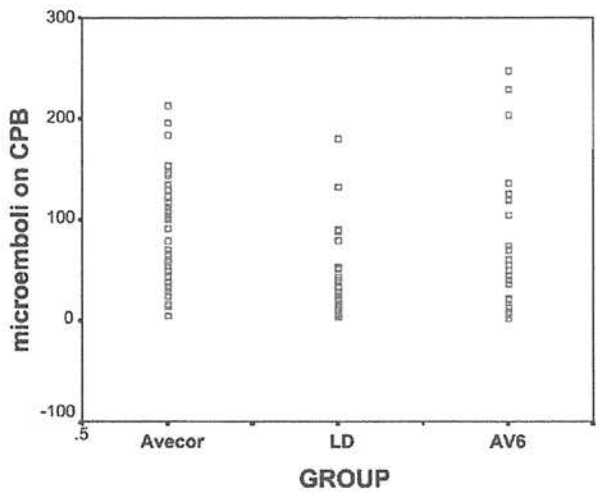

Fig. 3. Microemboli counts on CPB for filter groups. See text for medians and range for each group. Mann–Whitney test: LG-6 vs. Avecor $P < 0.0001$, LG-6 vs. AV-6 $P < 0.0001$ and Avecor vs. AV-6 $P = 0.79$.

As the LD filters significantly reduced microemboli but had a non-significant effect on NP outcome, the data were looked at within each group to detect any relationship between emboli counts and NP scores. The correlation coefficients ranged from 0.03 to 0.16 none being significant. Combining all three groups however showed that those with less than the median count of 35 microemboli had a higher Z change score than the others ($Z = 1.98$, SD 2.11 compared to $Z = 0.74$, SD 1.35, $P < 0.005$ one-tailed t-test). Using the median split is somewhat arbitrary but it suggests that a lower total microemboli count is associated with a better NP performance.

4. Discussion

This study has shown that LD filters further reduce the number of microembolic events recorded by TCD during CABG, and that NP test results strongly suggest an accompanying degree of neuroprotection.

The study has the advantage of a randomised format with demonstrated matching of pre- and intra-operative parameters which can influence cerebral outcome (age, operation time, bypass time). Recent studies of new surgical techniques such as off-pump surgery [12] and epiaortic scanning with Y-grafting [13], for example have lacked this.

Interpretation of the TCD records however must be circumspect. The monitoring of one vessel can only be a very incomplete measure of total cerebral embolic load although there is little difference between sides [14], and more emboli enter the middle cerebral artery than the anterior cerebral artery [15]. Also the consensus criteria for embolus detection are set on an arbitrary size limit which

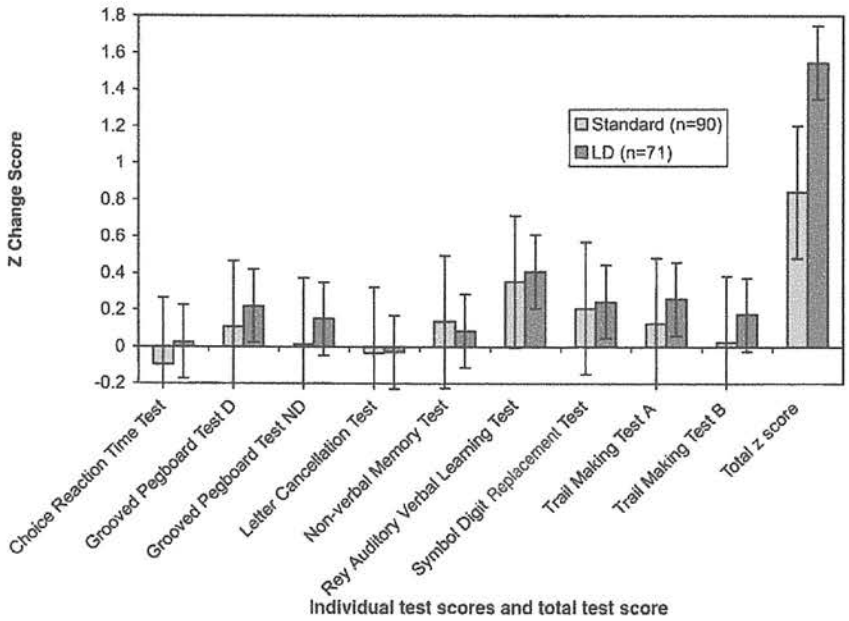

Fig. 4. Neuropsychological outcome as mean Z score changes.

may not reflect the most pathologically important dimensions. Furthermore the methodology current at the time of this study cannot distinguish between solid and gaseous particles. Histological study suggests many emboli which enter cerebral capillaries are lipid rich. Brooker [16] showed that such emboli were more common in dogs put on bypass when cardiotomy suction was used, and Kincaid [17] confirmed these results by reducing their number by processing the suction blood with a cell saver. Arterial line filtration, including leucocyte filters did not reduce the histological evidence of embolism however. This is of interest in the present context where leucocyte filters reduced the total number of microemboli. It is unlikely that the LD filter removed more gaseous microemboli as it is the screen filter in both types of filter that is designed to remove bubbles. However, it is theoretically possible that the LD mesh traps some bubbles that pass through the screen. Although repeated cross clamping can theoretically increase microemboli production there is no reliable evidence that intermittent cross clamp fibrillation produces more microemboli than cardioplegic techniques. Indeed, it is possible that since intermittent cross clamping tends to result in shorter cross clamp and bypass times, total microemboli production could be less than with cardioplegia. The two methods have only ever been compared in one randomised study in terms of microemboli production or NP outcome. This study reported no difference but was underpowered [18].

The next question to consider is whether the reduction in microemboli seen with the LD filter can confer a reduction in NP deficit post-operatively. Although a number of studies have linked microemboli to NP outcome, the relationship between the two remains unclear. Pugsley initially demonstrated a correlation between number of microemboli detected intra-operatively and incidence of post-operative NP deficits. In his study 8.6% of patients with microemboli counts <200 had deficits compared to 43% of those with counts >1000. Four of 49 filtered patients had deficits and 12 of 45 non-filtered patients had deficits. However, the overall numbers of microemboli in Pugsley's study were much greater, probably as a result of the use of bubble oxygenators which produce more microemboli [19] and pH stat control which may increase cerebral blood flow and so microemboli delivery [20]. A later study by Clark [21] using alpha stat and membrane oxygenators divided patients into microemboli count of <30, 30–60 and >60 and found that those with higher counts had worse NP outcome. Barbut [22] showed that perioperative stroke was associated with a higher microemboli count. Fearn et al. [23] found a weak but significant ($r = 0.3$, $P < 0.01$) correlation between emboli and memory loss in a group of 70 patients who had NP testing pre-operation and at 2 months post-operation. Neville et al. [24] seem to provide evidence to the contrary. In a non-randomised study, 193 patients having CABG were compared to 73 patients having valve replacement surgery. NP testing was performed pre-surgery and at 5–7 days, 1 and 6 months post-surgery and intra-operative left common carotid Doppler detection of microemboli was performed. Neville et al. confirmed previous findings that there were more microemboli detected during valve surgery but there was no difference found in NP outcome. Their result suggests that the number of microemboli may not always be the most significant determinant of NP outcome.

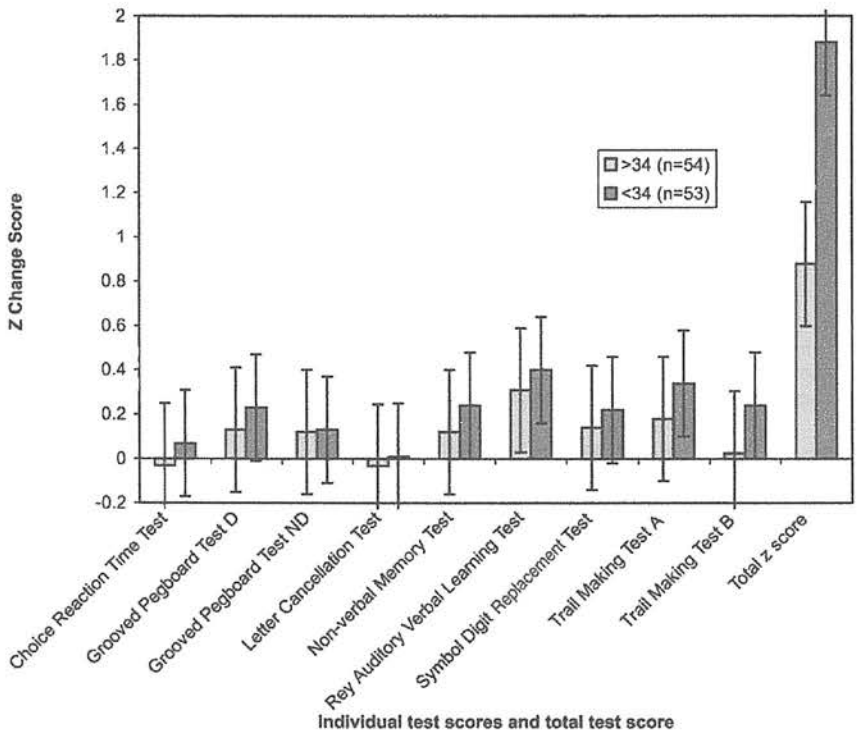

Fig. 5. Z scores for <34 and >34 microemboli.

In our study it can be seen that the effect of the LD filter on NP outcome is less striking than the effect on microemboli. Fig. 4 shows that although there is a trend towards an improvement in NP outcome with the LD filters compared to controls, this does not reach statistical significance ($P = 0.07$). However, the fact that this trend extends across almost all the tests in the battery suggests that this is a real effect that has not reached significance due to statistical under powering, rather than an insignificant effect. Power calculation for this study using NP outcome were calculated and based on an estimated deficit incidence of 20%. This was based on current studies but turned out to be an overestimate as the incidence was found to be much lower. The incidence of deficits was 7.8% in the control group and 5.6% in the LD group. The low incidence may have been due both to improvements in operative, anaesthetic and perfusion techniques as well as strict inclusion criteria of this study. Excluding patients with insulin-dependent diabetes or a history of previous stoke or TIA will have reduced the risk of poor NP outcome in our study population. The finding that increased microemboli is associated with worse NP outcome (Fig. 5) suggests that at least some of the neuroprotective effect of the LD filters is due to a reduction in microemboli.

Loss of patients to follow-up may also lead to an underestimate of the true deficit rate. If there is selective attrition of those patients with the worse deficits then the NP performance of those returning will be higher than that of the study population as a whole. As can be seen from Table 1, the follow-up rate in our study was good (84%) with no difference in follow-up rates or complication rates between filter groups.

In conclusion, this study shows that LD filters are able to reduce the number of cerebral microemboli detected by TCD. However, due to the limitations of TCD, we do not know the type or size of the microemboli affected. Elucidation of the mechanisms by which the LD filters reduce microemboli will require further study. The reduction in microemboli associated with the LD filters has not been shown to lead to a statistically significant neuroprotective effect but the data show that all but one of the tests were in the direction of improved performance with the LD filter. It is likely that in the presence of low morbidity the study was underpowered despite recruiting 198 patients.

Acknowledgements

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References

[1] Newman SP, Stygall J. Neuropsychological outcome following cardiac surgery. In: Newman SP, Harrison MJG, editors. The brain and cardiac surgery, 2nd ed. London: Harwood Academic Press; 2000. p. 21–49.
[2] Pugsley W, Klinger L, Paschalis C, Treasure T, Harrison M, Newman SP. The impact of microemboli during cardiopulmonary bypass on neuropsychological functioning. Stroke 1994;25:1393–9.
[3] Clark RE, Brillman J, Davis DA. Microemboli during coronary artery bypass grafting. J Thorac Cardiovasc Surg 1995;109:249–58.
[4] Neville MJ, Butterworth J, James RL, Hammon JW, Stump DA. Similar neurobehavioural outcome after valve or coronary artery operations despite differing embolic counts. J Thorac Cardiovasc Surg 2001;109:249–58.
[5] Allen S. The role of leucocytes in the systemic inflammatory response and the potential impact of leucocyte depletion. Cardiovasc Engng 1997;2:34–54.
[6] Johnson D, Thomson D, Mycyk T, Burbridge B, Mayers I. Depletion of neutrophils by filter during aortocoronary bypass surgery transiently improves cardiorespiratory status. Chest 1995;107:1253–9.
[7] Hachida M, Hanayama N, Okamura T, Akasawa T, Maeda T, Bonkohara Y, Endo M, Hashimoto A, Koyanagi H. Role of leucocyte depletion in reducing injury to myocardium and lung during cardiopulmonary bypass. Am Soc Artif Intern Organs J 1995;41: M291–4.
[8] Ringlestein EB, Droste DW, Babikan VL, Evans DH, Grosset DG, Kaps M, Markus HS, Russell D, Siebler M. Consensus on microemboli detection by TCD. International Consensus Group on Microembolus Detection. Stroke 1998;29:725–9.
[9] Kong RS, Butterworth J, Aveling W, Stump D, Harrison MJG, Hammon J, Stygall J, Rorie KD, Newman SP. Clinical trial of the neuroprotectant clomethiazole in coronary artery bypass graft surgery. A randomized controlled trial. Anesthesiology 2002;97:585–91.
[10] Radloff L. The CES-D scale. A self-report depression scale for research in the general population. Appl Psychosoc Meas 1977;1: 385–401.
[11] Speilberger CD, Gorusch RL. Manual for the State–Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press; 1983.
[12] Bowles BJ, Lee JD, Dang CR, Taoka SN, Johnson EW, Lau EM, Nekomoto K. Coronary artery bypass performed without the use of cardiopulmonary bypass is associated with reduced cerebral microemboli and improved clinical results. Chest 2001;119:25–30.
[13] Royse AG, Royse CF, Ajani AE, Symes E, Maruff P, Karagiannis S, Gerraty RP, Grigg LE, Davis S. Reduced neuropsychological dysfunction using epiaortic scanning and the exclusive Y graft. Ann Thorac Surg 2000;69:1431–8.
[14] Moser DJ, Ferneyhough KC, Bauer RM, Arndt S, Schultz SK, Haynes WG, Staples ED, Alexander J, Davies LK, O'Brien DJ. Unilateral vs. bilateral ultrasound in the monitoring of cerebral microemboli. Ultrasound Med Biol 2001;27:757–60.
[15] Wijman CA, Babikian VL, Winter MR, Pochay VE. Distribution of cerebral microembolism in the anterior and middle cerebral arteries. Acta Neurol Scand 2000;101:122–7.
[16] Brooker RF, Brown WR, Moody DM, Hammon JW, Reboussin DM, Deal DD, Ghazi-Birry HS, Stump DA. Cardiotomy suction: a major source of brain lipid emboli during cardiopulmonary bypass. Ann Thorac Surg 1998;65:1652–5.
[17] Kincaid EH, Jones TJ, Stump DA, Brown WR, Moody DM, Deal DD, Hammon JW. Processing scavenged blood with a cell saver reduces cerebral lipid microembolisation. Ann Thorac Surg 2000;70: 1296–300.
[18] Musumeci F, Feccia M, MacCarthy PA, Ellis GR, Mammana L, Brinn F, Penny WJ. Prospective randomized trial of single clamp technique versus intermittent ischaemic arrest: myocardial and neurological outcome. Eur J Cardiothorac Surg 1998;13:702–9.
[19] Padayachee TS, Parsons S, Theobold R, Linley J, Gosling RG, Deverall PB. The detection of microemboli in the middle cerebral artery during cardiopulmonary bypass: a transcranial Doppler ultrasound investigation using membrane and bubble oxygenators. Ann Thorac Surg 1987;44:298–302.
[20] Plochl W, Cook DJ. Quantification and distribution of cerebral emboli during cardiopulmonary bypass in swine. Anesthesiology 1999;90: 183–90.
[21] Clark RE, Brillman J, Davis DA. Microemboli during coronary artery bypass grafting. J Thorac Cardiovasc Surg 1995;109:249–58.
[22] Barbut D, Lo Y-W, Gold JP, Trifiletti RR, Yao FSF, Hager DN, Hinton RB, Isom OW. Impact of embolization during coronary artery bypass grafting on outcome and length of stay. Ann Thorac Surg 1997;63:998–1002.
[23] Fearn SJ, Pole R, Wesnes K, Faragher EB, Hooper TL, McCollum CN. Cerebral injury during cardiopulmonary bypass: emboli impair memory. J Thorac Cardiovasc Surg 2001;121:1150–60.
[24] Neville MJ, Butterworth J, James RL, Hammon JW, Stump DA. Similar neurobehavioural outcome after valve or coronary artery operations despite differing embolic counts. J Thorac Cardiovasc Surg 2001;109:249–58.

Appendix A. Conference discussion

***Dr S. Hagl** (Heidelberg, Germany)*: Are these two cohorts of patients compared really comparable in terms of bypass time and the severity of disease?

Dr Whitaker: I didn't have time to show you that data, but there was no differences in preoperative and intraoperative data. And the cross-clamp time was approximately 30 minutes in each group, and the bypass time was approximately 60 minutes.

***Dr M. Grimm** (Vienna, Austria)*: Could you identify specific subgroups, such as patients with diabetes or patients with a history of a cerebrovascular event, who benefit more than others from using such a filter?

Dr Whitaker: That is certainly possible. In this study we excluded patients with diabetes or with previous strokes to reduce confounding variables in the study. But future studies should certainly include these patients to see if they receive greater benefit.

***Dr R. Bonser** (Birmingham, United Kingdom)*: What was the group incidence in each group of neuropsychological deficit by a definition of 20% reduction in 20% of the tests or by one standard deviation? What was the group incidence?

Dr Whitaker: We used the 20% cutoff level. And it was 5.6 in the leukocyte-depleting group and 7.5. This did not reach statistical significance on chi-squared testing. So that's a low incidence.

***Dr S. Westaby** (Oxford, United Kingdom)*: I know of at least one study currently that's looking at neuropsychological testing after cardiopulmonary bypass versus off-pump surgery versus interventional cardiology. And these comparisons are not demonstrating significant changes between these groups. You didn't demonstrate statistical significance with your filter or not. Given that off-pump work gives you the same element of neuropsychological dysfunction, and it's now appearing that catheter

techniques do too, could you comment on why you think you were going to find a difference in these patients.

Dr Whitaker: We had two hypotheses, either by reducing the inflammatory response with these filters or reducing microemboli. We didn't find any difference in inflammatory response in this study.

Dr P. Boonstra *(Groningen, The Netherlands)*: How did you handle cardiotomy suction? Because a lot of microemboli are formed in that area. Can you comment on that?

Dr Whitaker: We used routine cardiotomy suction. But I agree, it is an important factor

Dr Boonstra: And didn't filter it?

Dr Whitaker: No. Well, there was a routine 20-micron filter in the cardiotomy suction line, but this was not leukocyte-depleting filtration. It was standard filtration.

Dr P. Punjabi *(London, United Kingdom)*: According to you there are two theories: One was the mechanical theory and the other was the activated neutrophils theory.

I suppose the mechanical theory you don't really need to have an activated leukocyte depletion, you can use any proper filter.

As far as activation, what is your opinion about the pharmacological agents, especially as we are working quite a lot on aprotinin where we are hoping that we will be able to reduce the inflammatory response and that may have an effect on the neuropsychological outcome?

Dr. Whitaker: There has been a lot of work using pharmacological agents over the last 10 years. And as far as I'm aware, there is no convincing evidence that any are beneficial. And that was part of the reason why we decided to use a physical method to try and reduce inflammation.

Dr Punjabi: I think you're quite right. We are just in the process of starting a major study to look at that.

Dr J. Svennevig *(Oslo, Norway)*: Was the drop in the number of circulating leukocytes significant, or was it just transient?

Dr Whitaker: We did detect a significant reduction in neutrophils at 30 minutes on bypass. This was only between the leukocyte-depleting group and the Avecor group. There wasn't a difference between the leukocyte-depleting group and the other control group.

Dr Svennevig: So in your opinion, the effect that you achieved, is that based more on, let's say, physical removal of particles, or do you think it has more to do with the inflammation therapy?

Dr Whitaker: It's difficult to say from the information we have. We didn't measure activated neutrophils in this study; although other studies have shown a reduction in activated neutrophils with this filter. It's possible that we're filtering out activated neutrophils and then the bone marrow is replenishing them. And that's the explanation why these filters often don't reduce the overall number of neutrophils.

Appendix 5 - Role of the Author

Role of the Author in This Study

Under the supervision of Professor Newman and Professor Harrison, the author designed and implemented this study. The author wrote the study protocol and submitted this for ethical approval and research and development committee approval. During the design of the study the author liaised with Pall Biomedical who manufacture the LG-6 leucocyte-depleting filter and the clinical Perfusionists at the Middlesex Hospital. The bypass circuit is manufactured as a ready made circuit with the arterial line filters in place and so they had to be ordered and made. Discussion was carried out with the cardiothoracic surgeons whose patients were to be recruited into the study to ensure that they approved of the protocol. Several previous studies investigating various aspects of neuropsychological outcome in cardiac surgical patients had been carried out at the Middlesex hospital and so the surgeons were familiar with this type of study. The author made contact with the collaborating physicians Dr Green and Dr Mackie who carried out the S100b and neutrophil elastase assays.

At the beginning of the project the author was employed as a part time (one day per week and one weekend per month) resident medical officer in a nearby private hospital so as to have a salary. The author therefore applied to a variety of sources for funding for the project. All applications were written by the author and submitted to The British Heart Foundation, The Royal College of Surgeons of Edinburgh, The British Medical Association, The Medical Research Council, The Welcome Institute, The Stroke Association (UK), The Jules Thorn Institute among others. Following the award of grants from The Royal College of Surgeons of Edinburgh, The British Medical Association and The Stroke Association (UK) the author worked on the project full time for two years.

The author constructed the case report files (CRFs) for recoding the study data, designed the patient information sheet and once ethical approval was given, began recruiting patients.

The author wrote to all patient scheduled for surgery informing them of the study, enclosing the information sheet. When patients arrived the day prior to surgery the author introduced himself and explained the study verbally. Patients were invited to ask questions and take part if they wished. The author consented all patients who agreed to participate and then introduced them to Mrs Stygall who was the psychologist performing the neuropsychological

tests. At this stage the author made cups of tea or coffee as requested by the patients and Mrs Stygall. When a patient had been recruited it was necessary for the author to inform the perfusionists so that they could set up the bypass circuit, the anaesthetists so that they were prepared for the study anaesthetic protocol (and did not for example prepare Propofol). The author examined all patients and took the required pre-operative blood samples.

On the day of surgery, the author recorded all data into the CRF, set up the transcranial Doppler machine as the patient left the anaesthetic room and entered theatre. This had to be done in the short period of time before the patient was prepped and draped for surgery. The author had to monitor the probe to ensure that it was not dislodged during recording and also take the intra-operative blood tests as described in Materials and Methods. All peri-operative blood samples were spun in the centrifuge by the author, decanted into small tubes and then placed in the freezer. The author then monitored the inpatient post-operative progress of all patients. When discharged, the author was responsible for coordinating the return outpatient visit with an appointment to see the psychologist. The author wrote to all patients again to confirm this and also phoned all patients himself to encourage attendance.

The author recorded all the data from the CRFs onto a personal computer for the subsequent analysis. The author also reviewed all the transcranial Doppler tapes. With supervision, the author performed all the statistical analysis of the results and wrote this thesis.