The effect of leucocyte-depleting arterial line filters on cerebral microemboli and neuropsychological outcome following coronary artery bypass surgery

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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
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 bruises 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 × 10⁹/mm³ 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 paco₂ 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/m² per min at 37 °C reducing to 1.8 l/m² per min at 32 °C. Perfusion pressure was maintained between 50 and 70 mmHg using phenylephrine and phenolamine. Paco₂ 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.
To make optimal use of the NP data each patient's 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 \( \frac{X_2 - X_1}{\mu SD1} \)). 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 \( \pm 1 \) SD from their pre-operative performance in two or more tests were compared (\( \chi^2 \)). 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>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical outcomes and attendance at follow-up</th>
</tr>
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<tbody>
<tr>
<td>All, n = 192</td>
<td>%</td>
</tr>
<tr>
<td>Dead</td>
<td>9</td>
</tr>
<tr>
<td>CVA</td>
<td>2</td>
</tr>
<tr>
<td>Medically unable to FU</td>
<td>11</td>
</tr>
<tr>
<td>Unwilling to attend FU</td>
<td>8</td>
</tr>
<tr>
<td>Completed</td>
<td>162</td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
</tr>
</tbody>
</table>

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 (\( \chi^2 \)-test).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Demographic and intra-operative data</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (198)</td>
<td>LD (82)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.1 (8.7)</td>
</tr>
<tr>
<td>Sex (% m:f)</td>
<td>86:14</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>27.8 (4.5)</td>
</tr>
<tr>
<td>Parsonnet score</td>
<td>6.13 (5.6)</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>169 (30.5)</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>67.7 (19.1)</td>
</tr>
<tr>
<td>Cross clamp time (min)</td>
<td>30.9 (9.2)</td>
</tr>
<tr>
<td>Number of grafts</td>
<td>2.88 (0.7)</td>
</tr>
</tbody>
</table>

Figures are expressed as means with SD in brackets. There were no significant differences found in demographic data between groups. Mann–Whitney U-test.
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 (χ² = 0.18).

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
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.
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 stroke 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.

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References


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. Grimmer (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 with your filter or not. Given that off-pump work gives you the same element of neuroprotective benefit, I think that we have to look at these results with caution.
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.