S100 protein and its relation to cerebral microemboli in on-pump and off-pump coronary artery bypass surgery

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Abstract

Objectives: S100 protein has been used as a marker for cerebral injury. Studies have reported lower levels in off-pump coronary artery surgery (CABG) compared to on-pump surgery. However, most of these are flawed as S100 from extracerebral sources was included (e.g. blood from cardiomyotomy suckers). Microemboli (high-intensity transient signals or HITS) during CABG have been implicated as a cause of postoperative neurocognitive dysfunction. The aim of this study was to compare the number of HITS during on-pump and off-pump CABG, measure S100 accurately by excluding extracerebral sources, and assess whether any changes in S100 were related to HITS.

Methods: Thirty-five patients admitted for CABG were randomised to on-pump (n = 20) or off-pump (n = 15) surgery. Bilateral transcranial Doppler ultrasonography was performed on the middle cerebral artery to detect HITS. S100 was measured preoperatively, at completion of anastomoses, and at termination of bypass in on-pump surgery, and at completion of anastomoses in off-pump surgery, and 48 h postoperatively. A cell saver was used instead of cardiotomy suction in the on-pump group in order to limit extracerebral contamination of the S100 assay.

Results: The number of HITS was 2016 during on-pump and 1897 during off-pump surgery (P < 0.0001). In on-pump surgery S100 increased from 0.05 ± 0.03 to 0.50 ± 0.28 μg/l (P < 0.0001) at termination of bypass. In off-pump surgery S100 increased from 0.08 ± 0.05 to 0.35 ± 0.20 μg/l (P < 0.0001) at completion of anastomoses. The mean intraoperative S100 in the on-pump group was 1.6 times greater compared to that in the off-pump group (95% CI 0.88–2.8; P = 0.1). There was no evidence of a relationship between S100 and HITS in both groups. By 48 h S100 decreased to 0.22 ± 0.14 μg/l in the on-pump and 0.21 ± 0.09 μg/l in the off-pump group (P < 0.0001, compared to the preoperative value).

Conclusions: We have demonstrated a significantly higher number of cerebral microemboli in patients undergoing on-pump compared to off-pump CABG. By limiting contamination from extracerebral sources, we have shown S100β levels during on-pump CABG one and a half times greater than that in off-pump, although this did not reach statistical significance. In addition, we have shown no correlation between S100β and the total microemboli count, possibly because of the small numbers in this study.

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1. Introduction

Neurocognitive injury is a significant problem after cardiac surgery, with an incidence as high as 50% at discharge from hospital [1]. Apart from diffuse cerebral injury as a result of hypoperfusion and the systemic inflammatory response, cerebral emboli are thought to be the main cause of neurocognitive dysfunction and stroke [2–5]. The majority of microemboli are generated as a result of manipulation and instrumentation of the heart and aorta, and from the pump circuitry [6]. With the advent of off-pump coronary artery bypass surgery (CABG), generation of microemboli from these sources is avoided [7].

A number of biochemical markers have been investigated as a possible marker of cerebral injury, of which S100β protein is thought to be a sensitive, albeit non-specific, indicator of central nervous damage [8,9]. S100β is
were recorded (Table 1).

Previous studies have investigated the role of S100β as an indicator of cerebral injury after cardiac surgery [11–13]. However, early studies did not account for other sources of S100β, such as mediastinal fat and shed blood in the mediastinum. These extracerebral sources of S100β are returned to the patient via cardiotomy suckers and as a result serum S100β levels are falsely elevated [14,15].

The aim of this study was to compare the number of microemboli (high-intensity transient signals or HITS) between on-pump and off-pump CABG, to accurately measure levels of S100β by excluding contamination from extracerebral sources, and to determine if there is a correlation between intraoperative cerebral microemboli and S100β levels.

2. Materials and methods

2.1. Patient population and selection criteria

Approval for the study was obtained from the local ethics committee and all patients had given written informed consent. Between July and October 2002, 35 patients admitted for first time elective CABG were randomised to on-pump (group A; n = 20) or off-pump (group B; n = 15) surgery. Block randomisation was carried out by an independent observer on the morning of surgery. This individual would open sealed opaque envelopes containing a card that would indicate treatment allocation. Exclusion criteria for entry into the study were carotid artery stenosis; previous cerebrovascular or psychiatric disease; absence of criteria for entry into the study were carotid artery stenosis; previous cerebrovascular or psychiatric disease; absence of a temporal acoustic window for transcranial Doppler monitoring; concomitant surgery; q-wave myocardial infarction (MI) in the past 6 weeks, and very poor left ventricular function. Baseline characteristics including age, sex, history of hypertension, diabetes, myocardial infarction (MI), myocardial infarction (MI), peripheral vascular disease, New York Heart Association (NYHA) class, and EuroSCORE were recorded (Table 1).

2.2. Anaesthesia

Premedication was administered with morphine and hyoscine. Anaesthesia was induced with either fentanyl and vecuronium or alfentanil, morphine and pancuronium. Maintenance anaesthesia was provided with isoflurane or propofol.

2.3. On-pump CABG

On-pump CABG was performed with a Stöckert S3 roller pump (Stöckert, Munich, Germany), membrane oxygenators (Avant Sorin, Mirandola, Italy), and a 40 µm arterial blood filter (Dideco, Mirandola, Italy). A microbubble sensor (Stöckert) was incorporated in the pump circuit to detect microbubbles. Microbubbles greater than 250 µm in diameter would trigger an alarm. Moderate hypothermia (32 °C) and pH-stat management were used. Myocardial protection was achieved with cold antegrade blood-based cardioplegia. Perfusion pressure was kept at ≥60 mmHg and a pump flow of 2–2.4 l/min per m² was maintained throughout cardiopulmonary bypass (CPB). Blood from cardiotomy suckers was separated from the pump circuit and washed with a cell saver (Dideco). In this way recirculation of extracerebral sources of S100β was minimised. After the distal anastomoses had been completed, the aortic cross-clamp was removed and the proximal anastomoses were carried out using a side-clamp on the aorta.

2.4. Off-pump CABG

Off-pump CABG was conducted via a median sternotomy. A heparin dose of 150 IU/kg was given to maintain an activated clotting time (ACT) ≥400 s. Occlusion and stabilisation of the target coronary artery was achieved with the use of silastic snare and the CTS Retractor (Cardio Thoracic Systems Inc, Cupertino, CA). All distal anastomoses were performed first. The proximal anastomoses were subsequently fashioned onto the aorta with the use of a single side-clamp. Near normothermia (35 °C) was maintained using warmed fluids and a heating mattress. Systolic blood pressure was kept ≥70 mmHg.
2.5. Transcranial Doppler ultrasound (TCD)

All patients underwent bilateral middle cerebral artery TCD monitoring using a Nicolet/EME companion II machine (Eden Medizinische Elektronik GmbH, Kleinheim, Germany), which uses a 128-point colour-coded fast Fourier transform. The middle cerebral arteries (MCA) were isonated with two 2-MHz EME probes that were fixed to the transtemporal widows with a Welder fixation headset (SciMed, Bristol, UK). The MCA was identified at a depth of 48-58 mm. Power was set at 48%, sweep speed maintained at 5.1 s and the sample volume kept constant at 10 mm. The Doppler sound was turned off during monitoring so that the surgeon was blinded to the number and timing of microemboli. The Doppler signals were recorded onto digital audio tape (Sony TCD-D8 DAT recorders) and then played back through the TCD system for off-line analysis at a later date. A single observer, who was blinded to patient details and treatment group, carried out analysis of the recordings for microemboli. Embolic signals were defined as short duration HITS and identified according to standard consensus criteria previously published [16]. An intensity threshold of 7 dB was one of the criteria used to distinguish HITS from artefact. Times of various surgical manipulations were noted. During on-pump CABG the times noted were as follows: aortic cannulation and decannulation, initiation and termination of CPB, application and removal of cross-clamp and side-clamps. In off-pump CABG, times when the heart was lifted, when the stabiliser was applied and removed, and when the side-clamp was applied and released were noted. HITS were counted within 1 min of each surgical manipulation and the total number of HITS for the whole procedure was determined for each patient. Monitoring commenced on opening of the pericardium and continued throughout the operation.

2.6. Serum S100β analysis

Venous blood samples for S100β were obtained prior to induction of anaesthesia, at termination of CPB in group A, upon completion of anastomoses in group B, and at 48 h. The samples were centrifuged, cooled and stored within 1 h and subsequently analysed in batches. All samples were coded and thus analysis of S100β was carried out without knowledge of patient details or treatment group. Serum S100β levels were measured by a monoclonal 2-site immunoradiometric assay (Sangtec 100 LIA assay: Sangtec Medical AB, Bromma, Sweden). The functional detection limit of the assay is 0.02 µg/l. A concentration greater than 0.15 µg/l was considered to be elevated.

2.7. Statistical analysis

All statistics were performed using Stata version 8.0 for windows (Stata Corporation, TX, USA). Results for continuous variables are represented as mean ± SD and results for categorical variables are expressed as number (percent). Age and EuroSCORE are shown as median with interquartile range (IQR). Continuous variables that were normally distributed were compared with unpaired t-tests and non-normally distributed variables were compared with Mann–Whitney tests. The χ² test was used to compare categorical variables. Intraoperative and postoperative S100β values were compared to preoperative levels using paired t-tests on raw or log transformed data. Comparison of intraoperative S100β between the two groups was carried out by analysis of covariance, with the preoperative level as a covariate. Correlation between intraoperative S100β level and HITS count was calculated using the Spearman correlation test.

3. Results

Baseline characteristics of the two groups are shown in Table 1. Although there were more patients in group A with moderate left ventricular function, the median EuroSCORE was the same for both groups. One patient in group A suffered a postoperative stroke involving the cerebellum, pons and thalamus on the ninth postoperative day.

More patients in group A had triple vessel coronary artery disease, and more bypass grafts were performed in this group (Table 2). No patients in the off-pump group were converted to on-pump surgery. There were no jump grafts or pedicled grafts used in either group.

The mean number of HITS was significantly greater during on-pump CABG (2016 ± 1897 vs 16 ± 21, P < 0.0001). In group A the largest number of HITS was noticed on initiation of CPB, followed by removal of the aortic cross-clamp and side-clamp (Fig. 1). The patient who succumbed to a postoperative stroke on day nine had a total HITS count above 1700. In group B, release of the side-clamp resulted in the highest number of HITS (Fig. 2).

The values for S100β for the two groups are shown in Table 3. For both groups there was a significant rise in intraoperative S100β titre from preoperative levels (0.05 ± 0.03 to 0.50 ± 0.28 µg/l, P < 0.0001 in group A; 0.08 ± 0.05 to 0.35 ± 0.20 µg/l; P < 0.0001 in group B). Although the intraoperative S100β level during on-pump CABG was 1.6 times greater than that during off-pump, the difference was not statistically significant. In addition there was no correlation between the HITS count and intraoperative S100β level in either group (Fig. 3).

Table 2

<table>
<thead>
<tr>
<th>Comparison of coronary bypass procedures</th>
<th>Group A</th>
<th>Group B</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of side-clamps used/patient</td>
<td>1.15 ± 0.99</td>
<td>0.8 ± 0.41</td>
<td>NS</td>
</tr>
<tr>
<td>No. of proximal anastomoses</td>
<td>2.2 ± 1.06</td>
<td>1.2 ± 0.86</td>
<td>0.01</td>
</tr>
<tr>
<td>No. of grafts</td>
<td>3.2 ± 0.99</td>
<td>2.2 ± 0.94</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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4. Discussion

Although the incidence of overt stroke after elective CABG remains about 3%, neurocognitive impairment after cardiac surgery is an increasingly important problem, especially as the population of patients undergoing CABG becomes older [17]. One of the main mechanisms underlying neurocognitive dysfunction is thought to be cerebral microemboli [5].

We have shown that there is a significantly higher number of intraoperative cerebral microemboli in patients undergoing on-pump CABG compared to off-pump surgery. The few HITS that were detected in the off-pump group arose during lifting of the heart and removal of the side-clamp. The mean HITS count of this study in the on and off-pump groups is similar to that reported by Bowles and colleagues [7]. There is, however, a wide variation in the number of HITS within the on-pump group. One reason could be due to different degrees of aortic atherosclerosis amongst these patients. A recent study by Mackensen has shown that patients with extensive atherosclerosis of the ascending aorta are more prone to cerebral embolisation during CABG. Therefore, manipulations of the aorta such as cannulation and clamping will result in increased cerebral embolisation in patients who have a significant degree of aortic atheroma [18].

In addition, the number of HITS upon applying and removing the side-clamp in the on-pump group is higher than that in off-pump CABG. One reason might be that the ‘sandblasting’ effect from the high-velocity jet exiting the aortic cannula will exacerbate any injury to the aortic wall e.g. dislodgment of atheroma after removal of a side-clamp. As a result the combined process of the sandblasting effect and application/removal of the side-clamp will lead to a higher number of HITS in on-pump CABG, compared to that in off-pump, at the corresponding time points.

Measurement of S100β protein has been used to identify cerebral injury after CABG [19]. However, S100β from non-cerebral sources is thought to elevate the overall S100β level, thus limiting usefulness of this assay [14,15]. One of the main mechanisms whereby extracerebral sources of S100β are introduced is via cardiotomy suckers. Cardiotomy suckers return shed mediastinal blood to the patient and it has been shown that mediastinal blood contains a high concentration of S100β [20]. In addition, experiments in dogs have shown that cardiotomy suckers are probably responsible for passage of lipid microemboli to the brain and formation of small capillary arterial dilatations [21]. We have avoided direct return of blood from cardiotomy suckers in this study and used a cell-saver instead to collect and wash mediastinal blood before transfusing it back to the patient. In this way the non-cerebral contribution to the S100β assay is kept to a minimum, and unnecessary allogenic blood transfusion avoided. We measured S100β 48 h postoperatively as a previous study by Jönsson has
shown correlation of S100β level measured at this time with size of cerebral infarction identified on CT scanning [19].

The mean intraoperative levels of S100β achieved in our study is much lower than that reported in previous work comparing on-pump with off-pump CABG [13,22]. It is possible that in Diegeler’s study the high S100β level in the on-pump group is due to recirculation of activated leucocytes and mediastinal fat via cardiotomy suckers [13]. Anderson and colleagues study have reported S100β levels during on-pump CABG 10 times the order of magnitude than that found after off-pump surgery [22]. Even though no autotransfusion was carried out post-operatively, a limitation of Anderson’s study is that no arterial filter was used in the on-pump circuity. There is strong evidence to show that arterial filters reduce the number of microemboli passing to the brain and protect against neuropsychological impairment. [5,23].

Our S100β results concur with work done by Jönsson’s group who showed that using a cell-saver in on-pump surgery, and not directly autotransfusing blood from cardiotomy suckers, limits extracerebral contamination of the assay [20].

Having accounted for factors such as cardiotomy suckers and arterial filters in our study, we found intraoperative S100β levels during on-pump CABG one and a half times greater than that in off-pump, although this did not reach statistical significance. In addition, even though the difference in microemboli counts between the two groups was highly significant, there was no correlation between S100β and microemboli count, possibly because of the small numbers in our study. Grocott et al. have reported that there is a correlation between intraoperative microemboli and serum S100β [24]. However, the mean S100β at the end of bypass achieved in that study is almost five times greater than the equivalent level here, possibly as a result of use of cardiotomy suckers.

Despite randomisation of patients, the off-pump group had fewer bypass grafts compared to the on-pump group. This finding has been reported by others [25]. It may be that the off-pump technique raises the threshold for grafting smaller vessels.

It is possible that cerebral microemboli are not the cause of S100β elevation, and release of S100β could occur as a result of a generalised increased permeability of the blood-brain barrier during CPB. In addition, as Anderson and colleagues have shown, there is a small rise in S100β soon after sternotomy and before CPB is instituted [14,15]. This implies that not all the extracerebral sources of S100β are eliminated by avoiding cardiotomy suckers and that the surgical procedure itself causes an increase in S100β. However, before discounting the link between embolic induced ischemia and rise of S100β, a larger study with the same precautions taken in this investigation to limit extracerebral contamination of the S100β assay is required. Detailed neurocognitive testing can be conducted to correlate any elevation of S100β with neurocognitive dysfunction.

References


Appendix A. Conference discussion

Dr R. Poston (Baltimore, Maryland, USA): Did you use the multirange Doppler, talked about by the previous presenter, that’s been validated to differentiate air from other embolic debris?

Dr Motallebzadeh: No. We have used a transcranial Doppler machine that does not distinguish between air and particulate emboli. Our department of Neurology has extensive experience in transcranial Doppler ultrasound and is currently validating a multirange machine.

Dr Poston: That might be why you didn’t see a correlation between neurologic injury represented by S100 and the HITS data. “Traditional” HITS doesn’t represent anything pathologic: mainly air emboli occurring at the onset of cardiopulmonary bypass that basically don’t have harmful neurologic effects.

Dr Motallebzadeh: Sure. That is a possibility. If anything, the machine used has overestimated the number of emboli and should give a positive correlation if one exists. As these are the preliminary results of a larger study, we cannot assume that air emboli have no neurologic sequelae.

Dr A. Liebold (Regensburg, Germany): I wonder a little bit about your time point of measurement. Because others have shown that the maximum, the peak, of S100-beta is about 4 hours after injury. So your difference between the on-pump and off-pump group should be rather higher when you measured it at that time point. Why did you choose 48 hours and immediately after declamping?

Dr Motallebzadeh: We chose, actually, the end of bypass in the on-pump group. In essence, we tried to find roughly similar time points in both groups.

In answer to your second part of the question, for 48 hours, there are two reasons why we chose this. First, the S100β at 48 hours has been shown to be a predictor of stroke in a previous paper by Jönsson, and a marker of prognosis.

Secondly, some of the cell-saved blood was obviously given back to the patients. As S100β has a relatively short half-life, the contribution by this source to the overall S100β serum level would be small at 48 hours. Therefore any elevations of S100β at 48 hours is from the brain rather than from any other source.

Dr M. Elahi (Leicester, United Kingdom): I would like to know, for the off-pump and on-pump surgery, have you compared the temperature differences between both types of surgery? Because off-pump would be normothermia and on-pump would be hypothermia.

Dr Motallebzadeh: In the on-pump group, the patients were cooled and bypass performed at 32°C and in the off-pump group near normothermia was maintained. I don’t believe that’s a difference that it’s possible to get around. But sure, the temperature in the on-pump group will have to increase slowly up to 37 degrees centigrade and we ensured patients were not warmed beyond that.