Establishment of an early embolus-related cerebral injury model after cardiopulmonary bypass in miniature pigs

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Abstract

Embolus-related cerebral injury is still a serious adverse event after cardiopulmonary bypass (CPB). But there is no stable animal model for basic and clinical research purposes. We chose miniature pig to establish a stable animal model of embolus-related cerebral injury after CPB and verified the validity of results by correlating the histopathological findings with those of diffusion-weighted magnetic resonance imaging (DW-MRI). Based on different treatment regimens, 24 male miniature pigs were randomly assigned into four groups: Control, CPB, embolus, and CPB-embolus groups. DW-MRI was performed before and after surgery to diagnose and locate the brain lesions. Histopathological changes in brain tissues were examined using H&E and Nissl staining. All surgical procedures were uneventful with 100% postoperative survival of pigs. Two animals in the Embolus group and six animals in the CPB-embolus group showed signs of ischemic penumbra on DW-MRI performed 6 h after surgery. Consistent with the results of DW-MRI, histopathological examination showed necrosis and ischemic lesions. In this paper, we demonstrate the feasibility and validity of a pig model of embolus-related cerebral injury associated with CPB. This model may be used in the future for basic and translational research.

Keywords: Animal models, cerebral injury, embolus, cardiopulmonary bypass, diffusion-weighted magnetic resonance imaging, miniature pig

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Introduction

The first successful cardiopulmonary bypass (CPB) procedure was performed in 1968,¹ and since then, it has been widely used in heart surgery. Cerebral ischemic injury is a serious complication of cardiac surgeries that involve establishment of CPB, more so in the elderly patients. Previous studies have established a close relationship between CPB-induced cerebral injury and vascular micro-embolization.^{2,3} A large number of brain capillaries and small arteries get dilated during CPB with a gradual increase in their permeability. This increases the risk of ischemic brain injury resulting from extravasation of thrombi across the microvasculature. Elderly patients are at a higher risk of producing large-sized thrombi and have a poor tolerance to microvascular embolization during CPB. This may be due to underlying primary pathological conditions like atherosclerosis, diabetes, and hypertension.⁴ Although there are some established animal models of cerebral injury, a stable animal model of cerebral

injury after CPB, for basic and clinical research purposes has not been standardized.^{5,6}

Currently, most animal models of CPB have been established in bigger animals like dogs, pigs, and sheep. The anatomy of the pig heart is closest to that of a human heart and is thus suited to the establishment of an experimental CPB model. In a preliminary experiment, none of pigs receiving thoracotomy with only CPB had detectable brain damage. The probable reason was that we used six-month-old pigs which had a good collateral circulation and no atherosclerotic plaque in the vasculature. As microvascular dysfunction and microthrombi formation are believed to be instrumental in the pathogenesis of early brain injury,⁷ an additional element of microembolization was included in this study to enhance the characterization of the pathophysiological changes in the experimental model.

Diffusion-weighted magnetic resonance imaging (DW-MRI) has a high sensitivity for detection of movement of

water molecules within a few hours of ischemia. It is particularly useful for detecting cerebral infarction in acute and hyper acute stages.⁸ Being very sensitive to early changes of cerebral ischemia, DW-MRI confers a distinct advantage in detecting lesions when compared with the conventional MRI T1WI and T2WI. DW-MRI, which is known to have a high sensitivity for detecting small and early infarcts, was used to detect early signs of postoperative cerebral injury by us.

In this study, we sought to establish a stable animal model of embolus-related cerebral injury after CPB and assess the feasibility and validity by corroborating the histopathological findings with the corresponding imaging findings on DW-MRI.

Materials and methods

Animals

A total of 24 six-months-old miniature pigs were obtained from experimental animal center of Fuzhou general hospital of the Nanjing military region and housed according to the National Laboratory Animal Regulations. All experimental procedures involving pigs were approved by the Institutional Animal Care and Use Committee at Fujian Medical University.

Study groups

The animals were randomly divided into four equal groups: Control, CPB, embolus, and CPB-embolus groups ($N=6$ in each group). Animals assigned to the CPB and the CPB-embolus groups were subjected to thoracotomy with CPB, while those in the control and embolus groups were subjected to thoracotomy without CPB. After the surgical procedures, the right internal carotid artery of animals in embolus and CPB-embolus groups was located 3 cm below the skin right side of trachea immediately, and 1 mL of microemboli of 100–300 μm diameter (S210GH, Merit Medical Systems, Inc., USA) were slowly injected into the distal artery.

Surgical procedures

All pigs were kept fasting overnight but were allowed free access to water prior to the surgery. General anesthesia was administered by intramuscular injection of ketamine 300 mg, midazolam 10 mg, and anisodamine 0.6 mg, followed by intravenous injection of pipecuronium (4 mg) into the ear vein. General anesthesia was maintained by continuous intravenous infusion of fentanyl, 0.02–0.05 mg/kg; pipecuronium, 1–8 mg/kg; and propofol, 0.02 g/kg. A 16G endotracheal tube was advanced into the trachea, and mechanical ventilation with room air was initiated using a volume-controlled ventilator (750, Bear, USA). Ventilator parameters were as follows: tidal volume: 10 mL/kg; respiratory rate (RR): 16 breaths/min; oxygen concentration: 100%; and positive end expiratory pressure (PEEP): 5 cm water (H_2O) (1 cm H_2O = 0.098 kPa). The RR was adjusted to maintain an end-tidal CO_2 pressure of 35–45 mmHg. A flow-directed Swan-Ganz catheter (CCOmbo V-774HF75, Edwards Life Sciences,

Irvine, CA, USA) was inserted through the right femoral vein and into the pulmonary artery for monitoring the pulmonary arterial pressure and pulmonary capillary wedge pressure. Heart rate, arterial pressure, venous pressure, and peripheral oxygen saturation were monitored with electrocardiogram monitor and blood-gas analyzer (GEM Premier 3000, Instrumentation laboratory, USA) during the surgery.

The animals in the CPB and CPB-embolus groups were dissected in the middle of sternum with due care to ensure optimal hemostasis followed by opening of the pericardium. Heparin (100 mg intravenous) was administered for anticoagulation. After activation of thromboplastin (activated clotting time [ACT] > 200 s), an aortic cannula (18 F) was rapidly inserted into a double purse made in the anterior wall of aorta ascendens using 6×14 suture, while a 28 F venous line was placed in the atrium dextrum. These were fixed and connected to the pipes of the CPB machine (Extracorporeal membrane oxygenation 90, Xijing medical supplies Co. Ltd, China), which were connected correctly, filled with priming solution (hydroxyethyl starch 20 sodium chloride injection 500 mL, sodium lactate ringer's injection 500 mL, urbason 500 mg, heparin sodium 20 mg, and mannitol 0.5–1.0 g/kg) to exhaust gas in pipes previously. Blood gas analysis was conducted at different time points, i.e. before proceeding to CPB, 15 min after bypass, and prior to shutting off. The body temperature decreased to 26°C after ACT > 480 s, following by CPB with 2.1–2.4 L*min of flow rate, 60–90 mmHg (1 mmHg = 133.3 Pa) of angiotension and α steady state of blood gas levels. Without blocking the aorta ascendens and arresting the heart, the breath was stopped. Temperature as measured in the epipharynx and rectum was maintained at 26°C for 2 h, followed by a gradual increase to 32°C. With respiratory support, the CPB machine was shut down and the venous line extracted after maintaining the temperature (37°C) for 30 min. Protamine (100–150 mg intravenous) was administered; aortic cannula was extracted. After ensuring adequate hemostasis, the drainage tubes were placed in the pericardium and mediastinum and the chest closed. Heart rate, arterial blood pressure, venous pressure, peripheral oxygen saturation, urine output, blood gas, and ACT were monitored during the procedure. The animals in the control and embolus groups were subjected to mid-sternal thoracotomy without CPB for a period of 2 h. All medication and monitoring during the procedure are the same with them in the CPB and CPB-embolus groups.

Animals were laid on their sides and kept sedated with continued catheterization, fluid replacement, nutritional support, and remnant blood infusion after the surgery. Pre- and postoperative antibiotic therapy was administered. The dose of vasoactive drugs was gradually reduced according to the cycling conditions of animals. Ventilatory support was calibrated to maintain a RR of 15 breaths/min, tidal volume 10 mL/kg, 100% oxygen saturation, and PEEP at 5 cm H_2O till the animals recovered from anesthesia. On initiation of spontaneous breathing, the ventilator was switched to synchronized intermittent mandatory ventilation mode. The RR was reduced gradually to 4 breaths/min and the endotracheal tube removed after attainment of

normal blood gas levels. The drainage tubes in the pericardium and mediastinum were extracted once the drainage flow was <10 mL/h. All animals were kept under intensive care for a 4 h period and subsequently weaned off controlled ventilation. Animals were placed in observation cages.

Pathology and imaging

All animals underwent DW-MRI one day prior to and 6 h after surgery. The animals were sacrificed after the postoperative DW-MRI. The brain tissues of sacrificed animals were harvested and fixed with 10% formalin for a period of 48 h. Histopathological examination of brain tissue was done using H&E and Nissl stain (C0117, Beyotime, China).

Statistical analysis

All variables are expressed as mean $\pm$ standard deviation. Statistical analyses were performed using SPSS version 19.0. Intragroup and intergroup differences were assessed using Student's *t*-test, one way Analysis of Variance (ANOVA), or rank-sum test. $P < 0.05$ and κ (Kappa) > 0.4 were considered indicative of a statistically significant difference.

Results

All surgical procedures were uneventful with 100% survival of the animals. Data pertaining to surgery-related variables associated with (CPB and CPB-embolus groups) and without (control and embolus groups) CPB were recorded and intergroup differences assessed. The mean duration of CPB was 118.90 ± 12.03 min. Postoperatively, signs of brain dysfunction like limb asthenia, astasia, and weak response to environmental stimuli were monitored in all animals. There were no significant differences with respect to weight, urine output, and intraoperative fluid balance between the CPB and the control group ($P > 0.05$) (Table 1). The heart rate and results of blood gas analyses are presented in Table 2.

All animals showed normal preoperative MRI findings in DWI modes. Animals in the control and CPB groups showed no abnormal signs on DW-MRI at 6 h after the surgery. Six hours after surgery two animals in the embolus group and all six animals in the CPB-embolus group showed signs of ischemic penumbra on DW-MRI. The lesions identified on MRI study were largely confined to

the cerebral cortex, internal capsule, basal ganglia, and the cerebellum. The postoperative positive signals associated with ischemic brain lesions in animals are shown in Figure 1.

To further evaluate the ischemic lesions, brain tissues were dissected from the cerebral cortex, internal capsule, basal ganglia, and cerebellum, based on the DW-MRI findings. A total of 52 paraffin-embedded tissues with positive and 52 ones with negative images of ischemic lesions were stained with H&E and Nissl stain (Table 3). H&E staining showed numerous necrotic spots in the positively stained tissues, while only mild inflammatory cell infiltration was observed in the negatively stained tissues. Nissl staining showed swollen neurons, pale cytoplasm with vacuolization, eccentric nuclei, attenuated dendrites, and significant gliosis in the positive stained tissues. In the negatively stained tissues, the neuronal cells appeared normal with uniform staining and clear dendrites (Figure 2). The histopathological findings were consistent with those of DW-MRI ($\kappa = 0.692$, $P < 0.01$ for H&E and $\kappa = 0.712$, $P < 0.01$ for Nissl staining).

Discussion

For manageability and availability, we chose six-month-old pigs as experimental objects but failed in creating detectable brain injury in pre-experiments. Cerebral emboli may be composed of atherosclerotic debris, calcium, air, fat, platelet thrombi, or CPB tubing. Atherosclerosis has been recognized as the most important risk factor for perioperative stroke and postoperative neurobehavioral changes and could be responsible for spontaneous embolic stroke during the intraoperative and postoperative periods.⁹ To increase the likelihood of ischemic cerebral insult, we added an element of postoperative injection of microemboli into the right internal carotid artery of young pigs in the present study to simulate the thromboembolism from atherosclerotic plaques in elderly patients. Consequently, we documented a significantly enhanced cerebral insult according to the results in CPB and CPB-embolus groups.

Cerebral injury in cardiac surgery with CPB may result not only from microemboli but also from postischemic reperfusion injury, systemic inflammatory response syndrome, CPB-related operative procedures (like aortic cannulation), and so on.¹⁰⁻¹² In the embolus group, animals underwent thoracotomy without CPB but were injected microemboli into the brain. Only two out of the six animals showed evidence of brain damage. The ischemic injury caused by microemboli of 100–300 μ m diameter could have easily been prevented by the rich collateral circulation in young animals. This is one of the important drawbacks of using young pigs to establish a model of cerebral injury after CPB. But CPB may produce a period of vulnerability during which otherwise benign insults may produce significant injury. The CPB procedure proved to be an important contributor to the causation of postoperative cerebral injury. The CPB procedure used in the present study closely simulated that practiced in clinical settings and did not control for the external predisposing factors (such as CPB pipe without endothelial cell and activation of endotoxemia) and

Table 1 General monitoring indicators (mean $\pm$ standard deviation)

	Weight (kg)	Urine output (mL)	Liquid balance (mL)
With CPB (N = 12)	15.45 $\pm$ 1.51	490.26 $\pm$ 85.66	154.15 $\pm$ 84.32
Without CPB (N = 12)	15.52 $\pm$ 1.59	528.33 $\pm$ 64.89	183.70 $\pm$ 71.63
<i>P</i> value	0.9201	0.2327	0.3649

CPB: cardiopulmonary bypass.

Table 2 Heart rate and blood gas analysis (mean $\pm$ standard deviation)

	With CPB		Without CPB			
	Before CPB	15 min after CPB	Before CPB shutting off	Before thoracotomy	15 min After thoracotomy beginning	2 h after thoracotomy beginning
Heart rate (bpm)	100.42 $\pm$ 7.82	60.05 $\pm$ 3.58*	125.83 $\pm$ 9.20*	94.75 $\pm$ 9.29	101.33 $\pm$ 6.34	90.00 $\pm$ 4.18
PH	7.47 $\pm$ 0.07	7.58 $\pm$ 0.06	7.59 $\pm$ 0.06	7.49 $\pm$ 0.06	7.62 $\pm$ 0.06	7.63 $\pm$ 0.04
Hct (%)	38.31 $\pm$ 3.97	17.80 $\pm$ 3.13*	20.81 $\pm$ 3.01*	36.74 $\pm$ 5.26	36.40 $\pm$ 5.78	36.63 $\pm$ 5.20
PO ₂ (mmHg)	462.55 $\pm$ 26.44	681.45 $\pm$ 48.91*	475.33 $\pm$ 52.27	449.48 $\pm$ 25.70	484.40 $\pm$ 38.19	439.69 $\pm$ 62.89
PCO ₂ (mmHg)	32.39 $\pm$ 2.91	26.08 $\pm$ 3.92	31.71 $\pm$ 5.48	31.93 $\pm$ 4.37	29.63 $\pm$ 5.02	27.68 $\pm$ 5.83
HCO ₃ ⁻ (mmol/L)	28.69 $\pm$ 3.52	26.97 $\pm$ 3.19	28.42 $\pm$ 3.47	28.56 $\pm$ 3.53	27.69 $\pm$ 3.05	27.68 $\pm$ 3.23
BE (mmol/L)	5.54 $\pm$ 3.23	3.99 $\pm$ 1.49	4.43 $\pm$ 2.02	5.02 $\pm$ 2.91	4.58 $\pm$ 1.36	3.34 $\pm$ 1.43
K ⁺ (mmol/L)	4.49 $\pm$ 0.71	2.86 $\pm$ 0.28*	4.08 $\pm$ 0.45	4.51 $\pm$ 0.77	3.28 $\pm$ 0.19	4.24 $\pm$ 0.48
Lac (mmol/L)	1.42 $\pm$ 0.68	3.48 $\pm$ 0.07*	2.63 $\pm$ 1.05	1.09 $\pm$ 0.60	1.88 $\pm$ 0.43	2.96 $\pm$ 0.63

CPB: cardiopulmonary bypass.

*Compare with and without CPB, $P < 0.05$. **Figure 1** Representative findings of brain DW-MRI of miniature pigs. (a) Negative findings on DW-MRI, (b) positive findings on DW-MRI: ischemic penumbra with lesions largely located in the cerebral cortex, internal capsule, basal ganglia, and cerebellum. DW-MRI: diffusion-weighted magnetic resonance imaging**Table 3** Consistency analysis of pathology and imaging

		H&E*		Nissl*	
		Positive	Negative	Positive	Negative
DW-MRI	Positive	40	12	42	10
	Negative	4	48	5	47
Total		44	60	47	57

DW-MRI: diffusion-weighted magnetic resonance imaging.

*Consistency analysis with DW-MRI, $P < 0.01$.

intrinsic predisposing factors (such as intraoperative tissue injury, endothelial cell activation, and ischemia reperfusion injury), which can induce CPB to initiate systemic response syndrome. The observed indicators were normal during the surgery, and postoperative pathological evaluation confirmed the necrosis and nerve cell changes in the brain

tissue after CPB. These results indicate the successful creation of an experimental animal model of brain injury after hypothermic CPB.

DW-MRI method is sensitive in detecting changes in a movement of water molecules. All the factors causing cerebral injury reduce the diffusion of water molecules, thereby showing a high signal on DW-MRI. DW-MRI is widely used for establishing a diagnosis of cerebral infarction in the department of neurology, but it has only been rarely used for assessment of brain injury after cardiac surgery. Postoperative cerebral injury after cardiac surgery with CPB can potentially be caused by several mechanisms that are relatively distinct from that of pure cerebral infarction.^{2,3} To further establish the validity of our findings, we assessed the consistency between histopathological findings and the imaging results. Pathological evaluation further confirmed the accuracy of DW-MRI results in making an early diagnosis of cerebral injury after CPB.

Figure 2 H&E and Nissl staining showed ischemic brain injury associated with CPB in pigs. Paraffin-embedded section of brain tissues was examined after H&E and Nissl staining. H&E stained sections showed multiple necrotic spots indicative of ischemic injury. Only mild inflammatory cell infiltration was observed in the negatively stained tissues. Nissl staining showed the significant swelling in neuronal cells, pale stained cytoplasm with vacuolization, a side pushed nucleus, shorter or even disappeared dendrite, gliosis in positive tissues, while normal cellular morphology and clear dendrite in negative tissues. The results shown are representatives of pathological staining images (200 $\times$). (A color version of this figure is available in the online journal.)

To make the model more convenient and efficient, we did not block the ascending aorta or arrest and open the heart, thus avoiding the risk of myocardial injury and failure of heart resuscitation. Maintaining a beating heart is not likely to reduce the brain damage caused by CPB but may improve the success rate of surgery and that rate of survival of the animals. Since an allogeneic blood transfusion experiment has some limitations such as high cost, production of large thrombus (that heparin failed to solve), increase in occurrence of infection, resulting in a high death rate of animals, we used non-blood priming solution without blood transfusion in this study. That made Hct decreased after bypass but did not significantly affect the postoperative survival of animals despite Hct could only be increased to 20% before shutting down the CPB machine. Although the levels of electrolytes during CPB tend to vary from the baseline, we successfully maintained the electrolyte levels within the normal range. Careful hemostasis was maintained to minimize the intraoperative blood loss as the blood for back transfusion was heparinized. Close monitoring of ACT allows for administration of nucleoproamine to counteract the effect of heparin, if required, in our model.

Besides the good collateral circulation, young pigs have a higher metabolic rate and a higher tolerance to anesthesia. We had to increase the dosage of sedatives, analgesics, and muscle relaxants depending upon the actual condition in order to ensure a smooth operation. Many studies have failed to confirm the association between anesthesia and postoperative cognitive dysfunction (POCD),^{13–17} and it is

also not clear whether high concentration of drugs can cause irreversible brain damage. Negative findings in control group may help us to exclude the effects of anesthesia on experimental results.

Available evidence suggests that CPB is not an independent risk factor for POCD^{2,18} and we actually did not damage the brain in CPB group in our study. However, CPB tends to aggravate the damage caused by microemboli as indicated in the results in the embolus and CPB-embolus groups. These findings suggest there is a period of vulnerability conveyed by CPB which accentuates the brain injury associated with embolus. The study was not designed to demonstrate or negate the relevance of CPB as an independent risk factor for POCD because of the small sample size and the added element of injection of solid microspheres. Further experiments may be needed to assess their relationship in detail.

Authors' contributions: GW conceived and designed the experiments. WZ, ML, SY, JB, XC, ZD, HW, and HC performed the experiments. WZ, ML, and SY analyzed the data. WZ wrote the paper and GW polished the manuscript. All authors have read and approved the final manuscript.

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DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

- Gibbon JH. Development of the artificial heart and lung extracorporeal blood circuit. *JAMA* 1968;**206**:1983–6
- Liu YH, Wang DX, Li LH, Wu XM, Shan GJ, Su Y, Li J, Yu QJ, Shi CX, Huang YN, Sun W. The effects of cardiopulmonary bypass on the number of cerebral microemboli and the incidence of cognitive dysfunction after coronary artery bypass graft surgery. *Anesth Analg* 2009;**109**:1013–22
- Motallebzadeh R, Bland JM, Markus HS, Kaski JC, Jahangiri M. Neurocognitive function and cerebral emboli: randomized study of on-pump versus off-pump coronary artery bypass surgery. *Ann Thorac Surg* 2007;**83**:475–82
- Moller JT, Cluitmans P, Rasmussen LS, Houx P, Rasmussen H, Canet J, Rabbitt P, Jolles J, Larsen K, Hanning CD, Langeron O, Johnson T, Lauven PM, Kristensen PA, Biedler A, van Beem H, Fraidakis O, Silverstein JH, Beneken JE, Gravenstein JS. Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. International Study of Post-Operative Cognitive Dysfunction. *Lancet* 1998;**351**:857–61
- Agematsu K, Korotcova L, Scafidi J, Gallo V, Jonas RA, Ishibashi N. Effects of preoperative hypoxia on white matter injury associated with cardiopulmonary bypass in a rodent hypoxic and brain slice model. *Pediatr Res* 2014;**75**:618–25
- Blue ME, Wilson MA, Beaty CA, George TJ, Arnaoutakis GJ, Haggerty KA, Jones M, Brawn J, Manmohan S, Lange MS, Johnston MV, Baumgartner WA, Troncoso JC. Brain injury in canine models of cardiac surgery. *J Neuropathol Exp Neurol* 2014;**73**:1134–43
- Andereggen L, Neuschmelting V, von Gunten M, Widmer HR, Fandino J, Marbacher S. The role of microclot formation in an acute subarachnoid hemorrhage model in the rabbit. *Biomed Res Int* 2014;**2014**:161702
- Rodriguez RA, Rubens FD, Wozny D, Nathan HJ. Cerebral emboli detected by transcranial Doppler during cardiopulmonary bypass are not correlated with postoperative cognitive deficits. *Stroke* 2010;**41**:2229–35
- Mukherji J, Hood RR, Edelstein SB. Overcoming challenges in the management of critical events during cardiopulmonary bypass. *Semin Cardiothorac Vasc Anesth* 2014;**18**:190–207
- Gold JP, Charlson ME, Williams-Russo P, Szatrowski TP, Peterson JC, Pirraglia PA, Hartman GS, Yao FS, Hollenberg JP, Barbut D. 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;discussion 1311–4
- Mathew JP, Shernan SK, White WD, Fitch JC, Chen JC, Bell L, Newman MF. Preliminary report of the effects of complement suppression with pexelizumab on neurocognitive decline after coronary artery bypass graft surgery. *Stroke* 2004;**35**:2335–9
- Pugsley W, Klinger L, Paschalis C, Treasure T, Harrison M, Newman S. The impact of microemboli during cardiopulmonary bypass on neuro-psychological functioning. *Stroke* 1994;**25**:1393–9
- Rasmussen LS, Johnson T, Kuipers HM, Kristensen D, Siersma VD, Vila P, Jolles J, Papaioannou A, Abildstrom H, Silverstein JH, Bonal JA, Raeder J, Nielsen IK, Korttila K, Munoz L, Dodds C, Hanning CD, Moller JT. ISPOCD2 (International Study of Postoperative Cognitive Dysfunction) Investigators. Does anaesthesia cause postoperative cognitive dysfunction? A randomised study of regional versus general anaesthesia in 438 elderly patients. *Acta Anaesthesiol Scand* 2003;**47**:260–6
- Rasmussen LS, Schmehl W, Jakobsson J. Comparison of xenon with propofol for supplementary general anaesthesia for knee replacement: a randomized study. *Br J Anaesth* 2006;**97**:154–9
- Höcker J, Stapelfeldt C, Leiendecker J, Meybohm P, Hanss R, Scholz J, Bein B. Postoperative neurocognitive dysfunction in elderly patients after xenon versus propofol anesthesia for major noncardiac surgery: a double-blinded randomized controlled pilot study. *Anesthesiology* 2009;**110**:1068–76
- Steinmetz J, Funder KS, Dahl BT, Rasmussen LS. Depth of anaesthesia and post-operative cognitive dysfunction. *Acta Anaesthesiol Scand* 2010;**54**:162–8
- Patel N, Minhas JS, Chung EM. Risk factors associated with cognitive decline after cardiac surgery: a systematic review. *Cardiovasc Psychiatry Neurol* 2015;**2015**:370612
- Farhoudi M, Mehrvar K, Afrasiabi A, Parvizi R, Khalili AA, Nasiri B, Hashemzadeh K, Ghabili K. Neurocognitive impairment after off-pump and on-pump coronary artery bypass graft surgery – an Iranian experience. *Neuropsychiatr Dis Treat* 2010;**6**:775–8

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