Journal of Cardiothoracic and Vascular Anesthesia 000 (2024) 1–9

Contents lists available at ScienceDirect

Journal of Cardiothoracic and Vascular Anesthesia

journal homepage: www.jcvaonline.com

Original Article

Standard versus High Cardiopulmonary Bypass Flow Rate: A Randomized Controlled Subtrial Comparing Brain Injury Biomarker Release

Anna Corderfeldt Keiller, EBCP, PhD[*,†,‡,1], Markus Axelsson, MD, PhD[§], Gudrun Bragadottir, MD, PhD[‖], Lukas Lannemyr, MD, PhD[‖,¶], Johanna Wijk, MD[‖,¶], Kaj Blennow, MD, PhD[**,††,‡‡,§§], Henrik Zetterberg, MD, PhD[**,††,‖‖,¶¶,***,†††], Roger Olofsson Bagge, MD, PhD[‡,‡‡‡,§§§]

[*]*Department of Perfusion, Sahlgrenska University Hospital, Gothenburg, Sweden*
[†]*Department of Surgery, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*
[‡]*Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden*
[§]*Department of Clinical Neuroscience, Institute of Neuroscience and Physiology at Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*
[‖]*Department of Cardiothoracic Anesthesia and Intensive Care, Sahlgrenska University Hospital, Gothenburg, Sweden*
[¶]*Department of Anesthesiology and Intensive Care Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden*
[**]*Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*
[††]*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*
[‡‡]*Paris Brain Institute, ICM, Pitié-Salpêtrière Hospital, Sorbonne University, Paris, France*
[§§]*Neurodegenerative Disorder Research Center, Division of Life Sciences and Medicine, and Department of Neurology, Institute on Aging and Brain Disorders, University of Science and Technology of China and First Affiliated Hospital of USTC, Hefei, P.R. China*
[‖‖]*Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK*
[¶¶]*UK Dementia Research Institute at UCL, London, UK*
[***]*Hong Kong Center for Neurodegenerative Diseases, Clear Water Bay, Hong Kong, China*
[†††]*Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI*
[‡‡‡]*Department of Surgery, Sahlgrenska University Hospital, Gothenburg, Sweden*
[§§§]*Sahlgrenska Center for Cancer Research, Department of Surgery, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*

Objectives: To compare brain injury biomarker release levels between two different cardiopulmonary bypass (CPB) flow rates in elective cardiac surgery and to explore differences in postoperative delirium between groups and associations between age, sex, CPB time, oxygen levels, and near-infrared spectroscopy, and biomarker levels.
Design: A randomized controlled substudy trial
Setting: Sahlgrenska University Hospital, Sweden
Participants: Forty patients undergoing elective cardiac surgery with CPB
Intervention: Patients were assigned at random to either a standard (2.4 L/min/m^2) or a high (2.9 L/min/m^2) CPB flow rate.

[1]*Address correspondence to* Anna Corderfeldt Keiller, PhD, Department of Perfusion, Sahlgrenska University Hospital, 413 45 Gothenburg, Sweden
E-mail address: anna.corderfeldt@vgregion.se (A.C. Keiller).

https://doi.org/10.1053/j.jvca.2024.07.017

Measurements and Main Results: Glial fibrillary acidic protein, neurofilament light chain, total-tau, and phosphorylated-tau217 were sampled in plasma before anesthesia induction, after 60 minutes on CPB, and at 30 minutes, 24 hours, and 72 hours post-CPB. Mixed models for repeated measures were used to analyze differences in biomarker levels between groups and to assess relationships, which showed no differences between the 2 flow rate groups. There also was no difference in the occurrence of delirium between the 2 groups. Associations were found between age and increased neurofilament light chain levels. Female sex, oxygen delivery >330 mL/min/m^2, and near-infrared spectroscopy level >60% were associated with lower biomarker levels.

Conclusions: An increased flow rate did not have any significant effects on biomarker levels compared to a standard flow rate. Several associations were identified between treatment characteristics and biomarker levels. No difference in delirium was seen.

Key Words: cardiopulmonary bypass; cardiac surgery; neuronal biomarkers; brain injury; oxygen delivery; oxygen extraction ratio; near-infrared spectroscopy; postoperative delirium

Concerns about potential postoperative neurologic side effects arose shortly after the introduction of the heart-lung machine in the 1950s. Even in cases of successful and otherwise uneventful surgery, some patients exhibited impaired cognitive function. Approximately 60% of patients undergoing cardiac surgery experience early cognitive dysfunction or delirium within the first week, and after 12 months, as many as 20% to 40% still show lingering signs of cognitive decline.[1] The cognitive decline observed after heart surgery with CPB is believed to be a multifactorial phenomenon with various contributing factors, including impaired delivery of adequate oxygenated blood during CPB, which can adversely impact the brain.[2]

Historically, a CPB blood flow rate of 2.2 to 2.5 L/min/m^2, mimicking the cardiac output of an unsedated adult, has been considered adequate for normothermic CPB.[3] The use of goal-directed perfusion strategies to ensure sufficient oxygen delivery (DO_2) to the tissue rather than adhering to fixed indexed flow rates has been suggested.[4] This strategy recommends a nadir-indexed oxygen delivery (DO_2i) threshold of ≥330 mL/min/m^2 during CPB at 36 °C.[5] Near-infrared spectroscopy (NIRS), a real-time, noninvasive, continuous measurement tool for measuring tissue oxygen saturation, is often used during cardiac surgery as a guide for cerebral oxygenation, adequate perfusion, and oxygen delivery.[6]

Biomarkers indicating brain injury become evident as neurons suffer damage. Neuronal biomarkers can be measured in various matrices, including cerebrospinal fluid and blood (serum or plasma).[7] Neurofilament light chain (NfL) is a neuronal cytoplasmic protein highly expressed in large, myelinated axons, providing structural stability to neurons. Elevated NfL levels correlate with the extent of axonal damage in various neurologic disorders, including inflammatory, neurodegenerative, traumatic, and cerebrovascular diseases.[8] Total tau is a microtubule-associated protein important for stabilizing the axonal cytoskeleton and facilitating vesicle transport in neuronal synapses. It plays a vital role in maintaining axonal integrity, and elevated t-tau levels are indicative of neuronal damage.[9] Plasma phosphorylated tau (p-tau217) has demonstrated one of the most promising levels of diagnostic accuracy and sensitivity among emerging biomarkers for Alzheimer's disease.[10] Glial fibrillary acidic protein (GFAP) serves as the primary intermediate filament protein in mature astrocytes, playing a vital role in maintaining the astrocyte cytoskeleton for cell integrity and resilience,[11] and serves as a biomarker for glial activation and neuroinflammation.[12]

The hypothesis behind this study posits that a higher CPB flow rate would lead to superior perfusion parameters, resulting in reduced release levels of biomarkers associated with brain injury. In this randomized controlled trial, the aim was to examine whether there is a difference in the pattern of neuronal biomarker release associated with brain injury during cardiac surgery between a standard CPB flow rate of 2.4 L/min/m^2 and a high CPB flow rate of 2.9 L/min/m^2.

Methods

Patients and Randomization

This is a preplanned substudy of the ICAROX2 single-center randomized controlled trial (ClinicalTrials.gov NCT04084301). The main study included 101 patients, of whom the first 40 patients also were included in this substudy. Patients were randomized at a 1:1 ratio to either a standard (2.4 L/min/m^2) or high (2.9 L/min/m^2) CPB flow rate. Patients age ≥18 years who provided written informed consent and planned for elective open cardiac surgery with an expected CPB duration ≥60 minutes in normothermia and left ventricular ejection fraction ≥30% were included. Patients with verified previous cerebral infarction, advanced grown-up congenital disease correction, or a body mass index >32 kg/m^2 were excluded. Patients were screened for participation the day before surgery. Randomization was conducted using the Sealed Envelope online randomization program, and was stratified according to estimated glomerular filtration rate above or below 60 mL/minute and preoperative left ventricular ejection fraction above or below 40%.

Sample Size

In this substudy, the sample size was calculated on a previous study examining oxygen delivery during different CPB flow rates.[13] Where a CPB flow rate of 2.4 L/min/m^2 delivered a mean DO_2i of 322 ± 45 and a CPB flow rate of 2.7 L/min/m^2 delivered a mean DO_2i of 374 ± 32, a sample size of 12 patients per group was deemed necessary to detect differences

in DO_2i (with an α value of 0.05 and power of 0.80). To test the hypothesis that DO_2i affects the biomarker levels associated with brain injury, 20 patients in each group were included in this substudy to account for potential dropouts.

Study Objectives

The primary objective was to examine whether there is a difference in the pattern of neuronal biomarker release associated with brain injury during cardiac surgery between a standard CPB flow rate of 2.4 L/min/m^2 and a high CPB flow rate of 2.9 L/min/m^2. Secondary objectives were to investigate whether there is any difference in the occurrence of postoperative delirium between the 2 different CPB flow rate groups, as well as whether age, sex, CPB time, NIRS, oxygen delivery, and oxygen extraction ratio had any relationship with the level of biomarker release.

Ethics

Ethical approval was granted by the Swedish Ethical Board (Dnr: 2019-01423) in June 2019. The study adhered to the International Committee of Medical Journal Editors Recommendations for the Protection of Research Participants and followed the principles outlined in the Declaration of Helsinki. The trial was registered at ClinicalTrials.gov (NCT04084301).

Clinical Management

Vasodilators, such as angiotensin-converting enzyme and calcium channel blockers, were discontinued on the day of surgery. Beta-receptor antagonists were administered at reduced doses according to clinical routine. NIRS measurement was initiated before anesthesia induction in all patients. The anesthesia protocol followed standard clinical procedure, involving induction with propofol (Baxter) and fentanyl (Kalceks), followed by maintenance of anesthesia with sevoflurane (Baxter), except during CPB, when propofol was used.

CPB

A cardiopulmonary roller pump system (LivaNova S5) was used together with an Inspire 8F oxygenator with arterial filter and reservoir (LivaNova) primed with 1300 mL of Ringer acetate (Fresenius Kabi AB). The patient's core temperature was urine bladder monitored and held at 36 to 37 ˚C during CPB using a heater-cooler (T3; LivaNova). Cannula size was chosen based on the safety pressure drop limits provided by the manufacturer, and heparin was given with a target activated clotting time of >480 seconds before initiating CPB.

The target flow rate, measured with a flow sensor on the arterial line, remained constant throughout the CPB period, except for instances of flow reduction necessitated by the placement or removal of the aortic cross-clamp or when a lower flow rate was required for shorter periods due to the surgical procedure. If increased volume was needed to maintain the flow rate, Ringer acetate or albumin 200 g/L (Baxalta) was added. Blood transfusion was given sparingly to patients to maintain a hematocrit above 24%. Mean arterial pressure (MAP) was kept within 60 to 80 mmHg using norepinephrine or sodium nitroprusside, as necessary. During CPB, the target arterial partial pressure of oxygen (pO_2) was 20 kPa and target partial pressure of carbon dioxide (pCO_2) was 5.0 to 6.0 kPa. A CO_2 flush of the chest was used at the surgeon's discretion.

Measurements and Blood Sampling

CPB flow rate, hemodynamic data, and blood gases (CDI500; Terumo) were analyzed continuously, as was NIRS data (INVOS 5100C; Medtronic). Blood gas samples were drawn every 15 minutes (Rapid Point 500e; Siemens). Data collection for all parameters was done every 15 minutes during CPB. In all patients, blood samples were collected before anesthesia induction (baseline), at 60 minutes on CPB, and at 30 minutes, 24 hours, and 72 hours after CPB. Blood samples were collected into EDTA tubes for plasma and centrifuged within 20 to 60 minutes. Plasma was separated, aliquoted, and approved by Biobank Sverige (PS-0531-5-002#0531) for storage at -80 ˚C until biochemical analysis.

Biomarker Analysis

NfL, t-tau, and GFAP concentrations were measured in plasma using ultrasensitive single molecule array technology (Simoa) with commercially available kits on an HD-X instrument (Quanterix). Plasma p-tau217 was measured using the in-house Simoa method described by Gonzalez-Ortiz et al.[10] All samples were analyzed in a single run at the Clinical Neurochemistry Laboratory at the University of Gothenburg by board-certified laboratory technicians blinded to clinical data using a single batch of reagents for each assay. The intra-assay coefficient of variation was <10% for all assays.

Nursing Delirium Screening Scale

Postoperatively, in the thoracic intensive care unit, patients were evaluated for delirium using the Nursing Delirium Screening Scale (NuDESC) once during every 8-hour shift. This screening tool incorporates the scoring of particular behaviors linked to delirium. The assessment included evaluation of the level of consciousness, orientation, memory, psychomotor activity, sleep−wake cycle disturbances, and perceptual disturbances on a 3-point scale (0-2), with a score of ≥2 points indicating the presence of delirium.[14]

Statistical Evaluation

Variables were described by mean ± standard deviation, median and range or interquartile range, or number and percent. The 2-sample *t* test was used to compare treatment characteristics between the 2 groups for normally distributed continuous variables. A lognormal distribution was applied together with the *t* test for non-normally distributed continuous variables and the Fisher exact test for binary variables.

Changes from baseline to the highest biomarker levels were evaluated using the Wilcoxon signed- rank test. Mixed models for repeated measures (MMRM) with a lognormal distribution (due to biomarkers being non-normally distributed) were used to test for differences in biomarker levels between the 2 CPB flow rate groups. Analysis was adjusted for baseline value, and an unstructured covariance pattern was used for each treatment arm for the relationship of data over different time points. Risk ratios (RRs) with 95% CIs were obtained with associated p values and adjusted for multiple testing using Bonferroni-Holm adjustment. All other analyses were exploratory, and a significance level of 0.05 was applied. MMRM also were to study the relationships between biomarkers and various baseline/patient characteristics, dichotomized at the median or clinically important cutoffs over study time points. These analyses were adjusted for baseline values of the biomarker and the randomized treatment arm. All analyses were performed using SAS version 9.4 (SAS Institute) and SPSS version 28 (IBM).

Results

Between October 2019 and September 2021, 40 patients were included in this randomized trial. One patient was excluded owing to rupture of the right ventricle and the need for redo surgery, leaving 39 patients for analysis. The median patient age was 72 years (range, 32-84 years), with a male-to-female ratio of 30:9. Patient demographics and baseline characteristics are detailed in Table 1. The mean duration of CPB was 130 ± 56 minutes, with a mean cross-clamp time of 99 ± 44 minutes (Table 2).

When comparing the biomarkers between the 2 flow rate groups, the only numerical difference between the 2 CPB flow rate groups over time was seen in GFAP levels at 72 hours after CPB (RR, 1.19; 95% CI, 1.01-1.42; p = .042). This difference was no longer statistically significant following Bonferroni-Holm adjustment (Table 3).

As an exploratory analysis, the relationships between released biomarkers and baseline/treatment characteristics were analyzed. Patients age ≥72 years had elevated NfL levels after 60 minutes on CPB (RR, 1.29; 95% CI, 1.04-1.58; p = 0.02) and increased levels of t-tau (RR, 1.26; 95% CI, 1.06-1.50; p = 0.011) and p-tau217 (RR, 1.70; 95% CI, 1.15-2.50; p = 0.009) after 72 hours. Women had 52% lower t-tau levels at 60 minutes on CPB compared with men (RR, 0.48; 95% CI, 0.32-0.71; p < 0.001). Further findings showed that being on CPB for >117 minutes was associated with average increases in p-tau217 levels at 30 minutes after CPB (RR, 1.43; 95% CI, 1.16-1.77; p = 0.002). However, NfL levels and GFAP levels were reduced by 21% and 30%, respectively, when CPB exceeded 117 minutes (RR, 0.79 [95% CI, 0.66-0.95; p = 0.02] and RR, 0.70 [95% CI, 0.53-0.93; p = 0.02) at 60 minutes on CPB. Measured mean NIRS ≥60% during CPB was associated with a 41% lower level of p-Tau217 (RR, 0.59; 95% CI, 0.38-0.90; p = 0.02) at 24 hours after CPB. Further findings revealed that DO_2i >330 mL/min/m^2 during CPB resulted in a 28% lower t-tau release at 72 hours after CPB (RR, 0.72; 95% CI. 0.58-0.89; p = 0.004). At 24 hours after CPB, p-tau217 levels were 34% lower when O_2ER was ≥25% (RR, 0.66; 95% CI, 0.45-0.97; p = 0.03) (Table 4).

Four patients (20%) in the standard CPB flow rate group were identified with postoperative delirium in the intensive care unit compared to none in the high CPB flow rate group (p = 0.11) (Table 2). When comparing patients with postoperative delirium and those without postoperative delirium, NfL was numerically increased at all time points for the delirium group but significantly increased at only 24 hours after CPB (55.6 ng/L vs 26.1 ng/L; p = 0.007). Furthermore, the CPB flow rate was significantly lower in the delirium group (4.5 L/min vs 5.3 L/min; p = 0.048) and NIRS levels measured during CPB also were significantly lower in the delirium group (53% vs 70%; p = 0.003) (Table 5).

Table 1
Baseline patient characteristics

Characteristic	Standard-flow group (N= 20)	High-flow group (N=19)
Age, y, median (IQR)	71 (32-82)	72 (44-84)
Male sex, n (%)	14 (70)	16 (84)
Weight, kg, mean ± SD	78±13	84±13
Height, cm, mean ± SD	175±9	177±9
BSA Dubois, m^2, mean ± SD	1.93±0.20	2.01±0.19
Creatinine, μmol/L, mean ± SD	80±18	89±24
Hemoglobin, g/L, mean ± SD	120±16	125±14
NfL ng/L, median (IQR)	22.1 (12.8-35.3)	23.8 (13.5-;38.6)
GFAP, ng/L, median (IQR)	212 (155;312)	235 (172;307)
T-tau, ng/L, median (IQR)	10.5 (8.5;12.6)	10.7 (9.3;13.3)
P-tau217, ng/L, median (IQR)	3.6 (2.4;4.6)	5.2 (3.6;7.1)
NIRS right, %, mean ± SD	66±10	70±13
NIRS left, %, mean ± SD	65±11	68±12
CABG, n (%)	0	3 (16)
AVR, n (%)	7 (35)	6 (32)
MVR, n (%)	7 (35)	4 (21)
Combination valve, n (%)	3 (15)	0
CABG + valve replacement, n (%)	2 (10)	5 (26)
Supracoronary graft, n (%)	1 (5)	1 (5)

Abbreviations: AVR, aortic valve replacement; BSA, body surface area; CABG, coronary artery bypass grafting; GFAP, glial fibrillary acidic protein; MVR, mitral valve replacement; NIRS, near-infrared spectroscopy; NfL, neurofilament light chain.

Discussion

No previous studies have investigated biomarkers associated with brain injury with different CPB flow rates during cardiac surgery. The primary finding of the present study is that no significant differences in biomarker levels were identified between the 2 different CPB flow rate groups.

A bit surprisingly, contrary to the stated hypothesis, there was no difference in the biomarker release pattern between the 2 flow rate groups, even though crucial perfusion parameters during CPB showed significantly superior performance in the high CPB flow rate group in terms of oxygen delivery, venous oxygen saturation, and NIRS. However, the analysis showed that on average, the critical DO_2i threshold was exceeded in both groups. This might have contributed to the inability to

Table 2
Treatment During CPB

Parameter	Standard-flow group (N = 20)	High-flow group (N = 19)	p value
CPB flow rate, L/min	4.6 ± 0.5	5.8±0.5	<0.001***
CPB time, min	132 ± 43	129±68	0.87
CPB cross-clamp, min	101 ± 36	98±53	0.84
MAP, mmHg	60 ± 9	63±7	0.14
Temperature, ˚C,	36.1 ± 0.6	36.3±0.4	0.37
Hematocrit, %,	30 ± 3	31±3	0.47
Hemoglobin *g/L*	103±13	105±11	0.69
pCO_2 *kPa*	5.5±0.4	5.6±0.3	0.30
PaO_2 *kPa*	27±4	25±4	0.07
SvO_2 %	76±4	79±3	0.02*
DO_2i *ml/min/m²*	341±44	419±43	<.001***
VO_2 *ml/min/m²*	76±18	80±14	0.44
O_2ER %	22±4	20±4	0.07
NIRS right %	65±12	72±8	0.05
NIRS left %	65±15	71±8	0.16
Total volume added *mL*	1748±900	1970 ± 1240	0.53
Diuresis *mL*	225 (100;428)	390 (183;1450)	0.047*
Postoperative delirium on ICU *n*	4 (20)	0 (0)	0.11

Non-normally distributed data were log-transformed. The Student t test was used for numerical data, reported as mean ± SD or median and IQR, and the Fisher exact test was used for binary data, reported as number and percentage. Oxygen parameters, MAP, hematocrit, hemoglobin, and NIRS were measured every 5 minutes. The table shows a merged mean during CPB.
Abbreviations: CPB, cardiopulmonary bypass; DO_2i, indexed oxygen delivery; ICU, intensive care unit; MAP, mean arterial pressure; NIRS, near-infrared spectroscopy; O_2ER, oxygen extraction ratio; PaO_2,partial pressure of arterial oxygen; pCO_2, partial pressure of carbon dioxide; SvO_2, mixed venous oxygen saturation; VO_2, oxygen consumption.
* $p < 0.05$.** $p < 0.01$.
*** $p < 0.001$

Table 3
MMRM for biomarkers comparing standard and high CPB flow rates

Biomarker	Samples	Standard-flow group (N = 20), median (IQR)	High-flow group (N =19), median (IQR)	RR (95% CI)	p value
NfL, ng/L	Baseline	22.1 (12.8-35.3)	23.8 (13.5-38.6)		
	60 min on CPB	18.8 (11.4-24.7)	23.7 (15.1-37.9)	1.18 (0.92-1.52)	0.19
	30 min post-CPB	23.4 (17.4-29.4)	28.7 (17.1-38.3)	1.16 (0.91-1.48)	0.22
	24 h post-CPB	28.5 (19.3-51.2)	26.1 (23.0-36.6)	0.98 (0.77-1.24)	0.83
	72 h post-CPB	35.8 (24.0-45.3)	34.8 (25.4-66.3)	1.07 (0.85-1.35)	0.53
GFAP, ng/L	Baseline	212 (155-312)	235 (172-307)		
	60 min on CPB	184 (127-275)	259 (168-325)	1.27 (0.95-1.69)	0.10
	30 min post-CPB	242 (158-413)	270 (243-374)	1.23 (0.95-1.60)	0.11
	24 h post-CPB	282 (186-379)	294 (208-356)	0.96 (0.78-1.19)	0.72
	72 h post-CPB	221 (168-340)	293 (210-339)	1.19 (1.01-1.42)	0.042*
t-tau, ng/L	Baseline	10.5 (8.5-12.6)	10.7 (9.3-13.3)		
	60 min on CPB	38.4 (31.0-57.9)	30.7 (22.7-46.4)	0.88 (0.63-1.23)	0.44
	30 min post-CPB	66.7 (53.8-79.8)	57.8 (45.1-80.1)	0.96 (0.72-1.27)	0.76
	24 h post-CPB	15.1 (10.8-24.8)	14.3 (12.6-19.2)	0.95 (0.70-1.28)	0.72
	72 h post-CPB	10.2 (8.8-12.2)	11.1 (9.4-13.4)	1.12 (0.93-1.34)	0.22
p-tau217, ng/L	Baseline	3.6 (2.4-4.6)	5.2 (3.6-7.1)		
	60 min on CPB	12.7 (9.0-16.1)	11.4 (8.3-16.0)	0.86 (0.63-1.17)	0.33
	30 min post-CPB	10.6 (7.5-13.5)	9.9 (8.2-12.4)	0.98 (0.78-1.23)	0.84
	24 h post-CPB	7.0 (4.6-10.5)	7.1 (5.1-9.9)	1.04 (0.81-1.34)	0.77
	72 h post-CPB	4.8 (3.1-8.7)	5.8 (4.5-8.4)	1.19 (0.81-1.74)	0.37

MMRM were used to test the CPB flow rate effect over time, with lognormal distribution. The analysis was adjusted for baseline values. An unstructured covariance matrix was applied by treatment group.
Abbreviations: GFAP, glial acidic fibrillary protein; IQR, interquartile range; MMRM, mixed models for repeated measures; NfL, neurofilament light chain; RR, risk ratio.
* Nonsignificant following Bonferroni-Holm adjustment.

Table 4
MMRM for biomarker relationships to various baseline and treatment characteristics over time

		NfL (ng/L)		GFAP (ng/L)		Tau (ng/L)		Tau217 (ng/L)	
Patient characteristics	Samples	RR (95% CI)	p value	RR (95% CI)	p value	RR (95% CI)	p value	RR (95% CI)	p value
Age, ≥72 y vs <72 y	60 min on CPB	1.29 (1.04-1.58)	.02*	1.29 (0.92-1.81)	.13	0.73 (0.51-1.05)	.09	0.79 (0.57-1.08)	.13
	30 min post-CPB	1.08 (0.90-1.28)	.40	1.04 (0.76-1.41)	.80	0.82 (0.62-1.09)	.17	0.96 (0.74-1.23)	.71
	24 h post-CPB	1.02 (0.84-1.24)	.83	1.13 (0.84-1.52)	.39	1.17 (0.86-1.60)	.30	1.20 (0.92-1.56)	.17
	72 h post-CPB	1.03 (0.86-1.23)	.76	1.17 (0.93-1.48)	.18	1.26 (1.06-1.50)	.011*	1.70 (1.15-2.50)	.009**
Sex, female vs male	60 min on CPB	0.80 (0.62-1.03)	.09	0.81 (0.57-1.15)	.23	0.48 (0.32-0.71)	<.001***	1.02 (0.70-1.49)	.90
	30 min post-CPB	0.98 (0.81-1.20)	.88	1.02 (0.74-1.41)	.91	0.79 (0.56-1.13)	.19	1.10 (0.81-1.50)	.52
	24 h post-CPB	0.88 (0.69-1.12)	.30	0.89 (0.66-1.20)	.43	0.77 (0.52-1.14)	.19	0.86 (0.62-1.20)	.36
	72 h post-CPB	0.92 (0.74-1.13)	.40	0.83 (0.67-1.02)	.08	0.88 (0.70-1.10)	.24	1.26 (0.76-2.11)	.36
CPB time, ≥117 min vs <117 min	60 min on CPB	0.79 (0.66-0.95)	.02*	0.70 (0.53-0.93)	.02*	1.18 (0.80-1.75)	.39	1.18 (0.86-1.61)	.31
	30 min post-CPB	0.99 (0.84-1.18)	.95	0.96 (0.73-1.26)	.76	1.15 (0.84-1.56)	.37	1.43 (1.16-1.77)	.002**
	24 h post-CPB	0.91 (0.75-1.09)	.29	0.87 (0.68-1.11)	.25	1.25 (0.93-1.70)	.14	1.23 (0.95-1.60)	.11
	72 h post-CPB	1.08 (0.92-1.28)	.33	0.98 (0.82-1.18)	.87	1.12 (0.92-1.36)	.25	1.03 (0.67-1.57)	.90
NIRS, ≥60% vs <60%	60 min on CPB	0.94 (0.59-1.49)	.77	1.13 (0.73-1.75)	.57	0.78 (0.42-1.45)	.41	0.85 (0.52-1.39)	.49
	30 min post-CPB	1.07 (0.72-1.60)	.71	1.38 (0.94-2.03)	.10	0.70 (0.45-1.09)	.11	1.00 (0.63-1.58)	.99
	24 h post-CPB	0.96 (0.55-1.70)	.89	0.83 (0.58-1.20)	.31	0.66 (0.36-1.20)	.16	0.59 (0.38-0.90)	.02*
	72 h post-CPB	1.14 (0.70-1.86)	.58	1.02 (0.73-1.42)	.90	0.85 (0.63-1.15)	.28	1.22 (0.59-2.49)	.58
DO_2i, ≥330 mL/ min/m^2vs <330 mL/min/m^2	60 min on CPB	0.95 (0.66-1.36)	.78	1.15 (0.81-1.63)	.42	0.88 (0.54-1.44)	.59	0.90 (0.61-1.34)	.59
	30 min post-CPB	1.12 (0.82-1.53)	.44	1.29 (0.94-1.77)	.11	0.89 (0.60-1.32)	.55	0.76 (0.55-1.06)	.10
	24 h post-CPB	1.05 (0.67-1.63)	.82	1.15 (0.89-1.49)	.27	0.74 (0.46-1.18)	.20	0.71 (0.49-1.02)	.06
	72 h post-CPB	1.01 (0.68-1.50)	.96	1.26 (0.97-1.64)	.08	0.72 (0.58-0.89)	.004**	0.65 (0.39-1.08)	.09
O_2ER, ≥25% vs <25%	60 min on CPB	1.13 (0.82-1.56)	.44	1.46 (0.97-2.21)	.07	1.10 (0.64-1.89)	.73	0.84 (0.55-1.27)	.39
	30 min post-CPB	1.06 (0.83-1.34)	.66	1.42 (0.97-2.07)	.07	1.35 (0.90-2.02)	.14	0.84 (0.58-1.20)	.32
	24 h post-CPB	0.95 (0.68-1.32)	.75	1.00 (0.71-1.40)	>.99	0.91 (0.56-1.47)	.68	0.66 (0.45-0.97)	.03*
	72 h post-CPB	0.97 (0.73-1.29)	.85	0.89 (0.68-1.16)	.38	1.04 (0.80-1.36)	.74	0.76 (0.40-1.45)	.40

MMRM were used to test the relationship between baseline characteristics and biomarkers over time, with lognormal distribution, adjusted for baseline value and randomization group. An unstructured covariance matrix was applied by treatment group. Exploratory analyses were not adjusted for multiple testing; that is, a significance level of 0.05 was applied.
Abbreviations: CPB, cardiopulmonary bypass; DO_2i, indexed oxygen delivery; MMRM, mixed models for repeated measures; NIRS, near-infrared spectroscopy; O_2ER, oxygen extraction ratio; RR, risk ratio.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$

support the hypothesis underlying this study. Substantial research has been done on determining the optimal MAP during CPB and comparing low MAP and high MAP during CPB,[15] with the general agreement that perioperative stroke incidence, neurocognitive outcome, or acute kidney injury do not change significantly with different MAP regimens.[16] Research on optimal pump flow during CPB, also including optimal hematocrit, temperature, and DO_2, remains lacking, however. The findings of this study show that a high CPB pump flow of 2.9 L/min/m^2 does not negatively impact the brain injury biomarkers compared to a standard CPB pump flow of 2.4 L/min/m^2, which supports ongoing discussions on the importance of efficient flow rather than MAP for optimal organ perfusion during CPB.

The results for the secondary endpoints showed a significant relationship between several biomarker measure points and treatment characteristics. A notable observation was the associations between age and elevated levels of biomarkers. Across various measurement points, individuals age ≥72 years exhibited noticeably higher levels of NfL, t-tau, and p-tau217 after cardiac surgery. A similar finding was confirmed by Wiberg et al,[17] who found that increasing age was significantly associated with higher levels of NfL during cardiac surgery. Another noteworthy finding was that female sex was associated with significantly lower t-tau levels, >50% lower compared to men. Bridel et al[18] reported similar findings regarding the diagnostic value of NfL in neurology, where the magnitude of the increase varied widely and was higher in men than in women, even among the healthy controls. Furthermore, the findings revealed an association between DO_2i >330 mL/min/m^2 and lower t-tau levels, which aligns with studies supporting the superiority of a goal-directed perfusion flow rate strategy over a fixed indexed CPB flow rate, particularly in terms of kidney protection.[4] This aligns with another of the study's finding that when NIRS exceeded 60% during CPB, a significant decrease in p-tau217 levels was seen.

Interestingly, there was a numerical reduction in postoperative delirium in the group with a high CPB flow rate. Despite the limited sample size, the noteworthy difference in the incidence of postoperative delirium merits consideration of the potential benefits associated with a higher CPB flow rate. However, the findings also show numerically increased

Table 5
Patient, treatment, and biomarker characteristics in the presence and absence of postoperative delirium

Characteristic	No postoperative delirium (N = 35)	Postoperative delirium (N = 4)	p value
Male sex, n (%)	28 (80)	2 (50)	0.22
Age, y, median (IQR)	72 (32-84)	73 (64-78)	0.63
CPB flow rate, L/min, mean ± SD	5.3 ± 0.8	4.5 ± 0.6	0.048*
CPB time, min, mean ± SD	126 ± 56	165 ± 53	0.20
DO_2i, mL/min/m^2, mean ± SD	383 ± 59	345 ± 47	0.22
O_2ER, %, mean ± SD	21 ± 4	24 ± 5	0.20
NIRS CPB, %, mean ± SD	70 ± 10	53 ± 12	0.003**
NfL at baseline, ng/L	20.9 (13.4-36.7)	39.3 (30.0-49.1)	0.10
NfL at 60 min of CPB, ng/L, median (IQR)	19.8 (12.5-29.4)	33.1 (20.6-58.3)	0.09
NfL at 30 min post-CPB, ng/L, median (IQR)	25.0 (17.4-36.2)	36.5 (26.3-49.5)	0.18
NfL at 24 h post-CPB, ng/L, median (IQR)	26.1 (22.5-47.0)	55.6 (50.0-67.6)	0.007**
NfL at 72 h post-CPB, ng/L, median (IQR)	32.6 (25.4-46.2)	47.0 (39.6-57.1)	0.22
GFAP at baseline, ng/L, median (IQR)	232 (164-308)	227 (190-284)	0.87
GFAP at 60 min of CPB, ng/L, median (IQR)	222 (145-293)	215 (161-328)	0.90
GFAP at 30 min post-CPB, ng/L, median (IQR)	256 (191-390)	289 (197-380)	0.99
GFAP at 24 h post-CPB, ng/L, median (IQR)	270 (207-365)	322 (282-372)	0.43
GFAP at 72 h post-CPB, ng/L, median (IQR)	279 (181-337)	232 (172-294)	0.41
T-tau at baseline, ng/L, median (IQR)	10.5 (9.1-12.9)	11.7 (10.7-12.8)	0.40
T-tau at 60 min of CPB, ng/L, median (IQR)	35.0 (26.2-50.7)	38.5 (27.9-56.5)	0.81
T-tau at 30 min post-CPB, ng/L, median (IQR)	59.3 (48.5-79.6)	74.4 (64.4-126.1)	0.12
T-tau at 24 h post-CPB, ng/L, median (IQR)	14.3 (12.1-21.2)	24.8 (19.1-30.8)	0.09
T-tau at 72 h post-CPB, ng/L, median (IQR)	10.7 (9.2-12.5)	11.8 (10.5-15.0)	0.41
P-tau217 at baseline, ng/L, median (IQR)	4.4 (2.9-5.6)	3.4 (2.9-7.1)	0.99
P-tau217 at 60 min of CPB, ng/L, median (IQR)	12.1 (8.4-16.0	9.6 (5.9-14.8)	0.51
P-tau217 at 30 min post-CPB, ng/L, median (IQR)	10.3 (8.0-12.9)	10.5 (7.7-12.9)	0.74
P-tau217 at 24 h post-CPB, ng/L, median (IQR)	6.6 (4.8-10.0)	10.2 (9.5-10.5)	0.19
P-tau217 at 72 h post-CPB, ng/L, median (IQR)	5.7 (4.0-8.3)	6.1 (2.4-8.8)	0.26

Patients identified with postoperative delirium were tested against patients without postoperative delirium. Non-normally distributed data were log-transformed. The Student t test was used for numerical data, and the Fisher exact test was used for categorial data.
Abbreviations: CPB, cardiopulmonary bypass; GFAP, glial fibrillary acidic protein; DO_2i, indexed oxygen delivery; NIRS, near-infrared spectroscopy; NfL, neurofilament light chain; O_2ER, oxygen extraction ratio.
* $p < 0.05$.
** $p < 0.01$.***p < 0.001.

baseline levels of NfL and significantly increased NfL levels at 24 hours in the delirium group, which might indicate a preexisting neurologic decline even before surgery.

The present study has some notable limitations as well as strengths. The results are based on a limited number of patients and constitute a substudy to the main investigation exploring perioperative kidney function in relation to CPB flow rates. Advanced postoperative neurocognitive examinations were not performed; only NuDESC was used to identify behaviors linked to delirium, which could be less sensitive to patients with hypoactive and mixed delirium, which potentially could underestimate the proportion of patients with delirium.[19] Moreover, brain imaging was not performed, and thus the study could not provide insight into the correlations between biomarker levels and neurocognitive function and structural brain injuries following cardiac surgery.

Strengths of the study include the large number of sampled neuronal biomarkers, and the high specificity and sensitivity of the Simoa technology for biomarker analysis, along with the strict standardization of CPB conduct and sample collection.

In conclusion, this study of patients who underwent uncomplicated elective cardiac surgery with CPB found no significant difference in the levels of brain injury biomarkers between the 2 CPB flow rate groups. Associations were identified between biomarker levels and age, sex, CPB time, NIRS, DO_2, and oxygen extraction rate. Further research is warranted to explore the relationships between perioperative biomarker release and clinical outcomes, including postoperative delirium and cognitive decline.

Declaration of competing interest

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests. M.A. reports compensation for lectures and advisory boards from Biogen, Genzyme, and Novartis. L.L. reports consultancy honoraria from XVIVO Perfusion AB. K. B. is a founder of Brain Biomarker Solutions in Gothenburg AB, which is a part of the GU Ventures Incubator Program, that includes board membership. Supported by the Swedish Research Council (Grants 2017-00915 and 2022-00732), the Swedish Alzheimer Foundation (Grants AF-930351, AF-939721, AF-968270, and AF-994551), Hjärnfonden Sweden (Grants FO2017-0243 and ALZ2022-0006), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF agreement (ALFGBG-715986 and ALFGBG-965240), the European Union Joint Program for

Neurodegenerative Disorders (JPND2019-466-236), the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495), the Alzheimer's Association 2022-2025 Grant (SG-23-1038904 QC), La Fondation Recherche Alzheimer, and the Kirsten and Freddy Johansen Foundation. K.B. reports serving as a consultant and on advisory boards for AC Immune, Acumen, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers; serving on data monitoring committees for Julius Clinical and Novartis; and participating in educational programs for AC Immune, Biogen, Celdara Medical, Eisai and Roche Diagnostics. He is a cofounder of Brain Biomarker Solutions in Gothenburg AB. H.Z. declares a financial interest in Biomarker Solutions in Gothenburg AB, which includes board membership. He is a Wallenberg Scholar and a Distinguished Professor at the Swedish Research Council supported by grants from the Swedish Research Council (2023-00356, 2022-01018, and 2019-02397), the European Union's Horizon Europe research and innovation program under Grant 101053962, Swedish State Support for Clinical Research (ALFGBG-71320), the Alzheimer Drug Discovery Foundation (201809-2016862), the AD Strategic Fund and the Alzheimer's Association (ADSF-21-831376-C, ADSF-21-831381-C, ADSF-21-831377-C, and ADSF-24-1284328-C), the Bluefield Project, Cure Alzheimer's Fund, the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (FO2022-0270), the European Union's Horizon 2020 research and innovation program under Marie Skłodowska-Curie Grant 860197, the European Union Joint Programme–Neurodegenerative Disease Research (JPND2021-00694), the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre, and the UK Dementia Research Institute at UCL (UKDRI-1003). He has served on scientific advisory boards and/or as a consultant for AbbVie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche, and is a cofounder of Brain Biomarker Solutions in Gothenburg AB. R. O.B. reports receiving institutional research grants from Bristol-Myers Squibb, Endomag, SkyLineDx, and NeraCare GmbH; receiving speaker's honoraria from Roche, Pfizer, and Pierre-Fabre; and serving on advisory boards for Amgen, BD/BARD, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Roche, and Sanofi Genzyme; and is a shareholder in SATMEG Ventures AB.

CRediT authorship contribution statement

Anna Corderfeldt Keiller: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Markus Axelsson:** Writing – review & editing, Validation, Supervision, Conceptualization. **Gudrun Bragadottir:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization. **Lukas Lannemyr:** Writing – review & editing, Validation, Conceptualization. **Johanna Wijk:** Writing – review & editing, Validation, Investigation. **Kaj Blennow:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Data curation. **Henrik Zetterberg:** Writing – review & editing, Validation, Resources, Funding acquisition, Data curation. **Roger Olofsson Bagge:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

References

1 Newman MF, Mathew JP, Grocott HP, et al. Central nervous system injury associated with cardiac surgery. Lancet 2006;368:694–703. https://doi.org/10.1016/s0140-6736(06)69254-4.
2 Jufar AH, Lankadeva YR, May CN, et al. Renal and cerebral hypoxia and inflammation during cardiopulmonary bypass. Compr Physiol 2021;12:2799–834. https://doi.org/10.1002/cphy.c210019.
3 Murphy GS, Hessel EA 2nd, Groom RC. Optimal perfusion during cardiopulmonary bypass: An evidence-based approach. Anesth Analg 2009;108:1394–417. https://doi.org/10.1213/ane.0b013e3181875e2e.
4 Gao P, Liu J, Zhang P, et al. Goal-directed perfusion for reducing acute kidney injury in cardiac surgery: A systematic review and meta-analysis. Perfusion 2023;38:591–9. https://doi.org/10.1177/02676591211073783.
5 Oshita T, Hiraoka A, Nakajima K, et al. A better predictor of acute kidney injury after cardiac surgery: The largest area under the curve below the oxygen delivery threshold during cardiopulmonary bypass. J Am Heart Assoc 2020;9:e015566. https://doi.org/10.1161/jaha.119.015566.
6 Ali J, Cody J, Maldonado Y, et al. Near-infrared spectroscopy (NIRS) for cerebral and tissue oximetry: Analysis of evolving applications. J Cardiothorac Vasc Anesth 2022;36(8 Pt A):2758–66. https://doi.org/10.1053/j.jvca.2021.07.015.
7 Tobieson L, Zetterberg H, Blennow K, et al. Extracellular fluid, cerebrospinal fluid and plasma biomarkers of axonal and neuronal injury following intracerebral hemorrhage. Sci Rep 2021;11:16950. https://doi.org/10.1038/s41598-021-96364-x.
8 Gaetani L, Blennow K, Calabresi P, et al. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry 2019;90:870–81. https://doi.org/10.1136/jnnp-2018-320106.
9 Evered L, Silbert B, Scott DA, et al. Association of changes in plasma neurofilament light and tau levels with anesthesia and surgery: Results from the CAPACITY and ARCADIAN studies. JAMA Neurol 2018;75:542–7. https://doi.org/10.1001/jamaneurol.2017.4913.
10 Gonzalez-Ortiz F, Ferreira PCL, González-Escalante A, et al. A novel ultrasensitive assay for plasma p-tau217: Performance in individuals with subjective cognitive decline and early Alzheimer's disease. Alzheimers Dement 2024;20:1239–49. https://doi.org/10.1002/alz.13525.
11 Eng LF. Glial fibrillary acidic protein (GFAP): The major protein of glial intermediate filaments in differentiated astrocytes. J Neuroimmunol 1985;8:203–14. https://doi.org/10.1016/s0165-5728(85)80063-1.
12 Simrén J, Elmgren A, Blennow K, et al. Fluid biomarkers in Alzheimer's disease. Adv Clin Chem 2023;112:249–81. https://doi.org/10.1016/bs.acc.2022.09.006.
13 Lannemyr L, Bragadottir G, Hjärpe A, et al. Impact of cardiopulmonary bypass flow on renal oxygenation in patients undergoing cardiac surgery. Ann Thorac Surg 2019;107:505–11. https://doi.org/10.1016/j.athoracsur.2018.08.085.
14 Gaudreau JD, Gagnon P, Harel F, et al. Fast, systematic, and continuous delirium assessment in hospitalized patients: The nursing delirium

screening scale. J Pain Symptom Manage 2005;29:368–75. https://doi.org/10.1016/j.jpainsymman.2004.07.009.

15 Kotani Y, Kataoka Y, Izawa J, et al. High versus low blood pressure targets for cardiac surgery while on cardiopulmonary bypass. Cochrane Database Syst Rev 2022;11:CD013494. https://doi.org/10.1002/14651858.CD013494.pub2.

16 Ranucci M, Cotza M, Di Dedda U. The conundrum of systemic arterial pressure management on cardiopulmonary bypass. J Clin Med 2023;12:806. https://doi.org/10.3390/jcm12030806.

17 Wiberg S, Holmgaard F, Blennow K, et al. Associations between mean arterial pressure during cardiopulmonary bypass and biomarkers of cerebral injury in patients undergoing cardiac surgery: Secondary results from a randomized controlled trial. Interact Cardiovasc Thorac Surg 2021;32:229–35. https://doi.org/10.1093/icvts/ivaa264.

18 Bridel C, van Wieringen WN, Zetterberg H, et al. Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: A systematic review and meta-analysis. JAMA Neurol 2019;76:1035–48. https://doi.org/10.1001/jamaneurol.2019.1534.

19 Kotfis K, Marra A, Ely EW. ICU delirium - a diagnostic and therapeutic challenge in the intensive care unit. Anaesthesiol Intensive Ther 2018;50:160–7. https://doi.org/10.5603/AIT.a2018.0011.