Cardiac surgery is associated with biomarker evidence of neuronal damage

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Abbreviated title: Neuronal damage in cardiac surgery

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Abstract

BACKGROUND: Anesthesia and surgery is commonly associated with central nervous system sequelae and cognitive symptoms, which may be caused by neuronal injury. Neuronal injury can be monitored by plasma concentrations of the neuronal biomarkers tau and neurofilament light protein (NFL). Currently, there are no studies examining whether neuronal injury varies between surgical procedures.

OBJECTIVE: Our aim was to investigate if neuronal damage is more frequent after cardiac than after otolaryngeal surgery, as estimated by tau and NFL concentrations in plasma.

METHODS: Blood samples were drawn before, during and after surgery and concentrations of tau, NFL, Aβ40 and Aβ42 were measured in 25 patients undergoing cardiac surgery (9 off-pump and 16 on-pump) and 26 patients undergoing otolaryngeal surgery.

RESULTS: Tau increased during surgery (1752%, P=0.0001) and NFL rose seven days post-surgery (1090%, P<0.0001) in patients undergoing cardiac surgery; even more in patients on-pump than off-pump. No changes were observed in patients undergoing otolaryngeal surgery and only minor fluctuations were observed for Aβ40 and Aβ42.

CONCLUSION: Cardiac surgery is associated with neuronal injury, which is aggravated by extracorporeal circulation. Analyses of NFL and tau in blood may guide development of surgical procedures to minimize neuronal damage.

Introduction

Initially, it was thought that once the post-op period passed, any effects by anaesthesia and surgery on cognition would be reversed. However, several studies have shown that problems with cognition are common after surgery and this was already acknowledged in the 1960s [1, 2]. Post-operative cognitive dysfunction (POCD) is frequent after general surgery [3] and possibly even more so after cardiac surgery [4]. Research into POCD has focused on causes external to the CNS such as physiological perturbations including hypoxemia, hypotension [3], microembolism [5], or inflammation [6]. Clinical studies have also tried to evaluate the effect of anaesthesia, by comparing regional versus general anaesthesia but only found no or minor effect [7-9].

It has been suggested that cognitive problems after anaesthesia and surgery may be caused by neuronal damage. One study investigating this found small and transient changes in the markers NSE and S100B after cardiac surgery [10], but neither of these markers are specific to CNS injury [11]. Other markers which better reflect neuronal damage include the microtubule-tubule associated protein tau and the neurofilament light protein (NFL). Tau is a protein specifically expressed in neurons and is currently used as a clinical biomarker primarily of cortical neuronal damage [12], but is also very sensitive to acute neuronal injury [11, 13]. NFL together with its medium and heavy counterpart make up the intermediate axonal filaments called neurofilaments that determine axonal calibre and in part axonal velocity [14]. NFL is also a well-established biomarker for acute neuronal damage, both in cerebrospinal fluid (CSF) and in blood [11, 15]. Both tau and NFL can be analysed in blood by sensitive analyses [16, 17].

Recently, Evered et al found that tau increased 250% and NFL increased 67% during anaesthesia and surgery indicating neuronal injury [18]. However, since that cohort was mixed it is presently unclear whether neuronal injury is more common in any type of surgery.

To address this we collected samples before, during and after cardiac surgery and otolaryngeal surgery and measured plasma tau and NFL to test the hypothesis that surgery in general and cardiac surgery in particular is associated with neuronal injury reflected by changes in these biomarkers. Furthermore, we compared cardiac surgery patients on and off pump to investigate the impact of extracorporeal circulation on neuronal injury. We also measured plasma levels of the AD markers Aβ40, which is thought to reflect the individual production of Aβ with normal levels in Alzheimer's disease (AD) [12], and Aβ42 which in CSF is an AD biomarker with lower levels due to deposition into amyloid plaques [12, 19], to test that there is no general, unspecific release of brain-specific proteins by general anaesthesia and surgery. We also measured NFL and tau in patients with myocardial infarction to rule out that these biomarkers are released from the myocardium during damage.

Material and methods

Study subjects undergoing cardiac or otolaryngeal surgery

The study consisted of 26 patients who underwent otolaryngeal surgery and 25 subjects who underwent cardiac surgery at the University Teaching Hospital, Bialystok, Poland (Tables 1 and 2). The study was approved by the Ethical Committee, Medical University of Bialystok Clinical Hospital, Poland, and all patients gave informed consent.

Inclusion criteria included an ejection fraction (EF) above 40% and that the one and the same anesthesiologist was scheduled for the planned surgery. Exclusion criteria were type 1 diabetes, non-stable endocrine disease or thyrotoxicosis, active infectious disease, a severely

impaired immunological function (i.e. post-transplantation, autoimmune disease or cancer treatment), severe psychiatric illness, severe depression, or acute surgical procedure.

Blood samples were taken from the radial artery at baseline (sample 1), during surgery (sample 2), at the end of surgery (sample 3), 24 hours post-surgery (sample 4), and 7 days post-surgery (sample 5) and collected into standardized polypropylene collection test tubes containing EDTA. Within 30 minutes the blood was centrifuged (1200 g, 5 min) and plasma was aliquoted and frozen at -80 C.

Subjects sampled during myocardial infarction
Blood samples from sixteen individuals (7 females and 9 men; age range 45-89) with myocardial infarction were collected at the Sahlgrenska University Hospital in Mölndal.

Anaesthesia and surgery
Oral benzodiazepine (midazolam, 0,2. mg/kg) was used as premedication. All patients undergoing either cardiac or otolaryngeal surgery were given general anesthesia with the same set of pharmacological agents: sulfentanil, pancuronium and etomidate as induction and sevoflurane as maintenance anesthesia. The concentrations of respiratory gases (oxygen, air, and sevoflurane) were continuously monitored by real-time spectroscopy. The depth of sevoflurane anesthesia was assessed by clinical and hemodynamic parameters and drug concentrations in the respiratory mixture was measured spectrophotometrically by gas analyzer and adjusted to the level of minimum alveolar concentration (MAC), calculated with the use of algorithm for Dräger Optima. MAC was maintained between 1.1-1.5. Normal arterial blood gas values were secured by mechanical ventilation. pH was maintained between 7.43 and 7.45. Arterial acid-base balance assessment was performed according to the calculated Siemens algorithm for Siemens Rapid Lab 348. During the extra-corporeal circulation, the gasometric values were recalculated according to the alpha-stat algorithm. All

patients with extracorporeal circulation had gas values assessed every 15-30 minutes; patients without extra-corporeal circulation – every 30 minutes. CO_2 was assessed by capnography at the same time points and were maintained between 30-40 mmHg. Multi-parameter ECG with ST segment and rhythm disturbance analysis of five precordial leads was recorded. Measurements of carbon dioxide by capnometry plethysmography, heart rate, hemoglobin saturation were also monitored.

Peripheral blood pressure measurements were performed using continuous invasive measurements with a canula inserted into right radial artery in all patients. In both study groups, central venous pressure (CVP) were measured in vena cava superior (VCS) using a canula inserted to VCS via the right internal jugular vein. In addition, patients undergoing valvular surgery, had a Swan-Ganz catheter (Braun AG) inserted in a small pulmonary artery that also measured blood pressure, pulmonary artery pressure, wedge pressure and calculated cardiac projection by thermodilution.

CABG was performed at normothermia and AVR at 32 degrees Celsius. Cardioplegic and potassium solutions were used according to the method of Calafiore [20] with warm cardioplegia for CABG procedures.

Extracorporeal circulation was carried out on a Stockert camera using Medtronic Fusion oxygenators. The flow of respiratory gases was calculated from a rotameter (Sechrist, Air/Oxygen gas mixers Model 20090, USA). Standard doses of unfractionated heparin (UHF) were used as an anticoagulant during extracorporeal circulation with doses calculated according to body weight and antithrombin coagulation time (ACT).

Patients prophylactically received two doses of intraoperative cefazolin and low molecular weight heparin and the patients undergoing coronary surgery also received aspirin.

To minimize blood loss and to improve surgical conditions mean systemic arterial pressure was maintained between 60 to 70 mmHg in patients undergoing cardiac and otolaryngeal surgery alike.

Biomarker analysis

Plasma levels of Aβ40 and Aβ42 were measured with a multiplex assay from Fujirebio (Gent, Belgium) on Luminex IS100 and plasma levels of tau and NFL were measured with an ultra-sensitive single molecule array (Simoa, Lexington, MA) technology, as previously described [16, 17]. Troponin T concentration was measured in clinical routine (Roche, Diagnostics, Vienna, Austria). All plasma levels were corrected for IgG levels to compensate for possible differences in plasma volume due to fluid replacement during surgery. This did not change results or significances as compared with uncorrected values (data not shown). Biomarker measurements were performed in clinical laboratory using methods and procedures accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC). The measurements were performed by board-certified laboratory technicians who were blinded to clinical data.

Statistical analysis

Analysis of differences in neuronal protein levels was performed using linear mixed effects models, with procedure and time included as fixed effects and participants as a random effect. In order to better compare plasma biomarker levels between patients undergoing the two different forms of surgery, we normalized the values by setting baseline (i.e. before surgery) levels to 100%, and dividing concentrations in longitudinal samples with the baseline value in each patient (raw data values are given Table 3). We included age and sex as covariates and evaluated main and interaction effects using the Wald test. Comparisons between-time/within-procedure (difference between time points within the cardiac surgery or the otolaryngeal

surgery group) and within-time/between-procedure (difference between the cardiac surgery and the otolaryngeal surgery group at a specific time point) were evaluated by contrasting least-square means. We report an effect size for each comparison as the percent difference in neuronal protein levels between compared groups as estimated by the least-square means, along with P values adjusted for multiple comparisons using the Tukey method.

Associations between neuronal protein levels and troponin T levels in myocardial infarction patients were calculated using partial Spearman's correlation adjusted for age and sex.

All tests were pre-planned and two-sided. Statistical analysis was performed using the R programming language, version 3.4.3, along with the *nlme,* version 3.1, and *lsmeans,* version 2.27, packages.

Results

The effect of surgery on serum levels of tau

Six out of 200 (3%) measurements were missing for tau due to lack of sample volume. Tau levels were significantly elevated in the patients undergoing cardiac surgery both during (mean difference, 1701%; P=0.0001) and at the end of surgery compared to baseline (mean difference, 1752%; P<0.0001; Figure 1A). However, there were no significant change in tau levels over time in the patients undergoing otolaryngeal surgery. Tau levels were significantly increased during and at the end of surgery in patients undergoing cardiac surgery compared to the same time points in otolaryngeal surgery (mean difference, 1413%;, P=0.004; mean difference, 1416%; P=0.004, respectively, Figure 1A).

The effect of surgery on serum levels of NFL

Six out of 200 (3%) measurements were also missing for NFL due to lack of sample volume. NFL levels in the cardiac surgery group seven days post-surgery were significantly increased compared to all other time points – baseline (mean difference, 1090%; P<0.0001), during

surgery (mean difference, 1042%; P<0.0001), at the end of surgery (mean difference, 991%; P<0.0001), and 24 hours post-surgery (mean difference, 933%; P<0.0001, Figure 1B). As for tau, there were no significant differences over time for NFL in the otolaryngeal surgery group. NFL levels 24 hours and seven days post-surgery were significantly increased in the cardiac group compared to the corresponding time points in the otolaryngeal group (mean difference, 174%, P=0.01, and mean difference, 1065%, P<0.0001, respectively; Figure 1B).

Aβ40

Aβ40 levels were significantly elevated during surgery compared to baseline (mean diff., 64.18 pg/mL; P<0.0001), 24 hours post-surgery (mean diff., 34.57 pg/mL; P=0.0001), and seven days post-surgery (mean diff., 36.96 pg/mL; P=0.0001) in the cardiac group (Figure 1C). Also, Aβ40 levels in the cardiac group at the end of surgery were significantly elevated compared to baseline (mean diff., 56.75 pg/mL; P<0.0001), 24 hours post-surgery (mean diff., 27.15 pg/mL; P=0.005), and seven days post-surgery (mean diff., 29.53 pg/mL; P=0.004; Figure 1C). Similarly, Aβ40 levels in the cardiac group remained elevated seven days post-surgery compared to baseline (mean diff., 27.22 pg/mL; P=0.01). Interestingly, we found qualitatively similar results in the otolaryngeal group, except that Aβ40 levels seven days post-surgery returned to baseline levels.

We also found that Aβ40 levels after induced hypotension were significantly elevated in the cardiac group compared to the otolaryngeal group (mean diff., 27.79 pg/mL; P=0.02; Figure 1C).

Aβ42

Aβ42 levels during surgery were elevated compared to baseline (mean diff., 59.3 pg/mL; P=0.0001), and 24 hours post-surgery (mean diff., 32.6 pg/mL; P=0.04) in the cardiac group (Figure 1D). The Aβ42 levels were also elevated at the end of surgery compared to baseline

(mean diff., 79.8 pg/mL; P<0.0001), 24 hours post-surgery (mean diff., 53.0 pg/mL; P=0.0001), and seven days post-surgery (mean diff., 39.6 pg/mL; P=0.01) in the cardiac group (Figure 1D). As with Aβ40, changes in Aβ42 levels in the otolaryngeal group followed a similar profile to the cardiac group between time points. Furthermore, Aβ42 levels at the end of surgery were significantly elevated in the cardiac group compared to otolaryngeal group (mean diff., 44.1 pg/mL; P=0.04; Figure 1C).

Extracorporeal circulation and neuronal protein levels

Of the 25 patients undergoing cardiac surgery, 16 were on extracorporeal circulation (on-pump) and 9 underwent off-pump coronary artery bypass. On-pump cardiac surgery patients had elevated tau levels compared to off-pump cardiac surgery patients during surgery (mean difference, 2099%; P=0.02) and at the end of surgery (mean difference, 2213%; P=0.01, Figure 2A). The within-group profile of NFL change over time was similar for both on-pump and off-pump cardiac surgery patients. However, NFL levels for those on-pump were increased compared to those off-pump seven days post-surgery (mean difference, 431%; P=0.006; Figure 2B).

Furthermore, after comparing neuronal protein levels between the on-pump cardiac surgery group and the otolaryngeal surgery group, tau levels were found to be significantly elevated in the on-pump surgery group, particularly during surgery (mean difference, 2362%; P<0.0001), at the end of surgery (mean difference, 2453%; P<0.0001), and 24 hours post-surgery (mean difference, 1227%; P=0.009). Similarly, NFL levels were elevated in the on-pump cardiac surgery group compared to the otolaryngeal surgery group 24 hours post-surgery (mean difference, 202%, P=0.009) and seven days post-surgery (mean difference, 1184%; P<0.0001). Differences were also analysed between the off-pump cardiac surgery group and the otolaryngeal surgery group. Tau levels were still elevated in the off-pump cardiac surgery group compared to the otolaryngeal surgery group during surgery (mean difference, 136%;

P=0.009), at the end of surgery (mean difference, 152%; P=0.003), and 24 hours post-surgery (mean difference, 138%; P=0.006; Figure 3A). Similarly, NFL levels seven days post-surgery were still significantly elevated in the off-pump cardiac surgery group compared to the otolaryngeal surgery group (mean difference, 716%; P<0.0001; Figure 3B).

No effect of myocardial infarction on neuronal markers
To rule out that the increases in the neuronal biomarkers tau and NFL were due to release from the myocardium, these proteins were measured in blood samples from patients with myocardial infarction. However, myocardial infarction was not associated with increased release of tau as there was no correlation between plasma levels of troponin T and tau (rho=0.08, P=0.78); nor was there any significant correlation between plasma levels of troponin T and NFL following myocardial infarction (rho=0.50, P=0.07).

Discussion

Patients undergoing cardiac surgery had markedly increased plasma levels of the neuronal protein tau during surgery and at the end of surgery compared to baseline levels in the same patient, as well as compared to the same time points in patients undergoing otolaryngeal surgery. Furthermore, patients undergoing cardiac surgery also had greatly increased plasma levels of NFL one week post-surgery, which was not observed in patients undergoing otolaryngeal surgery. In addition, extracorporeal circulation worsened the increase in plasma levels of both tau and NFL within the group of cardiac surgery patients.

Our findings are in concert with a previous study showing minor to moderate increases in tau and NFL in patients undergoing surgery [18]. However, that study examined a mixed population of mainly orthopaedic patients, with data presented at the group level and only a minority (6 out of 30) being cardiac surgery patients. Indeed, the changes were also much less pronounced than in the present study (250% vs 1750% for tau and 67% vs 1100% for NFL).

Importantly, no time-points after 48 hours were included in that study, meaning that any NFL peak around 7-10 days may have been overseen given the slow temporal course for the increase in blood NFL levels after an acute brain insult [21].

The most likely interpretation of our findings is that neuronal injury occurs to a greater extent in patients undergoing cardiac surgery than otolaryngeal surgery. It is foreseeable that an increase in tau could be due to a transient insult which in some cases may be reversible since these levels normalize after one week post-surgery. In contrast, since NFL is a part of neurofilaments, that are the main intermediary filament in axons, it is unlikely that the marked release of NFL reflects anything but neuronal damage. Supporting this are findings from neurodegenerative diseases where the highest levels of NFL are found in amyotrophic lateral sclerosis (ALS) where motor neurons with the longest axons in the body are irreversibly lost [22]. Increased levels of NFL have also been found in multiple sclerosis (MS) and they correlate with brain volume reduction and the number of contrasting enhanced lesions on MRI [23]. Further support of increased tau and NFL being related to the severity of neuronal damage comes from several studies that have shown marked increases in blood concentrations of these biomarkers after acute traumatic brain injury (TBI), with manifold higher levels in severe than in mild TBI, where higher levels predict functional and neurological outcomes [21, 24]. Similarly, marked increases in tau and NFL are also found in patients with cardiac arrest, where higher levels are highly predictive of poor neurological outcome and survival [25, 26]. Last, the time-course for the increase in tau and NFL was clearly different, with a rapid increase in plasma tau (peaking during the surgical procedure) but a slow gradual increase in NFL (with the highest levels one week post-surgery). This difference in temporal profile between tau and NFL is in agreement with previous studies [18, 21]. The reason behind the difference in the temporal profile of tau and NFL is unknown but likely caused by different mechanisms where the release of tau may be caused by stress to neurons and active

secretion, whereas the delayed release of NFL may occur when the neurons, and especially the axons, are damaged and neurofilaments are broken down slowly and released passively. Together with the lack of change in plasma Aβ levels, the tau and NFL results suggest that these changes are not due to unspecific release of neurological proteins during or after general anaesthesia and surgery. Thus, our results indicate that cardiac surgery results in neuronal damage.

Why does neuronal damage occur to a greater extent in cardiac than otolaryngeal surgery? Mean arterial pressure was kept at 60-70 mm Hg throughout both forms of surgeries in the present study ruling out hypoperfusion as a reason for the differences in tau and NFL levels. Extracorporeal circulation has been proposed as a cause of confusion, POCD and CNS damage in cardiac surgery patients [27, 28]. In our study, the majority of cardiac surgery patients (n=16) were on-pump, while a minority were off-pump (n=9). Indeed, plasma levels of tau were greatly elevated during surgery and at the end of surgery, as were NFL levels seven days post-surgery in the patients on-pump compared to those off-pump. Thus, our data indicate that extracorporeal circulation aggravates the neuronal damage caused by cardiac surgery.

We did not perform any a priori power calculation since this is the first study of its kind and therefore the expected effect size was unclear.

Neither tau nor NFL are expressed in the heart according to the expression database BioGPS (http://biogps.org). Thus, the increases in tau and NFL were not caused by direct damage to the heart during cardiac surgery but rather reflect neuronal damage. This conclusion was further supported by the lack of correlation between plasma levels of tau and NFL with plasma levels of troponin T in patients with myocardial infarction.

Both tau and NFL levels are almost ten-fold higher in CSF compared to serum/plasma levels suggesting that the neuronal damage may be underestimated by studying serum levels [21, 29].

Regarding the difference in length of surgery between the otolaryngeal and cardiac groups, we cannot rule out that this may have affected the results. However, within both the cardiac and otolaryngeal groups, no association between tau or NFL levels and length of surgery was observed (data not shown), suggesting that the effect was minor.

Since tau and NFL are indicative of neuronal damage and death, future studies may analyse these biomarkers when developing new surgical methods to identify regimes with minimal effects on the CNS, aiming for reducing post-operative cognitive decline especially in elderly patients.

In conclusion, cardiac surgery was associated with a marked release of the neuronal markers tau during and at the end of surgery and NFL seven days post-surgery. This is likely indicative of neuronal damage, which was not observed in the contrast group of patients undergoing otolaryngeal surgery. Importantly, the increases in levels of these biomarkers reflecting neuronal were further aggravated by extracorporeal circulation. Thus, analyses of NFL and tau may in the future help to improve surgical procedures to minimize neuronal damage.

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Table 1 Cardiac surgery patients

ID	Procedure	ECT	Age	Sex
1	MVI	128	59	F
2	AVR, MVR	166	77	F
3	OPCAB	NA	63	M
4	AVR	89	64	M
5	AVR	127	61	F
6	OPCAB	NA	62	M
7	AVR, MVR, TVP	185	70	F
8	Bentall	109	76	F
9	OPCAB	NA	80	M
10	CABG	102	72	M
11	OPCAB	NA	76	M
12	AVR, MVR, CABG	196	79	M
13	AVR, MVR	181	70	M
14	MVR	190	45	M
15	OPCAB	NA	70	F
16	CABG, SVR	70	52	F
17	MVR, TVP	192	58	F
18	CABG	89	67	M
19	CABG	130	67	F
20	CABG	85	76	M
21	CABG	101	80	F
22	OPCAB	NA	60	M
23	OPCAB	NA	62	F
24	OPCAB	NA	68	M
25	OPCAB	NA	66	F

ECT: extracorporeal circulation time, MVI: mitral valve implantation, AVR: aortic valve replacement, MVR: mitral valve replacement, OPCAB:

off pump coronary artery bypass, TVP: tricuspid valve procedure, SVR: surgical ventrical restoration, CABG: coronary artery bypass grafting.

Table 2 Otolaryngeal surgery patients

ID	Procedure	ECT	Age	Sex
1	FESS, SS	NA	51	F
2	FESS	NA	50	F
3	FESS	NA	33	M
4	FESS, SS, INF, CP	NA	34	M
5	FESS, EOD, SS	NA	35	F
6	FESS, CP	NA	43	M
7	FESS, SS, CP	NA	31	F
8	FESS	NA	31	F
9	FESS, SS	NA	48	M
10	FESS	NA	69	M
11	FESS	NA	47	M
12	FESS	NA	51	M
13	FESS	NA	59	M
14	FESS, SS	NA	55	M
15	FESS, SS, INF	NA	25	F
16	FESS, SS	NA	18	F
17	FESS	NA	65	F
18	FESS, SS	NA	63	M
19	FESS	NA	25	F
20	FESS	NA	51	M
21	EOD	NA	56	F
22	FESS, SS	NA	53	M
23	FESS	NA	23	F
24	FESS	NA	54	M
25	FESS	NA	33	F
26	FESS	NA	52	F

ECT: extracorporeal circulation time, FESS: functional endoscopic sinus surgery, SS: sinus surgery (extended), INF: infundibulotomy, CP: conchoplasty, EOD: endoscopic orbital decompression.

Table 3 Raw values for tau, NFL, Aβ40 and Aβ42 during otolaryngeal and cardiac surgery on and off pump.

Biomarker	Otolaryngeal surgery	Cardiac surgery combined	Cardiac surgery on-pump	Cardiac surgery off-pump
Tau 1	2.8 (1.6-4.6)	3.2 (2.3-4.1)	3.2 (2.4-4.1)	3.1 (2.3-3.9)
Tau 2	3.2 (2.1-6.4)	16.4 (11.3-51.6)	33.9 (15.9-60.3)	7.2 (6.5-60.3)
Tau 3	3.5 (2.6-4.8)	21.8 (8.8-50.5)	47.1 (23.4-60.6)	7.9 (7.1-9.0)
Tau 4	2.0 (1.6-3.0)	6.6 (5.0-11.2)	8.3 (6.4-13.2)	4.7 (3.6-6.8)
Tau 5	1.7 (1.6-2.2)	3.1 (2.4-5.4)	3.1 (2.4-5.0)	2.9 (2.5-6.2)
NFL 1	9.0 (7.0-13.1)	21.2 (13.4-35.7)	19.3 (12.9-27.5)	29.5 (16.5-54.8)
NFL 2	9.2 (6.9-17.2)	22.8 (13.4-34.9)	20.5 (13.4-30.7)	29.5 (19.0-65.0)
NFL 3	11.4 (8.0-20.6)	25.5 (20.4-38.4)	28.3 (21.3-36.1)	22.4 (17.7-43.8)
NFL 4	10.6 (8.2-21.5)	40.9 (26.6-57.6)	41.8 (33.1-57.4)	38.1 (22.0-83.1)
NFL 5	16.4 (12.6-25.1)	188.8 (154.3-288.4)	188.8 (166.4-299.4)	219.3 (142.2-284.4)
Aβ40 1	114.9 (104.0-147.9)	194.9 (175.2-221.9)	192.8 (178.4-225.0)	201.2 (149.1-204.9)
Aβ40 2	137.6 (126.0-162.1)	214.3 (174.9-244.6)	184.8 (168.5-229.8)	238.3 (220.5-308.3)
Aβ40 3	137.9 (113.0-153.6)	192.4 (153.8-232.2)	184.1 (153.0-219.6)	202.4 (169.2-232.2)
Aβ40 4	123.7 (105.7-132.5)	184.3 (161.7-253.6)	185.0 (172.6-255.2)	184.3 (153.5-253.6)
Aβ40 5	125.7 (109.1-141.0)	196.4 (174.6-223.2)	196.9 (179.1-250.3)	167.7 (148.7-216.2)
Aβ42 1	36.0 (29.6-41.9)	43.3 (35.7-52.7)	45.2 (36.1-55.9)	39.3 (35.7-42.3)
Aβ42 2	45.4 (37.2-49.6)	44.9 (35.1-49.0)	42.0 (31.5-45.8))	48.2 (44.1-51.9)
Aβ42 3	42.7 (35.1-48.0)	44.1 (39.2-51.2)	40.6 (35.2-51.5)	45.3 (41.9-50.0)
Aβ42 4	37.2 (30.6-42.8)	38.5 (28.1-49.9)	38.9 (28.0-50.2)	38.3 (34.5-42.5)
Aβ42 5	36.3 (32.7-42.0)	44.9 (41.0-51.2)	46.4 (42.5-53.5)	41.7 (35.2-44.0)

Timepoints: 1 baseline, 2 during surgery, 3 at the end of surgery, 4 24-hours post-surgery and 5 seven days post-surgery. Data are presented as median and interquartile range.

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Figure legends

Figure 1. Plasma levels of tau, NFL, Aβ40 and Aβ42 before, during and after cardiac and otolaryngeal surgery. (A) Changes in plasma levels of tau, (B) NFL, (C) Aβ40 and (D)

Aβ42 before, during and after cardiac surgery and otolaryngeal surgery. The levels are normalized in each figure and the baseline is set to 100%. The P-values are adjusted for age and sex whereas the plasma levels in the graphs are the raw data values. Data are presented as mean±SEM. Baseline (sample 1), during surgery (sample 2), at the end of surgery (sample 3), 24 hours post-surgery (sample 4), and 7 days post-surgery (sample 5).

Figure 2. Plasma levels of tau and NFL before, during and after cardiac surgery on or off pump. (A) Tau and (B) NFL before, during and after cardiac surgery on or off extracorporeal circulation. The levels are normalized in each figure and the baseline is set to 100%. The P-values are adjusted for age and sex whereas the plasma levels in the graphs are the raw data values. Data are presented as mean±SEM. Baseline (sample 1), during surgery (sample 2), at the end of surgery (sample 3), 24 hours post-surgery (sample 4), and 7 days post-surgery (sample 5).

Figure 3. Plasma levels of tau and NFL before, during and after cardiac surgery off-pump and otolaryngeal surgery. (A) Tau and (B) NFL before, during and after cardiac surgery off extracorporeal circulation and otolaryngeal surgery. The P-values are adjusted for age and sex whereas the plasma levels in the graphs are the raw data values. The levels are normalized in each figure and the baseline is set to 100%. Data are presented as mean±SEM. Baseline (sample 1), during surgery (sample 2), at the end of surgery (sample 3), 24 hours post-surgery (sample 4), and 7 days post-surgery (sample 5).
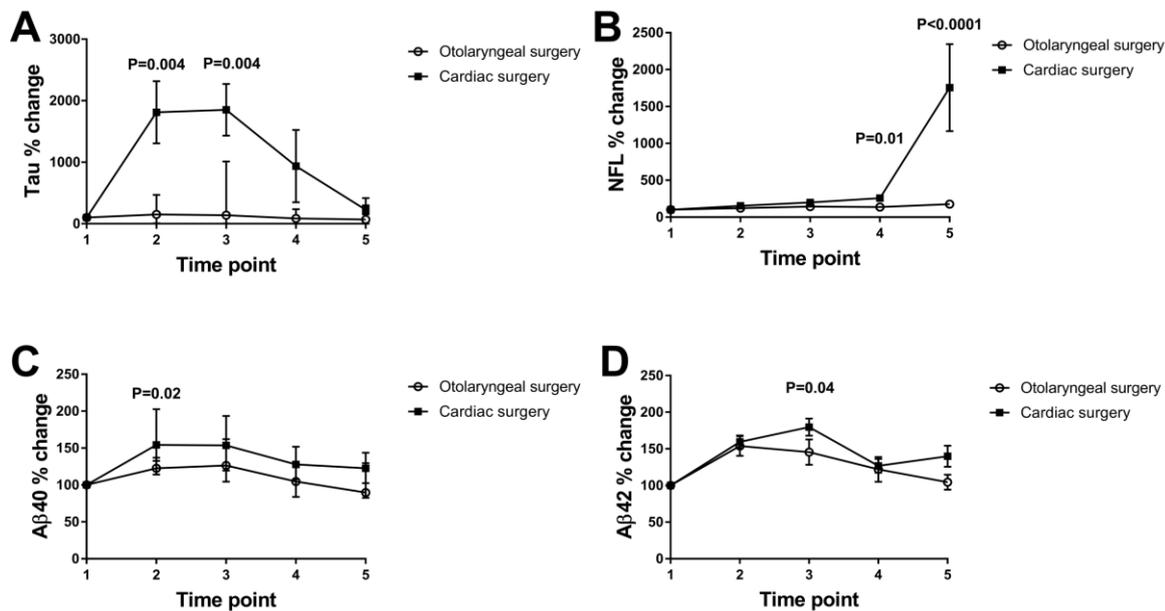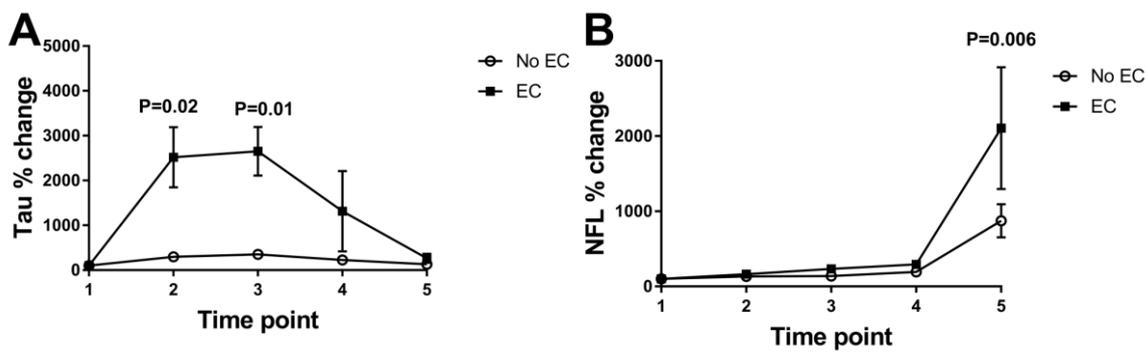
Figures

Figure 1

Figure 2

Figure 3