

Perioperative ischaemic brain injury and plasma neurofilament light: a secondary analysis of two prospective cohort studies

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Abstract

Background: Ischaemic brain infarction can occur without acute neurological symptoms (covert strokes) or with symptoms (overt strokes), both associated with poor health outcomes. We conducted a pilot study of the incidence of preoperative and postoperative (intraoperative or postoperative) covert strokes, and explored the relationship of postoperative ischaemic brain injury to blood levels of neurofilament light, a biomarker of neuronal damage.

Methods: We analysed 101 preoperative (within 2 weeks of surgery) and 58 postoperative research MRIs on postoperative days 2-9 from two prospective cohorts collected at the University of Wisconsin (NCT01980511 and NCT03124303). Participants were aged >65 yr and undergoing non-intracranial, non-carotid surgery.

Results: Preoperative covert stroke was identified in 2/101 participants (2%; Bayesian 95% confidence interval [CI], 0.2-5.4). This rate was statistically different from the postoperative ischaemic brain injury rate of 7/58 (12%, 4.9-21.3%; $P=0.01$) based on postoperative imaging. However, in a smaller group of participants with paired imaging ($n=30$), we did not identify the same effect ($P=0.67$). Patients with postoperative brain injury had elevated peak neurofilament light levels (median [inter-quartile range], 2.34 [2.24-2.64] \log_{10} pg ml⁻¹) compared with those without (1.86 [1.48-2.21] \log_{10} pg ml⁻¹; $P=0.025$). Delirium severity scores were higher in those with postoperative brain injury (19 [17-21]) compared with those without (7 [4-12]; $P=0.01$).

Conclusion: Although limited by a small sample size, these data suggest that preoperative covert stroke occurs more commonly than previously anticipated. Plasma neurofilament light is a potential screening biomarker for postoperative ischaemic brain injury.

Keywords: brain injury; cognition; delirium; stroke; surgery.

Introduction

Perioperative ischemic brain infarction can occur as ‘overt strokes’ with neurological symptoms, or as ‘covert strokes’, acute cerebral ischaemic infarction without clinical manifestations. Although historically classified as “silent”, these covert strokes significantly impact long-term health outcomes, with increased neurological deficits, cognitive dysfunction, overt stroke, and mortality.^{1,2} Strong risk factors for covert stroke include age (>70 years), hypertension, metabolic syndrome, carotid artery disease, and chronic kidney disease.³

The NeuroVISION-1 study recently reported that the incidence of perioperative covert stroke, detected on postoperative magnetic resonance imaging (MRI), was 7% (95% Confidence Interval [CI] 6-9%) based on 78 participants with covert stroke among 1114 participants ≥ 65 years of age

who underwent elective non-cardiac surgery.² We also reported that perioperative covert stroke was associated with a doubling of the adjusted odds ratio (1.98, 95% CI 1.20-3.22) of cognitive decline (defined as a reduction of two points from baseline on the Montreal Cognitive Assessment scale) at 1-year follow-up. MRI scans were conducted from days 2-9 postoperatively as diffusion-weighted lesions on MRI typically remain obvious on imaging for up to nine days after the event. While pragmatic, this design does not exclude the possibility that some lesions arose in the days before surgery.

Covert stroke in the community is rare, with an estimated incidence of 0.3%.⁴ However, surgical patients are at higher risk of cognitive decline than non-admitted patients,⁵ so it is unclear if we can assume such a low incidence applies preoperatively. The overall incidence of covert stroke in the background population is strongly correlated with age and is estimated to be substantially higher in those aged 70 years and older.⁶ Hence preoperative scanning will help exclude the possible misclassification of pre-existing lesions as new perioperative covert strokes. Due to the size of the NeuroVISION cohort and the costs involved with MRI, preoperative scanning was not routinely conducted. Instead, in a single centre, parallel study, we sought to identify the incidence of preoperative covert stroke in the Interventions in Perioperative Delirium (IPOD-B3) cohorts. Through dual-enrolling in the NeuroVISION-1 and IPOD-B3 studies, we sought to confirm that lesions identified as postoperative covert strokes were not present on preoperative imaging.

The standard for detecting acute brain infarction is a brain MRI with diffusion-weighted imaging. However, its use outside of research is not practical for all patients undergoing noncardiac surgery. The use of biomarkers of neuroaxonal damage potentially offers a more accessible method for measuring neuronal injury. Neurofilament light chain (NfL) is an intermediate filament protein that forms part of the neuronal cytoskeleton and is exclusively expressed in neurons.⁷ It is released into the peripheral blood and cerebrospinal fluid circulation in response to neuroaxonal damage subsequent to ischaemia and other neurological conditions that are associated with neurodegeneration.^{8,9} Increases in NfL are associated with postoperative delirium.^{3,9,10}

In summary, at the University of Wisconsin, we enrolled patients into NeuroVISION-1 and our on-going perioperative cohort study (IPOD-B3) that included preoperative magnetic resonance imaging and plasma biomarkers. Some patients were dual enrolled in these two studies allowing

us to compare preoperative and postoperative MRI scans. We have undertaken analyses to inform the following objectives:

1. to determine the incidence of preoperative covert stroke;
2. to confirm that lesions identified as postoperative covert stroke were not present preoperatively; and
3. to determine the association between perioperative covert stroke and peak postoperative NfL.

Methods

Study Design

This is an analysis of data from two prospective cohort studies (i.e., IPOD-B3 and NeuroVISION-1) undertaken at the University of Wisconsin, Madison, Wisconsin, USA. Ethic approvals were obtained from the University of Wisconsin-Madison (UWM) Institutional Review Board (2015-0374), and the studies were registered on clinicaltrials.gov (NCT01980511 and NCT03124303). Data are reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.¹¹

Participants

Participants were eligible if aged 65 years or older and undergoing elective, non-intracranial, non-carotid artery surgery with a minimum anticipated hospital stay of two days. Participants were excluded if they had a documented history of dementia, resided in a nursing home, or were unable to complete neurocognitive testing due to language, vision, or hearing impairment.

Study procedure

Preoperatively, participants provided demographic and clinical information, a blood sample, and underwent cognitive assessments. Cognitive assessments included the Montreal Cognitive Assessment (MoCA), which was the primary outcome of the NeuroVISION-1 study, the Digital Symbol Substitution Test (DSST), and Trail Making Test B (TMT-B). They also underwent a preoperative and/or postoperative MRI scan. At the University of Wisconsin, postoperative MRI

scanning was implemented first as part of the NeuroVISION-1 protocol, with the preoperative MRI scanning implemented later as the IPOD-B3 study.

For participants in this analysis, researchers assessed delirium postoperatively twice daily during hospitalisation in days 1-4 using the 3-minute Diagnostic interview for Confusion Assessment Method-defined delirium (3D-CAM)¹² and the Delirium Rating Scale Revised (DRS-R-98)¹³. Postoperative blood samples were collected on inpatient days 1-4 and on the day of postoperative scanning (between day 2 and 9).

Classification of Covert Stroke

As per the methods from NeuroVISION-1, MRI scans were reviewed by two blinded neuroradiologists (EI and VP) and classified for covert stroke based on review of fluid-attenuated inversion recovery, diffusion- and susceptibility-weighted images with apparent diffusion coefficient map. The details of the sequences are: susceptibility-weighted imaging (TR: 7500ms, TE: 25ms, slice thickness 2.9mm, spacing 0mm, 53 slices, Flip Angle: 90°, bandwidth: 250kHz, acquisition time 1 minute), diffusion-weighted imaging (TR: 8000, TE: 82.8 -106 m, slice thickness 3 mm, inter slice gap 0mm, matrix 128 × 128, FOV: 240mm, NEX 2, frequency direction right to left, b values 1000 s mm⁻² along the three orthogonal directions); and fluid-attenuated inversion recovery (sagittal T2-weighted FLAIR cube anatomical scan [1mm³ isotropic]).

Neurofilament light measurement

Blood samples were taken preoperatively, once daily for postoperative inpatient days 1-4 and at the time of the postoperative MRI. NfL concentration was measured using a Single molecule array (Simoa) method, as we have previously described.^{8,14} As covert strokes detected in NeuroVISION-1 could occur at any point until day 9, *a priori* we evaluated whether the peak postoperative neurofilament light levels differed in patients with covert stroke versus those that did not have covert stroke. We preferred peak postoperative NfL, rather than pre-postoperative postoperative day 1 change in NfL, as it is unclear when postoperative covert strokes may occur. We hypothesized that as NfL levels are stable after postoperative day 1, a discharge blood test could be associated with covert stroke and indicate the need for further intervention or follow up and hence may be used to modify an individual's health trajectory. This investigation is the first step in exploring our hypothesis.

Statistics

Eighty-five patients were required to provide sufficient power to ensure we detected a preoperative stroke rate with a 95% confidence interval (0-2%). Based on community covert stroke rates of 0.3%, we hypothesized a 0% incidence of preoperative covert stroke on imaging in a cohort of 100 patients. According to the prior logic of the NeuroVISION-1 pilot study,¹⁵ we decided to recruit 100 patients to ensure a reasonable estimate of the incidence. However, once we had cued the scans for blinded review, 103 preoperative scans had been completed, so all were reviewed.

A Fisher's exact test was planned to compare the incidence of preoperative covert stroke with postoperative covert stroke rates at our institution. Otherwise, Shapiro tests were conducted to determine the data distribution with subsequent Mann-Whitney or t-tests as appropriate.

Data analysis was conducted in R (RStudio version 2021.09, R base version 4.1). Assay results, image diagnoses and study data were inspected, cleaned, and joined by participant identifier, timepoint, and consistent batch where available. Binomial confidence intervals were calculated using Bayesian inference with Jeffery's interval method. NfL was reported in picograms per millilitre (pg/ml) and normalised by logarithm (base 10). We used maximum DRS score for peak delirium severity and maximum NfL over postoperative days 1-9 for peak postoperative NfL. Time courses were plotted for the preoperative to postoperative change in NfL. Boxplots used dichotomous covert stroke diagnosis compared with a Mann-Whitney U / Wilcoxon rank sum exact test. One outlier was observed and excluded from NfL analysis with a standardized Pearson residual >2 and another for alcohol withdrawal. After confirmation of normal distribution of peak postoperative NfL using Shapiro-Wilks, generalised linear regression was conducted on this dataset.

Results

At the University of Wisconsin, we recruited 103 patients into the IPOD-B3 study. Two participants lacked complete preoperative diffusion weighted imaging, leaving 101 participants with preoperative scans. We enrolled 59 participants into NeuroVISION-1 and one participant declined the MRI at the time of scanning, leaving 58 postoperative scans. Of these, 48 participants provided postoperative biomarker samples and one was excluded due to alcohol withdrawal, leaving 47 participants with postoperative imaging and biomarkers. Paired preoperative and

postoperative imaging was available for 31 participants. A STROBE diagram is available in **Figure 1** and patient demographics are available in **Table 1**.

Objective 1: Incidence of preoperative covert stroke

Preoperative covert strokes were detected in 2/101 (2%, Bayesian 95% Confidence Interval 0.2-5.4) participants in the IPOD-B3 cohort. Neither participant had neurological symptoms and their surgery was not delayed, though carotid duplex scans were conducted to confirm the absence of carotid pathology. Both patients were undergoing vascular procedures with a history of peripheral vascular disease but not atrial fibrillation.

One patient with preoperative covert stroke was a 68-year-old female who underwent vascular surgery. She was a smoker with a history of chronic obstructive pulmonary disease (COPD) and peripheral vascular disease. The surgery lasted for 390 minutes and there was minimal blood loss (300ml). Her baseline DRS score was 0 and reached 19 at its peak and her Montreal Cognitive Assessment (MoCA) was 29 at baseline and 22 at one year, an overall decline of 7 points.

The other patient with a preoperative covert stroke was a 70-year-old male who underwent a vascular procedure. He was a smoker with a history of hypertension and peripheral vascular disease. His surgery lasted 350 minutes with minimal blood loss (100ml). His baseline DRS score was 2 and reached at peak of 12 and his baseline MoCA was 22 and remained unchanged at one year.

Objective 2: Confirm postoperative lesions were not present preoperatively

The preliminary incidence of postoperative overt and covert stroke at our centre was 7/58 (12%, 95% CI 4.9-21.3) and was statistically different from the 2% preoperative rate of covert stroke (Fisher's exact test, $p=0.014$).

Next, we compared preoperative and postoperative imaging available for 31 participants. In figure 2a we show scans of one of the participants with a preoperative covert stroke that was evident on preoperative, but not postoperative, scanning. Another participant had a postoperative lesion that was also identified preoperatively (though it was not classified as a preoperative covert stroke but rather as an artefact, Figure 2b).

We did not anticipate having a postoperative overt stroke in our cohort but the scans for this participant are shown in **Figure 2c**. This patient was a 70-year-old male undergoing vascular surgery and was a smoker with a history of hypertension, COPD, and peripheral vascular disease. His surgery lasted 435 minutes with 2700ml blood loss. His baseline DRS was 3 and postoperative peak DRS was 30. His baseline MoCA was 22 and he was lost to follow-up at one year.

In comparison to the preoperative covert stroke rate of 2%, the incidence of covert and overt stroke was 5/31 (16% (5.4-30.2%), Fishers $p=0.008$). After exclusion of the overt stroke, the covert stroke rate was 4/30 (7% (3.6-26.8) fishers $p=0.025$).

Aim 3: Association between Postoperative Ischaemic Brain Injury and Peak Neurofilament Light

Patients with postoperative ischaemic brain injury (overt and covert stroke ($n=5$)) had higher peak postoperative levels of NfL than those without ($p=0.025$, **Figure 3D**). The one participant who was misclassified as a postoperative covert stroke, based on the retrospective identification of the same lesion on the preoperative scan, did not show a rise in NfL from baseline. Based on the available data, the time courses for NfL are plotted in **Figure 3A and 3B**. Peak levels of NfL correlated with delirium severity in Figure 3C. The patient with an overt stroke showed a moderate postoperative peak change in NfL (Log₁₀ pg/ml) of 0.44 compared to the other male participant of the same age with a covert stroke and postoperative peak change in NfL of 0.144.

We validated these findings in linear regressions with peak change in NfL as the outcome. Postoperative ischaemic brain infarction remained significant in models unadjusted ($\beta=0.58$, $p=0.007$) and adjusted for age and sex ($\beta=0.55$, $p=0.01$), explaining up to 30% of the variance in NfL (Pseudo $R^2=0.30$, **Table 2**). These effects were sustained in sensitivity analyses adjusting for the postoperative day of NfL peak ($\beta=0.37$, $p=0.034$, **Supplementary Table 1**). We also confirmed an interaction between postoperative covert stroke and a preoperative medical history of stroke/transient ischaemic attack, causing covert stroke to lose significance as a predictor due to collinearity ($\beta=1.2$, $p=0.024$, **Supplementary Table 2**).

Discussion

Due to our small sample size, and the low incidence of preoperative covert stroke, a larger study would be required to identify if preoperative covert stroke is more frequent than the incidence of covert stroke in the community.. Our data highlight that a baseline measure of neuronal injury could help identify new onset injury. A key finding from our study is that postoperative ischaemic brain infarction was associated with increases in NfL, suggesting that perioperative neuronal infarction of this form can be detected by a plasma measure. If true, this could represent a more cost-effective way to screen for potential postoperative covert stroke than performing brain MRI on all patients. Given the relatively stable kinetics of NfL after injury, a discharge plasma sample may detect individuals who have incurred a perioperative covert stroke and/or more routine NfL sampling can be implemented for patients at high risk of covert stroke. However, MRI scanning cannot detect all the neuronal injury detected by NfL, as patients without covert stroke also showed large rises in NfL. Our prior analyses suggest this may be mediated by inflammation, which may cause a widespread diffuse injury as opposed to the more circumscribed injury of covert stroke.

These data have key strengths and limitations. Strengths include rigorous approaches to data capture including: MRI scans and blinded review of the imaging; thorough delirium assessments; and collection of biomarkers. However, there were many weaknesses. Our recruitment into NeuroVISION-1 was slower than anticipated and hence there was less overlap of preoperative and postoperative scanning than planned. Our cohort is also not representative of the whole NeuroVISION-1 cohort. In particular, the higher delirium and covert stroke rates than most centres may reflect the patient comorbidities and high-risk surgeries conducted at the University of Wisconsin. In particular, we recruited a greater proportion of vascular surgeries in Wisconsin. Larger cohorts with preoperative MRI scanning are required to firmly identify the incidence of preoperative covert stroke and compare to a cross-sectional community study. While not part of the objectives of this study, another limitation of our work is that, with only two preoperative covert strokes, we are underpowered to investigate whether preoperative or postoperative covert stroke may be associated with subsequent cognitive decline. Nonetheless, it is important to reflect that classification of scan artefacts as postoperative covert strokes (as may have occurred in NeuroVISION-1) would bias any finding to the null. Hence, if anything, the results of

NeuroVISION-1 may underestimate cognitive decline that may occur in patients who get postoperative covert stroke.

In summary, our data suggest that preoperative covert strokes may occur, though further research is required to obtain more reliable estimates. Furthermore, NfL may be a cost-effective alternative to MRI scanning in the detection of perioperative covert stroke.

Details of authors' contributions

RDS designed the research with input from VP, MM, PJD, RAP and RL. RDS, HL, RM and VN collected data. Data analysis was conducted by RDS, LE, JT, JB, HZ and KB, and VP. RDS and JT drafted the manuscript and all authors contributed to interpretation and review of the article.

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None.

Declaration of interests

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. The other authors report no conflict of interest. PJD is member of a group with a policy of not accepting payments from industry for their own financial gain. They do, however, take money from industry to fund research endeavours. Based on ideas he originated and grants he has written Dr. Devereaux has received grants from Abbott Diagnostics, Bayer, Boehringer Ingelheim, CloudDX, Philips Healthcare, Roche Diagnostics, Siemens, Striker, and Trimedica, outside the submitted work.

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Figure 1: STROBE diagram

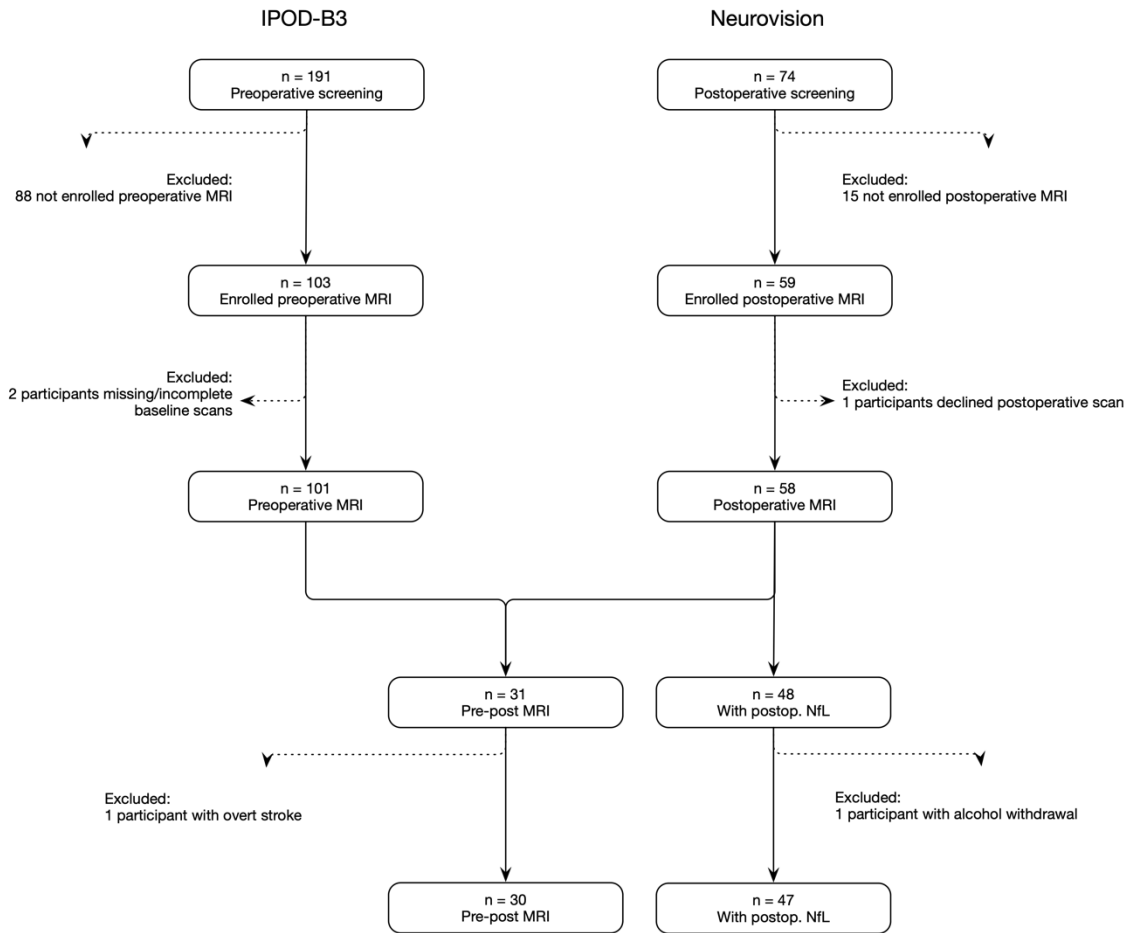


Table 1: Demographic table

	Preoperative MRI n=101	Pre-postoperative MRI n=58
Age	72 (69-76)	70 (67 – 74)
Sex Female	41 (41%)	23 (40%)
Hypertension	75 (74%)	47 (81%)
Perivascular disease	28 (28%)	23 (40%)
Smoker ever	71 (70%)	42 (72%)
Prior medical history of stroke	3 (3%)	1 (2%)
Prior medical history of transient ischaemic attack	9 (9%)	5 (9%)
Operating time (minutes)	216 (173 - 350)	327 (208 – 434.25)
Surgery type	Cardiac 13 (13%) ENT 1 (1%) General 7 (7%) Orthopaedic 43 (43%) Urological 4 (4%) Thoracic 2 (2%) Vascular 31 (31%)	ENT = 1 (2%) General = 9 (16%) Orthopedic = 14 (24%) Urological = 7 (12%) Vascular = 27 (47%)
MoCA total score (n=99)	24 (22 – 26)	23.5 (23.25 – 23.75)
TMTB	78 (60 – 108)	70 (55 – 85)
DSST	52 (41 – 61)	42 (32.5 – 51.5)

NSQIP-D = American College of Surgeons National Quality Improvement Program risk of death, MoCA = Montreal Cognitive Assessment, TMTB = Trail Making Test B, DSST = Digit Symbol Substitution Test.

Figure 2: Preoperative and postoperative ischaemic brain infarction. (a) a patient with a preoperative covert stroke that is not evident on postoperative scanning. (b) a patient with a lesion evident on both preoperative and postoperative scanning. (c) a patient with a new onset overt stroke captured as part of this cohort.

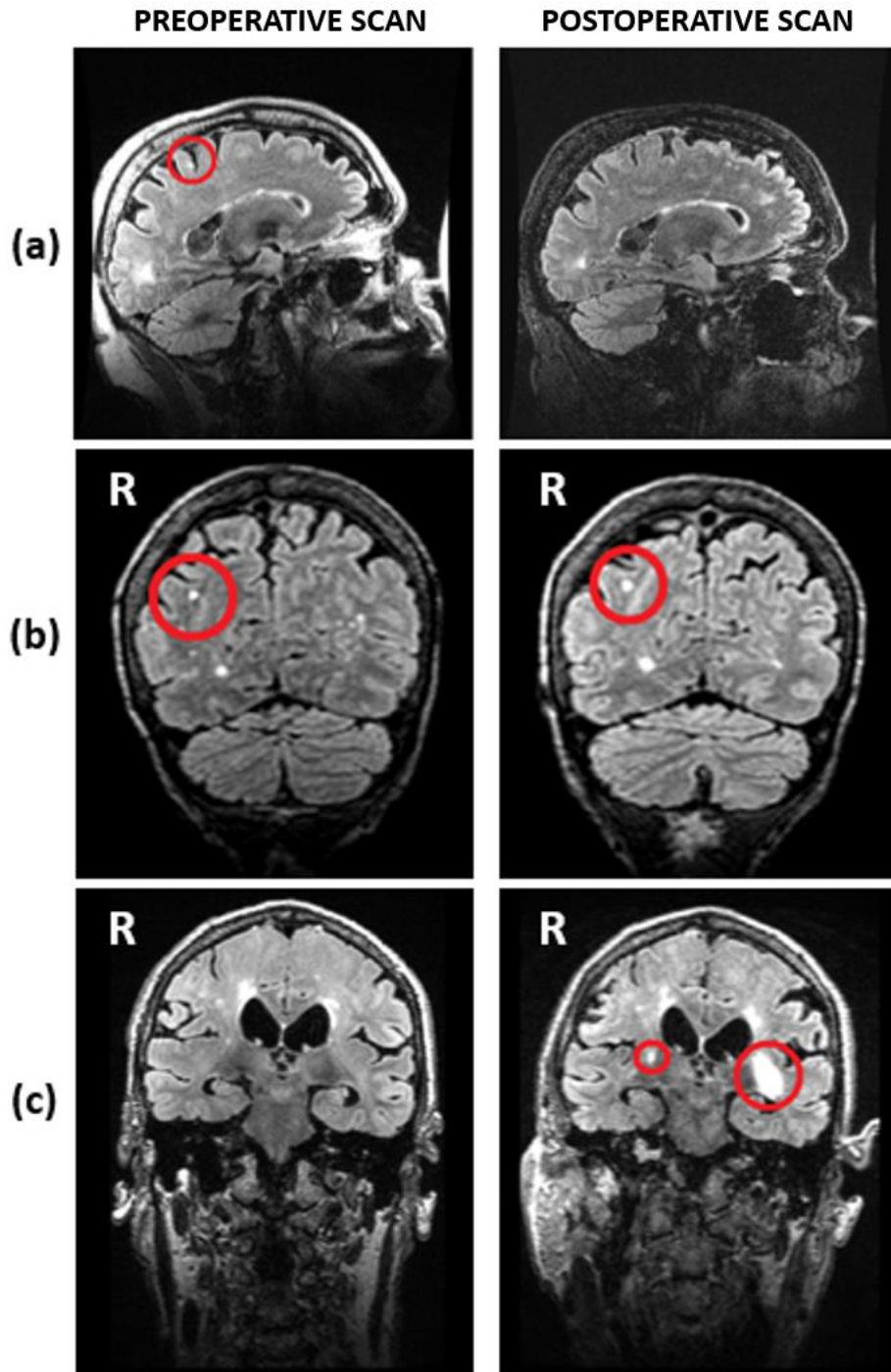
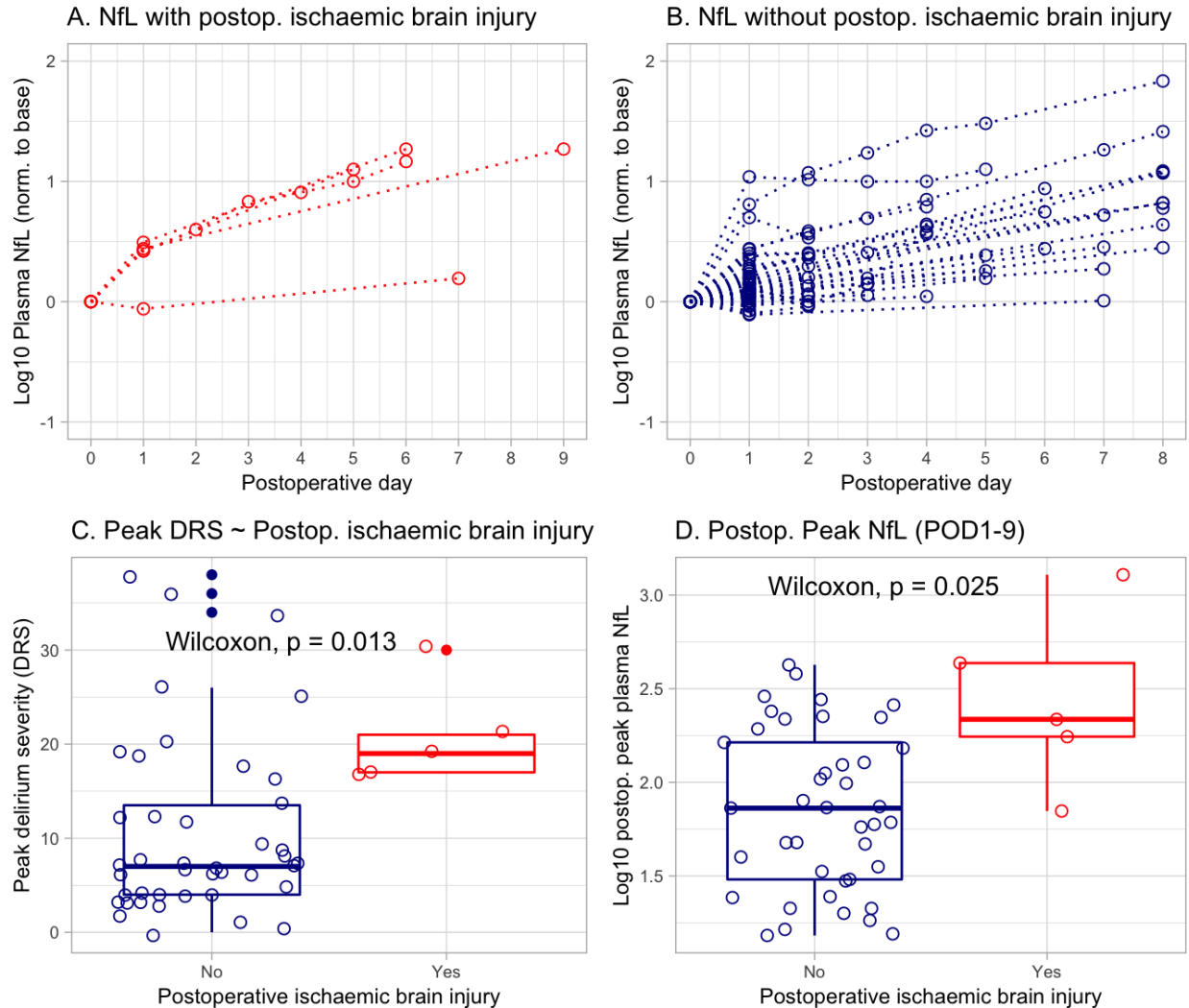


Figure 3: Associations of Peak change in Neurofilament Light with postoperative ischaemic brain infarction



Dataset includes all participants with postoperative scan and postoperative plasma NfL and excludes one participant with delirium rating while in alcohol withdrawal (n = 47).

A. Change in Log10 NfL normalised to baseline with postoperative ischaemic brain injury (n=5).

B. Change in Log10 NfL normalised to baseline without postoperative ischaemic brain injury (n=42).

C. Peak delirium severity by ischaemic brain injury. D. Log10 postoperative peak plasma NfL (excludes one additional outlier with standardised residual >2, n=46). DRS=Delirium Rating Scale Revised-98. NfL=Neurofilament Light.

Table 2: Peak post NfL linear regression

Characteristic	Beta ¹	SE ²	95% CI ²	p-value
Unadjusted				
(Intercept)	1.852	0.068	1.719, 1.985	<0.001***
Postoperative ischaemic brain infarction	0.582	0.206	0.180, 0.985	0.007**
Adjusted				
(Intercept)	1.824	1.02	-0.182, 3.830	0.082
Postoperative ischaemic brain infarction	0.547	0.204	0.147, 0.947	0.010*
Age	0.002	0.014	-0.026, 0.030	0.877
Sex male	-0.234	0.127	-0.483, 0.016	0.073

¹ *p<0.05; **p<0.01; ***p<0.001

² SE = Standard Error, CI = Confidence Interval

Unadjusted Model No. Obs. = 46; Log-likelihood = -25.8; AIC = 57.681; BIC = 63.167; Residual df = 44

Model Fit: $\chi^2(1) = 1.512$, p = 0.005 Pseudo-R² (Cragg-Uhler) = 0.213

Adjusted Model No. Obs. = 46; Log-likelihood = -25.8; AIC = 58.127; BIC = 67.271; Residual df = 44

Model Fit: $\chi^2(3) = 2.127$, p = 0.009 Pseudo-R² (Cragg-Uhler) = 0.300

Dataset includes all participants with postoperative scan and postoperative plasma NfL, excluding one participant in alcohol withdrawal and one outlier with standardized residual >2 (n=46).

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Supplementary (optional)

Supplementary Table 1: Peak post NfL linear regression with NfL peak POD

Characteristic		Beta ¹	SE ²	95% CI ²	p-value
(Intercept)		1.409	0.102	1.209, 1.610	<0.001***
Postoperative stroke	covert	0.370	0.169	0.039, 0.702	0.034*
NfL postoperative day	peak	0.099	0.019	0.061, 0.137	<0.001***

¹ *p<0.05; **p<0.01; ***p<0.001

² SE = Standard Error, CI = Confidence Interval

No. Obs. = 46; Log-likelihood = -15.0; AIC = 37.918; BIC = 45.232; Residual df = 43

Model fit: $\chi^2(2) = 4.634$, p<0.001 Pseudo-R² (Cragg-Uhler) = 0.653

Supplementary Table 2: Peak post NfL linear regression with Stroke/TIA interaction

Characteristic	Beta ¹	SE ²	95% CI ²	p-value
(Intercept)	1.897	0.99	-0.044, 3.838	0.063
Postoperative covert stroke	0.318	0.219	-0.111, 0.747	0.154
Age	0.002	0.014	-0.026, 0.029	0.905
Sex male	-0.265	0.130	-0.521, -0.010	0.048*
Stroke/TIA	-0.151	0.227	-0.595, 0.294	0.510
Postoperative covert stroke * Stroke/TIA				
Yes * Stroke/TIA	1.193	0.508	0.197, 2.189	0.024*

¹ *p<0.05; **p<0.01; ***p<0.001

² SE = Standard Error, CI = Confidence Interval

No. Obs. = 46; Log-likelihood = -21.1; AIC = 56.1; BIC = 68.9; Residual df = 40

Model fit: $\chi^2(5) = 3.066$, p<0.001, Pseudo-R² (Cragg-Uhler) = 0.432