HHS Public Access

Author manuscript

Ann Neurol. Author manuscript; available in PMC 2021 November 01.

Published in final edited form as:

Ann Neurol. 2020 November ; 88(5): 984–994. doi:10.1002/ana.25889.

Association of Plasma Neurofilament Light with Postoperative Delirium

Tamara G. Fong, MD, PhD[*,1,2,3], **Sarinnapha M. Vasunilashorn, PhD**[*,4,6], **Long Ngo, PhD**[4,7], **Towia Libermann, PhD**[8,9], **Simon Dillon, PhD**[8,9], **Eva Schmitt, PhD**[2,3], **Alvaro Pascual-Leone, MD, PhD**[3,11,12], **Steven E. Arnold, MD**[13], **Rich Jones, ScD**[10], **Edward R. Marcantonio, MD, SM**[*,4,5], **Sharon K. Inouye, MD, MPH**[*,2,3,5], **SAGES Study Group**

[1]Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA

[2]Aging Brain Center, Hebrew SeniorLife, Boston, MA

[3]Hinda and Arthur Marcus Institute for Aging Research, Hebrew SeniorLife, Boston, MA

[4]Division of General Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA

[5]Division of Gerontology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA

[6]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

[7]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA

[8]Division of Interdisciplinary Medicine and Biotechnology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA

[9]Beth Israel Deaconess Medical Center Genomics, Proteomics, Bioinformatics and Systems Biology Center, Boston, MA.

[10]Departments of Psychiatry and Human Behavior and Neurology, Warren Alpert Medical School, Brown University, Providence, RI, USA.

[11]Department of Neurology, Harvard Medical School, Boston, MA

[12]Guttmann Brain Health Institut, Institut Guttmann, Universitat Autonoma Barcelona, Spain

[13]Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

Corresponding Author: Tamara G. Fong, MD, PhD, Aging Brain Center, Institute for Aging Research, Hebrew SeniorLife, 1200 Centre Street; Boston, MA 02131 USA, Phone: (617) 971-5390; Fax (617) 971-5309, tfong@bidmc.harvard.edu (not for publication).

*these authors contributed equally to the manuscript

Author Contributions

TGF, SV, SA, RJ, ERM and SKI contributed to the conception and design of the study; TGF, SV, LN, TL, SD, ES, SA, RJ, ERM and SKI contributed to the acquisition and analysis of data; TGF, SV, LN, TL, SD, ES, APL, SA, RJ, ERM and SKI contributed to drafting the text and preparing the figures. Members of the SAGES Study Group and their institutional affiliations are contained in Supplementary Table 1, online.

Potential Conflicts of Interest

The authors declare that there is no conflict of interest

Abstract

OBJECTIVE ——To examine the association of plasma neuroaxonal injury markers neurofilament light (NfL), total tau, glial fibrillary acid protein and ubiquitin carboxyl-terminal hydrolase L1 and delirium, delirium severity, and cognitive performance.

METHODS ——Delirium case-no delirium control (n=108) pairs were matched by age, sex, surgery type, cognition, and vascular comorbidities. Biomarkers were measured in plasma collected preoperative(PREOP), 2 days(POD2) and 30 days postoperative(PO1MO) using Simoa technology (Quanterix). The Confusion Assessment Method (CAM) and CAM-S(severity) was used to measure delirium and delirium severity, respectively. Cognitive function was measured with General Cognitive Performance(GCP) scores.

RESULTS ——Delirium cases had higher NfL on POD2 and PO1MO (median matched pair difference 16.2pg/ml and 13.6pg/ml, respectively ($P < .05$)). Patients with PREOP and POD2 NfL in the highest quartile (Q4) had increased risk for incident delirium (adjusted OR= 3.7(95% CI,1.1–12.6), and 4.6(95% CI, 1.2–18.2), respectively) and experienced more severe delirium, with sum CAM-S scores 7.8 points (95%CI,1.6, 14.0), and 9.3 points higher (95%CI, 3.2, 15.5). At PO1MO, delirium cases had continued high NFL (adjusted OR of 9.7; 95% CI,2.3,41.4), and those with Q4 NfL values showed −2.3 point decline on GCP score (−2.3 points, 95% CI, −4.7, −0.9).

INTERPRETATION ——Patients with highest PREOP or POD2 NfL levels were more likely to develop delirium. Elevated NfL at PO1MO was associated with delirium and greater cognitive decline. These findings suggest NfL may be useful as a predictive biomarker for delirium risk and long-term cognitive decline, and once confirmed would provide pathophysiological evidence for neuroaxonal injury following delirium.

I Introduction

Delirium, an acute decline in cognition and attention, is a common complication of illness, acute trauma, and surgery in older adults that is associated with increased morbidity and mortality[1–3]. Knowledge of delirium pathophysiology is limited, but emerging evidence suggests neuronal injury may play a role[4,5]. Increasingly, fluid biomarkers of neuronal injury have been studied in a range of conditions, including traumatic brain injury (TBI)[6], multiple sclerosis[7,8] stroke[9] and neurodegenerative disease[7]. In delirium, prior studies examining plasma markers of neuronal injury have been mixed. For example, S100B, a calcium-binding peptide and measure of glial activation and/or death, has been found to be elevated[10], or unchanged in patients with persistent delirium compared to those with probable Alzheimer's disease (AD)[11]. Results with neuron specific enolase (NSE), a highly specific marker for neurons and peripheral neuroendocrine cells, have been variable, with one study showing NSE levels were lower in CSF of patients with delirium[11], others reporting higher plasma NSE concentrations[11,12] and yet others finding no significant difference between delirium and control groups[10].

With the development of ultra-sensitive immunoassays, additional markers of neuronal injury that can be measured from blood have emerged and are being investigated. For example, neurofilament light (NfL), a highly expressed cylindrical intermediate filament

protein that provides structural support for myelinated axons has been found to be elevated in blood in multiple conditions including stroke, TBI, multiple sclerosis, AD, progressive supranuclear palsy, and frontotemporal dementia, and appears to increase in both cerebrospinal fluid (CSF) and blood proportionally to the degree of axonal damage[7]. NfL has been shown to increase after delirium, in a case-series report[13] and a cohort study of older adults undergoing elective surgery[14]. Tau, a microtubule-stabilizing protein primarily localized in neurons, but also expressed at low levels in astrocytes and oligodendrocytes, and phosphorylated tau (p-tau) have been observed to be elevated in the CSF of patients with AD and other neurodegenerative diseases and TBI, suggesting that tau is released from cells during neuronal damage and may serve as a brain-specific biomarker[15]. Serum NfL and tau both increase over 48 hours after surgery, suggesting that general anesthesia and surgery may be associated with neuronal damage in the short term[16] Glial fibrillary acidic protein (GFAP), expressed by astrocytes and used as a marker of astrocytosis in neurodegeneration, and ubiquitin carboxyl-terminal hydrolase L1(UCHL-1), a neuron specific enzyme, are two other potential biomarkers of TBI[17]. While these four biomarkers of neuroaxonal injury, NfL, total tau, GFAP, and UCHL-1 have been examined previously in the context of neurodegenerative diseases or TBI, in this paper, our aim is to measure these 4 potential markers of different aspects of neuronal injury in the plasma using state-of-the-art technology, and determine whether they are associated with delirium incidence, severity and duration in a cohort of older patients undergoing elective orthopedic surgery. Given the previously established association of delirium with postoperative cognitive decline[18,19], we also examine whether these biomarkers predict one-month cognitive function after delirium.

II Methods

a. Study population

The Successful Aging after Elective Surgery (SAGES) study is a prospective, observational cohort study of older adults without dementia undergoing major elective surgery. The study design and methods have been described in detail previously[20,21]. In brief, eligible participants were age≥70 years, English speaking, with an anticipated hospital length of stay ≥3 days. Eligible surgical procedures included: total hip or knee replacement, lumbar, cervical, or sacral laminectomy, lower extremity arterial bypass surgery, open abdominal aortic aneurysm repair, and open or laparoscopic colectomy. Exclusion criteria were evidence of dementia, delirium, or hospitalization within 3 months, terminal condition, legal blindness, severe deafness, history of schizophrenia or psychosis, and history of alcohol abuse or withdrawal. A total of 560 patients met all eligibility criteria and were enrolled between June 18, 2010 and August 8, 2013. Written informed consent for study participation was obtained from all participants according to procedures approved by the institutional review boards of Beth Israel Deaconess Medical Center and Brigham and Women’s Hospital, the two study hospitals, and Hebrew SeniorLife, the coordinating center for the study.

From the full cohort, a nested matched case-control (delirium-no delirium) sample was created and used in prior studies examining cytokines and post-operative delirium[22]. The nested matched case-control study offers a powerful design, with advantages of: 1) improved

efficiency with reduced numbers of samples to be analyzed; 2) strong control of confounding, and 3) statistical power nearly identical to the full cohort study[23]. Delirium cases were identified as participants who had their peak delirium on the second post-operative day. Controls had no evidence of delirium or subsyndromal delirium throughout their hospitalization. The case-control pairs were matched on six variables: age within five years, baseline General Cognitive Performance (GCP)[24] within five points (see below for details), and an exact match for sex, surgery type, presence of vascular comorbidity, and Apolipoprotein E ε4 carrier status. Vascular comorbidity was present if the participant had at least one Charlson diagnosis[25] related to vascular disease (myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, hemiplegia, and diabetes or diabetes with end organ damage). For Apolipoprotein E genotype, DNA was extracted from cellular material in the blood and genotyped at the Partners Center for Personalized Medicine. We classified individuals with ≥1 ε4 allele as Apolipoprotein E ε4 carriers. For this paper, to limit heterogeneity of surgical procedure, we selected only participants who had undergone an orthopedic procedure (66 matched pairs). Twelve pairs with low plasma volume in the biorepository were excluded, yielding n=54 matched pairs (108 participants) for inclusion in this study.

b. Data Collection

Participants underwent a 90-minute baseline assessment in their homes, conducted by trained research associates two weeks prior to the index surgery[20,21]. Demographic information, including age, sex, race, marital status, years of education, neuropsychological testing and functional assessments were collected at this time. The neuropsychological battery included the Modified Mental Status Exam (3MS), Hopkins Verbal Learning Test (HVLT), Digit Span Forwards and Backwards, Category Fluency, Phonemic (F-A-S) Fluency Tasks, Boston Naming Test, Visual Search and Attention Test (VSAT), Trail Making Test (A and B), and the Digit Symbol Test, from which a General Cognitive Performance (GCP) score was created[24]. The GCP is a weighted composite summary measure calibrated to a nationally representative sample of adults age ≥70 years[26] to yield a mean score=50 and standard deviation=10[27]. A GCP cut-point of 45, corresponding with an MMSE of 23/24, optimally discriminated participants with dementia[27]. The Geriatric Depression Scale[28] was administered to all participants. The Informant Questionnaire on Cognitive Decline in the Elderly[29] was administered to a family member of each participant. Following surgery, from the first postoperative day through discharge, participants underwent a daily delirium assessment, which included a brief cognitive testing and family and nurse interviews. Attempts were made to conduct the assessment at a similar time each day, but clinical care and patient preferences were prioritized and interviews were conducted accordingly. Delirium was defined by CAM criteria[21], supplemented by nurse interviews and a validated chart-based delirium diagnosis[30]. Delirium days was the sum of total postoperative days the patient was delirious by either CAM or chart review over the duration of hospitalization.

The current nested-case control cohort study intentionally maximized the contrast between cases and controls. Cases had to have delirium minimally on post-operative day 2 (POD2), meaning that someone delirious only on day 3 would be ineligible, but someone with

delirium on days 1–3 could be included. This ensured that blood collected on POD2 corresponded to the presence of delirium. Controls were defined as patients with no delirium, including no subsyndromal delirium on any post-operative day (POD) over the entire study. Characteristics of the n=134 participants with delirium in the full SAGES cohort were similar to the n=54 participants with delirium for the nested-study. Delirium severity was scored using the Confusion Assessment Method-Severity (CAM-S long form), an instrument with good psychometric properties, high inter-rater reliability, and strong association with clinical outcomes related to delirium[31]. In the current study, we examined peak CAM-S, the highest single CAM-S rating across all hospital days for each patient, and the sum of CAM-S over all days in hospital[32].

After discharge from the index hospitalization, a comprehensive medical record abstraction was conducted to collect details about the hospitalization, including surgical procedure and anesthesia type; Charlson score and vascular comorbidity were calculated from information extracted from the medical record. Follow-up interviews and neuropsychological testing were conducted in person at one-month post-surgery. All study interviews were conducted by experienced interviewers, who underwent two to four weeks of intensive training and standardization[21]. Standardization and inter-rater reliability testing of all key study variables, including delirium and neuropsychological assessment, was conducted every six months continually throughout the study and coding questions were addressed in weekly meetings of all study staff.

c. Blood Collection

Blood was collected during the preoperative clinic visit (PREOP), on the morning of POD2, and at 1 month following the hospitalization (PO1MO). Blood was collected in heparinized tubes, placed immediately on ice, and transported to the Clinical Research Center, where it was processed within four hours of collection. Tubes underwent low speed centrifugation to separate out cellular and plasma components. Plasma was aliquoted and stored at −80°C[21].

d. Quanterix 4-plex assay for tau, NfL, UCHL-1, GFAP

The assays were conducted using the Simoa Human Neurology 4-Plex A, a 2-step digital immunoassay using the Simoa HD-1 Analyzer and Single Molecule Array (Simoa) technology (Quanterix Accelerator Program, Lexington, MA)[32]. Samples were delivered to Quanterix on dry ice and were stored at −80°C upon arrival. Prior to analysis, samples were thawed completely at room temperature, then were vortexed and spun down for 3 minutes at 14,000xg to pellet any debris. Samples were processed in order from the provided manifest and tested in duplicate. Plasma samples were diluted on-board the instrument at 4x dilution using N4PA Sample Diluent (included in kit) prior to testing. Ready-to-use calibrators were tested in triplicate and ready-to-use controls tested in duplicate. The concentration of total tau, NfL, GFAP, and UCHL-1 was determined from a correspondent standard curve.

e. Statistical analysis

The distributions of total tau, NfL, UCHL-1, or GFAP overall and by delirium status were examined using descriptive statistics, including mean, standard deviation, median, and range. As the distributions of the biomarkers were non-normal (data not shown), we decided

to employ non-parametric approaches, and that we would analyze biomarker values by quartiles, defined by the 25th, 50th, and 75th percentile concentrations, as we have done in prior studies[33,34]. To examine the relationship between the biomarkers and delirium incidence, because of the nested matched case-control study design, we performed two types of matched pair analyses. First, the Wilcoxon signed-rank test was used to compare the matched (delirium case versus no delirium control) samples to assess whether the population mean ranks differed. Second, we used conditional logistic regression to estimate the odds of having delirium (incidence) given the relative level (i.e., comparison between quartiles) of the biomarker proteins at a specific timepoint (i.e., PREOP or POD2) or whether the biomarker level at PO1MO was associated with delirium. In these models, the major confounders were adjusted by the nested case-control design. We used six factors in the construction of the 54 matched pair sample: gender, surgery type, vascular comorbidity, APOE ε4 status, age at surgery, and baseline GCP. By definition all patients with delirium were delirium-positive on POD2. We required the case and control within a matched pair to have identical value on the first four factors. For age and GCP, we required the caliper (difference between case and control) to be five or less. Thus, the quality of the match (the total difference from age and GCP) is better with values closer to zero. On average, the match quality was excellent with the median difference of less than 2 for both age and GCP (data not shown). We report delirium incidence by odds ratio (OR) estimates and the corresponding 95% confidence interval (CI), and the estimated area under the receiver-operating-characteristic curve (c-statistic).

For delirium severity, we also examined whether PREOP or POD2 biomarker level, or the change in biomarker level from PREOP to POD2 correlated with delirium severity, as measured by peak-CAM-S and sum CAM-S. We also considered the number of delirium days over the course of the hospitalization as a measure of delirium duration. We used linear mixed effects models to account for the within-pair correlation, and modeled this correlation via the use of the compound symmetry structure of the variance-covariance matrix. Restricted maximum likelihood estimation was used in these models.

Lastly, we examined the association between biomarker levels at all timepoints and the GCP at one month corrected for baseline GCP, using a linear mixed effects model taking the within-pair correlation (compound symmetry structure on the variance-covariance matrix) into account. As with other analyses, we controlled for confounders by entering the six matching variables into the model. SAS software version 9.4 (Cary, N.C.) was used for all analyses.

III Results:

a. Demographics

Characteristics of the full sample and the matched case-controls are described in Table 1. The matched sample (n=108) included older adults who were on average (standard deviation, SD) 77(5) years old at the time of study enrollment; 56(53%) were women and 10(9%) non-white. The sample was generally healthy with an average Charlson score of 1.3. Participants had 15(3) years of education and an average baseline cognitive performance of

55.5(5.4) points on the GCP. Eighty-four percent of patients had their surgical procedure under general anesthesia.

b. Plasma biomarker concentrations

Due to missing samples or insufficient plasma volume, a total of 304 samples (PREOP, n=107; POD2, n=98 and, n=99) were tested in duplicate for each biomarker; of these 304 plasma samples tested, 291(95.7%) samples had data for both replicates. For tau, NfL, and GFAP, all 291(100%) samples with data available from both replicates were in linear range, with a mean concentration coefficient of variation (CV) of 5.1%, 4.6%, and 4.2%, respectively. For UCHL-1, 153 samples (50.3%) were in the linear range and 146 samples (48.0%) were between the limits of detection and the lower limit of quantification with an overall mean concentration CV of 13.7%. Five UCHL-1 samples were below the limits of detection and were excluded from analysis.

c. The relationship with delirium incidence, severity, and duration.

We examined the relative risk of postoperative delirium by quartiles of biomarker levels at PREOP and POD2. After matching for baseline GCP, age, sex, surgery type, presence of vascular comorbidity, and APOE ε4 status, patients with PREOP NfL values in the highest quartile (Q)4 had a significantly increased risk of developing postoperative delirium relative to patients in the lowest quartile (Q1) [adjusted odds ratio (OR) of 3.71 (95%CI 1.09–12.58, $P<.05$)] (Table 2). On POD2, patients with NfL levels in Q3 and Q4, had an adjusted OR (95%CI) of 4.67 (1.20–18.15) and 8.83 (1.88–41.36), respectively, for delirium relative to those with NfL levels in Q1 ($P<.05$) (Table 2). No association was found for PREOP or POD2 GFAP, Tau, or UCHL-1 levels with postoperative delirium, although GFAP did show a non-significant trend (Table 3).

We examined the association of NfL with delirium severity. Participants with NfL levels in the highest quartile (Q4) at PREOP or POD2 relative to Q1 experienced more severe delirium, with sum CAM-S scores that were 7.8 points higher (95%CI,1.60,14.04), and 9.34 points higher (95%CI, 3.22,15.46) respectively. On POD2, participants with NfL in Q4 were more likely to have a delirium that lasted on average 1.04 days longer (95%CI 0.5, 1.6) (Table 2) and a peak CAM-S score 3.0 points higher (95%CI, 0.8,5.1) than those with Q1 NfL values. The change in NfL level from PREOP to POD2 correlated with delirium severity as measured by peak CAM-S (rank correlation coefficient=.31, $P=.003$), sum CAM-S (0.29, $P=.005$) and delirium-days (0.37, $P<.001$).

d. Changes in biomarkers following surgery

Using the matched pair design of the study, paired differences between the delirium and no delirium (i.e, NfL level in delirium – NfL level in no delirium) were examined over time (Figure 1). At baseline, the median paired difference (MPD) for NfL (5.6 pg/ml) was not significant, however after surgery the MPD was significant at both POD2 (16.2 pg/ml, $P<.05$, Wilcoxon signed-rank test) and PO1MO (13.6 pg/ml, $P<.05$, Wilcoxon signed-rank test), reflecting higher NfL levels in the delirium pairs, and persistently elevated at PO1MO compared to baseline. Absolute NfL values increased in all subjects (data not shown).

At one month, patients who had been delirious were more likely to have NfL levels in the highest quartile (Q4) compared to the lowest (Q1) quartile (adjusted OR of 9.73; 95%CI 2.28,41.43). In addition, for every point higher on sum CAM-S the odds for PO1MO NfL being in Q4 vs. Q1 increased by 10% (adjusted OR 1.11, 95%CI 1.03,1.20) (Table 4). No association with GFAP, UCHL-1, or total tau and delirium or delirium severity was observed (data not shown).

We also examined NfL and cognitive performance at one month. In this study we found that patients with highest NfL (in Q4) at PO1MO had a mean GCP decline (the difference between GCP at 1-month and PREOP) of −2.31 points more (95%CI: −4.14, −0.48) than those with lowest NfL levels (Q1) where the mean change was 0.11 points (95%C −1.74,1.96). There was no difference in baseline GCP scores between those in the groups classified in Q4 versus Q1at PO1MO [median (range) 55.86 (45.66–68.69) vs. 53.98 (44.73–60.09), p =.10]. The proportion of patients with the highest NfL at one month who declined on the GCP and were positive for delirium on POD2 was 31.5% (17/54). NfL at PREOP and POD2 were not associated with a decline in GCP at one month (Table 5).

Discussion

We have previously hypothesized that neuronal injury may represent a final common pathway for disparate pathophysiologic processes that occur during acute delirium[4]. In delirium, synaptic or neuronal injury may occur through a variety of mechanisms, including excitotoxicity, oxidative stress, and/or inflammation among other effects[5,35,36]. In this study, we examined four potential plasma biomarkers of neuronal injury, NfL, GFAP, UCHL-1 and tau, and found only NfL to be altered in association with surgery and delirium.

Patients who had higher baseline levels of NfL (i.e., those with high values in Q4) were more likely to develop delirium. This suggests that patients with pre-existing levels of neuronal injury may be more vulnerable to developing delirium with surgery, consistent with observations that older persons, particularly those with underlying cognitive impairment[2,37], or neuroimaging markers of neurodegeneration[38] have a higher predisposing risk for developing delirium. A recent meta-analysis of 126 studies examining functional neuroimaging and electroencephalogram studies identified that strength and efficiency of connectivity appear to characterize structural brain networks of patients at risk for delirium[39], supporting the notion that underlying structural brain vulnerability is a key factor for delirium occurrence. Although patients with dementia were excluded from participation, 11% of the patients in the SAGES cohort had a mild cognitive impairment by clinical consensus diagnosis at baseline[40], and we assume, given the age of the cohort, that additional individuals likely have subtle cognitive impairment as well. Overall, we anticipate that neuronal injury due to underlying neurodegeneration at baseline will be subclinical to mild.

In the current study, we also identified that on POD2, NfL increased in both delirium cases and non-delirium controls. Increased NfL following surgery has been reported previously[16] however, NfL levels rose more in the delirious cases, and NfL levels in the 3rd and 4th quartiles were significantly associated with delirium. As the study design identified delirium

cases as being present on POD2, this finding that delirium is associated with increased NfL levels suggests that delirium and/or the insult triggering the delirium may potentially lead to neuroaxonal injury. Patients with higher PREOP NfL, reflecting neuronal injury prior to surgery, were more likely to have higher sum CAM-S scores reflecting greater severity of the delirium episode. This may occur in the presence of an underlying but subclinical neurodegenerative condition where neurons are more vulnerable to injury and cell death with just modest levels of neuroinflammation, and an exaggerated central inflammatory response[41] promotes even greater neuronal injury.

Interestingly, we saw that NfL remained elevated one month after hospitalization, but especially in patients who had experienced delirium. This persistence of elevated NfL after surgery and delirium to one month has not been previously reported in the literature. In our prior work we have found patients with delirium had a significantly greater decline in GCP scores (−1.0 points) in the first month after surgery compared to those without delirium[18]; in the current study we find that those patients with NfL values in the highest quartile at one month decline in GCP score by −2.31 points more than those patients who had NfL values in the lowest quartile at one month. Neither baseline nor POD2 NfL levels were associated with lower GCP scores one-month post-surgery, which may suggest that it is the persistence of elevated NfL levels that may affect cognitive function. A potential explanation for this observation is that following the acute phase of delirium (i.e., POD2), some patients recover, with normalization of NfL by PO1MO, but others have ongoing elevation in NfL and go on to have ongoing cognitive decline[4]. Not all patients with higher NfL at baseline and POD2 reach the highest level of NfL at PO1MO. However, while a nested case-control sample optimizes delirium analyses, the design limits the analyses of other outcomes, thus, the finding on one-month cognitive function should be interpreted with caution. If confirmed in a larger study, when taken together, this evidence might suggest that once triggered, delirium may lead to neuroaxonal injury, and for some patients this injury may persist well beyond the time frame of the acute illness or surgery, and may contribute to long-lasting effects on cognitive function.

We did not find significant changes in plasma tau, GFAP, or UCHL-1 in our cohort. In a prior case-series of nine individuals undergoing cardiac surgery in which the ability of serum NfL, tau and GFAP levels to predict delirium was examined[13] no differences were observed with GFAP. Another study of patients undergoing cardiac surgery found no association between GFAP levels and delirium[42]. In the case series, tau was observed to be increased pre-operatively in those who later developed delirium[13], and increased after surgery in all cases, with levels returning to baseline at post-operative day one[13]. It is possible that our study did not identify an association of baseline tau with delirium due to differences in patient population. Changes in tau after surgery may have been missed in our study due to the timing of of blood samples, and serum tau may be a less sensitive marker than serum NfL of neuroaxonal injury, as has been found previously in sports related concussion[43]. Serum UCHL-1 levels in our study were variable, with only about half the samples within the linear range of detection, and for this reason may not be a useful marker of neuronal injury in delirium.

A number of prior studies have found associations of NfL with delirium. A study of patients presenting with hip fractures found that those with delirium had significantly higher preoperative and postoperative serum NfL compared with patients without delirium[44]. Likewise, our study found similar associations, and our cohort of elective surgical patients eliminates potential confounding effects associated with hip fracture. In another study, NfL was found to be increased after cardiopulmonary bypass, with the highest post-operative levels among those with delirium. A recent study by Casey et. al. showed in a cohort of 108 older patients (36.1% delirium rate) that NfL levels gradually and continued to increase 4 days after surgery in all patients, although the increase in NfL was more profound in patients with delirium[14]. We also observed NfL increased from baseline values across the whole cohort, even up to 1 month postoperatively, with higher NfL seen in patients with delirium at all timepoints. However, neither the case studies reported by Saller et. al., or the study by Casey et. al. found that PREOP NfL predicted delirium in their surgical cohort[14] as we saw in ours. These discrepant findings could be due to differences in study design, patient population, and sample size, and certainly additional studies are warranted.

Evered et. al. have reported, in a cohort of 30 patients 60 years and older undergoing general anesthesia for elective surgeries, a significant and rapid increase plasma tau from baseline values, peaking at 6 hours, and a slower and more continuous rise in plasma NfL with continued increase thru at least 48 hours, regardless of type of surgery or anesthesia[16], although their study did not report delirium assessments. While our study included participants (n=17) who received spinal anesthesia with intravenous sedation, without general anesthesia, there was insufficient power to assess the effect of general anesthesia on NfL levels (data not shown).

Strengths of our study include our ability to leverage the existing resources of the SAGES study, which included biobanked plasma over multiple timepoints. The full cohort was well-characterized and utilized rigorous measures of delirium, with detailed longitudinal follow-up. Clinically evident dementia was excluded using a rigorous process. We used a nested case-control substudy from SAGES which allowed us to select delirium cases and delirium-free controls who were carefully matched. This is a powerful study design, which maximizes efficiency for evaluating potential biomarkers[45], and the matching helps control for multiple confounding variables, including those not part of the original matching scheme (see Table 1).

A few limitations should be noted. First, the study was conducted in a relatively small, matched sample, which while suitable for biomarker discovery, may compromise interpretation of some outcome measures due to unintentional biases. For example, the matching may inadvertently select a control sample that is non-representative of the full SAGES cohort or even the general surgical population. Second, our sample was relatively homogenous in terms of race/ethnicity and educational attainment. Validation of our NfL findings is recommended in larger, more diverse cohorts. Third, in conditions such as AD where there is good correlation between CSF and plasma NfL, the low concentrations of NfL detected in plasma is presumed to have leaked from the CSF[7]. In delirium there is emerging evidence for endothelial cell dysfunction and increased blood brain barrier (BBB) permeability[46] but whether this contributes to plasma NfL is uncertain, as there are some

limited data that plasma NfL is not confounded by BBB permeability[47]. Studies which include both plasma and CSF measures of NfL would help address these issues. Likewise, as we did not find any associations between plasma tau, GFAP, and UCHL-1 with delirium, examination of these biomarkers in the CSF may provide greater sensitivity for neuronal injury in delirium. Lastly, different NfL polypeptide gene mutations have been identified and whether such NEFL gene variations, or other genes, contribute a genetic susceptibility to delirium is not known, and should be considered in further studies.

In summary, we found that higher baseline plasma levels of the neuronal injury marker NfL were associated with delirium in a matched case-control cohort of older adults undergoing major surgery, suggesting that NfL may have value as a predictive risk marker for delirium. While NfL levels rose in all patients after surgery, delirium, and more severe delirium, were associated with a greater incrase in NfL levels immediately postoperatively, which remained elevated at 1 month after surgery. This persistently elevated NfL was associated with postoperative cognitive decline. If confirmed with additional studies, these findings will help build support for the role of neuronal injury in linking delirium pathophysiology with long-term cognitive decline.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Ackowledgments

The authors gratefully acknowledge the contributions of the patients, family members, nurses, physicians, staff members, and members of the Executive Committee who participated in the Successful Aging after Elective Surgery (SAGES) Study. This work is dedicated to the memory of Joshua Bryan Inouye Helfand.

Funding: This manuscript was funded by R21AG057955(TGF), P01AG031720 (SKI), R24AG054259 (SKI), K07AG041835 (SKI), R01AG051658 (ERM), K24AG035075 (ERM), and R03AG061582 (SMV) and K01AG057836 (SMV) from the National Institute on Aging, and the Alzheimer's Association AARF-18-560786 (SMV). Dr. Inouye holds the Milton and Shirley F. Levy Family Chair at Hebrew SeniorLife/Harvard Medical School. The funding sources had no role in the design, conduct, or reporting of this study.

References

1. Marcantonio ER. Delirium in Hospitalized Older Adults. N Engl J Med. 2017 10 12;377(15):1456–66. [PubMed: 29020579]
2. Inouye SK, Westendorp RG, Saczynski JS. Delirium in elderly people. Lancet. 2014 3 8;383(9920):911–22. [PubMed: 23992774]
3. Gleason LJ, Schmitt EM, Kosar CM, et al. Effect of Delirium and Other Major Complications on Outcomes After Elective Surgery in Older Adults. JAMA Surg. 2015;150(12):1134–40. [PubMed: 26352694]
4. Fong TG, Vasunilashorn SM, Libermann T, et al. Delirium and Alzheimer disease: A proposed model for shared pathophysiology. Int J Geriatr Psychiatry. 2019 2 17.
5. Marcantonio ER. Postoperative delirium: a 76-year-old woman with delirium following surgery. JAMA. 2012 7 4;308(1):73–81. [PubMed: 22669559]
6. Zetterberg H, Blennow K. Fluid biomarkers for mild traumatic brain injury and related conditions. Nat Rev Neurol. 2016 10;12(10):563–74. [PubMed: 27632903]
7. Gaetani L, Blennow K, Calabresi P, et al. Neurofilament light chain as a biomarker in neurological disorders. Journal of neurology, neurosurgery, and psychiatry. 2019 4 9.

8. Kuhle J, Plavina T, Barro C, et al. Neurofilament light levels are associated with long-term outcomes in multiple sclerosis. Multiple Sclerosis Journal. 2019:1352458519885613. [PubMed: 31680621]
9. Glushakova OY, Glushakov AV, Miller ER, et al. Biomarkers for acute diagnosis and management of stroke in neurointensive care units. Brain Circ. 2016 Jan-Mar;2(1):28–47. [PubMed: 30276272]
10. van Munster BC, Korse CM, de Rooij SE, et al. Markers of cerebral damage during delirium in elderly patients with hip fracture. BMC neurology. 2009 5 27;9:21. [PubMed: 19473521]
11. Caplan GA, Kvelde T, Lai C, et al. Cerebrospinal fluid in long-lasting delirium compared with Alzheimer's dementia. J Gerontol A Biol Sci Med Sci. 2010 10;65(10):1130–6. [PubMed: 20530241]
12. Grandi C, Tomasi CD, Fernandes K, et al. Brain-derived neurotrophic factor and neuron-specific enolase, but not S100beta, levels are associated to the occurrence of delirium in intensive care unit patients. J Crit Care. 2011 4;26(2):133–7. [PubMed: 21106342]
13. Saller T, Petzold A, Zetterberg H, et al. A case series on the value of tau and neurofilament protein levels to predict and detect delirium in cardiac surgery patients. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia. 2019 9;163(3):241–6.
14. Casey CP, Lindroth H, Mohanty R, et al. Postoperative delirium is associated with increased plasma neurofilament light. Brain. 2020 1 1;143(1):47–54. [PubMed: 31802104]
15. Schraen-Maschke S, Sergeant N, Dhaenens C-M, et al. Tau as a biomarker of neurodegenerative diseases. Biomark Med. 2008;2(4):363–84. [PubMed: 20477391]
16. 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(5):542–7. [PubMed: 29459944]
17. Diaz-Arrastia R, Wang KKW, Papa L, et al. Acute biomarkers of traumatic brain injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein. J Neurotrauma. 2014;31(1):19–25. [PubMed: 23865516]
18. Inouye SK, Marcantonio ER, Kosar CM, et al. The short-term and long-term relationship between delirium and cognitive trajectory in older surgical patients. Alzheimers Dement. 2016 7;12(7):766–75. [PubMed: 27103261]
19. Saczynski JS, Marcantonio ER, Quach L, et al. Cognitive trajectories after postoperative delirium. N Engl J Med. 2012 7 5;367(1):30–9. [PubMed: 22762316]
20. Schmitt EM, Marcantonio ER, Alsop DC, et al. Novel risk markers and long-term outcomes of delirium: the successful aging after elective surgery (SAGES) study design and methods. J Am Med Dir Assoc. 2012 11;13(9):818 e1–10.
21. Schmitt EM, Saczynski JS, Kosar CM, et al. The Successful Aging after Elective Surgery (SAGES) Study: Cohort Description and Data Quality Procedures. J Am Geriatr Soc. 2015 12;63(12):2463–71. [PubMed: 26662213]
22. Dillon ST, Vasunilashorn SM, Ngo L, et al. Higher C-Reactive Protein Levels Predict Postoperative Delirium in Older Patients Undergoing Major Elective Surgery: A Longitudinal Nested Case-Control Study. Biol Psychiatry. 2017 1 15;81(2):145–53. [PubMed: 27160518]
23. Ngo LH, Inouye SK, Jones RN, et al. Methodologic considerations in the design and analysis of nested case-control studies: association between cytokines and postoperative delirium. BMC medical research methodology. 2017;17(1):88–. [PubMed: 28587598]
24. Jones RN, Rudolph JL, Inouye SK, et al. Development of a unidimensional composite measure of neuropsychological functioning in older cardiac surgery patients with good measurement precision. J Clin Exp Neuropsychol. 2010 12;32(10):1041–9. [PubMed: 20446144]
25. Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40(5):373–83. [PubMed: 3558716]
26. Langa KM, Plassman BL, Wallace RB, et al. The Aging, Demographics, and Memory Study: study design and methods. Neuroepidemiology. 2005;25(4):181–91. [PubMed: 16103729]
27. Gross AL, Jones RN, Fong TG, et al. Calibration and validation of an innovative approach for estimating general cognitive performance. Neuroepidemiology. 2014;42(3):144–53. [PubMed: 24481241]

28. Yesavage JA, Brink TL, Rose TL, et al. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res. 1982;17(1):37–49. [PubMed: 7183759]
29. Jorm AF. A short form of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): development and cross-validation. Psychol Med. 1994 2;24(1):145–53. [PubMed: 8208879]
30. Saczynski JS, Kosar CM, Xu G, et al. A tale of two methods: chart and interview methods for identifying delirium. J Am Geriatr Soc. 2014 3;62(3):518–24. [PubMed: 24512042]
31. Inouye SK, Kosar CM, Tommet D, et al. The CAM-S: development and validation of a new scoring system for delirium severity in 2 cohorts. Ann Intern Med. 2014 4 15;160(8):526–33. [PubMed: 24733193]
32. Rissin DM, Kan CW, Song L, et al. Multiplexed single molecule immunoassays. Lab Chip. 2013 8 7;13(15):2902–11. [PubMed: 23719780]
33. Vasunilashorn SM, Dillon ST, Inouye SK, et al. High C-Reactive Protein Predicts Delirium Incidence, Duration, and Feature Severity After Major Noncardiac Surgery. J Am Geriatr Soc. 2017 8;65(8):e109–e16. [PubMed: 28555781]
34. Vasunilashorn SM, Ngo LH, Inouye SK, et al. Apolipoprotein E genotype and the association between C-reactive protein and postoperative delirium: Importance of gene-protein interactions. Alzheimers Dement. 2020 3;16(3):572–80. [PubMed: 31761478]
35. Khan BA, Zawahiri M, Campbell NL, et al. Biomarkers for delirium--a review. J Am Geriatr Soc. 2011 11;59 Suppl 2:S256–61. [PubMed: 22091570]
36. Simone MJ, Tan ZS. The role of inflammation in the pathogenesis of delirium and dementia in older adults: a review. CNS Neurosci Ther. 2011 10;17(5):506–13. [PubMed: 20553303]
37. Inouye SK. Delirium in older persons. N Engl J Med. 2006 3 16;354(11):1157–65. [PubMed: 16540616]
38. Cavallari M, Dai W, Guttmann CR, et al. Neural substrates of vulnerability to postsurgical delirium as revealed by presurgical diffusion MRI. Brain. 2016 4;139(Pt 4):1282–94. [PubMed: 26920674]
39. van Montfort SJT, van Dellen E, Stam CJ, et al. Brain network disintegration as a final common pathway for delirium: a systematic review and qualitative meta-analysis. Neuroimage Clin. 2019 4 3;23:101809. [PubMed: 30981940]
40. Racine AM, Fong TG, Gou Y, et al. Clinical outcomes in older surgical patients with mild cognitive impairment. Alzheimers Dement. 2018 5;14(5):590–600. [PubMed: 29190460]
41. Cortese GP, Burger C. Neuroinflammatory challenges compromise neuronal function in the aging brain: Postoperative cognitive delirium and Alzheimer's disease. Behav Brain Res. 2017 3 30;322(Pt B):269–79. [PubMed: 27544872]
42. Gailiušas M, Andrejaitienė J, Širvinskas E, et al. Association between serum biomarkers and postoperative delirium after cardiac surgery. Acta Med Litu. 2019;26(1):8–10. [PubMed: 31281210]
43. Shahim P, Tegner Y, Marklund N, et al. Neurofilament light and tau as blood biomarkers for sports-related concussion. Neurology. 2018;90(20):e1780–e8. [PubMed: 29653990]
44. Halaas NB, Blennow K, Idland AV, et al. Neurofilament Light in Serum and Cerebrospinal Fluid of Hip Fracture Patients with Delirium. Dementia and geriatric cognitive disorders. 2018;46(5–6):346–57. [PubMed: 30522125]
45. Ernster VL. Nested case-control studies. Preventive medicine. 1994 9;23(5):587–90. [PubMed: 7845919]
46. Hughes CG, Pandharipande PP, Thompson JL, et al. Endothelial Activation and Blood-Brain Barrier Injury as Risk Factors for Delirium in Critically Ill Patients*. Critical Care Medicine. 2016;44(9).
47. Kalm M, Boström M, Sandelius Å, et al. Serum concentrations of the axonal injury marker neurofilament light protein are not influenced by blood-brain barrier permeability. Brain Res. 2017 8 1;1668:12–9. [PubMed: 28522263]

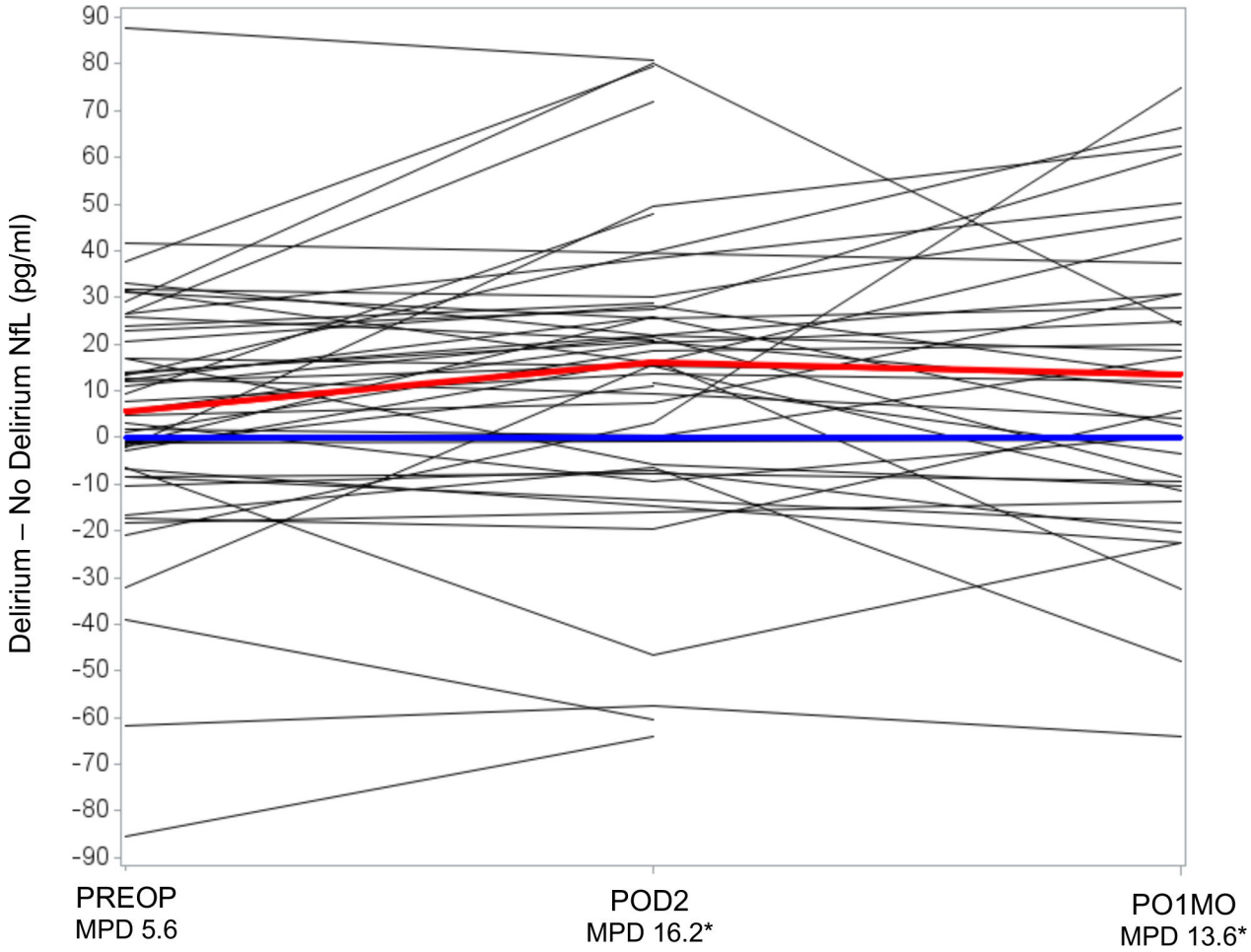

Figure 1.

Neurofilament light (NfL) values over time. Each black line represent the difference in NfL levels between the delirium and no delirium case in each of 54 pairs, matched by age, baseline GCP, sex, surgery type, presence of vascular comorbidity, and Apolipoprotein E ε4 carrier status. The red line is the median paired-difference (MPD) across the cohort wheras the blue line shows a difference of zero across all time points for reference. * $P < .05$ MPD=median paired difference, NfL=neurofilament light chain, PO1MO=postoperative 1 month, POD2=postoperative day 2, PREOP=preoperative

Table 1:

Study Sample Characteristics

Study Variables	Full sample (N=108)	Delirium Cases (N=54)	Matched No Delirium Controls (N=54)
Age, years (M, SD) *	77 (5)	77 (5)	77 (4)
Female (n, %) *	56 (53%)	28 (53%)	28 (53%)
Nonwhite (n, %)	10 (9%)	5 (9%)	5 (9%)
Education, years (M,SD)	15 (3)	15 (3)	15 (3)
Married, (n, %)	64 (60%)	30 (56%)	34 (63%)
GCP (M, SD) *	55.5 (5.4)	55.1 (5.6)	55.9 (5.5)
Charlson score (0–7)	1.3 (1.4)	1.3 (1.4)	1.3 (1.4)
Vascular comorbidity			
Presence (n, %) *	52 (49%)	26 (49%)	26 (49%)
ApoE ε4 carrier (n, %) *	23 (22%)	11 (21%)	12 (23%)
3MS score, (M, SD)	92.3 (5.9)	92.0 (5.67)	92.6 (6.1)
Proxy IQCODE >3.2, (n, %)	23 (22%)	13 (24%)	10 (19%)
Anesthesia type			
General alone	89 (84%)	44 (83%)	45 (85%)
Spinal alone	17 (16%)	9 (17%)	8 (15%)

* Variable used for matching.

Abbreviations: ApoE=Apolipoprotein E, GCP=general cognitive performance, IQCODE = Informant Questionnaire on Cognitive Decline in the Elderly M=mean, 3MS=Modified Mini-Mental State Examination, SD=standard deviation

Table 2.

Association between Neurofilament Light Chain and Postoperative Delirium Incidence, Days and Severity

Quartile	NFL(pg/ml)	Delirium Adjusted OR (95% CI)	Delirium Days* Days (95% CI)	Sum CAM-S* (per point) Score (95% CI)
PREOP (n=53 matched pairs)				
Q1	≤20.76	Reference	Reference	Reference
Q2	20.77–29.06	1.39 (0.41–4.74)	−0.04 (−0.57, 0.49)	0.03 (−5.22, 5.28)
Q3	29.07–44.38	1.05 (0.35–3.14)	0.03 (−0.54, 0.61)	1.17 (−4.56, 6.89)
Q4	≥44.39	**3.71 (1.09–12.58)**	0.47 (−0.16, 1.10)	**7.82 (1.60, 14.04)**
p for trend		0.06	0.35	0.02
POD2 (n=47 matched pairs)				
Q1	≤28.08	Reference	Reference	Reference
Q2	28.09–40.21	3.97 (0.90–17.47)	0.11 (−0.43, 0.65)	1.02 (−4.96, 7.00)
Q3	40.22–54.75	**4.67 (1.20–18.15)**	−0.14 (−0.74, 0.45)	1.40 (−5.04, 7.85)
Q4	≥54.76	**8.83 (1.88–41.36)**	**1.04 (0.49, 1.60)**	**9.34 (3.22, 15.46)**
p for trend		**<.01**	<.01	<.01

In all models, we adjusted for baseline covariables likely to be related to delirium and cognitive decline, including age, sex, vascular comorbidity, baseline general cognitive performance and Apolipoprotein E4 carrier status

Delirium is expressed as an adjusted odds ratio (null=1) whereas delirium days and delirium severity (sum of CAM-S) are expressed as the increase in delirium days or CAM-S points for patients in a given quartile (Q) relative to patients in Q1 (null=0)

Abbreviations: CAM-S=Confusion Assessment Method-Severity, GDS=Geriatric Depression Scale; IQCODE= Informant Questionnaire on Cognitive Decline in the Elderly, NfL=neurofilament light chain, POD2=postoperative day 2, PREOP=preoperative, Q=quartile

Bold indicates significant at $P < .05$

Table 3.

Associations Between Neuronal Injury Markers (GFAP, Tau, UCHL-1) and Postoperative Delirium

		Delirium			Delirium			Delirium
GFAP (pg/ml)		**OR (95% CI)**	**Tau (pg/ml)**		**OR (95% CI)**	**UCHL-1 (pg/ml)**		**OR (95% CI)**
PREOP (n=53 matched pairs)			PREOP (n=53 matched pairs)			PREOP (n=52 matched pairs)		
Q1	≤164.24	Reference	Q1	≤1.85	Reference	Q1	≤12.24	Reference
Q2	164.25–225.41	1.78 (0.54–5.87)	Q2	1.86–2.71	3.30 (0.93–11.75)	Q2	12.25–18.84	1.14 (0.36–3.71)
Q3	225.42–333.04	2.03 (0.60–6.94)	Q3	2.72–4.50	1.81 (0.63–5.24)	Q3	18.85–34.45	1.96 (0.57–6.66)
Q4	≥333.05	2.97 (0.72–2.20)	Q4	≥4.51	2.22 (0.78–6.32)	Q4	≥34.46	1.39 (0.46–4.20)
p for trend		0.14	p for trend		0.29	p for trend		0.54
POD2 (n=44 matched pairs)			POD2 (n=44 matched pairs)			POD2 (n=48 matched pairs)		
Q1	≤174.14	Reference	Q1	≤2.15	Reference	Q1	≤19.93	Reference
Q2	174.15–243.49	0.93 (0.29–2.97)	Q2	2.16–3.26	0.86 (0.30–2.48)	Q2	19.94–31.35	0.75 (0.22–2.60)
Q3	243.50–385.18	1.05 (0.31–3.58)	Q3	3.27–5.33	2.28 (0.62–8.45)	Q3	31.36–64.74	1.24 (0.37–4.19)
Q4	≥385.19	2.19 (0.56–8.53)	Q4	≥5.34	0.91 (0.32–2.60)	Q4	≥64.75	2.17 (0.63–7.44)
P for trend		0.25	*P* for trend		0.68	*P* for trend		0.19

Abbreviations: GFAP=glial fibrillary acidic protein, POD2=postoperative day 2, PREOP=preoperative, Q=quartiles, UCHL-1 = ubiquitin carboxyl-terminal hydrolase L1, Q=quartile

Table 4.

Associations of Postoperative Delirium, Days, and Severity with Neurofilament Light Chain Measured on Postoperative 1 Month

	NfL PO1MO (pg/ml)		
	Q2 vs. Q1	**Q3 vs. Q1**	**Q4 vs. Q1**
Delirium vs. No Delirium [OR (95% CI)]	2.45 (0.69–8.76)	2.19 (0.62–7.79)	**9.73 (2.28–41.43)**
Delirium Days (per day) [OR (95% CI)]	1.29 (0.67–2.49)	1.14 (0.58–2.22)	**2.05 (1.09–3.84)**
Sum CAM-S (per pt) [OR [95% CI)]	1.06 (0.99–1.15)	1.05 (0.98–1.14)	**1.11 (1.03–1.20)**

Abbreviations: CAM-S=Confusion Assessment Method-Severity, CI=confidence interval, NfL=neurofilament light chain, OR=odds ratio, PO1MO=postoperative 1 month, Q=quartile

Multinomial logistic regression adjusted for age, sex, vascular comorbidity, baseline general cognitive performance and Apolipoprotein ε4 carrier status

Bold indicates significant at $P < .05$

NfL quartiles (pg/ml): PO1MO - Q1 ≤28.08, Q2 28.09–40.21, Q3 40.22–54.75, Q4 ≥54.75

At PO1MO n=49 matched pairs

Table 5.

Association between Neurofilament Light Chain and 1 Month General Cognitive Performance

		Change Between 1 Month GCP and PREOP GCP
NfL (pg/ml)		**Score (95% CI)**
PREOP (n=53 matched pairs)		
Q1	≤20.76	Reference
Q2	20.77–29.06	−0.01 (−1.83, −1.84)
Q3	29.07–44.38	−0.17 (−2.02, 1.68)
Q4	≥44.39	−1.94 (−3.97, 0.09)
P for trend		0.09
POD2 (n=44 matched pairs)		
Q1	≤28.08	Reference
Q2	28.09–40.21	0.82 (−2.94, 4.57)
Q3	40.22–54.75	0.70 (−1.30, 2.70)
Q4	≥54.76	0.99 (−1.11, 3.10)
P for trend		0.39
PO1MO (n=46 matched pairs)		
Q1	≤32.30	Reference
Q2	32.31–44.34	0.11 (−1.74, 1.96)
Q3	44.35–64.11	−0.89 (−2.67, 0.90)
Q4	≥ 64.12	**−2.31 (−4.14, −0.48)**
P for trend		**<.01**

Abbreviations: CI=confidence interval, GCP=general cognitive performance, NfL=neurofilament light chain

Model adjusted for age, sex, baseline GCP, vascular comorbidity, and APOE E4 carrier status.

Bold indicates significant at $P < .05$