

Association between plasma tau and postoperative delirium incidence and severity: a prospective observational study

Tyler Ballweg 1, Marissa White 1, Margaret Parker 1, Cameron Casey 1, Amber Bo 1, Zahra Farahbakhsh 1, Austin Kayser 1, Alexander Blair 1, Heidi Lindroth 2, Robert A Pearce 1, Kaj Blennow 3, Henrik Zetterberg 4, Richard Lennertz 1, Robert D Sanders 5

Affiliations

1. Department of Anesthesiology, University of Wisconsin, Madison, WI, USA.
2. Department of Medicine, Indiana University School of Medicine, Center for Aging Research, Center for Health Innovation and Implementation, Indianapolis, IN, USA.
3. Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden.
4. Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden; Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden; Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK; UK Dementia Research Institute at UCL, London, UK.
5. University of Sydney, Sydney, NSW, Australia; Department of Anaesthetics, Royal Prince Alfred Hospital, Camperdown, NSW, Australia. Electronic address: robert.sanders@sydney.edu.au.

Abstract:

Background: Postoperative delirium is associated with increases in the neuronal injury biomarker, neurofilament light (NfL). Here we tested whether two other biomarkers, glial fibrillary acidic protein (GFAP) and tau, are associated with postoperative delirium.

Methods: A total of 114 surgical patients were recruited into two prospective biomarker cohort studies with assessment of delirium severity and incidence. Plasma samples were sent for biomarker analysis including tau, NfL, and GFAP, and a panel of 10 cytokines. We determined a priori to adjust for interleukin-8 (IL-8), a marker of inflammation, when assessing associations between biomarkers and delirium incidence and severity.

Results: GFAP concentrations showed no relationship to delirium. The change in tau from preoperative concentrations to postoperative Day 1 was greater in patients with postoperative delirium ($P < 0.001$) and correlated with delirium severity ($\rho = 0.39$, $P < 0.001$). The change in tau correlated with increases in IL-8 ($P < 0.001$) and IL-10 ($P = 0.0029$). Linear regression showed that the relevant clinical predictors of tau changes were age ($P = 0.037$), prior stroke/transient ischaemic attack ($P = 0.001$), and surgical blood loss ($P < 0.001$). After adjusting for age, sex, preoperative cognition, and change in IL-8, tau remained significantly associated with delirium severity ($P = 0.026$). Using linear mixed effect models, only tau (not NfL or IL-8) predicted recovery from delirium ($P < 0.001$).

Conclusions: The change in plasma tau was associated with delirium incidence and severity, and resolved over time in parallel with delirium features. The impact of this putative perioperative neuronal injury biomarker on long-term cognition merits further investigation.

Clinical trial registration: NCT02926417 and NCT03124303.

Introduction

Postoperative delirium is a major healthcare issue affecting thousands of patients each year. Not only is delirium unpleasant, it is associated with increasing morbidity and mortality, as well as increased costs and subsequent cognitive decline^{1,2}. However, establishing that long-term changes in cognitive decline are causally attributable to delirium is challenging. A major hurdle is that pre-delirium cognitive impairment is associated with increased risk for delirium, and patients with this preoperative cognitive decline are likely declining faster than subjects who do not incur delirium^{3,4}. Hence any postoperative changes in cognition could merely be attributable to preoperative differences. We, and others, have supplied important evidence for a small long-term effect on cognition associated with surgery when accounting for the preoperative cognitive trajectory⁵⁻⁷ but it is unclear if this small effect may be – in part – attributable to delirium or not. In order to address whether delirium may itself have some neurotoxic component of its pathogenesis we recently undertook studies of postoperative covert strokes⁸ and the plasma biomarker, NfL⁹. Delirium was associated with postoperative covert strokes⁸ and rises in postoperative NfL⁹. While it remains to be seen if these rises in NfL correlate with changes in long-term cognition, like covert stroke may, it is also not clear that this is the only relevant biomarker to pursue. We therefore undertook further analyses to identify if alternate plasma biomarkers, tau and glial fibrillary acidic protein (GFAP) may show similar relationships with delirium.

Tau is a microtubule associated protein that is highly expressed in neurons and modulates the stability of axonal microtubules. It is increased in various neurodegenerative states¹⁰, as well as

following stroke¹¹, but has not been thoroughly studied in the perioperative period. However its rapid clearance from the blood relative to NfL may be advantageous for studying acute injury¹⁰. GFAP is an intermediate filament protein that is expressed in astrocytes and often used in their cellular identification on microscopy. Despite being relatively specific for astrocytes in the central nervous system, expression outside of the central nervous system does occur. This limitation aside, plasma levels of GFAP have been proposed as a nervous system injury biomarker in traumatic brain injury and stroke as well as for postoperative cognitive decline¹².

As postoperative delirium is frequently considered to be a response of the brain to inflammation, we hypothesized that any neuronal marker of injury will correlate to some extent with inflammation. Therefore, we additionally tested a panel of 10 cytokines to identify markers that may add additional information over inflammation itself (using our prior methods⁹). To do this, we tested all the cytokines and pursued the cytokine with the strongest relationship to peak delirium severity. In our prior work, we investigated rises in NfL on postoperative day 1 (POD1) as NfL levels rose with time and it was unclear if this related to selection bias (as healthier patients were discharged earlier), ongoing injury or accumulation in the plasma. In this paper, we pursued POD1 changes for similar logic but also report information on preoperative biomarkers and peak biomarker levels where appropriate.

Methods

The data are derived from an ongoing prospective perioperative cohort study registered with ClinicalTrials.gov (NCT03124303) and approved by the University of Wisconsin-Madison

Institutional Review Board (2015-0374). 102 adult patients were recruited who were scheduled for major elective non-intracranial, non-cardiac surgery which was defined as requiring at least a two-day hospital stay (described in our recent publication on NfL⁹). See **Supplementary Figure 1** for the STROBE diagram and **Supplementary Table 1** for inclusion and exclusion criteria.

Participants had blood draws preoperatively and on each of the first (up to) four postoperative days the patient was in hospital. Preoperatively, and twice daily postoperatively, participants underwent delirium assessments with the Confusion Assessment Method (CAM)/3D-CAM, or the CAM-ICU if the patient was intubated. Delirium severity was assessed with the validated Delirium Rating Scale-98 (DRS). No patient was intubated for greater than 96 hours postoperatively allowing collection of at least one DRS measure per participant without intubation.

Plasma samples were collected in EDTA-containing tubes preoperatively, and in the morning (06.00-10.00) of each postoperative day, stored at -80°C, and sent for cytokine multiplex assay for interleukin 1 (IL-1) beta, IL-1 receptor antagonist, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, monocyte chemoattractant protein 1 (MCP-1) and Tumor Necrosis Factor-alpha (Eve technology, Canada). In addition, samples were sent to the University of Gothenburg for analysis of tau and GFAP. **Tau and GFAP concentrations were measured using commercially available kits (the Tau Advantage and GFAP Discovery kits, respectively) on the Single molecule array (Simoa) HD-1 Analyzer (Quanterix, Billerica, MA).** We report some additional analyses with NfL which were not available for our prior publication. NfL concentration was also measured using a Simoa method, as previously described in detail ¹³.

Measured values below the range of detection were imputed as 0.001 for cytokines, 1.22 pg/mL for tau, 0.69 pg/mL for GFAP, and 6.7 pg/mL for NfL based on the lower limit of quantification of each assay. All biomarker data were then log-transformed to correct the strong rightward skew.). In total, 87 participants had both pre-operative and post-operative day 1 blood samples analyzable for GFAP and tau.

Statistical Analysis

The sample size was determined based on a prior study that showed a rise in postoperative NfL levels ¹⁵ however that study showed similar changes in tau to NfL hence we conducted this additional investigation. Assuming that delirium harboured the burden of the tau increase from preoperative levels, 87 patients would provide 80% power ($p=0.025$) to show a difference in tau levels of 2.1 pg/mL (standard deviation 3 pg/mL) assuming a delirium incidence of 33%. This report represents a secondary analysis of this dataset to verify whether we should test additional biomarkers in addition to NfL. The significance level for the primary outcome measure was set at $p<0.025$ for testing both the change in POD1 levels of GFAP or tau based on delirium incidence (Bonferroni correction for two tests using Mann-Whitney). However, a priori we determined that for additional stringency, any putative biomarker of delirium also had to vary proportionately to delirium severity (Spearman correlation) meaning the p-value was equivalent to 0.025^2 .

As a mechanistic/exploratory secondary step, *a priori* we determined that we would correlate GFAP or tau with each of the 10 cytokines and adjust p-values across cytokines for testing a correlation with GFAP or tau; we did not adjust for multiple comparisons across the 10

cytokines. Hence the p-value was again set at $p < 0.025$ but these analyses should be considered hypothesis generating. For mechanistic studies, IL-8 was selected as the marker of the inflammation with the strongest correlation to delirium severity based on our prior work and to allow direct comparison to our previous results with NfL⁹. Spearman correlations of POD1 change in tau or GFAP and peak DRS were correlated with Spearman. Linear regression was conducted in R with age, sex, preoperative Trail Making Test B (a measure of cognitive executive function) and POD1 change in IL-8 as covariates. Finally, we conducted linear mixed effect modelling to investigate whether change in any of the biomarkers may predict resolution of in delirium symptom severity from POD1 to POD4. All analyses were conducted in R.

Results

The cohort demographics have already been reported⁹, however in brief, the delirium incidence was 39/108 (36.1%) and the mean peak DRS was 21.2 for delirious subjects and 6.8 for non-delirious subjects. Age (69.5 vs 72.0 years old, $t=1.654$, $p=0.104$) and sex (proportion female – 46.2% vs. 39.1%, $\chi^2=0.506$, $p=0.477$) did not differ by postoperative delirium status. Delirious participants underwent higher risk surgery (National Surgical Quality Improvement Program Risk of Death; 5% vs 2%, $p=0.010$), particularly more vascular surgery (64.1% vs. 37.7%, $\chi^2=6.98$, $p=0.008$, see **Supplementary Table 2** for demographic information).

Preoperative Biomarker Associations with Postoperative Delirium

Neither preoperative GFAP or tau were associated with postoperative delirium or postoperative delirium peak severity (**Table 1**).

Time courses of changes in perioperative biomarkers

Figure 1 shows the time course for perioperative change tau or GFAP from preoperative levels divided by delirium status. Only tau showed clear changes in time from preoperative to postoperative levels. Tau changed from preoperative to POD1 levels in both delirious and non-delirious subjects though the rise was greater in delirious compared to non-delirious subjects (**Table 1**). Next, we tested the Receiver Operating Characteristic (ROC) features of the different biomarkers using the area under the ROC (AUROC). Tau showed a significant classification of delirium status (AUROC 0.70, 95% CI 0.58-0.81, $p=0.004$) while GFAP (AUROC 0.57, 95% CI 0.42-0.71, $p=0.324$; **Figure 2**) did not. IL-8 could classify delirium (AUROC 0.73, 95% CI 0.60-0.87, $p=0.0006$) but NfL could not (AUROC 0.63, 95% CI 0.48-0.78, $p=0.059$). The POD1 rise in tau also correlated with delirium severity while GFAP did not (**Figure 3**). There was no difference in the strength of correlation of tau and delirium severity and NfL and delirium severity (Williams Test $p = X$).

Next, we tested the relationships of tau and IL-8 (**Table 2**). We previously reported that NfL and IL-8 are correlated⁹. In our exploratory analyses, we found that tau and IL-8 are correlated at a threshold of $p<0.025$ (**Table 2**). In order to probe whether tau contributed to the pathogenesis of delirium independently of inflammation, we conducted linear regression for the change in IL-8, age, sex and preoperative Trail Making Test B to identify predictors of peak delirium severity (DRS). In this model, preoperative Trail Making Test B ($p=0.010$), change in IL.8 ($p=0.028$), and change in tau ($p=0.017$) were predictors of peak DRS (adjusted $r^2=0.25$, $p<0.001$, **Supplementary Table 3**).

We also investigated clinical predictors of tau. In a linear regression model including age, sex, type of surgery, operative time, blood loss, and area under the curve for intraoperative mean arterial pressures

less than 10%, (ml), age ($p=0.041$), vascular surgery ($p=0.041$) and blood loss ($p<0.001$) were all associated with increased POD1 plasma tau levels (adjusted $r^2=0.40$, $p<0.001$, **Supplementary Table 4**).

Biomarker correlations for the Resolution of Delirium Symptoms

Finally, we investigated whether changes in tau, NfL or IL-8 over time may correlate with resolution of delirium symptoms. Linear mixed effect models were constructed with age, sex, biomarker, time (POD) and an interaction for biomarker and time. Of the three models tested, the only biomarker that showed a significant interaction with time was tau ($p=0.007$). In this model, tau ($p=0.014$), time ($p=0.012$), sex ($p=0.043$) and the interaction of tau and time were significant but age was not ($p=0.429$). The interactions for NfL and time ($p=0.701$) or IL-8 and time ($p=0.848$) were not significant. Using complete datasets for all three models, we compared the models using the Akaike Information Criterion; this showed that the model with tau (992) was superior to NfL (996) or IL-8 (996). The linear mixed effects model including tau was then re-run with covariates of IL-8 and the interaction of IL-8 and time, and the significant interaction of tau and time was unchanged ($p=0.007$). GFAP was not tested as it showed no relationship to delirium.

Discussion

Plasma tau changes were associated with delirium incidence, severity and resolved with delirium symptoms, suggesting a possible causal role of neuronal injury with delirium pathogenesis. These changes were independent of changes in inflammation as assessed by IL-8. It is of interest that tau appeared to prove superior to NfL for classification of delirium incidence and for tracking the resolution of symptoms. This may be due to more rapid plasma clearance rather than altered neuronal injury detection and further studies are required to disambiguate these alternatives. These data support the accumulating data that neuronal injury may play a role in the

pathogenesis of delirium^{8,9} and suggest that future studies investigating the impact of perioperative tau rises on long-term cognition are warranted. In contrast, GFAP was not associated with delirium and did not rise perioperatively.

Our study has many strengths including modeling approaches and attention to p-values including the requirement that a biomarker changes with delirium incidence and proportionately to delirium severity. However, there are also multiple limitations. While we have attempted to control for confounding, in any observational study there are issues with unmeasured confounding. Nonetheless we have established that changes in plasma tau are associated with delirium severity and the resolution of delirium symptoms even when adjusting for inflammation as measured by IL-8. Of course, IL-8 may not capture all relevant inflammation and further studies, investigating other potential biomarkers, are warranted. Our study was also of modest size, and larger studies are required, particularly if they are to probe associations with long-term cognition. Most importantly we are unable to establish causality with our observational research, and experimental studies are required to probe causal relationships between changes in neuronal injury biomarkers and delirium.

Table 1: Preoperative and POD1 change associations of tau, GFAP and cytokines with delirium incidence.

Table 2: Correlations of tau and GFAP with cytokines

Figure 1: Perioperative time course of tau and GFAP

Figure 2: Receiver Operating Curves for classifying delirium for tau, GFAP, NfL and IL-8.

Figure 3: Correlations of delirium severity with tau and GFAP

References

1. Sanders RD, Pandharipande PP, Davidson AJ, Ma D, Maze M. Anticipating and managing postoperative delirium and cognitive decline in adults. *BMJ*. 2011;343:d4331.
2. Witlox J, Eurelings LS, de Jonghe JF, Kalisvaart KJ, Eikelenboom P, van Gool WA. Delirium in elderly patients and the risk of postdischarge mortality, institutionalization, and dementia: a meta-analysis. *JAMA*. 2010;304(4):443-451.
3. Inouye SK, Marcantonio ER, Kosar CM, et al. The short-term and long-term relationship between delirium and cognitive trajectory in older surgical patients. *Alzheimer's & dementia : the journal of the Alzheimer's Association*. 2016.
4. Vasunilashorn SM, Fong TG, Albuquerque A, et al. Delirium Severity Post-Surgery and its Relationship with Long-Term Cognitive Decline in a Cohort of Patients without Dementia. *J Alzheimers Dis*. 2018;61(1):347-358.
5. Krause BM, Sabia S, Manning HJ, Singh-Manoux A, Sanders RD. Association between major surgical admissions and the cognitive trajectory: 19 year follow-up of Whitehall II cohort study. *BMJ*. 2019;366:l4466.
6. Schulte PJ, Roberts RO, Knopman DS, et al. Association between exposure to anaesthesia and surgery and long-term cognitive trajectories in older adults: report from the Mayo Clinic Study of Aging. *Br J Anaesth*. 2018;121(2):398-405.
7. Whitlock EL, Diaz-Ramirez LG, Smith AK, Boscardin WJ, Avidan MS, Glymour MM. Cognitive Change After Cardiac Surgery Versus Cardiac Catheterization: A Population-Based Study. *Ann Thorac Surg*. 2018.
8. Neuro VI. Perioperative covert stroke in patients undergoing non-cardiac surgery (NeuroVISION): a prospective cohort study. *Lancet*. 2019;394(10203):1022-1029.
9. Casey CP, Lindroth H, Mohanty R, et al. Postoperative delirium is associated with increased plasma neurofilament light. *Brain*. 2019;143(1):47-54.
10. Bateman RJ, Blennow K, Doody R, et al. Plasma Biomarkers of AD Emerging as Essential Tools for Drug Development: An EU/US CTAD Task Force Report. *J Prev Alzheimers Dis*. 2019;6(3):169-173.
11. Pase MP, Himali JJ, Aparicio HJ, et al. Plasma total-tau as a biomarker of stroke risk in the community. *Ann Neurol*. 2019;86(3):463-467.
12. Rappold T, Laflam A, Hori D, et al. Evidence of an association between brain cellular injury and cognitive decline after non-cardiac surgery. *Br J Anaesth*. 2016;116(1):83-89.

13. Gisslen M, Price RW, Andreasson U, et al. Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. *EBioMedicine*. 2016;3:135-140.
14. Garrett RG. The chi-square plot: a tool for multivariate outlier recognition. . *Journal of Geochemical Exploration* 1989;32((1-3)): 319-341.
15. Evered L, Silbert B, Scott DA, Zetterberg H, Blennow K. 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):542-547.