Postoperative delirium is associated with increased plasma neurofilament light

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Abstract

While delirium is associated with cognitive decline and dementia, there is limited evidence to support causality for this relationship. Clarification of how delirium may cause cognitive decline, perhaps through evidence of contemporaneous neuronal injury, would enhance plausibility for a causal relationship. Dose-dependence of neuronal injury with delirium severity would further enhance the biological plausibility for this relationship. We tested whether delirium is associated with neuronal injury in 114 surgical patients recruited to a prospective biomarker cohort study. Patients underwent perioperative testing for changes in neurofilament light, a neuronal injury biomarker, as well as a panel of 10 cytokines, with contemporaneous assessment of delirium severity and incidence. A subset of patients underwent preoperative MRI. Initially we confirmed prior reports that neurofilament light levels correlated with markers of neurodegeneration [hippocampal volume ($\Delta R2 = 0.129$, $P = 0.015$)] and white matter changes including fractional anisotropy of white matter ($\Delta R2 = 0.417$, $P < 0.001$) with similar effects on mean, axial and radial diffusivity) in our cohort and that surgery was associated with increasing neurofilament light from preoperative levels [mean difference (95% confidence interval, CI) = 0.240 (0.178, 0.301) log10 (pg/ml), $P < 0.001$], suggesting putative neuronal injury. Next, we tested the relationship with delirium. Neurofilament light rose more sharply in participants with delirium compared to non-sufferers [mean difference (95% CI) = 0.251 (0.136, 0.367) log10 (pg/ml), $P < 0.001$]. This relationship showed dose-dependence, such that neurofilament light rose proportionately to delirium severity ($\Delta R2 = 0.199$, $P < 0.001$). Given that inflammation is considered an important driver of postoperative delirium, next we tested whether neurofilament light, as a potential marker of neurotoxicity, may contribute to the pathogenesis of delirium independent of inflammation. From a panel of 10 cytokines, the pro-inflammatory cytokine IL-8 exhibited a

strong correlation with delirium severity (ΔR2 = 0.208, P < 0.001). Therefore, we tested

whether the change in neurofilament light contributed to delirium severity independent of

IL-8. Neurofilament light was independently associated with delirium severity after adjusting

for the change in inflammation (ΔR2 = 0.040, P = 0.038). These data suggest delirium is

associated with exaggerated increases in neurofilament light and that this putative

neurotoxicity may contribute to the pathogenesis of delirium itself, independent of changes

in inflammation.

Introduction

Delirium is an acute confusional state, characterized by inattention and cognitive failure, that arises as a physiological consequence of a medical or surgical condition[1]. It is associated with increased morbidity and mortality[2,3], higher rates of institutionalization[3] and is estimated to cost up to $160 billion per annum in the USA[4]. Perhaps most critically, it can herald a decline in cognition[5,6] with a descent to neurodegenerative diseases such as dementia[3,7]. Clarifying how delirium may contribute to the pathology of dementia is a major focus of the National Institute of Aging (Bethesda, USA). One possibility is that delirium results as a failed "stress test" for the brain[8], revealing underlying neurodegeneration in the setting of an acute physiological stressor. An alternative is that delirium is associated with neuronal injury and this accelerates any cognitive decline. The dominant hypothesis for the pathogenesis of delirium is that inflammation drives the precipitous change in cognition[9-12]. By extension we hypothesize that inflammation may drive neuronal injury, as it can do in diverse neurological disorders ranging from multiple sclerosis[13,14], to cognitive decline and dementia[15,16]. In this brief communication, we first validate a prior finding that major surgery is associated with a rise in the neuronal injury biomarker, plasma neurofilament light[17] (NFL). Next we tested our primary hypothesis (end point), that postoperative delirium is associated with a greater rise in NFL on postoperative day 1, that in subjects who did not incur delirium. To provide supporting evidence suggest some causal role, we proposed that NFL levels should correlate with peak delirium severity (DRS). Given that inflammation is considered the primary precipitant for delirium[11,18], we also hypothesized that inflammation would correlate with NFL levels and extended this to test in mediation analysis whether inflammation may drive delirium severity through neuronal injury.

Methods

The data are derived from an ongoing perioperative cohort study registered with ClinicalTrials.gov (ref: NCT03124303, NCT01980511) and approved by the University of Wisconsin-Madison Institutional Review Board (#2015-0374). In brief, 100 patients were recruited who were scheduled for major non-cardiac non-neurological surgery (defined as requiring at least a two day hospital stay) and were over 65 years old. Subjects had blood draws preoperatively and on each of the postoperative days the patient was in hospital for the first four postoperative days. Preoperatively and twice daily postoperatively subjects underwent delirium assessments with the 3D-confusion assessment method (CAM or CAM-ICU) and delirium rating scale-98 (DRS) if not intubated. No patient was intubated for greater than 96 hours postoperatively allowing collection of at least one DRS measure per subject.

Samples were collected in the morning (06.00-10.00) on each postoperative day, stored at -80°C and sent for cytokine multiplex assay (Eve technology, Canada) with detection limits for the following cytokines: IL-8 etc. In addition, samples were sent to the University of Gothenburg for analysis of NFL using an in house Single molecule array (Simoa) method, as previously described in detail (ref: PMID: 26870824). The measurements were performed by board-certified laboratory technicians in one round of experiments using one batch of reagents. Intra-assay coefficients of variation were 5.1% for a quality control (QC) sample with an NfL concentration of 10.9 pg/mL and 9.6% for a QC sample with a concentration of 150 pg/mL. The lower limit of quantification was 6.7 pg/mL.

Data Analysis

Measured values below the range of detection were imputed as 0.001 for cytokines and 6.7 pg/mL for NFL based on the lower limit of quantification. Data were then log-transformed to correct the strong rightward-skew and examined for multivariate outliers using the robust Mahalanobis Distance (MD) (Garrett, 1989). Six samples were excluded from analysis due to extreme deviation of MD values from the expected chi-squared distribution. After outlier exclusion, 113 subjects remained, 95 of whom had both pre-op and POD1 blood samples.

Mediation analysis was performed using the mediation package in R (Tingley, 2014), which implements a non-parametric bootstrapping procedure for calculating the confidence intervals of the effects in the presented mediation models. Bootstrapping was run using 5000 iterations.

Results

Of the final 95 subject dataset, the delirium incidence was 32/95 (34%). Subjects who were delirious were not older (70.75 vs 74.1 years old, p = 0.063), or more likely to be female (46.88% vs. 39.68%, p= 0.652), did not incur more vascular surgery (62.5% vs. 39.68%, p= .05906), but had higher surgical risk (National Surgical Quality Improvement Program risk score) of death 4.859% vs. 1.94%, p=.021) than subjects who did not become delirious. Initially we confirmed a prior report that surgery is associated with a rise NFL[17]. NFL levels rose with time postoperatively (Figure 1A) and were higher on POD1 (p < 0.001, Figure 1B). It is unclear whether this continued postoperative rise is due to on-going neuronal injury, progressive release of NFL from the brain or due to selection bias as patients with more minor operations were likely discharged earlier than those with more severe operations. For the latter reason, we concentrate on the POD1 data for the rest of the paper. Next we tested which markers of

inflammation would correlate with NFL in our dataset using Bonferroni correction across the cytokines tested (Table 1). After Bonferroni correction, our results showed strong correlations between POD1 rises in IL-8 and IL-10 with NFL (Table 1, p<0.001). IL-10 is an anti-inflammatory cytokine and expected to be elevated in response to the large inflammatory insult induced by surgery. We therefore consider that IL-10 is tracking the pro-inflammatory signal of IL-8 and chose to focus our analyses on IL-8 for biological plausibility[11,18].

Having established these principles in the data, we tested our primary hypothesis that NFL increases more in subjects with delirium than those who did not develop delirium (Figure 1D). Preoperatively there was no difference in NFL between subjects who would later incur delirium or not (data not shown, p>0.05). Notably while NFL rose in both groups, the rise was more profound in the delirious group (p = 0.001, Figure 2A). Next we showed similar time courses in NFL for delirious and non-delirious subjects indicating a gradual rise over time in both groups (Figure 2B) and that POD1 NFL rises correlate with delirium severity (r = 0.4456, p < 0.001, Figure 2C). We saw similar variations for IL-8 (Figure 2D-F), indicating that they may share underlying biological effects in delirium and hence we undertook mediation analysis to test whether the IL-8 effect on delirium severity may be mediated by NFL, a marker of neuronal injury. We found statistically significant direct and indirect effects of IL-8 on delirium severity, b = 5.602 [1.77, 9.62], p = 0.004, and b = 3.247 [1.16, 5.77], p < 0.001, respectively. Due to our inability to experimentally manipulate IL-8 and NFL in this cohort, we also tested the reverse mediation model in which IL-8 mediates the effect of NFL on delirium severity. This model likewise showed significant direct and indirect effects, b = 6.077 [2.56, 9.98], p < 0.001, and b = 2.968 [0.93, 5.33], p = 0.006, respectively. The relative plausibility of these models will be discussed below. The mediation analysis demonstrated that our data are

consistent with a model in which IL-8 causally influences delirium severity, with 37% of the effect being mediated through neuronal injury, as indexed by NFL.

Discussion

These data provide preliminary evidence that delirium is associated with neuronal injury as revealed by increased NFL levels postoperatively. These NFL levels rose over time, perhaps indicating on-going neuronal injury and also occurred in subjects who did not incur delirium but to a lesser extent. NFL, IL-8 and delirium severity were correlated providing biological gradients and hence increasing the plausibility for causal relationships between these variables. A potential causal relationship between the three factors was further supported by mediation analysis. While we recommend our data are confirmed in future studies, they are highly suggestive that delirium is associated with neuronal injury which may explain how delirium can drive acceleration in cognitive decline[6,7] even years later[5]. Furthermore, our data suggest that inflammation may drive this neuronal injury and delirium severity suggesting that modulating the inflammatory response may protect against both these endpoints. A randomized controlled trial of such an intervention is required to evaluate this hypothesis further. Our study does have some limitations, first it is a relatively small study that should be verified in future biomarker cohorts. Secondly while we have conducted focussed hypothesis driven tests, with rigorous adjustment for multiple comparisons (Bonferroni) where appropriate, we have not attempted to adjust for confounding in our analyses. Given that many confounders will be collinear with our variables of interest, it is nigh impossible to adjust for them. We argue that establishing the principle that delirium is associated with neuronal

injury and providing a possible mechanism (inflammation) that may be manipulated in a randomized controlled trial is the best way of testing causality in this setting.

Conclusion

Delirium is associated with increases in NFL, a neuronal injury biomarker that detects degeneration in other neurological diseases. Our data suggest that this is driven by inflammation, offering a potential therapeutic avenue for manipulation of any putative neuronal injury.

Author Contributors

RDS designed the study and collected and analyzed data, CC collected and analyzed data, HL, ST, TB, and ZF collected data, HZ and KB analyzed samples. RDS and CC wrote the paper with input from all co-authors.

Competing Interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no competing interests that may be relevant to the submitted work. HZ has served at scientific advisory boards for Roche Diagnostics, Wave, Samumed and CogRx, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg (all unrelated to the submitted work).

Figure Legends

See figures below.

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Table 1

POD1-Baseline Cytokines Correlated with POD1-Baseline NFL

| Cytokine	Pearson's Correlation	t-Statistic	DF	p-value	Bonferroni
IL-1B	0.2691 [0.0657, 0.4511]	2.62	88	0.010	0.103
IL-1ra	0.2545 [0.05, 0.4384]	2.47	88	0.016	0.155
IL-2	0.2477 [0.0428, 0.4326]	2.4	88	0.019	0.186
IL-4	0.1192 [-0.0901, 0.3184]	1.13	88	0.263	1.000
IL-6	0.2332 [0.0275, 0.42]	2.25	88	0.027	0.269
IL-8	0.5215 [0.3524, 0.6575]	5.73	88	< 0.001	< 0.001
IL-10	0.4937 [0.3193, 0.6358]	5.33	88	< 0.001	< 0.001
IL-12p70	0.1412 [-0.0679, 0.3384]	1.34	88	0.184	1.000
MCP-1	0.2881 [0.0862, 0.4673]	2.82	88	0.006	0.059
TNFa	0.1742 [-0.0342, 0.368]	1.66	88	0.101	1.000
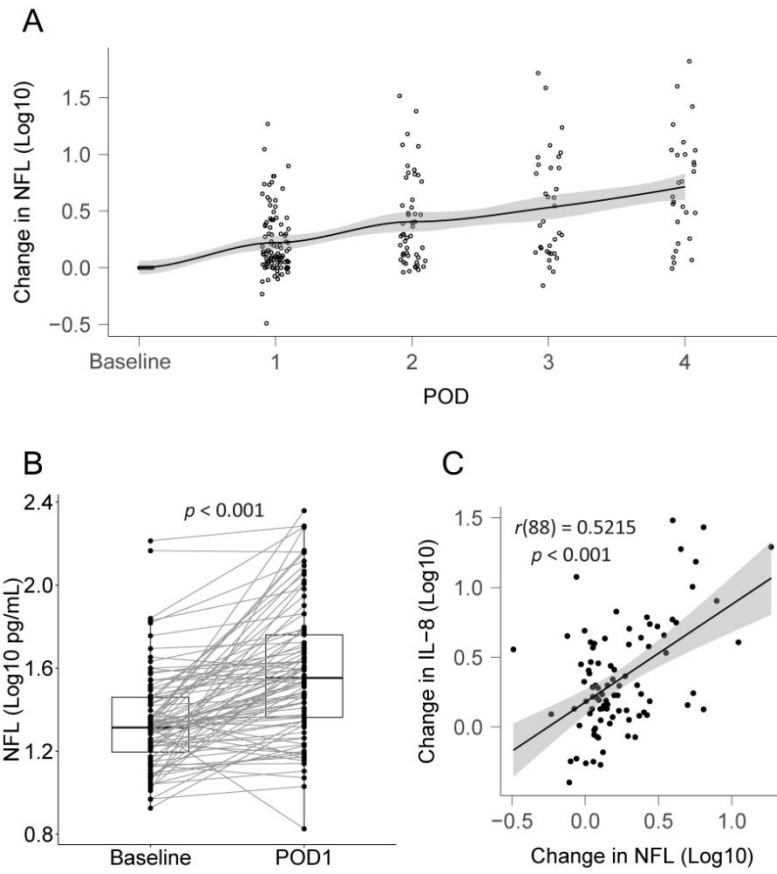
Figure 1. Relationship of NFL and IL-8 to surgery. (A) Time-course of NFL change from baseline levels over first 4 days after surgery. (B) Change in NFL from baseline to POD1. (C) Correlation between the change in values from baseline to POD1 for NFL and IL-8.
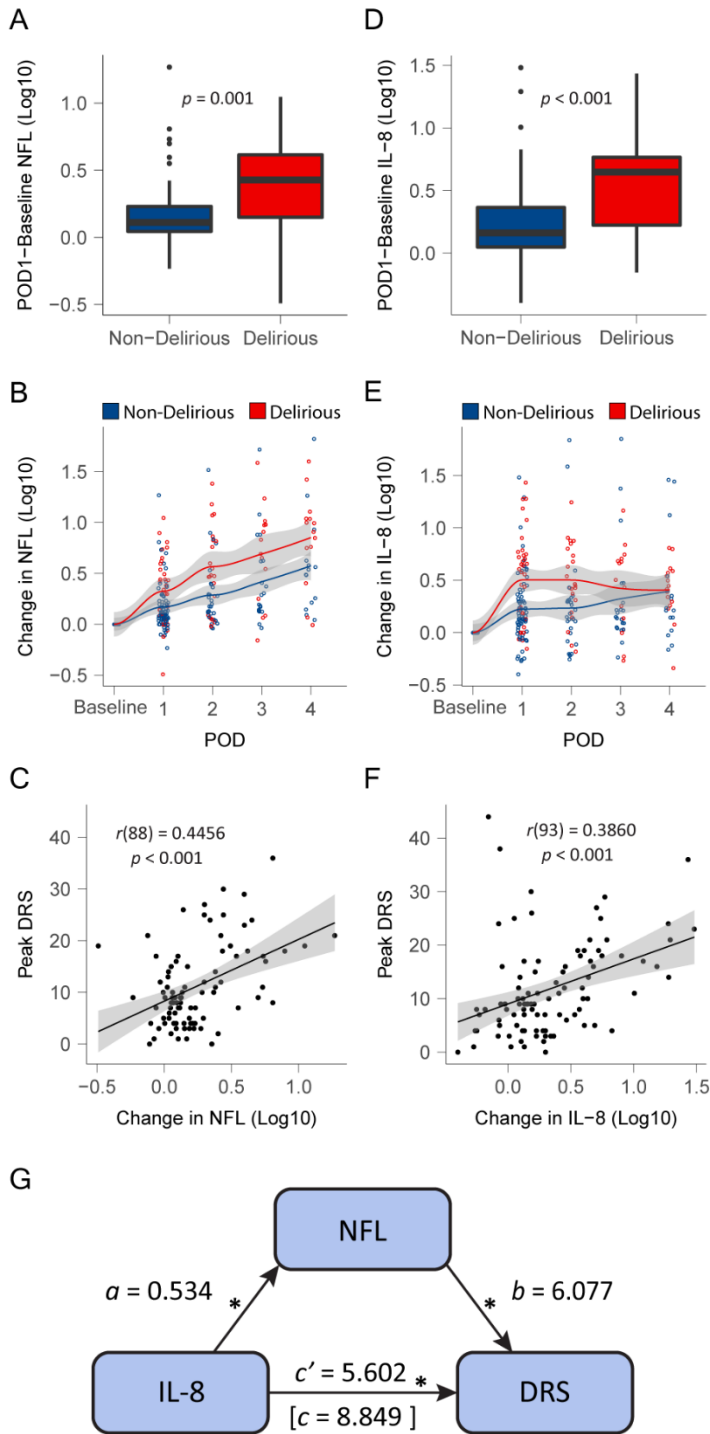
Figure 2. Relationship of NFL and IL-8 to delirium. (A, D) Difference in NFL/IL-8 from baseline to POD1 by delirium outcome. (B, E) Time-course of NFL/IL-8 change from baseline levels over first 4 days after surgery by delirium outcome. (C, F) Correlation between change in NFL/IL-8 from baseline to POD1 and peak DRS scores. (G) Forward mediation model in which the effect of inflammation, as indexed by peak IL-8, on peak DRS is mediated by neuronal injury, as indexed by peak NFL.