Postoperative delirium and changes in the blood–brain barrier, neuroinflammation, and cerebrospinal fluid lactate: a prospective cohort study

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Word Count (? limit): XXX excluding abstract and tables.

BJA*: Clinical investigations:* up to 3000 words, 40 references, and 6 tables or figures.

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Abstract

Background
Case-control studies have associated delirium with blood–brain barrier (BBB) permeability. However, this approach cannot determine whether delirium is attributable to high pre-existing permeability or to perioperative changes. We tested whether perioperative changes in cerebrospinal fluid/plasma albumin ratio (CPAR) and plasma S100B were associated with delirium severity.

Methods
Participants were recruited to two prospective cohort studies of non-intracranial surgery (NCT01980511, NCT03124303, and NCT02926417). Delirium severity was assessed using the Delirium Rating Scale-98. Delirium incidence was diagnosed with the 3D-Confusion Assessment Method (3D-CAM) or CAM-ICU (CAM for the ICU). CSF samples from 25 patients and plasma from 78 patients were analysed for albumin and S100B. We tested associations between change in CPAR (n=11) and S100B (n=61) and delirium, blood loss, CSF interleukin-6 (IL-6), and CSF lactate.

Results
The perioperative increase in CPAR and S100B correlated with delirium severity (CPAR ρ=0.78, P=0.01; S100B ρ=0.41, P<0.001), delirium incidence (CPAR P=0.012; S100B P<0.001) and CSF IL-6 (CPAR ρ=0.66 P=0.04; S100B ρ=0.75, P=0.025). Linear mixed-effect analysis also showed that decreased levels of S100B predicted recovery from delirium symptoms (P=0.001). Linear regression demonstrated that change in plasma S100B was independently associated with surgical risk, cardiovascular surgery, blood loss, and hypotension. Blood loss also correlated with CPAR (ρ=0.64, P=0.04), S100B (ρ=0.70, P<0.001), CSF lactate (R=0.81, P=0.01), and peak delirium severity (ρ=0.36, P=0.01).

Conclusion
Postoperative delirium is associated with a breakdown in the BBB. This increased permeability is dynamic and associated with a neuroinflammatory and lactate response. Strategies to mitigate blood loss may protect the BBB.

INTRODUCTION

Delirium is a disturbance in attention, cognition, and consciousness, an acute physiological consequence of medical events, such as hospital admission, surgery, sepsis, and pharmacological intervention.[1] It is characterised by a sudden onset and fluctuating course not otherwise explained by a pre-existing neurological disorder or medical condition.[2,3] Common in older adults, it affects up to 50% of those in hospital aged over 65 years with a healthcare burden estimated at $152 billion per annum in the USA.[4] Perioperative delirium has been associated with increased mortality,[5] comorbidity,[6,7] functional and neurocognitive decline,[8,9] hospital readmission,[10] and institutionalisation.[11] A recent meta-analysis (k=71) found older inpatients with delirium experienced a mortality risk three times that of those without delirium.[5]

The pathogenesis of postoperative delirium likely involves systemic inflammation [12,13] and the breakdown of the blood-brain barrier (BBB).[14] Prima facie it would appear that these two processes should be linked but perioperative steroids reduce systemic inflammation without preventing damage to the blood brain barrier. Understanding the mechanisms of BBB injury maybe key to preventing delirium as animal studies suggest that the influx of inflammatory cells and mediators likely drive the change in cognition.[15]

The BBB is a series of physical and chemical boundaries, or rather interfaces, between the central nervous system (CNS) and other body systems.[16] The BBB is mostly made up of vascular endothelial cells, joined by intracellular adhesion molecules known as ‘tight junctions’, as well as ependymal cells lining the ventricles and spinal cord in direct contact with cerebrospinal fluid.[17] However, the heavy metabolic demands of the CNS require the BBB to be dynamic and permeable to direct blood flow, influx and store energy, and efflux waste via neurovascular coupling.[18] As demonstrated in animal and human studies, the BBB is selective and adaptive to specific brain regions, physiological functions such as sleep, age, genetic and environmental factors, such as stress, illness, and injury, while maintaining the primary CNS functions of cellular signalling and glucose metabolism.[17,19]

Despite its role in ionic homeostasis and healthy ageing[16], BBB deterioration or dysfunction has been implicated in a number of neurological conditions such as Alzheimer’s disease and other dementias.[20] Case-control studies have demonstrated that delirium is associated with a

breakdown in the BBB.[21,22] To our knowledge, there is only one other study demonstrating an association between cognitive decline and a temporal *change* in the BBB and this study did not report on perioperative delirium.[23] While inflammation is known to be associated with BBB disruption, recent data strongly argue that plasmin activation leads to BBB breakdown though both directs effects on astrocytes[24] and exacerbating inflammation[25]. Given that blood loss is associated with delirium[26], we sought to understand if blood loss (with the ensuring fibrinolytic response) was associated with BBB breakdown.

Cerebrospinal fluid (CSF) plasma albumin ratio (CPAR) is the gold-standard measure of BBB permeability, directly measuring the physical breakdown in the barrier.[27] We conducted parallel investigations using CPAR and S100-beta (S100β), a well validated surrogate biomarker for BBB that is a calcium-binding protein in glial cells with a role in astrocytic cytoskeleton morphology. Elevated S100β in biofluids is a proposed marker of neuronal injury and BBB disruption. It has been shown in a number of neurological conditions, including traumatic brain injury[28], dementia,[21], and delirium.[29] We investigated two complementary biomarkers as (1) CPAR is the gold standard but hard to obtain, (2) S100β is a surrogate but can be measured in plasma and hence can easily be accessed in the perioperative period facilitating statistical power for analysis of consecutive patients in our cohort. *A priori* we decided that convergent associations on delirium *and* delirium severity would be required to be confident in our findings and reduce the chance of a false positive finding (equivalent to a $p=0.05^2$), similar to our prior approach with the electroencephalogram (EEG).[30] We also considered that S100β and CPAR should show similar direction for effects for results to be biologically interpretable and convergence adds credibility to our work.

Finally, recent case-control studies have also suggested that CSF lactate is associated with delirium,[31,32] however it is unknown if there are within-subject changes in CSF lactate paralleling the onset of delirium. This demonstration is required to identify a mechanistic association that is worth further interrogation. Increases in lactate are consistent with the metabolic insufficiency hypothesis, that propose that delirium results from impaired oxygen and glucose delivery.[33] An alternative interpretation is that CSF lactate is a marker of the central inflammatory inhibition whereby aerobic glycolysis generates lactate to induce localised immunosuppression via hydroxycarboxylic acid receptor 2.[34] As an exploratory

analysis we embarked upon investigation of a working hypothesis, that infiltrating monocytes through a permeable BBB, provoke a central inflammatory response, but CSF lactate biofeedback is induced to contain this inflammation. As a byproduct of this process, the neuroprotective somnogen, PGD2 is produced.[35] We have concomitantly implicated PGD2 in the EEG changes of delirium in our recent mouse study, enhancing plausibility for this relationship.[36]

To investigate our hypotheses of increased permeability of the BBB with delirium and systemic inflammation, we address the following research questions in this paper:

1. *Change* in **CSF to plasma albumin ratio**, as a fluid biomarker of BBB permeability, is associated with delirium incidence and severity.
2. C*hange* in S100β, a plasma biomarker of astrocytic injury/activation, is associated with delirium incidence and severity.
3. These changes are consistent with the *change* in CSF interleukin-6, a key biomarker of delirium.[37]
4. *Change* in **CSF lactate** is associated with inflammation and delirium severity.

METHODS

Study Design

This is an analysis of two ongoing observational, cohort studies of perioperative delirium at UW Health, Madison Country, Wisconsin, USA. Recruitment commenced in July 2015. Ethical approvals were obtained from the University of Wisconsin-Madison (UWM) Institutional Review Board (2015-0374 and 2015-0960) and the trials were registered on clinicaltrials.gov (NCT01980511, NCT03124303 and NCT02926417). Data are reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[38]

Participants

Participants, recruited from clinical lists or information provided in clinics at UW Health, are eligible aged 21 years or over, and undergoing elective, non-intracranial surgery with a minimum anticipated hospital stay of two days. Participants are excluded if they have a documented history of dementia, reside in a nursing home, or are unable to complete neurocognitive testing due to language, vision, or hearing impairment. Those with

contraindication to high-density electroencephalography (HD-EEG) are excluded from those measurements.

Study procedure

Prospects are screened by telephone and, if eligible, meet in-person with researchers to ensure written, informed consent prior to hospital admission. At baseline, participants provide demographic and clinical information, a blood sample, and undertake cognitive assessments and HD-EEG if appropriate. At surgery, researchers collect updated procedure details from the medical record, perform delirium assessments, and obtain blood and cerebrospinal fluid (CSF). CSF is only collected if a spinal drain is placed for clinical reasons for vascular surgery. During hospitalisation days 1-4, researchers perform further delirium assessments and HD-EEG, if appropriate.

Measurements

For participants in this analysis, researchers assessed delirium postoperatively on hospitalisation days 0-4 using the 3-minute Diagnostic interview for Confusion Assessment Method-defined delirium (3D-CAM)[39,40] and the Delirium Rating Scale (DRS-98).[41] The DRS has similarly demonstrated validity and specificity for delirium severity, and maximum (peak) DRS is the primary outcome used in this analysis.[42,43] Researchers also collected the results of the National Surgical Quality Improvement (NSQIP) surgical risk assessment with procedure details from the medical record.[44,45]

Researchers collected biofluid samples using standard venipuncture or indwelling line, obtaining a maximum of 80ml (8 x 10ml) of blood and 25ml (5 x 5ml) of CSF during the study. Samples were de-identified, anonymized, labelled, processed and stored at -80˚C in a laboratory at the Clinical Sciences Center, UWM. Researchers obtained HD-EEG data using a Philips-EGI 256-channel Geodesic Sensor Net. Readings take approximately 30 minutes.

Analysis processing and analysis

Consecutive patient plasma and all available CSF biofluids were analyzed by techniques such as enzyme-linked immunosorbent assay (ELISA). Albumin concentrations were measured by immunoturbidimetry on a Cobas instrument (Roche Diagnostics, Penzberg, Germany). Blood pressure was calculated using area under the curve (AUC) for intraoperative mean arterial pressures <10% of preoperative levels.

Data analysis for this paper was conducted in R: a language and environment for statistical computing.[46] Data was inspected, cleaned and data sources consolidated by patient identifier, timepoint, and consistent batch where available. Postoperatively, day one samples were used unless unavailable, then the next available inpatient sample was used. All biomarkers were normalised by logarithm (base 10). Unless otherwise specified, biomarker units are in picograms per millilitre (pg/ml)

Analysis included summary statistics, plots, and Shapiro-Wilk's test for normality[47]. A Mann Whitney/Wilcoxon rank sum test with continuity correction was used to differentiate delirious and non-delirious groups in univariate analysis.[48] Correlations used Pearson's method or Spearman's method where not normally distributed.[49] Outliers were validated by Cook's distance, using a conservative (4 x mean) threshold .[50,51] Continuous data are reported as mean (m) and standard deviation (SD), or median (M) and median absolute deviation (mad) where not normally distributed. Biomarker analysis used pre-postoperative change rather than postoperative values. Categorical data are reported as counts (n) and percentage.

Missing data were not imputed and models are based on complete cases. While CSF is a convenience sample of vascular surgery patients, we conducted power analysis on plasma S100β. Consistent with previous studies,[52] we determined a power threshold of 56 participants (α=0.05), based on a 300±400 nanogram (m±SD) difference in S100β between those with/without delirium. We performed linear regression modelling for delirium severity with maximum (peak) DRS and a Poisson distribution family as confirmed by histogram, Shapiro-Wilks test ($p<0.001$) and skewness (1.32). We also conducted a linear mixed model for delirium severity (peak DRS), fit with random effects maximum likelihood (REML) for individual patient and time. P-values were calculated using Satterthwaite degrees of freedom.

RESULTS

From a cohort of 179 patients with biofluids, samples of CSF Plasma Albumin ratio (CPAR) (n=25) and plasma S100β (n=78) were analysed. Missing data were due to the clinical restrictions on the collection of CSF. As seen in the STROBE diagram at Figure 1, two (2/25, 8%) patients were excluded at from CPAR baseline and three (3/78, 4%) from plasma S100β due to no contemporaneous DRS assessments with biofluid sampling. One further patient was excluded due to delirium assessment while intubated that prevented DRS data being collected. This resulted in 22 patients with CPAR at surgery, of which 11 (11/22, 50%) had

pre- and postoperative samples. Seventy-four patients had plasma S100β samples at either baseline or surgery. Of these, 63 (63/74, 85%) patients had pre- and postoperative samples with a further two excluded following Cook's distance outlier analysis. Demographic information for the cohorts are summarised in **Error! Reference source not found.**.

Primary Outcome

There were no between-group differences in preoperative levels of CPAR or plasma S100β between patients who experienced postoperative delirium versus those that did not ($p>0.05$). As seen in Figure 2A and 2B, there were greater increases in CPAR and plasma S100β in those with delirium (red) compared to those without (blue) postoperatively over time. Importantly, in our analysis of postoperative change to baseline, Figure 2C and 2D boxplots show an association of delirium incidence with changes in both CPAR (Wilcoxon $p=0.012$) and plasma S100β (Wilcoxon $p=0.001$). Similarly, **Error! Reference source not found.**E and 2F show that peak delirium severity is correlated with change in CPAR ($\rho=0.78$, $p=0.005$), plasma S100β ($\rho=0.41$, $p<0.001$) and CSF Interleukin 6 (CSF IL-6, $\rho=0.75$, $p=0.025$).**Error! Reference source not found.**E and 2F show that an established CSF biomarker of delirium, IL-6, also correlates with CPAR and plasma S100β, linking BBB permeability to central inflammation.

Having seen that plasma S100β rises proportionately with delirium severity, we tested whether this effect was robust to confounding. In a Poisson regression model, adjusting for age, sex and baseline cognition (Trail Making Test B), we identified that plasma S100β predicted delirium severity (**Supplementary Table 1**). Using a linear mixed effects model, with random effects for patient/subject and time, we show that S100β levels predicted delirium severity changes over time (**Supplementary Table 2**). In summary, S100β changes correlated with both increases in postoperative delirium severity from baseline and recovery of delirium symptoms.

Secondary outcome: associations with blood loss

As blood loss is associated with delirium and we hypothesized that plasmin activation was associated with exacerbated inflammation and blood-brain barrier breakdown,[24] we then tested whether blood loss was associated with markers of: (1) BBB breakdown; and (2) systemic inflammation. As seen in **Error! Reference source not found.**, blood loss

correlated with: change in CPAR (ρ=0.71, p=0.02); change in plasma S100β (ρ=0.43, p<0.001). Blood loss correlated with both delirium severity in peak DRS (ρ=0.29, p<0.001), EEG SWA (Oz 0.5 – 0.6 Hz, ρ=0.35, p=0.001) and change in plasma neurofilament light (NfL, ρ=0.64, p<0.001). Blood loss also correlated with two cytokine markers of peripheral inflammation we have previously associated with delirium[53,54]: plasma IL-8 (ρ=0.34, p<0.001); and plasma IL-10 (ρ=0.34, p<0.001).

We also investigated clinical predictors of blood-brain barrier breakdown, using the surrogate of change in plasma S100β, using a linear regression model with factors: age; female gender; blood loss; NSQIP-D, stroke/transient ischemic attack (TIA); and surgery type. As seen in Supplemental Table , blood loss (p=0.04), low blood pressure (p=0.001), surgical risk (NSQIP-D, p=0.01), were associated with change in plasma S100β (adjusted R^2=0.41, p < 0.001).

Secondary outcome: Associations with CSF Lactate

Finally, we hypothesized that CSF lactate is a biomarker of a central inflammatory response and hence would be associated with delirium, inflammation and BBB metrics (and hence also blood loss). Change in CSF lactate was associated with delirium severity (peak DRS, ρ=0.66, p=0.038), blood loss (R=0.81, p=0.005), blood brain barrier permeability CPAR (R=0.78, p=0.008) and central inflammation, CSF IL-10 (R=0.77, p=0.010). It was also associated with peripheral inflammation, plasma IL-8 (ρ=0.87, p=0.003) and plasma IL-10 (ρ=0.76, p=0.016). However, CSF Lactate did not correlate with plasma S100β or CSF NfL in this small sample.

DISCUSSION

Herein we demonstrate a dynamic breakdown in the blood brain barrier associated with delirium and delirium severity using both gold standard (CPAR) and surrogate (S100β) measures. This dual approach was undertaken to reduce the possibility of false positive results from a single biomarker. The fact that delirium severity also resolves in parallel to reducing levels of S100β, further argues that there is biological gradient supporting causal associations between BBB injury and delirium. Our data are consistent with a model, whereby trauma induces a peripheral inflammatory response and surgical blood loss exacerbates that response (

Figure 5). These data are consistent with multiple univariate and multivariable regression analyses. They are also convergent with many animal studies suggesting that surgery-induced cognitive dysfunction is associated with an influx of immune cells through a damaged BBB[55,56] and our data showing that BBB breakdown is associated with increases in CSF IL-6 (an established biomarker of delirium). A recent study demonstrated proof of principle for this in humans[23] and we extend this concept directly to delirium. Our mechanistic link to blood loss, as a critical clinical mediator, highlights a potential therapeutic opportunity for preventing fibrinolysis-plasmin activation in reducing postoperative delirium. For example, tranexamic acid is reported to be anti-inflammatory and reduce BBB breakdown in animal models and hence may reduce postoperative delirium. This concept should be tested in clinical trials and could provide the necessary causal link between delirium and BBB breakdown.

We extended these findings based on exploratory analyses of CSF lactate. Firstly, we demonstrated that CSF lactate *changes* with delirium, this is critical as prior data were based on case-control studies and could not exclude pre-delirium differences in lactate. Secondly, we propose an alternative explanation for the rise in CSF lactate, that as an immune moderating effector. This idea is consistent with both inflammatory models of delirium, and specifically prostaglandin models, that have gained substantial weight recently[36,57,58]. Increases in prostaglandins have been linked to both cognitive impairment[58] and EEG slowing[36] in animal models of inflammation. PGD2, a powerful somnogen, is the likely prostaglandin effector that drives EEG slowing[36,57]. Given that PGD2 is also neuroprotective following stroke[35], the possibility that suppression of brain activity contributes to this effect needs to be clarified. Our proposed pathway links these different concepts, providing hypotheses that can be tested in animal models (

Figure 5). Critically, this interpretation implies that lactate and prostaglandin release may represent endogenous protective mechanisms, and tinkering with them may lead to exacerbated inflammation and loss of neuroprotective effects.

By extension, this work suggests that a re-appraisal of inflammatory, metabolic insufficiency and cognitive disintegration hypotheses. Notably, inflammation, not neuronal injury, correlated with changes in CSF lactate. If metabolic insufficiency was occurring in the setting of neuronal injury, we would have expected there to be a correlation of neurofilament light and CSF lactate. The closer relationship with inflammation highlights the known biological mechanism of lactate as an anti-inflammatory effector. However, further studies are required to understand whether lactate in this context reflects metabolic failure or an active response to dampen inflammation and possibly to dampen neuronal energetic requirements, possibly via PGD2.

There are several limitations to our work. These data are observational and causality cannot be ascribed however we have sought multiple ways to test our hypotheses, including stringent criteria for accepting our primary endpoint. We also included parallel biomarker studies to ensure that the results are not unique to a single marker. Biological gradients for the effects support causality and we have proposed an intervention through which we can directly test the hypothesis (Tranexamic acid). We have also adjusted for confounders where possible. Nonetheless our sample sizes are small and we would caution about extrapolating our findings to other clinical situations, in particular where blood loss may be limited. Though evidence from multiple sources suggest that inflammation, in the absence of blood loss, may induce similar effects.[56]

This analysis confirms a known association between systemic inflammation, blood loss, and neuronal injury supported by factors such as surgical risk and operating times.[59] It is consistent with the one available study on the temporal change of the BBB in response to perioperative inflammation followed by postoperative cognitive decline.[23] Our analysis adds to this work by demonstrating increasing postoperative permeability of the BBB and its' association with delirium incidence and severity. Our mechanistic analyses highlight possible opportunities and pitfalls for delirium science and demonstrate that mechanistic research in delirium is urgently required.

Author's Contributions

RS designed the study with RAP and RL. MP, ST, DK and CR, CC collected, processed, and prepared data and biofluids. JT performed data analysis and drafting. HZ and KB conducted CPAR analyses. All authors reviewed the manuscript.

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Declaration of interests

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Eisai, Denali, Roche, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx, and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS).

TABLES AND FIGURES

Figure 1 - STROBE diagram

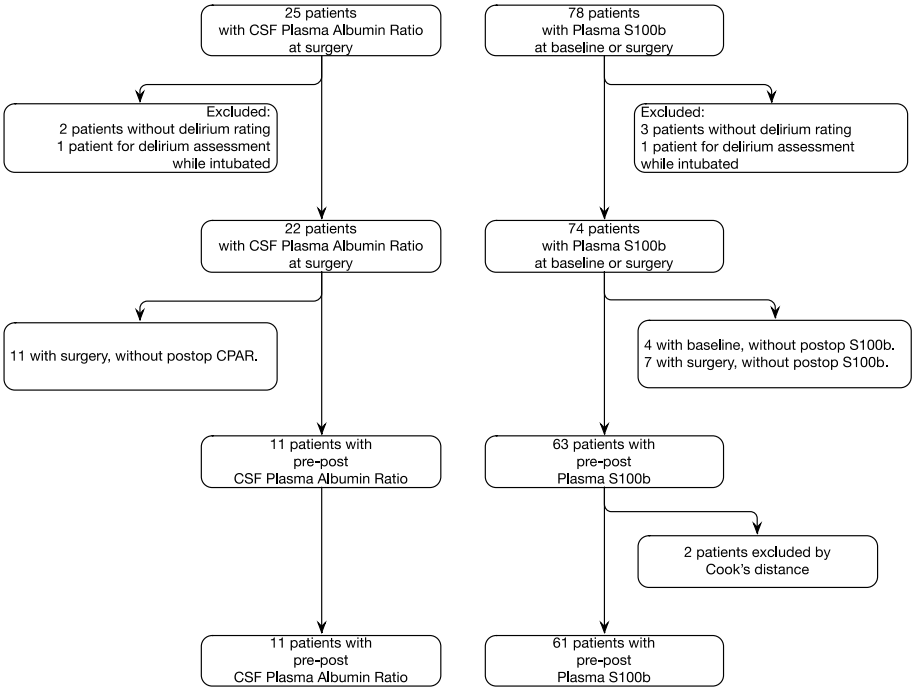
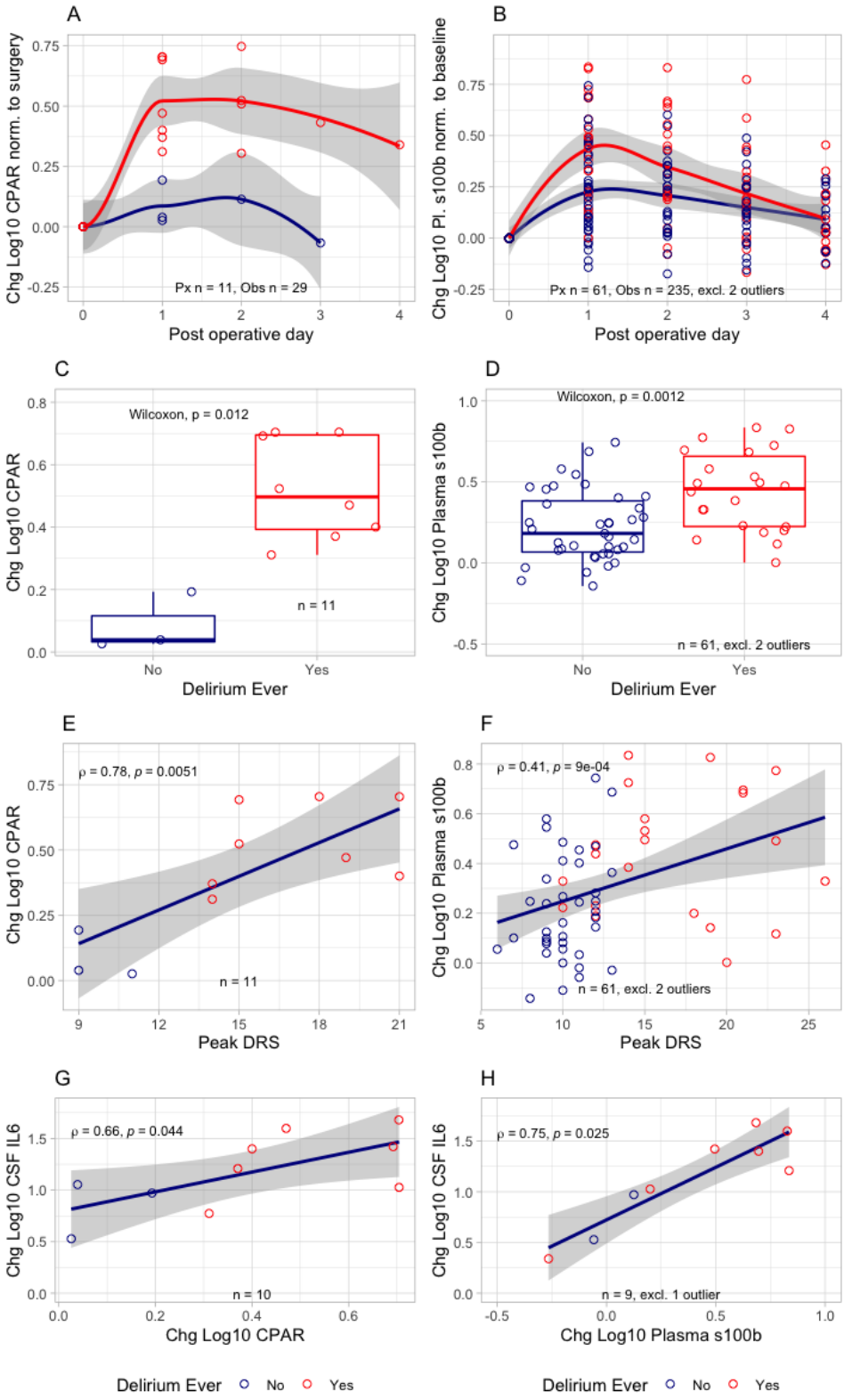


Table 1 - Demographic table

	TOTAL CPAR n = 22	**CPAR WITH DELIRIUM n = 15**		**CPAR WITHOUT DELIRIUM n = 7**		**TOTAL PLASMA S100β n = 74**	**PLASMA S100β WITH DELIRIUM n = 29**		**PLASMA S100β WITHOUT DELIRIUM n = 45**	
Variable	**M±mad[b] (range)**	**n (%)**	**M±mad (range)**	**n (%)**	**M±mad (range)**	**M±mad[b] (range)**	**n (%)**	**M±mad (range)**	**n (%)**	**M±mad (range)**
Age (years) *Gender	66 ± 8 (38 to 82)	15 (68)	64 ± 8 (38 to 78)	7 (32)	72 ± 9 (59 to 82)	71 ± 6 (38 to 85)	29 (39)	69 ± 5 (38 to 77)	45 (61)	73 ± 6 (59 to 85)
Female	70 ± 10 (38 to 82)	8 (80)	70 ± 10 (38 to 78)	2 (20)	74 ± 11 (67 to 82)	71 ± 4 (38 to 83)	14 (52)	69 ± 6 (38 to 77)	13 (48)	72 ± 7 (65 to 83)
Male	64 ± 7 (47 to 78)	7 (58)	63 ± 4 (47 to 67)	5 (42)	72 ± 9 (59 to 78)	71 ± 6 (47 to 85)	15 (32)	67 ± 6 (47 to 77)	32 (68)	73 ± 6 (59 to 85)
NSQIP-D[a]	4 ± 4 (0 to 33)	14 (70)	5 ± 3 (1 to 33)	6 (30)	2 ± 3 (0 to 8)	2 ± 2 (0 to 15)	29 (40)	4 ± 4 (0 to 15)	43 (60)	2 ± 2 (0 to 8)
Operating time (min)	456 ± 136 (180 to 863)	15 (68)	532 ± 128 (185 to 863)	7 (32)	295 ± 117 (180 to 531)	295 ± 153 (90 to 863)	29 (39)	408 ± 250 (185 to 863)	45 (61)	267 ± 129 (90 to 557)
Blood loss (ml) *Surgery Type										
Cardiac or vascular	5000 ± 3876 (7 to 12000)	14 (19)	6000 ± 5646 (7 to 12000)	7 (9)	200 ± 886 (50 to 5000)	300 ± 445 (0 to 12000)	21 (41)	2500 ± 3706 (0 to 12000)	30 (59)	150 ± 204 (0 to 5000)
Other	-	-	-	-	-	400 ± 408 (5 to 3000)	7 (32)	500 ± 371 (250 to 1100)	15 (68)	250 ± 326 (5 to 3000)
Blood pressure (log10 AUC[e] 10%)	5 ± 1 (4 to 6)	15 (68)	5 ± 1 (4 to 6)	7 (32)	5 ± 0 (4 to 6)	5 ± 1 (3 to 6)	28 (38)	5 ± 1 (4 to 6)	45 (62)	5 ± 1 (3 to 6)
Baseline TMTB	76 ± 24 (40 to 144)	6 (67)	76 ± 24 (50 to 144)	3 (33)	54 ± 21 (40 to 85)	72 ± 27 (31 to 189)	20 (32)	78 ± 22 (36 to 143)	42 (68)	66 ± 27 (31 to 189)
Baseline MoCA	23 ± 1 (20 to 26)	5 (62)	23 ± 0 (23 to 26)	3 (38)	24 ± 0 (20 to 24)	23 ± 3 (13 to 29)	20 (32)	23 ± 3 (19 to 27)	42 (68)	23 ± 3 (13 to 29)
Baseline COWAT	37 ± 10 (26 to 51)	7 (64)	39 ± 15 (24 to 49)	4 (36)	33 ± 7 (26 to 51)	37 ± 13 (13 to 91)	23 (35)	40 ± 15 (17 to 58)	42 (65)	36 ± 13 (13 to 91)

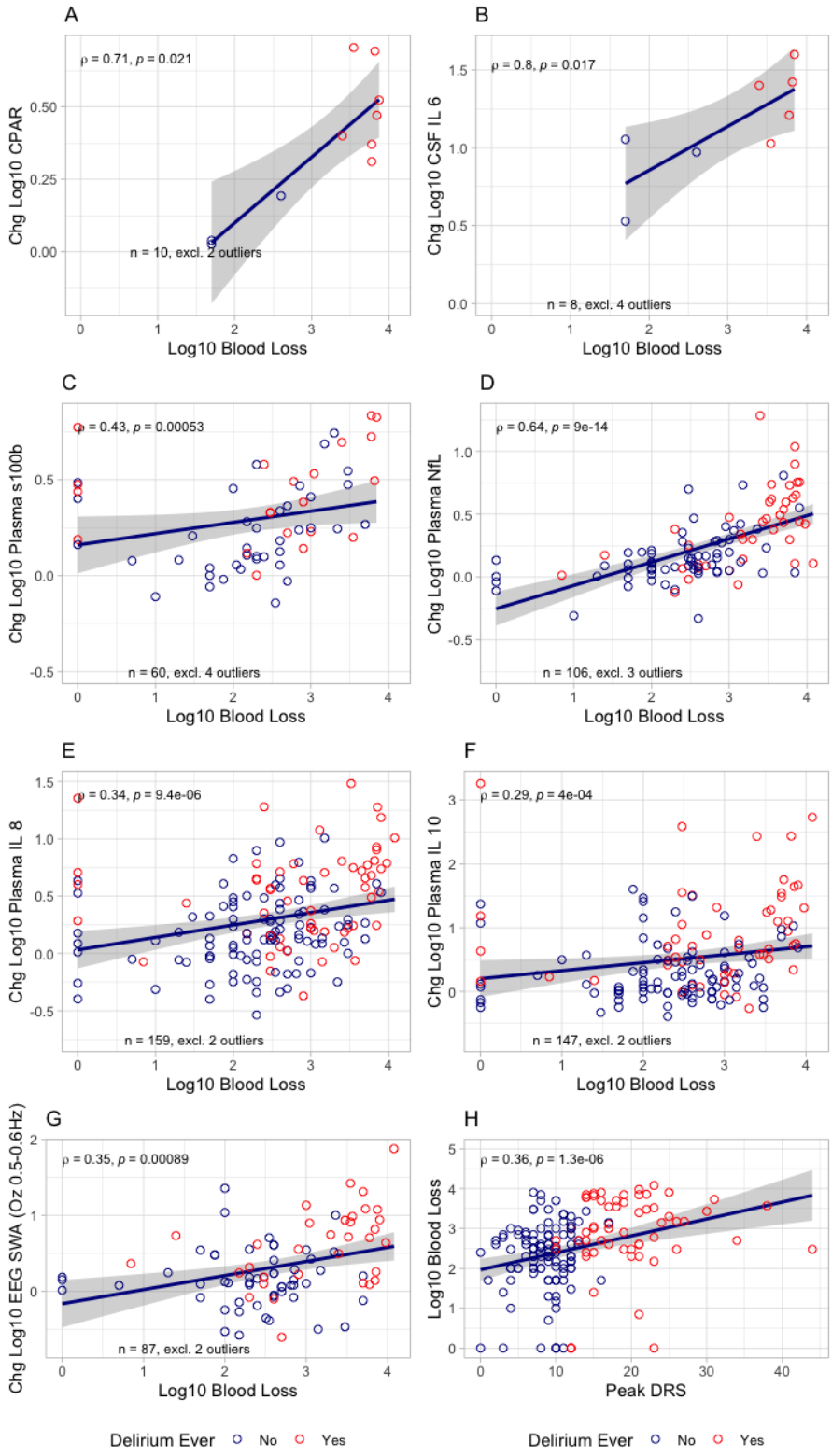
[a] National Surgical Quality Improvement Program (NSQIP-D) used to assess surgical risk. [b] Median (M) and median absolute deviation (mad) are reported as variables are not normally distributed. [c] Surgery type is grouped by cardiovascular (CV), and Other (Thoracic, General, Orthopedic, ENT, and Urological). Blood loss data excludes two outliers identified by Cook's distance. [d] TMTB and MoCA data exclude baseline assessments undertaken on the day of surgery. [e] Blood pressure is reported as baseline area under the curve (AUC) for intraoperative mean arterial pressures <10%.

Figure 2 – Perioperative time course of CPAR and Plasma S100β, and pre-postoperative change in Delirium Ever, and Peak DRS



CSF = Cerebrospinal Fluid. CPAR = CSF Plasma Albumin Ratio. CPAR and plasma S100β were normalised by log10 transformation and subtracting baseline from postoperative values. Plasma S100β excludes two outliers, and CSF IL-6 excludes one, based on Cook's distance (>4*mean). DRS = Delirium Rating Scale-98. Spearman's correlations were used given peak DRS is not normally distributed (Shapiro-Wilks' normality test $p < 0.001$). Units are picograms per millilitre (pg/ml).

Figure 3 – Pre-postoperative change in plasma S100β, CPAR and other biomarkers correlated with blood loss



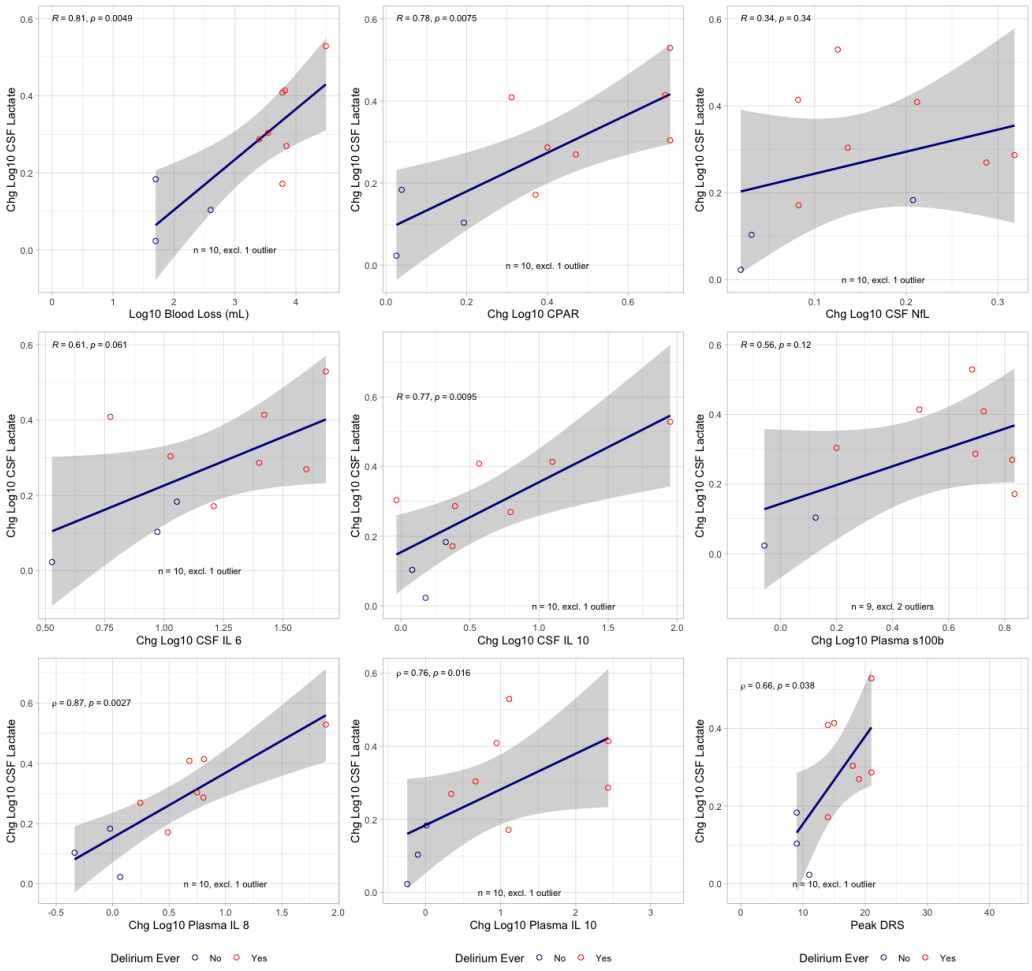
CPAR = CSF Plasma Albumin Ratio. NfL = Neurofilament Light. SWA = Slow Wave Activity. DRS = Delirium Rating Scale. Two outliers of blood loss were identified by Cook's distance (>4*mean) and excluded from all plots. Other outliers also identified by Cook's distance analysis for correlation. Blood loss is not normally distributed and Spearman's correlation method used. Units are picograms per millilitre (pg/ml) unless otherwise specified. Original unit of blood loss is millilitres (ml) and EEG SWA is reported in hertz (Hz).

Table 2 - Multiple linear regression for change in plasma S100β between baseline and perioperative day 1

DEPENDENT VARIABLE: CHANGE IN PLASMA S100β[a]	ESTIMATE	STD. ERROR (OLS[d])	T VALUE	P VALUE
(Intercept)	0.453	0.378	1.201	0.237
Blood Loss (ml)	0.000	0.000	2.073	0.044*
Blood Pressure (AUC[c] 10%)	0.000	0.000	3.479	0.001**
Age (years)	-0.004	0.005	-0.715	0.478
Gender - Female	-0.034	0.056	-0.616	0.541
NSQIP-D	0.029	0.011	2.691	0.010*
Surgery Type – Cardiovascular[b]	-0.140	0.055	-2.520	0.016*
TIA / Stroke	-0.074	0.097	-0.764	0.449
Observations	50 (10 missing obs. deleted)			
Model fit	$F(7,42) = 5.853$, $p < 0.001$***			
Multiple R^2 / Adjusted R^2	Multiple R^2: 0.494 / Adjusted R^2: 0.409			

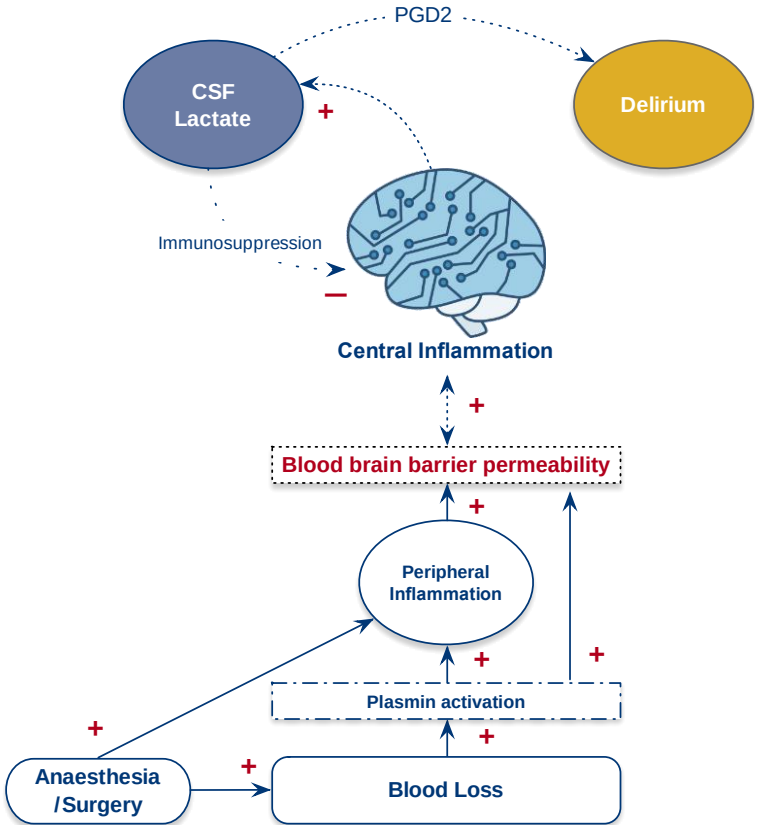
NSQIP-D = National Surgical Risk Quality Improvement Protocol – Death. TIA = Transient Ischaemic Attack. [a] Four outliers excluded from dataset; two each identified by Cook's distance for change in plasma S100β and blood loss. [b] Surgery type is grouped by cardiovascular (CV), and Other (Thoracic, General, Orthopedic, Ear Nose Throat (ENT), and Urological). Surgery type 'Other' used as the reference group. [c] Blood pressure is reported as the area under the curve (AUC) for intraoperative mean arterial pressures <10%. [d] Standard error of the estimate used ordinary least squares (OLS).

Figure 4 – Log change in CSF lactate and biomarkers



CSF = Cerebrospinal Fluid. CPAR = CSF Plasma Albumin Ratio. In addition to one outlier excluded based on Cook's distance for CSF Lactate, one outlier was excluded for plasma S100β. Plots use Pearson's correlation method, except for plasma IL 8 and IL 10 which use Spearman due to non-normal distribution.

Figure 5 – Blood brain barrier permeability hypothesis. In this figure we propose a mechanistic pathway through which peripheral inflammation, exacerbated by plasmin activation, leads to breakdown of the blood brain barrier. Subsequent inflammatory changes lead to a central anti-inflammatory response, including lactate induced activation of HCA receptors. The downstream effects on PGD2 lead to delirium though synaptic suppression. This results in reduced cerebral activity, as demonstrated by EEG slowing.



CSF = Cerebrospinal Fluid, PGD2 = Prostaglandin-D2

SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 1 – Linear regression for delirium severity (peak DRS) with plasma S100β

Dependent variable: Peak DRS (S100β dataset)		
	z	**p**
Intercept	3.491	<0.001***
Log10 Chg S100β	2.853	0.004**
Age (years)	0.001	0.999
Sex Female	-0.495	0.621
Log10 Blood pressure AUC 10%		
NSQIP-D		
TMTB Baseline[a]	1.546	0.122
Observations	55	
Model Fit	$\chi^2(4) = 10.176$, p = 0.038	
Pseudo R^2 (Cragg-Uhler)	0.170	
Pseudo R^2 (McFadden)	0.033	

Supplemental Table 2 - Linear mixed effects model for delirium severity within patient and over time.

DEP. VAR. DRS _TOTAL[e]	**EST.**	**STD. ERROR (MLE)**	**T VALUE**	**P VALUE[f]**
(Intercept)	-3.635	5.046	-0.720	0.474
Plasma S100β	4.360	1.292	3.375	0.001**
Time: Postop. day 1-4	-1.042	0.273	-3.820	<0.001***
Age (years)	0.090	0.051	1.768	0.082
Gender – Female	0.966	0.780	1.238	0.221
Observations	166 (Patients 59)			
Pseudo-R^2 (fixed effects)	0.187			
Pseudo-R^2 (total)	0.598			

[e] Linear mixed model fit by maximum likelihood estimate (MLE) with random effects for patient and time. [f] P-values calculated using Satterthwaite degrees of freedom.

REFERENCES

1. Inouye SK, Westendorp RG, Saczynski JS. Delirium in elderly people. *Lancet*. Mar 8 2014;383(9920):911-22. doi:10.1016/S0140-6736(13)60688-1
2. APA. *Diagnostic and statistical manual of mental disorders: DSM-5*. Fifth ed. American Psychiatric Association (APA) Publishing; 2013.
3. WHO. *ICD-11: international statistical classification of diseases and related health problems*. September, 2020 ed. World Health Organization (WHO); 2020.
4. Leslie DL, Marcantonio ER, Zhang Y, Leo-Summers L, Inouye SK. One-Year Health Care Costs Associated With Delirium in the Elderly Population. *Archives of Internal Medicine*. 2008;168(1):27-32. doi:10.1001/archinternmed.2007.4
5. Aung Thein MZ, Pereira JV, Nitchingham A, Caplan GA. A call to action for delirium research: Meta-analysis and regression of delirium associated mortality. *BMC Geriatr*. Sep 7 2020;20(1):325. doi:10.1186/s12877-020-01723-4
6. Hapca S, Guthrie B, Cvoro V, et al. Mortality in people with dementia, delirium, and unspecified cognitive impairment in the general hospital: prospective cohort study of 6,724 patients with 2 years follow-up. *Clin Epidemiol*. 2018;10:1743-1753. doi:10.2147/CLEP.S174807
7. Zhong X, Lin JY, Li L, Barrett AM, Poeran J, Mazumdar M. Derivation and validation of a novel comorbidity-based delirium risk index to predict postoperative delirium using national administrative healthcare database. *Health Serv Res*. Feb 2021;56(1):154-165. doi:10.1111/1475-6773.13565
8. Beishuizen SJ, Festen S, Loonstra YE, van der Werf HW, de Rooij SE, van Munster BC. Delirium, functional decline and quality of life after transcatheter aortic valve implantation: An explorative study. *Geriatr Gerontol Int*. Dec 2020;20(12):1202-1207. doi:10.1111/ggi.14064
9. Weng CF, Lin KP, Lu FP, et al. Effects of depression, dementia and delirium on activities of daily living in elderly patients after discharge. *BMC Geriatr*. Oct 11 2019;19(1):261. doi:10.1186/s12877-019-1294-9
10. Elsamadicy AA, Wang TY, Back AG, et al. Post-operative delirium is an independent predictor of 30-day hospital readmission after spine surgery in the elderly (>/=65years old): A study of 453 consecutive elderly spine surgery patients. *J Clin Neurosci*. Jul 2017;41:128-131. doi:10.1016/j.jocn.2017.02.040
11. Brummel NE, Boehm LM, Girard TD, et al. Subsyndromal Delirium and Institutionalization Among Patients With Critical Illness. *Am J Crit Care*. Nov 2017;26(6):447-455. doi:10.4037/ajcc2017263
12. Hughes CG, Patel MB, Brummel NE, et al. Relationships between markers of neurologic and endothelial injury during critical illness and long-term cognitive impairment and disability. *Intensive Care Med*. Mar 2018;44(3):345-355. doi:10.1007/s00134-018-5120-1
13. Clark IA, Vissel B. The Inflammatory Nature of Post-surgical Delirium Predicts Benefit of Agents With Anti-TNF Effects, Such as Dexmedetomidine. *Front Neurosci*. 2018;12:257. doi:10.3389/fnins.2018.00257
14. Mietani K, Sumitani M, Ogata T, et al. Dysfunction of the blood-brain barrier in postoperative delirium patients, referring to the axonal damage biomarker phosphorylated

neurofilament heavy subunit. *PLoS One*. 2019;14(10):e0222721. doi:10.1371/journal.pone.0222721
15. Terrando N, Eriksson LI, Ryu JK, et al. Resolving postoperative neuroinflammation and cognitive decline. *Ann Neurol*. Dec 2011;70(6):986-995. doi:10.1002/ana.22664
16. Banks WA, Reed MJ, Logsdon AF, Rhea EM, Erickson MA. Healthy aging and the blood-brain barrier. *Nat Aging*. Mar 2021;1(3):243-254. doi:10.1038/s43587-021-00043-5
17. Noe CR, Noe-Letschnig M, Handschuh P, Noe CA, Lanzenberger R. Dysfunction of the Blood-Brain Barrier-A Key Step in Neurodegeneration and Dementia. *Front Aging Neurosci*. 2020;12:185. doi:10.3389/fnagi.2020.00185
18. Kaplan L, Chow BW, Gu C. Neuronal regulation of the blood-brain barrier and neurovascular coupling. *Nat Rev Neurosci*. Aug 2020;21(8):416-432. doi:10.1038/s41583-020-0322-2
19. Montagne A, Nation DA, Sagare AP, et al. APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. *Nature*. May 2020;581(7806):71-76. doi:10.1038/s41586-020-2247-3
20. Li W, Lo EH. Leaky memories: Impact of APOE4 on blood-brain barrier and dementia. *J Cereb Blood Flow Metab*. Sep 2020;40(9):1912-1914. doi:10.1177/0271678X20938146
21. Hov KR, Bolstad N, Idland AV, et al. Cerebrospinal Fluid S100B and Alzheimer's Disease Biomarkers in Hip Fracture Patients with Delirium. *Dement Geriatr Cogn Dis Extra*. Sep-Dec 2017;7(3):374-385. doi:10.1159/000481853
22. Hughes CG, Pandharipande PP, Thompson JL, et al. Endothelial Activation and Blood-Brain Barrier Injury as Risk Factors for Delirium in Critically Ill Patients. *Crit Care Med*. Sep 2016;44(9):e809-17. doi:10.1097/CCM.0000000000001739
23. Danielson M, Wiklund A, Granath F, et al. Neuroinflammatory markers associate with cognitive decline after major surgery: Findings of an explorative study. *Ann Neurol*. Mar 2020;87(3):370-382. doi:10.1002/ana.25678
24. Keragala CB, Woodruff TM, Liu Z, et al. Tissue-Type Plasminogen Activator and Tenecteplase-Mediated Increase in Blood Brain Barrier Permeability Involves Cell Intrinsic Complement. *Front Neurol*. 2020;11:577272. doi:10.3389/fneur.2020.577272
25. Medcalf RL, Keragala CB. Fibrinolysis: A Primordial System Linked to the Immune Response. *Int J Mol Sci*. Mar 26 2021;22(7)doi:10.3390/ijms22073406
26. Whitlock EL, Behrends M. Blood Transfusion and Postoperative Delirium. *Current Anesthesiology Reports*. 2015;5(1):24-32. doi:10.1007/s40140-014-0085-2
27. Musaeus CS, Gleerup HS, Hogh P, Waldemar G, Hasselbalch SG, Simonsen AH. Cerebrospinal Fluid/Plasma Albumin Ratio as a Biomarker for Blood-Brain Barrier Impairment Across Neurodegenerative Dementias. *J Alzheimers Dis*. 2020;75(2):429-436. doi:10.3233/JAD-200168
28. Lindblad C, Nelson DW, Zeiler FA, et al. Influence of Blood-Brain Barrier Integrity on Brain Protein Biomarker Clearance in Severe Traumatic Brain Injury: A Longitudinal Prospective Study. *J Neurotrauma*. Jun 15 2020;37(12):1381-1391. doi:10.1089/neu.2019.6741
29. van Munster BC, Korevaar JC, Korse CM, Bonfrer JM, Zwinderman AH, de Rooij SE. Serum S100B in elderly patients with and without delirium. *Int J Geriatr Psychiatry*. Mar 2010;25(3):234-9. doi:10.1002/gps.2326
30. Tanabe S, Mohanty R, Lindroth H, et al. Cohort study into the neural correlates of postoperative delirium: the role of connectivity and slow-wave activity. *Br J Anaesth*. Jul 2020;125(1):55-66. doi:10.1016/j.bja.2020.02.027

31. Caplan GA, Kvelde T, Lai C, Yap SL, Lin C, Hill MA. Cerebrospinal fluid in long-lasting delirium compared with Alzheimer's dementia. *J Gerontol A Biol Sci Med Sci*. Oct 2010;65(10):1130-6. doi:10.1093/gerona/glq090
32. Kealy J, Murray C, Griffin EW, et al. Acute Inflammation Alters Brain Energy Metabolism in Mice and Humans: Role in Suppressed Spontaneous Activity, Impaired Cognition, and Delirium. *J Neurosci*. Jul 15 2020;40(29):5681-5696. doi:10.1523/JNEUROSCI.2876-19.2020
33. Haggstrom L, Welschinger R, Caplan GA. Functional neuroimaging offers insights into delirium pathophysiology: A systematic review. *Australas J Ageing*. Sep 2017;36(3):186-192. doi:10.1111/ajag.12417
34. Nolt B, Tu F, Wang X, et al. Lactate and Immunosuppression in Sepsis. *Shock*. Feb 2018;49(2):120-125. doi:10.1097/SHK.0000000000000958
35. Rahman M, Muhammad S, Khan MA, et al. The beta-hydroxybutyrate receptor HCA2 activates a neuroprotective subset of macrophages. *Nature communications*. May 21 2014;5:3944. doi:10.1038/ncomms4944
36. Sultan ZW, Jaeckel ER, Krause BM, et al. Electrophysiological signatures of acute systemic lipopolysaccharide-induced inflammation: potential implications for delirium science. *Br J Anaesth*. May 2021;126(5):996-1008. doi:10.1016/j.bja.2020.12.040
37. Hall RJ, Shenkin SD, Maclullich AM. A systematic literature review of cerebrospinal fluid biomarkers in delirium. *Dement Geriatr Cogn Disord*. 2011;32(2):79-93. doi:10.1159/000330757
38. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *The Lancet*. 2007;370(9596):1453-1457. doi:10.1016/s0140-6736(07)61602-x
39. Inouye SK, van Dyck CH, Alessi CA, Balkin S, Siegal AP, Horwitz RI. Clarifying confusion: the confusion assessment method. A new method for detection of delirium. *Ann Intern Med*. Dec 15 1990;113(12):941-8.
40. Marcantonio ER, Ngo LH, O'Connor M, et al. 3D-CAM: derivation and validation of a 3-minute diagnostic interview for CAM-defined delirium: a cross-sectional diagnostic test study. *Ann Intern Med*. Oct 21 2014;161(8):554-61. doi:10.7326/M14-0865
41. Trzepacz PT, Mittal D, Torres R, Kanary K, Norton J, Jimerson N. Validation of the Delirium Rating Scale-revised-98: comparison with the delirium rating scale and the cognitive test for delirium. *The Journal of neuropsychiatry and clinical neurosciences*. Spring 2001;13(2):229-42.
42. Adamis D, Slor CJ, Leonard M, et al. Reliability of delirium rating scale (DRS) and delirium rating scale-revised-98 (DRS-R98) using variance-based multivariate modelling. *J Psychiatr Res*. Jul 2013;47(7):966-71. doi:10.1016/j.jpsychires.2013.02.012
43. Gross AL, Tommet D, D'Aquila M, et al. Harmonization of delirium severity instruments: a comparison of the DRS-R-98, MDAS, and CAM-S using item response theory. *BMC Med Res Methodol*. Sep 10 2018;18(1):92. doi:10.1186/s12874-018-0552-4
44. Cohen ME, Ko CY, Bilimoria KY, et al. Optimizing ACS NSQIP modeling for evaluation of surgical quality and risk: patient risk adjustment, procedure mix adjustment, shrinkage adjustment, and surgical focus. *J Am Coll Surg*. Aug 2013;217(2):336-46 e1. doi:10.1016/j.jamcollsurg.2013.02.027

45. Cohen ME, Liu Y, Ko CY, Hall BL. Improved Surgical Outcomes for ACS NSQIP Hospitals Over Time: Evaluation of Hospital Cohorts With up to 8 Years of Participation. *Ann Surg*. Feb 2016;263(2):267-73. doi:10.1097/SLA.0000000000001192
46. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing; 2021. https://www.R-project.org/.
47. Shapiro SS, Wilk MB. An Analysis of Variance Test for Normality (Complete Samples). *Biometrika*. 1965;52(3/4):591-611. doi:10.2307/2333709
48. Wilcoxon F. Individual Comparisons by Ranking Methods. *Biometrics Bulletin*. 1945;1(6):80-83. doi:10.2307/3001968
49. Freedman D, Pisani R, Purves R. *Statistics*. 4th ed. / David Freedman, Robert Pisani, Roger Purves. ed. W.W. Norton & Co.; 2007.
50. Cook RD. Detection of Influential Observation in Linear Regression. *Technometrics*. 1977/02/01 1977;19(1):15-18. doi:10.1080/00401706.1977.10489493
51. Cook RD. Influential Observations in Linear Regression. *Journal of the American Statistical Association*. 1979;74(365):169-174. doi:10.1080/01621459.1979.10481634
52. van Munster BC, Bisschop PH, Zwinderman AH, et al. Cortisol, interleukins and S100B in delirium in the elderly. *Brain Cogn*. Oct 2010;74(1):18-23. doi:10.1016/j.bandc.2010.05.010
53. Casey CP, Lindroth H, Mohanty R, et al. Postoperative delirium is associated with increased plasma neurofilament light. *Brain*. Jan 1 2019;143(1):47-54. doi:10.1093/brain/awz354
54. Ballweg T, White M, Parker M, et al. Association between plasma tau and postoperative delirium incidence and severity: a prospective observational study. *Br J Anaesth*. Feb 2021;126(2):458-466. doi:10.1016/j.bja.2020.08.061
55. Degos V, Vacas S, Han Z, et al. Depletion of bone marrow-derived macrophages perturbs the innate immune response to surgery and reduces postoperative memory dysfunction. *Anesthesiology*. Mar 2013;118(3):527-36. doi:10.1097/ALN.0b013e3182834d94
56. Hu J, Feng X, Valdearcos M, et al. Interleukin-6 is both necessary and sufficient to produce perioperative neurocognitive disorder in mice. *Br J Anaesth*. Mar 2018;120(3):537-545. doi:10.1016/j.bja.2017.11.096
57. Saper CB, Romanovsky AA, Scammell TE. Neural circuitry engaged by prostaglandins during the sickness syndrome. *Nat Neurosci*. Jul 26 2012;15(8):1088-95. doi:10.1038/nn.3159
58. Griffin EW, Skelly DT, Murray CL, Cunningham C. Cyclooxygenase-1-dependent prostaglandins mediate susceptibility to systemic inflammation-induced acute cognitive dysfunction. *J Neurosci*. Sep 18 2013;33(38):15248-58. doi:10.1523/JNEUROSCI.6361-11.2013
59. Sanders RD, Craigova L, Schessler B, et al. Postoperative troponin increases after noncardiac surgery are associated with raised neurofilament light: a prospective observational cohort study. *Br J Anaesth*. Nov 3 2020;doi:10.1016/j.bja.2020.10.012