

Human Alzheimer and Inflammation Biomarkers after Anesthesia and Surgery

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ABSTRACT

Background: The prevalence of postoperative cognitive disturbance, coupled with growing *in vitro*, cell, and animal evidence suggesting anesthetic effects on neurodegeneration, calls for additional study of the interaction between surgical care and Alzheimer neuropathology. The authors studied human cerebrospinal fluid (CSF) biomarkers during surgery.

Methods: Eleven patients undergoing idiopathic nasal CSF leak correction were admitted to this Institutional Review Board-approved study. Lumbar subarachnoid catheters were placed before the procedure. Anesthesia was total intravenous propofol or remifentanyl or inhalational sevoflurane, depending on provider choice. CSF samples were taken after catheter placement (base), at procedure end (0 h), and then at 6, 24, and 48 h. CSF was analyzed using xMAP Luminex immunoassay (Luminex, Austin, TX).

Results: Of the 11 patients (age range, 53 ± 6 yr), 8 were women; 4 received intravenous anesthesia, 6 sevoflurane, and 1 mixed. Procedures lasted 6.4 ± 2 h. Mean CSF amyloid- $\beta(1-42)$ remained unchanged, but total-tau and phosphorylated-tau181P increased progressively until at least 48 h. Total-tau, phosphorylated-tau, or amyloid- $\beta(1-42)$ concentrations were not different between anesthetic groups. CSF interleukin-10, S100Beta, and tumor necrosis factor α

What We Already Know about This Topic

- Clinical observation of postoperative cognitive dysfunction and studies in animals suggest that anesthetics could interact with Alzheimer neuropathology

What This Article Tells Us That Is New

- In 11 patients without Alzheimer disease with intrathecal drains placed for surgery, the biomarkers for neuroinflammation (S100Beta and tumor necrosis factor α) and Alzheimer pathology (total tau and phosphorylated tau) increased in cerebrospinal fluid
- This increase was similar in patients receiving inhalational and intravenous anesthetics

were increased similarly in both anesthetic groups at 24 h, but interleukin-6 was increased more in the inhalational group.

Conclusion: These data indicate a robust neuroinflammatory response, including not only the usual markers (interleukin-6, tumor necrosis factor α , interleukin-10), but also S100Beta and tau, markers of injury. The total-tau/amyloid- $\beta(1-42)$ ratio increased in a pattern consistent with Alzheimer disease, largely because of an increase in total-tau rather than a decline in amyloid- $\beta(1-42)$. The differences in CSF interleukin-6 concentrations suggest that anesthetic management may make a difference in neuroinflammatory response.

THE possibility that anesthesia and surgery produces durable cognitive losses has gained attention over the last decades, but evidence remains ambiguous and controversial. Our patients and their families have long suspected that something about surgery accelerates age-related cognitive decline and are beginning to demand specific approaches and drugs in their preoperative visits. In addition, the possibility that anesthesia may specifically target Alzheimer disease pathways¹ has stimulated considerable research at all levels.² For example, anesthesia alone can accelerate amyloid- β (A β) production^{3,4} and aggregation,^{1,5} as well as tau phosphorylation and aggregation.⁶ Surgery may have an independent effect on these pathways.⁷ Much of this flows from studies in cell culture and animals. Human data are essential, but the decades-long refractory period, when Alzheimer pathology is developing in the absence of detectable cognitive symptoms, has made the research difficult to conduct when relying on cognitive tests. In fact, a recent state-of-the-science confer-

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ence on Alzheimer treatment⁸ concluded that, despite enormous numbers of studies, no definitive evidence exists for the ability of any pharmaceutical, environmental, or lifestyle factor to modulate the trajectory of the most common form of Alzheimer disease (late-onset type). This is due not only to the long time periods involved, but also to reliance on ambiguous and poorly defined outcome measures. Thus, validated biomarker and imaging outcomes are strongly needed to make any progress on the interaction between surgical care and Alzheimer neuropathology and dementia.

Currently, the only validated biomarker to aid a diagnosis of Alzheimer disease is the cerebrospinal fluid (CSF) total tau (t-tau) to $A\beta(1-42)$ ratio. In Alzheimer disease, the t-tau to $A\beta(1-42)$ ratio is greater than approximately 0.5.⁹ The consortium of Alzheimer's Disease Neuroimaging Initiative (ADNI) centers currently does not measure the other major form of amyloid β ($A\beta(1-40)$) but has found that increased concentrations of phosphorylated tau181P (p-tau_{181P}) is useful as a sensitive predictor of cognitive decline in initially cognitively normal patients.¹⁰ There is intense interest in the use of imaging modalities, but these are still considered experimental. Blood tests are desirable but currently are insufficiently validated. Obtaining CSF in surgical patients is challenging. First, many patients refuse an additional invasive procedure during the perioperative period unless it is part of their care. Second, CSF concentrations of these biomarkers may undergo diurnal variation.^{11,12} Studies suggest that $A\beta$ concentrations simply reflect synaptic activity¹³ and thus level of arousal. Technical and biologic variation in CSF biomarker measurements can be large,^{14,15} a matter to which the ADNI consortium has devoted considerable effort. Although there are few studies of the effect of an acute intervention on the CSF concentrations of Alzheimer disease biomarkers, a recent report showed that cardiac surgery patients showed an increase in injury biomarkers (S100Beta and tau) in their CSF, and decreases in $A\beta$ 6 months after surgery.¹⁶ This pattern is characteristic of Alzheimer patients. However, in the first hours or days after an intervention, it is not clear in which direction $A\beta$ peptides are likely to change. For example, $A\beta$ production *via* β -secretase activation⁴ might increase CSF $A\beta$ and add to brain amyloid burden. On the other hand, accelerated aggregation would decrease free $A\beta$ peptides. Thus, to begin addressing this question, we collected CSF from patients undergoing routine surgical procedures.

Materials and Methods

After recruiting for these somewhat uncommon procedures for 18 months, the single surgeon conducting them elected to discontinue the approach. Thus, 11 otherwise healthy patients scheduled for endoscopic nasal surgery to correct idiopathic CSF leaks were enrolled in this study approved by the Institutional Review Board (University of Pennsylvania, Philadelphia, Pennsylvania); all patients provided written/informed consent. There was no evidence of cognitive impairment and infection,

and no patients were taking central nervous system (CNS)-active medications. Normal care associated with these procedures included lumbar drains placed at the time of surgery by the anesthesiologist to infuse fluorescein to facilitate leak identification and maintain low CSF pressures to permit healing of the closure. The drains typically are left in place for 48–72 h after surgery and the drained CSF typically is discarded. Exclusion criteria included patient age younger than 40 yr, known dementia, epilepsy, or any CNS/intracranial process that might influence the CSF results.

Lumbar subarachnoid catheters were placed immediately before administration of the anesthetic and the surgical procedure. Anesthetic management depended entirely on provider choice and thus was not randomized. However, the providers for these cases fall neatly into two camps: those who always use inhalational agents for maintenance (usually sevoflurane) and those who always use total intravenous anesthesia (TIVA) (in a combination of propofol and remifentanyl). All patients were intubated with the aid of vecuronium and mechanically ventilated. The first, or baseline, CSF sample of 1–2 ml was taken at the time of lumbar drain placement. Another CSF sample was taken at the end of the procedure (0 time), and additional samples were taken at 6, 24, and 48 h after that or until the catheters were removed. All patients had at least four samples (baseline, 0, 6, and 24 h) and six had an additional sample at 48 h. Samples were collected roughly at the same time of day (± 3 h). All samples were divided into 1.5-ml plastic microcentrifuge tubes and immediately frozen at -80°C for subsequent batch analysis.

Alzheimer Biomarkers

Because of well-known interlaboratory variability and the effort undertaken to standardize the ADNI laboratories, we submitted aliquots of all our samples to the University of Pennsylvania ADNI biomarker laboratory.¹⁷ Briefly, $A\beta(1-42)$, t-tau, and p-tau_{181P} were measured in each of the aliquots using the multiplex xMAP Luminex platform (Luminex Corp., Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use-only reagents) immunoassay kit-based reagents. These kits included well-characterized capture monoclonal antibodies specific for $A\beta(1-42)$ (4D7A3), t-tau (AT120), and p-tau_{181P} (AT270), each chemically bonded to unique sets of color-coded beads, and analyte-specific detector antibodies (HT7, 3D6). Calibration curves were produced for each biomarker using aqueous buffered solutions that contained the combination of three biomarkers at concentrations ranging from 56 to 1,948 pg/ml for recombinant tau, 27 to 1,574 pg/ml for synthetic $A\beta(1-42)$ peptide, and 8 to 230 pg/ml for a synthetic p-tau peptide phosphorylated at the threonine 181 position.

Inflammatory Biomarkers

Other aliquots were analyzed for inflammatory biomarkers, also with Luminex xMAP technology¹⁸ (Luminex Corp) in the Human Immunology Core of the University of Pennsyl-

vania. Commercial MILLIPLEX® MAP kits (Millipore, Billerica, MA) were used in this study to quantify cytokines and neurodegenerative biomarkers in CSF samples, except for S100Beta, which was quantified with an enzyme-linked immunosorbent assay (ELISA) kit (Abnova, Taipei City, Taiwan). Interleukin-1 β , interleukin-6 (IL-6), interleukin-10, tumor necrosis factor α , and vascular endothelial growth factor were simultaneously quantified using Human Cytokines/Chemokines Panel 5 Plex kit (Millipore). Luminex bead assays were performed according to the manufacturer's instructions. After the CSF samples were thawed, they were added in duplicate to a 96-well, filter-bottom plate and incubated overnight at 4°C with antibody-coated beads that were internally coded with fluorescent dyes. After the samples were washed, biotinylated detection antibody was added, and 1 h later the streptavidin-phycoerythrin conjugate was added. After the samples were washed again, sheath fluid was added, and the plate was read on the BioPlex200 instrument (Bio-Rad, Hercules, CA). Standard curves with appropriate background media were run for every plate. Calibration curves were used to convert the median fluorescent intensity readings for each sample to concentrations (pg/ml) using a five-parameter logistic model.

Statistical Analysis

A repeated-measures one-way analysis of variance (ANOVA), with the Bonferroni *post hoc* test, was used to test statistical differences in the samples to 24 h, for which $n = 11$ at each time point. The 48-h time point ($n = 6$) is shown in the figures for illustrative purposes. For the stratification by anesthetic technique, a repeated-measures two-way ANOVA design, with the Bonferroni *post hoc* test, was used with GraphPad Prism 5.0 software (La Jolla, CA).

Results

Patients were 53 ± 6 yr old, 8 were women, and all were American Society of Anesthesiologists status I or II. Six patients received TIVA, four received sevoflurane for maintenance, and one received mixed. The procedures lasted 6.4 ± 2 h, much of the time being required for stereotactic imaging setup and the microdissection through the endoscope. Patients were euthermic throughout, and none experienced unusual changes in physiology. Procedures were without complications from either the surgery or the anesthesia or lumbar drain. Mean CSF $A\beta(1-42)$ concentrations fluctuated by less than 10% in either direction and were statistically unchanged throughout the 24-h postoperative period (fig. 1A). On the other hand, total tau was significantly increased after 6 h, more than 200% after 24 h (fig. 1B). The data suggest total tau may continue to increase even at 48 h after surgery. The ratio of t-tau to $A\beta(1-42)$ exceeded 0.5 at 48 h (fig. 1C), considerably higher than the 0.39 level used as a cutoff for mild cognitive impairment in the ADNI patient set.¹⁷ Although less dramatic, CSF concentrations of a pathologic p-tau_{181p} also were increased (~23%) after surgery (fig. 1D).

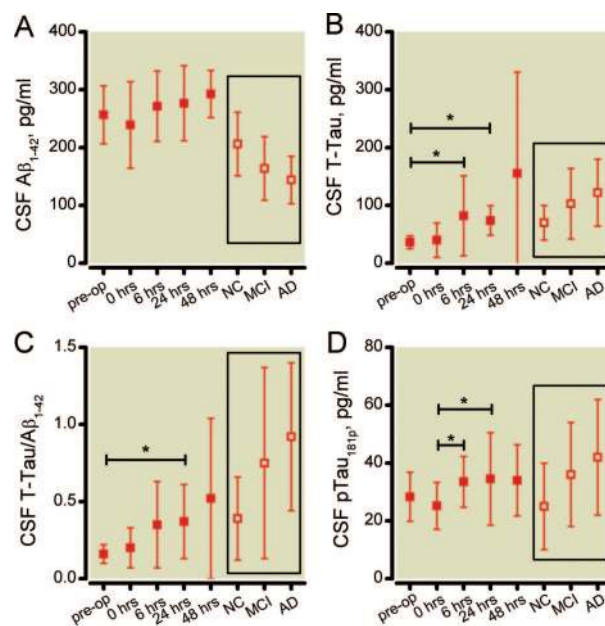


Fig. 1. Changes in Alzheimer biomarkers in the cerebrospinal fluid (CSF) during surgery. The boxes with open square data points contain data (mean \pm SD) from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort of patients¹⁷ for comparison purposes only (A-D). No change is seen in amyloid- $\beta(1-42)$ [$A\beta(1-42)$] CSF levels ($F = 2.39$, $P = 0.09$) over time (A), but a significant increase in total tau (T-tau) is detected ($F = 10.35$, $P = 0.0006$) (B), producing an increase in the T-tau/ $A\beta(1-42)$ ratio ($F = 2.96$, $P = 0.048$) (C). Phosphorylated tau181p (p-tau_{181p}) shows a significant time effect ($F = 4.71$, $P = 0.008$) (D). Points are mean \pm SD, $n = 11$ for all time points except 48 h, for which $n = 6$ (see Materials and Methods). The solid red squares are data from this study. * $P < 0.05$ using repeated-measures one-way ANOVA and the Bonferroni *post hoc* test. AD = Alzheimer disease ($n = 100$); MCI = mild cognitive impairment ($n = 196$); NC = normal cognition ($n = 114$).

Another "injury" biomarker, S100Beta, followed a similar course in the CSF as did t-tau (fig. 2). The inflammatory biomarkers Interleukin-10, IL-6, and tumor necrosis factor α also were significantly increased over time after surgery (fig. 3A-C), although no consistent change was observed in Interleukin-1 β or vascular endothelial growth factor (data not shown). Although the numbers of patients were small, we were able to detect a significant difference in IL-6 concentrations between the anesthetic management approaches. Maintenance with sevoflurane was associated with a higher postoperative CSF IL-6 concentration than was TIVA (fig. 4). There were too few men to make a gender comparison and too few patients overall to stratify the results according to procedure duration.

Discussion

Despite the small numbers of patients enrolled in this biomarker study, several observations are of interest. First, the standard, diagnostic measure of Alzheimer disease, $A\beta(1-$

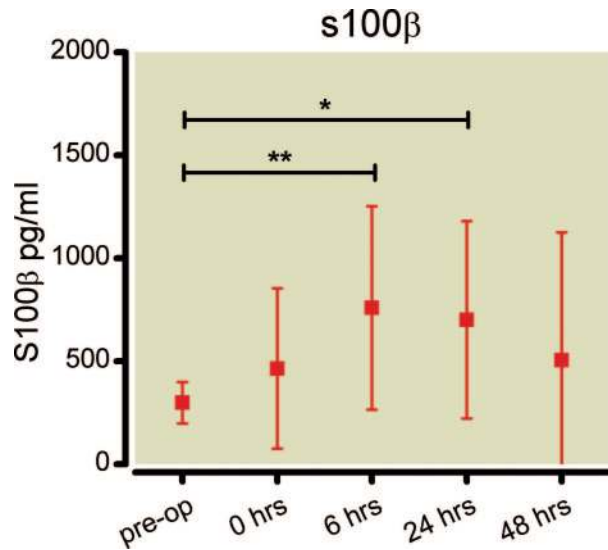


Fig. 2. Changes in cerebrospinal fluid levels of S100Beta perioperatively. Significant elevations in the injury biomarker S100Beta are seen at 6 (** $P < 0.01$) and 24 h (* $P < 0.05$) after surgery in the CSF using repeated-measures one-way ANOVA with the Bonferroni *post hoc* test. There is also an overall significant effect over time (repeated-measures one-way ANOVA, $F = 6.62$, $P = 0.0015$). Points are mean \pm SD; preoperative time period to 24 h ($n = 11$), 48 h ($n = 6$) (see Materials and Methods).

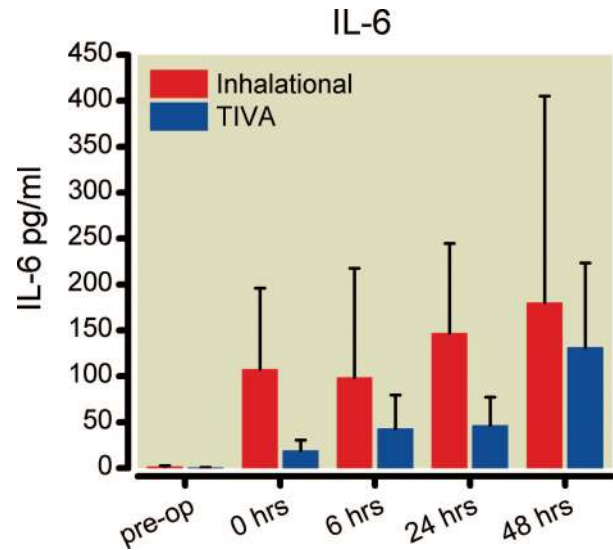


Fig. 4. Cerebrospinal fluid (CSF) Interleukin-6 (IL-6) response stratified by anesthetic technique. IL-6 levels in the CSF were found to have a significant treatment effect (by repeated-measures one-way ANOVA, $F = 5.50$, $P = 0.031$) in the postoperative period when sevoflurane ($n = 4$) was used instead of total intravenous anesthesia (TIVA) (propofol and remifentanyl) ($n = 6$). Because of the small n values and variation, no specific time point was significantly different by Bonferroni *post hoc* test.

42), remained unchanged throughout the perioperative period. In Alzheimer disease, this biomarker is decreased to 144 ± 41 pg/ml in the CSF,¹⁷ presumably because of sequestration into senile plaque, although there might also be less release into the extracellular space because of the synaptic destruction characteristic of this neurodegenerative process.¹⁹ Even mild cognitively impaired patients have CSF $A\beta(1-42)$ concentrations that are significantly less than any observed at any time point in the current study (164 ± 55 pg/ml). However, few studies on the effect of

an acute intervention have been conducted, so it is not clear whether any acute changes in CSF $A\beta(1-42)$ are to be expected, even if the intervention were known to enhance Alzheimer neurodegeneration.

On the other hand, the dramatic and progressive increase in CSF t-tau suggests that an acute CNS injury of some sort has occurred. Tau is a microtubule-associated protein that normally has an intracellular location. On hyperphosphorylation, it dissociates from microtubules and can aggregate to form paired helical filaments and, in excess, neurofibrillary

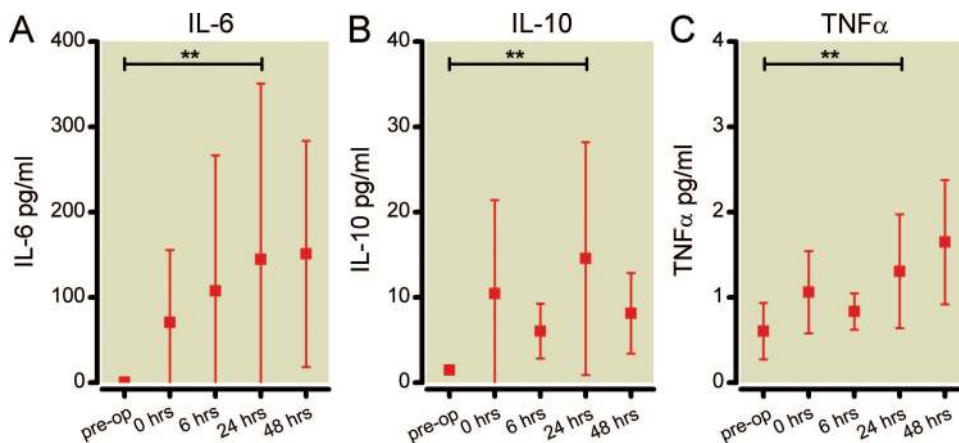


Fig. 3. Cerebrospinal fluid (CSF) inflammatory biomarkers in the perioperative period. Significant elevations are detected in interleukin-6 (IL-6) ($F = 4.59$, $P = 0.01$) (A), interleukin-10 (IL-10) ($F = 4.69$, $P = 0.008$) (B), and tumor necrosis factor α (TNF- α) ($F = 4.66$, $P = 0.009$) (C) postoperatively in the CSF. In each case, the 24-h point was significantly increased using repeated-measures one-way ANOVA with the Bonferroni *post hoc* test (** $P < 0.01$). Points are mean \pm SD; preoperative time period to 24 h ($n = 11$), 48 h ($n = 6$) (see Materials and Methods).

tangles, a hallmark intracellular lesion of Alzheimer disease. The appearance of tau in CSF is likely to reflect cellular damage and release, rather than the more complex $A\beta(1-42)$ process, which involves proteolysis, release, oligomerization, and extracellular plaque formation. This is consistent with the elevation in CSF S100Beta. Although S100Beta elevation is considered nonspecific, there exists consensus that it reflects CNS injury, most likely because of the release from astrocytes, Schwann cells, and other CNS cell types. Some may speculate that the anesthetic drug itself is responsible for the stress or cytotoxicity underlying the biomarkers, but similar changes observed with different anesthetic approaches render this somewhat less likely. It is perhaps more likely to be caused by the surgery-induced inflammatory cascade, for which we provide ample biochemical evidence, as has been observed in brain tissue from wild-type mice undergoing hepatectomy.⁷ However, we cannot rule out that the changes in tau and S100Beta are attributable to local tissue damage caused by the surgeon in the vicinity of the CSF leak repair. This seems unlikely because tau and S100Beta are considered to have a CNS origin and have a different time course after tissue injury than that observed here.²⁰

The ratio of t-tau to $A\beta(1-42)$ after postoperative 24 h approximates that seen in patients with diagnosed mild cognitive impairment.⁹ However, it is important to point out that in the ADNI study, the progressive increase in this ratio is driven primarily by a larger decrease in $A\beta_1-42$ and a smaller increase in t-tau. Because the increase in this ratio in our patients is driven almost entirely by an increase in t-tau, the significance with respect to Alzheimer neuropathology remains unclear.

Although it was smaller in magnitude than that of t-tau, a significant time effect in CSF p-tau_{181p} was seen during surgery, as indicated by the perioperative repeated-measures ANOVA (fig. 1D), with concentrations increasing more than 20% at 6 and 24 h compared with immediate postoperative samples. Tau phosphorylation is antecedent to microtubule detachment and destabilization, in addition to neurofibrillary tangle formation, a hallmark lesion of Alzheimer disease. Increases in CSF p-tau_{181p} have been found to be the most sensitive predictor of cognitive decline in otherwise cognitively normal patients.¹⁰ Thus, in future studies, CSF p-tau_{181p} should be considered as a potentially useful biomarker for postoperative cognitive and perhaps risk stratification. Whether it can be considered a predictor of Alzheimer dementia in these patients is unclear.

Our data clearly indicate that anesthesia and surgery initiate an acute pro-inflammatory event in the CNS, consistent with recent studies in both humans²¹ and animals.²² The proinflammatory cytokines IL-6 and tumor necrosis factor α increased in the 24–48 h after surgery, and the “antiinflammatory” interleukin-10 became modestly increased. Surgical initiation of inflammatory cascades is well known in the periphery²³ but has been less well documented in the CNS, where it would be considered “neuroinflammation.” En-

hanced neuroinflammation is hypothesized to worsen Alzheimer neuropathology, so our results may indicate one mechanism by which the perioperative period might modulate ongoing neurodegeneration. Most interestingly, we detected a significant difference in IL-6 concentrations between anesthetic management approaches. Maintenance with the inhalational anesthetic sevoflurane was associated with significantly higher CSF IL-6 concentrations than was maintenance with TIVA (propofol and remifentanyl). Although this finding is consistent with the repeated observation of inhaled anesthetic-induced neurotoxicity (usually isoflurane), it should be emphasized that few side-by-side comparisons with TIVA have been conducted, so it cannot yet be concluded that TIVA is less neurotoxic or neuroinflammatory than inhalational general anesthesia. Although the significance of an isolated CNS IL-6 change is not clear, the difference in IL-6 concentrations between these two approaches is not small and may indicate a more proinflammatory effect of sevoflurane or a more cytotoxic effect that secondarily triggers IL-6 release.

Summary

This biomarker study indicates that anesthesia and surgery evoke a robust neuroinflammatory response and an injury response marked by large increases in t-tau and S100Beta. Limited evidence suggests that anesthetic management may modulate this inflammatory response. The lack of effect of anesthesia and surgery on $A\beta(1-42)$ suggests the lack of a specific interaction with amyloidopathy pathways, although a small effect on CSF p-tau_{181p} suggests a potential interaction with tauopathy pathways. The relevance of these observations to long-term consequences must be tempered by the knowledge that few biomarker studies after an acute intervention have been performed and the evolution of biomarker changes remains unclear.

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