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Human plasma biomarker responses to inhalational general anaesthesia without surgery

Stacie Deiner[1,*], Mark G. Baxter[1,2,3], Joshua S. Mincer[4], Mary Sano[5], James Hall[6], Ismail Mohammed[1], Sid O'Bryant[6], Henrik Zetterberg[7,8,9,10], Kaj Blennow[7,8] and Roderic Eckenhoff[11]

[1]Department of Anesthesiology, Perioperative and Pain Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA, [2]Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA, [3]Department of Anesthesiology, Perioperative and Pain Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA, [4]Department of Anesthesiology, Memorial Sloan Kettering Cancer Center and Weill Cornell Medicine, New York, NY, USA, [5]Department of Psychiatry, James J. Peters VA Medical Center, Bronx, NY, USA, [6]University of North Texas Health Science Center, Fort Worth, TX, USA, [7]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden, [8]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden, [9]UK Dementia Research Institute at UCL, London, UK, [10]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK and [11]Department of Anesthesiology and Critical Care, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

*Corresponding author. E-mail: stacie.deiner@mssm.edu

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Abstract

Background: Postoperative neurocognitive disorders may arise in part from adverse effects of general anaesthetics on the CNS, especially in older patients or individuals otherwise vulnerable to neurotoxicity because of systemic disease or the presence of pre-existing neuropathology. Previous studies have documented cytokine and injury biomarker responses to surgical procedures that included general anaesthesia, but it is not clear to what degree anaesthetics contribute to these responses.

Methods: We performed a prospective cohort study of 59 healthy volunteers aged 40–80 yr who did not undergo surgery. Plasma markers of neurological injury and inflammation were measured immediately before and 5 h after induction of general anaesthesia with 1 minimum alveolar concentration of sevoflurane. Biomarkers included interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), C-reactive protein (CRP), and neural injury (tau, neurofilament light [NF-L], and glial fibrillary acidic protein [GFAP]).

Results: Baseline biomarkers were in the normal range, although NF-L and GFAP were elevated as a function of age. At 5 h after induction of anaesthesia, plasma tau, NF-L, and GFAP were significantly decreased relative to baseline. Plasma IL-6 was significantly increased after anaesthesia, but by a biologically insignificant degree (<1 pg ml^{-1}); plasma TNF-α and CRP were unchanged.

Conclusions: Sevoflurane general anaesthesia without surgery, even in older adults, did not provoke an inflammatory state or neuronal injury at a concentration that is detectable by an acute elevation of measured plasma biomarkers in the early hours after exposure.

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Editor's key points

- Postoperative neurocognitive disorders are common after surgical procedures in older patients, but the specific contribution of anaesthesia is unknown.
- The Trajectory of Recovery in the Elderly study enrolled 59 healthy volunteers aged 40–80 yr to receive 2 h of inhalational general anaesthesia with nothing more invasive than an i.v. catheter.
- In a prospective sub-study of this cohort, plasma biomarkers of neurological injury and inflammation were measured before and 5 h after 2 h of general anaesthesia with sevoflurane and no surgery.
- Sevoflurane anaesthesia did not provoke an inflammatory state or neuronal injury detectable by acute elevation of plasma biomarkers shortly after exposure.

Postoperative cognitive dysfunction (POCD) was initially conceptualised as an objective decline in cognitive function after anaesthesia and surgery.[1] Now termed perioperative neurocognitive disorders (PNDs), these conditions were thought to be related to anaesthesia medications or associated physiological derangement.[2,3] Decades of research have suggested that typical physiological derangements are minor contributors, but the extent to which anaesthetic drugs contribute remains unclear. There exists a large body of preclinical evidence that implicates volatile anaesthetics in PNDs. For example, volatile anaesthetics have been reported to enhance amyloid beta production and aggregation, tau phosphorylation and detachment from microtubules, calcium dysregulation, and neuroapoptosis.[4–9] Complicating the interpretation of clinical studies is the fact that almost all include a surgical procedure, which is well known to provoke a systemic inflammatory response. This inflammatory state alone could cause a cognitive syndrome of variable magnitude and duration, especially in the setting of a brain rendered vulnerable by age, genetics, or ongoing neuropathology.

Human biomarker studies hold promise for establishing causation and for risk stratification and monitoring progression of pathology. After surgery-induced tissue injury, there is an acute phase that occurs over minutes to hours marked by release of damage-associated molecular patterns (DAMPs), cytokines, and chemokines.[10] This acute phase may also include afferent vagal traffic that provokes neuroinflammation, amplified by acute-phase molecules that enter the brain parenchyma via a leaky blood–brain barrier.[11] Subsequently, vascular and neuronal injury occurs, releasing a number of different (injury) biomolecules into CSF and plasma. These molecules include tau, glial fibrillary acidic protein (GFAP), and neurofilament light (NF-L).[12] For example, several recent human studies have shown that tau and inflammatory cytokines were elevated in CSF up to 24 h after surgery.[13,14] Further, in a series of surgical patients, plasma tau and NF-L were significantly elevated from baseline at 6 h after the beginning of surgery.[15] These biomarkers have been associated with other forms of cerebral pathology, such as traumatic brain injury, Down's syndrome, Parkinson's disease, and Alzheimer's disease (AD).[16–18]

Clinical studies of surgery cannot isolate a contribution of anaesthesia to the biochemical outcome because of the concomitant effects of surgery. We had the unparalleled opportunity in the Trajectory of Recovery in the Elderly (TORIE) study that recruited healthy volunteers, aged 40–80 yr, to receive 2 h of inhalational general anaesthesia with nothing more invasive than an i.v. catheter.[19] This provided us the chance to investigate whether inhalational anaesthesia alone causes a similar biochemical response, such as the release of cytokines or markers of neuronal injury. We chose to evaluate biomarkers of both neural injury (NF-L, tau, and GFAP) and inflammation (interleukin-6 [IL-6], tumour necrosis factor alpha [TNF-α], and C-reactive protein [CRP]). Comparing this response with published data for patients receiving both anaesthesia and surgery provides insight into the major causes of biomarker release.

Methods

The parent study was approved by the institutional review board (IRB) of the Icahn School of Medicine at Mount Sinai (New York, NY, USA; IRB@mssm.edu) and registered at ClinicalTrials.gov (NCT02275026). The primary aim of the TORIE study was to delineate the age-specific trajectory of recovery from general anaesthesia in the absence of surgery and illness; full details of the parent study are described in the protocol paper.[19] The primary hypothesis was that older adults exposed to general anaesthesia alone achieve cognitive recovery within 30 days as measured by cognitive testing, but middle-aged adults recover more quickly than older adults. Secondary outcomes included the change in plasma biomarkers as reported herein. Specific inclusion criteria were adults aged 40–80 yr; ASA physical status 1 or 2; and no underlying cognitive dysfunction, which was determined from baseline cognitive testing before general anaesthesia. Exclusion criteria were contraindication to MRI scanning (parent study, implanted metal, claustrophobia) and pathophysiology seen on a pre-anaesthesia scan that could predispose to POCD, such as inflammatory conditions or cerebral microvascular disease as determined by a clinical radiologist.

Anaesthesia

Anaesthesia was induced in the MRI suite with propofol 2 mg kg^{-1} i.v., and a laryngeal mask airway (LMA) was placed. Anaesthesia was maintained with inhaled sevoflurane at an age-adjusted concentration of 1 minimum alveolar concentration (1.4–1.8 vol%). EEG leads were placed, and a bispectral index of 40–60 (BIS™ Complete 4 Channel Monitor System; Covidien, Mansfield, MA, USA) was assured after LMA placement to aid in assessment of depth of anaesthetic during equilibration of inhaled sevoflurane and washout of propofol.

EEG leads were then removed, and the subject was returned to the MRI bore for scanning. Depth of anaesthesia was then monitored by end-tidal sevoflurane concentration during scanning, along with physiological measures (blood pressure, ECG, oxygen saturation, ventilation, and temperature). Ventilation was maintained to achieve a target end-tidal carbon dioxide of 4.0–4.7 kPa. During the 2 h scan time, bolus administration of a vasopressor, such as ephedrine (5 mg i.v. or 25 mg i.m.) or phenylephrine 100 μg i.v. was administered as needed to maintain mean arterial blood pressure within 20% of baseline. The participant was then removed from the MRI bore and allowed to emerge from anaesthesia. The LMA was removed when the participant responded to commands. Ondansetron 4 mg i.v. was given before emergence for anti-emetic prophylaxis. No opioids, benzodiazepines, or neuromuscular blocking agents were administered.

Blood was obtained from the participants on the morning of the anaesthesia day immediately before induction during i.v. catheter placement, and then again 2 h into the PACU stay, ~5 h after the first blood draw and the induction of anaesthesia. Samples were collected in heparinised tubes, which were centrifuged, and plasma was aspirated, aliquoted, rapidly frozen, and stored at −80°C. After accumulation of samples from 59 subjects, parallel sets of samples were sent to two laboratories with substantial expertise in the measurement of biofluid biomarkers. To improve the rigour and reproducibility of our findings, we performed assays in two different well-established laboratories.

One set of samples was shipped frozen on dry ice to the University of North Texas Health Science Center (UNTHSC; Fort Worth, TX, USA). Samples were inspected and accessioned into the UNTHSC Institute for Translational Research Biomarker Core, where individual workflows for two platforms were created. The Meso Scale Discovery (MSD) platform has been used extensively to assay biomarkers associated with a range of human diseases, including AD[20–22]. Electrochemiluminescence technology uses labels that emit light when electronically stimulated, which improves the sensitivity of detection of many analytes at very low concentrations. Electrochemiluminescence measures have well-established properties of being more sensitive and requiring less volume than conventional enzyme-linked immunosorbent assays, which are the gold standard for most assays.[23] To improve assay performance, assay preparation was automated using a customised Hamilton Robotics STARplus system (Reno, Nevada).

Samples were also shipped on dry ice to the Clinical Neurochemistry Laboratory at the Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Mölndal Campus, Sweden. Similar to the above, samples were registered and assayed by the same two platforms, with the exception that plasma NF-L was measured using an in-house Single Molecule Array (Simoa®) method.[24] Single Molecule Array technology is a fully automated immunoassay platform utilising femtolitre reaction chambers to isolate and detect single proteins. Single Molecule Array technology utilises antibody conjugated to magnetic beads with biotinylated antibody detectors to generate a detection immunocomplex that is transferred to the Simoa disc array and sealed. The presence of labelling is visualised by single molecule 'on' or absence of single molecule 'off', allowing the concentration to be determined digitally rather than using traditional analogue detection. Single Molecule Array technology uses enzymatically labelled antibody combined with resorufin-β-d-galactopyranoside substrate to produce a fluorescence signal that is enriched in sealed microwells that each can hold a maximum of one bead.

Both assay platforms were reliable, showing excellent spiked recovery, dilution linearity, coefficients of variation (CV), reproducibility, and detection limits. Internal quality control protocols were implemented in addition to manufacturing protocols, including assaying consistent controls across batches and assay of pooled standards across lots. The analytical performance of each of these markers for >1300 samples across multiple cohorts and diagnoses was recently reported (normal cognition, mild cognitive impairment, and AD).[25]

A total of 500 μl of plasma was utilised to assay the following markers with CV and lowest levels of detection (LLOD) calculated from the automated systems: MSD plates: CRP (CV=2.4; LLOD=2.41 pg ml^{-1}), IL-6 (CV=4.6; LLOD=0.081 pg ml^{-1}), and TNF-α (CV=3.5; LLOD=0.077 pg ml^{-1}); Quanterix (Lexington, MA, USA) Simoa arrays: NF-L (CV=0.037; LLOD=0.038 pg ml^{-1}) and tau (CV=0.061; LLOD=0.019 pg ml^{-1}).

Tau and GFAP were measured using commercial Simoa assays (Quanterix) as described previously. Interleukin-6, TNF-α, and CRP were measured using MSD assays as described previously and by the manufacturer (Meso Scale Discovery, Rockville, MD, USA). Assay measurements were determined at both laboratories for all markers reported herein, except GFAP, and values were averaged for each participant and time point.

Data analysis

Statistical analyses were conducted in R is a collaborative open source project with many contributors (R version 3.6.1.) Packages lme4 and lmerTest were used for linear mixed-model analyses.[26,27]

Power analysis

We calculated our statistical power based on effect sizes from Evered and colleagues[15] for our sample of 57 participants for whom we had measures at both Time 0 (baseline) and Time 1 (5 h after anaesthesia). For tau difference between 0 and 6 h, we estimated an effect size of d=0.803 using the values in the table and assuming no association between measures (an unlikely scenario, but allowing the measures for each participant to correlate across time points only increases the effect size). With n=57 and d=0.803, we had 99.997% power to detect an effect as large as or larger than that reported for tau by Evered and colleagues[ref]. For NF-L, based on our own data, we had 0.89 correlation between NF-L values at Time 0 and Time 1. If r=0.8 effect size is d=0.385 (Evered and colleagues[ref]), we had 81.5% power with n=57 to detect an effect as large or larger.

Results

Participants

We screened 788 potential participants; 59 participants had completed assessments through Day 30 (Fig. 1) at the time the assays were conducted. There were 34 males and 25 females; mean (standard deviation [SD]) age 58.1 (11.6) yr; mean (SD) years of education 15.3 (2.2). Pre- and post-anaesthesia plasma samples were available for a total of 57 consecutive participants;

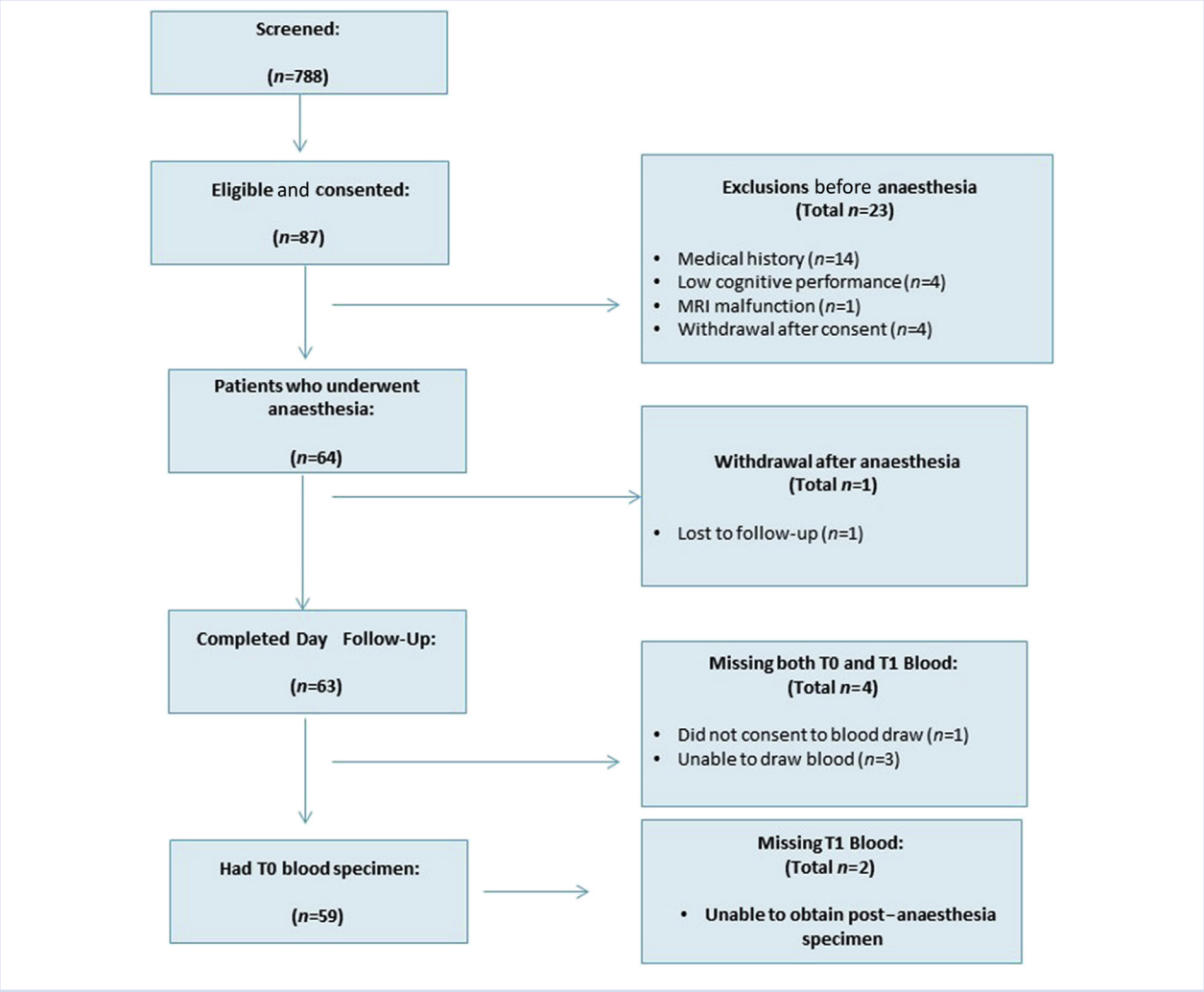

Fig 1. Strengthening the Reporting of Observational Studies in Epidemiology diagram.

Table 1 Characteristics of the Trajectory of Recovery in the Elderly cohort. SD, standard deviation.

Characteristics		Age group (yr) (n=59)			
		40–49 (n=18)	50–59 (n=16)	60–69 (n=7)	70–80 (n=18)
Sex	Male, n (%)	9 (50)	11 (69)	4 (57)	10 (56)
	Female, n (%)	9 (50)	5 (31)	3 (43)	8 (44)
Ethnicity	Hispanic or Latino, n (%)	4 (22)	3 (19)	0	1 (6)
	Not Hispanic or Latino, n (%)	14 (78)	13 (81)	7 (100)	17 (94)
	Unknown, not reported, n (%)	0	0	0	0
Race	American Indian/Alaskan native, n (%)	0	0	0	0
	Asian, n (%)	2 (11)	0	1 (14)	0
	Black or African American, n (%)	11 (61)	8 (50)	3 (43)	5 (28)
	Native Hawaiian or other Pacific Islander, n (%)	0	0	0	0
	White, n (%)	4 (22)	7 (44)	3 (43)	13 (72)
	More than one race, n (%)	1 (6)	0	0	0
	Unknown, not reported, n (%)	0	1 (6)	0	0
Age	Mean (SD) (yr)	45.3 (2.7)	53.8 (2.7)	63.3 (3.5)	72.8 (3.6)
ASA physical status	1, n (%)	13 (72)	14 (87)	6 (86)	12 (67)
	2, n (%)	5 (28)	2 (13)	1 (14)	6 (33)
Education	Mean (SD) (yr)	15.2 (2.0)	14.8 (2.6)	15.9 (1.5)	15.7 (2.2)

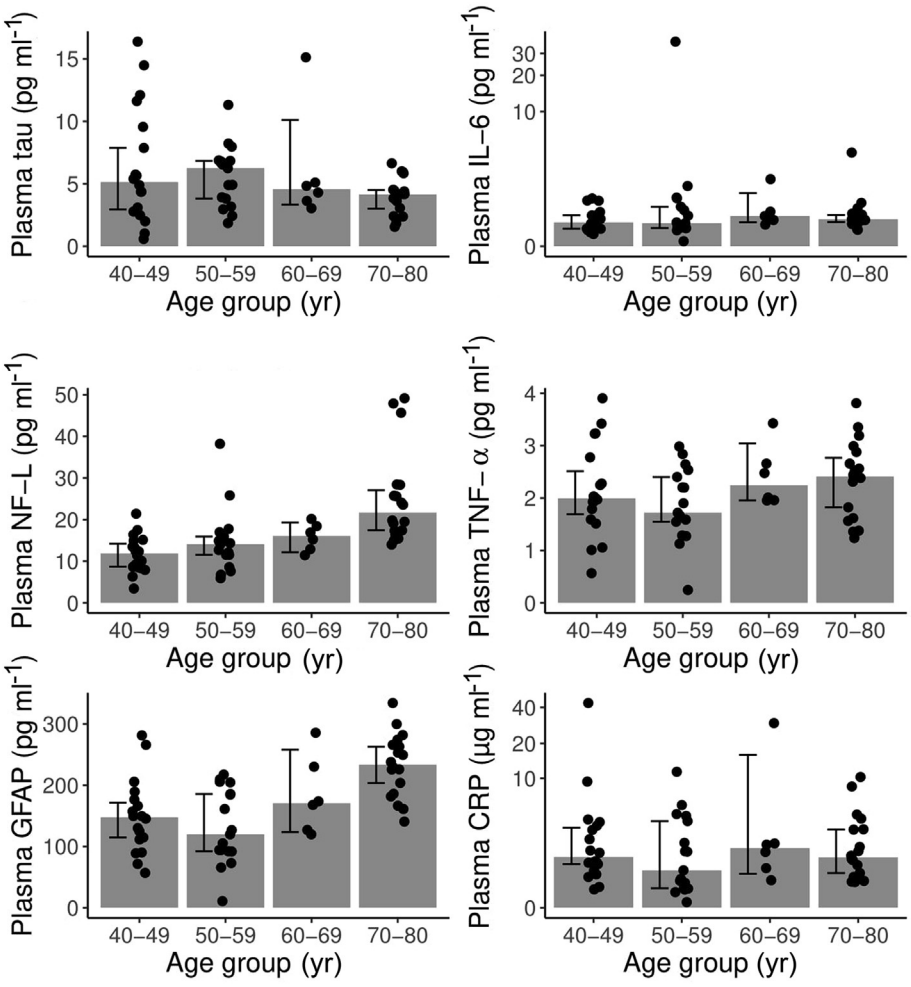

Fig 2. Serum biomarkers of neural injury and inflammation at baseline before anaesthesia by age group. CRP, C-reactive protein; GFAP, glial fibrillary acidic protein; IL-6, interleukin-6; NF-L, neurofilament light; TNF-α, tumour necrosis factor alpha.

post-anaesthesia samples were unavailable for two participants (Table 1).

We experienced a freezer thaw after collecting samples from the first 30 participants that resulted in them being warmer than −80°C for 24–36 h. Previous work by our collaborators has shown no effect of a single thaw or even multiple thaws on NF-L and tau.[28,29] Samples that had thawed had ~30% lower concentrations of TNF-α, but the concentrations of other markers were not significantly affected, and this amount of variation is not biologically significant given the enormous dynamic range (>100-fold) of TNF-α. Additionally, all comparisons were within subject and did not show elevation of Time 1 relative to Time 0. It was never the case that Time 1 samples were thawed and Time 0 were not (i.e. in all cases of thaw, both the pre- and post-anaesthesia specimens were thawed). Therefore, we included all samples, whether they had thawed or not, in all statistical analyses. Summary statistics on all markers are included (Supplementary Table S1). Both baseline and post-anaesthesia samples from each participant were either thawed or not, so thawing should not affect within-subject comparisons.

Cytokines

To examine inflammatory processes, we measured plasma IL-6 and TNF-α. These markers did not differ at baseline as a function of age: Kruskal–Wallis χ^2 (3) <4.21; $P>0.24$. Interleukin-6 showed a slight, but statistically significant, increase between baseline and post-anaesthesia, $F(1, \sim 54.3)=29.3$; $P<0.0005$; TNF-α was unchanged, $F(1, \sim 55.0)=0.005$; $P=0.94$ (Fig. 2). Median IL-6 was 0.60 pg ml^{-1} at baseline and 1.22 pg ml^{-1} post-anaesthesia; median TNF-α was 2.0 pg ml^{-1} at baseline and 2.0 pg ml^{-1} post-anaesthesia (Fig. 3).

Injury biomarkers

We measured plasma tau, NF-L, and GFAP as markers of neuronal injury, and CRP as a measure of vascular injury. Median (inter-quartile range [IQR]) baseline plasma tau concentrations were 4.47 (0.96–7.97) pg ml^{-1}; median plasma NF-L concentrations were 15.1 (7.36–22.84) pg ml^{-1}. Baseline tau did not differ as a function of age group (decade)

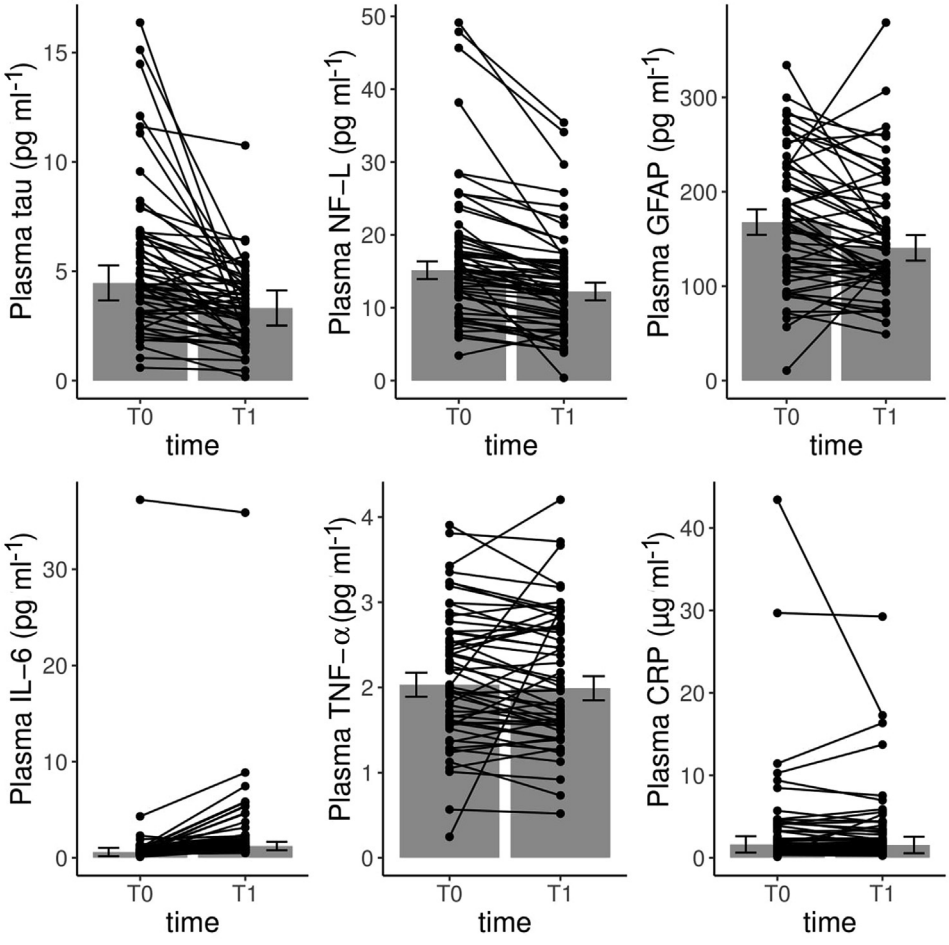

Fig 3. Serum biomarkers of neural injury before (T0) and 5 h after (T1) general anaesthesia. The height of bars shows the median value at each time point; points represent data for individual participants. Error bars are plus and minus two standard error of the difference in mean, representing the variability in within-participant differences. CRP, C-reactive protein; GFAP, glial fibrillary acidic protein; IL-6, interleukin-6; NF-L, neurofilament light; TNF-α, tumour necrosis factor alpha.

(Kruskal–Wallis χ^2 [3]=4.52; P=0.21), but baseline NF-L increased with age (Kruskal–Wallis χ^2 [3]=24.28; P<0.0005). Baseline NF-L concentrations (median; pg ml^{-1}) were 11.9, 14.1, 16.1, and 21.7 for participants who were 40–49, 50–59, 60–69, and 70–80 yr old, respectively (Fig. 2). The Spearman rank correlation of baseline NF-L with age in years was 0.65; P<0.0005. Median (IQR) baseline concentrations of GFAP were 168 (62–274) pg ml^{-1}. As with NF-L, plasma GFAP increased with age: Kruskal–Wallis χ^2 (3)=20.22; P<0.0005. Baseline GFAP concentrations (median; pg ml^{-1}) were 148, 120, 171, and 234 for participants who were 40–49, 50–59, 60–69, and 70–80 yr old, respectively (Fig. 2). The Spearman rank correlation of baseline GFAP with age in years was 0.51; P<0.0005. Median (IQR) baseline concentrations of CRP were 1.62 (2.69) μg ml^{-1} and did not differ between age groups; Kruskal–Wallis χ^2 (3)=1.51; P=0.68 (Fig. 2).

Compared with baseline, plasma tau and NF-L at 5 h after anaesthesia decreased or stayed the same for most participants (Fig. 3). All three markers showed decreases between baseline (T0) and ~5 h post-induction of anaesthesia (T1); linear mixed models for effect of time, Fs(1, ~54–56) >7.30; P<0.009. C-reactive protein did not change between baseline and 5 h post-anaesthesia, F(1, ~55)=0.37; P=0.54 (Fig. 3).

The correlation between the two sites was high for all markers: for tau, NF-L, IL-6, TNF-α, and CRP, the correlation coefficients were r=0.88, 0.94, 0.996, 0.82, and 0.95, respectively (Supplementary Table S2).

Discussion

The TORIE study gave us the rare opportunity to begin to understand the plasma biochemical response of adults to a significant exposure to the general inhalational anaesthetic sevoflurane alone. In these healthy volunteers, we found that plasma markers of neuronal injury, tau, NF-L, and GFAP were decreased, rather than increased, 5 h after induction of anaesthesia compared with baseline values. The inflammatory biomarker CRP was not changed. Two plasma markers that reflect the onset of inflammation were either unchanged (TNF-α) or very slightly increased (IL-6).

We chose to examine changes in NF-L and tau because they are considered sensitive markers of neuronal injury and neurodegenerative disease.[16–18] Although NF-L, tau, and GFAP largely arise from neuronal tissue, these biomarkers can be measured in the plasma and correlate with the onset of AD.[28,30–32] A recent study indicated that plasma NF-L and tau concentrations increased by 43% and 257%, respectively, 6 h after the beginning of surgery in older patients having noncardiac surgery.[33] We wanted to understand whether the injury reflected by these markers was attributable to anaesthesia, as suggested by considerable preclinical research, or to the surgical trauma itself. Although our baseline values are similar, we did not see an increase in either marker after anaesthesia alone. Our participants were on average younger (58 yr *vs* 69 yr); however, in the 19 TORIE participants over the age of 70 yr, we also detected no increase. Our study was limited to only two time points, whilst the previous study measured several values and found that plasma tau was maximally increased by 6 h, whereas NF-L continued to slowly rise up to 48 h.[33] Given that both biomarkers were elevated at the 6 h time point in the previous study, lack of an increase 5 h after anaesthesia alone strongly suggests a surgical origin for inflammation. This conclusion is further bolstered by an absence of increase in our study of the sensitive injury biomarkers GFAP and CRP.

Baseline values of these injury biomarkers for TORIE participants are in the normal range, although variation existed. Most interesting was that, for both NF-L and GFAP (but not tau or CRP), the concentrations were higher as a function of age. This has been observed before and may reflect subtle presymptomatic neurodegeneration, although not necessarily pathological.[34] Other investigators have found that these plasma biomarkers correlate with the degree of neurodegeneration on autopsy; thus, they may serve an important role in the risk stratification of patients before surgery, even when cognitive impairment is not detected.[35]

The significant decrease in plasma neuronal injury biomarkers after sevoflurane was unexpected. The mechanisms of this decrease can only be speculative, but might relate to a decrease in production of these biomolecules caused by a direct effect of the anaesthetic, or a decrease in clearance caused by an anaesthetic-induced decrease in cerebral blood flow. Related to this might be an effect of sevoflurane on glymphatic flow and thereby clearance, although anaesthetics may increase glymphatic flow similar to natural sleep.[36] It will be important to characterise this further with a more complete time course, as a consequence might be the CNS accumulation of toxic biomolecules (e.g. amyloid-β). This surprising decrease might suggest that increases in neuronal injury biomarkers as a result of surgery might be even larger in magnitude, but are masked, at least at early time points, by anaesthesia-induced decreases.[33]

The trigger for neuronal injury in the case of anaesthesia and surgery is thought to be inflammatory. Surgery-induced tissue trauma releases a host of cellular molecules, collectively called DAMPs, which activate the innate immune system. The activation is then characterised by the release of acute-phase cytokines, such as IL-6 and TNF-α, from various immune cells, both peripherally and centrally. The release after surgery is often several-fold and highly variable amongst individuals and procedures.[37] It has not been clear whether anaesthesia alone causes release of DAMPs or cytokines in older adults. Baseline values were in the normal range, consistent with the fact that TORIE participants were healthy. However, we were able to detect an increase in one of these cytokines (IL-6) 5 h after the induction of sevoflurane anaesthesia, but only by a very small degree (<1 pg ml^{-1}). This may reflect the minor tissue trauma of i.v. catheter insertion and confirms that our sevoflurane exposure, even in older adults, does not activate a biologically significant inflammatory cascade.

The principal limitation of this study is the single short interval of blood sampling. Further, it is possible that these markers were not sufficiently sensitive to detect a subtle stress or injury caused by the sevoflurane exposure. There are important differences between our population and the general surgical population. Our volunteers were healthy, had no prior history of cognitive issues before surgery, and had a uniform 2 h anaesthetic exposure. Patients with a surgical disease tend to have a higher co-morbidity burden than community-dwelling older adults. Given that pre-existing cognitive impairment is the most consistent risk factor for postoperative neurocognitive dysfunction, healthy volunteers may be less vulnerable. However, we investigated whether the anaesthetic exposure itself could induce markers of injury and inflammation, and cognitive impairment may not be related to biomarker elevation. Additionally, our volunteers were on average relatively young (58 yr old). Our sensitivity analysis found no difference in biomarker change between younger participants and those who were 65-80 yr old. Future study is needed to understand whether pre-existing cognitive impairment or vascular disease is related to changes in plasma biomarkers of injury after surgery.

In summary, 2 h of sevoflurane general anaesthesia alone in older adults produced little-to-no biochemical signature of either an inflammatory activation or neuronal injury when measured 5 h after induction. Baseline values in our subjects showed an increase in NF-L and GFAP as a function of age, which may indicate utility in risk stratification. A significant decrease in neuronal injury biomarkers may reflect anaesthetic-induced alterations in CNS production or clearance, which may have other implications for delayed forms of neurotoxicity.

Authors' contributions

Study design: SD, MGB, RE, MS
Data collection: JSM, IM, HZ, SO, JH, KB
Data analysis: SD, MGB, RE, HZ, SO, JH, IM, KB
Draft creation: all authors
Editing: all authors
SD has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Declarations of interest

The authors disclose the following relationships, none related to the work presented in this paper: KB has served as a consultant or at advisory boards for Alector, Biogen, Cognition Therapeutics (CogRx), Lilly, MagQu, Novartis, and Roche

Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg, Sweden, a GU Ventures-based platform company at the University of Gothenburg. HZ has served at scientific advisory boards for Roche Diagnostics, Wave, Samumed, and CogRx; has given lectures in symposia sponsored by Biogen and AlzeCure; and is a co-founder of Brain Biomarker Solutions. SO has received funding from the National Institute on Aging, Michael J. Fox Foundation, and Alzheimer's Association; has multiple patents pending on precision medicine for neurodegenerative diseases; is the founding scientist for Cx Precision Medicine; and has served as a consultant to Roche Diagnostics. SD has served as a consultant for Merck and Covidien, received product support from Covidien, Medtronic, Boulder, CO, USA, R01AG046634 and CASMED (processed EEG and oximetry monitors and sensors), and is an expert witness for legal affairs. The other authors have no conflicts to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2020.04.085.

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