Acta Anaesth. Bel., 2024, 75 (3): *157-169*

Original study

Serum neurofilament light release levels as marker of neurotoxicity in general anesthesia versus hypnosis: A prospective non-randomized trial

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Abstract

Background: **Whether general anesthetics induce neurotoxicity is unclear.**
Objectives: **We hypothesized that serum neurofilament light (NfL) release, a marker of neural injury, would not be different in patients receiving general anesthesia (GA) compared to hypnosis.**
Design: **Prospective, non-randomized.**
Setting: **Tertiary university hospital.**
Methods: **Patients undergoing breast cancer or thyroid/parathyroid surgery were enrolled. Propofol and remifentanil were used for GA. For hypnosis, only low dose remifentanil was allowed.**
Main outcome measures: **NfL was measured at baseline, at day 1 and at postoperative visit. At preoperative and postoperative visit, patients performed a Montreal Cognitive Assessment (MoCA) test. Primary outcome was absolute change in NfL at day 1 between groups.**
Results: **Among 100 included patients, 51 were in GA and 49 in hypnosis group. There were 26/49 (53%) breast cancer patients in hypnosis group and 13/51 (26%) in GA arm, p = 0.005. Baseline NfL was higher in the hypnosis group (p = 0.050). We subtracted log-transformed baseline NfL from postoperative NfL to analyze normalized values as absolute change. The mean normalized value at day 1 (primary endpoint) was 0.02 (± 0.11) in the GA and 0.03 (± 0.20) in the hypnosis group; p = 0.979 [d = 0.16, 95% CI: -0.40 to 0.39]. In a linear mixed-effects model including variables that influenced baseline NfL (advanced age, higher creatinine values, breast cancer), mean difference (95 % CI) in absolute change of log NfL at day 1 between both groups was 0.007 (- 0.04 to 0.06); p = 0.791. A change in MoCA as compared to baseline was not different between groups (p = 0.761).**
Conclusions: **Our results show that there was no statistical or clinical difference in postoperative NfL release between GA and hypnosis. GA with propofol was not associated with a different risk of neural injury compared to hypnosis.**
Trial registration: **NCT04500236.**

Keywords: **Breast cancer, General anesthesia, Hypnosis, Neurofilament light, Neurotoxicity.**

Presentation of the work: Preliminary results of this work have been presented at the virtual Euroanaesthesia meeting 2021, 17-19 December, Munich.
Internal Review Board: This study was approved on May 2020 by the "Comité d'Ethique Hospitalo-Facultaire" des Cliniques universitaires Saint-Luc (2020/14MAI/273, Brussels, Belgium – Chairman: Prof. J-M Maloteaux) and was registered on July 07, 2020 prior to patient enrolment at ClinicalTrials.gov (NCT04500236). Written informed consent was obtained from all patients, according to Declaration of Helsinki. Enrollment started on September 01, 2020 and was completed on July 25, 2022.

Introduction

The risk of developing neurocognitive disorders after anesthesia and surgery remains a public health issue[1]. Whether commonly used general anesthetics induce neurotoxicity and subsequently contribute to the development of perioperative neurocognitive disorders is not clear. Laboratory works have shown detrimental effects of anesthetic exposure on brain function of adult rodents[2,3]. However, surgery itself may be an important confounder. One study found that in animals, surgery plus anesthesia had a worse detrimental effect than anesthesia alone[4]. The acute phase after surgery-induced tissue injury may directly promote neuroinflammation and enhance the passage of acute phase molecules through blood brain barrier[5]. Nevertheless, anesthetics on their own may modulate the inflammation and alter this neuroinflammatory response[6].

Studies associating perioperative neurocognitive disorders with general anesthesia (GA) are not conclusive[7-12]. One study in healthy, nonsurgical participants with normal cognition at baseline showed that GA alone maintained with sevoflurane may not produce long-lasting cognitive decline[13]. In a prospective sub-study of that cohort, plasma markers of neural injury and inflammation did not increase 5 hours after induction of GA compared to baseline[14].

Although these studies indicate that there is no direct and immediate effect of anesthetic exposure on release of biomarkers of neuronal injury and inflammation, and no effect on cognitive recovery[13,14], their cohort included only healthy volunteers who were evaluated at a very early stage after anesthetic exposure. The latter limits the applicability of these results to clinical situations including patients with comorbidities, in whom the impact of surgery and anesthesia may manifest itself beyond the immediate postoperative period.

To address the potential neurotoxicity of anesthetic agents in the context of surgery and disease, we compared patients undergoing surgery with GA to a group of patients undergoing similar surgery under hypnosis and thus without anesthetic agents. We hypothesized that the extent of serum neurofilament light (NfL) release, a marker of neural injury, would not be different in both groups.

Methods

Population and study design

This prospective study was approved on May 2020 by the "Comité d'Ethique Hospitalo-Facultaire" des Cliniques universitaires Saint-Luc (2020/14MAI/273, Brussels, Belgium – Chairman: Prof. J-M Maloteaux) and was registered on July 07, 2020 prior to patient enrolment at ClinicalTrials.gov (NCT04500236). Written informed consent was obtained from all patients, according to Declaration of Helsinki. Enrollment started on September 01, 2020 and was completed on July 25, 2022. The study enrolled all patients > 18 y undergoing breast cancer or thyroid/parathyroid surgery and requiring at least one night of hospital stay. In our institution these surgeries are also regularly performed under hypnosis and were as such a suitable model for this study. The choice of the anesthetic technique was at the discretion of the patient as a hypnosis session requires the patient to be convinced of the technique to enter into a state of hypnosis. After informed consent to the study protocol and group allocation, the patients who chose a hypnosis session met an anesthesiologist certified in hypnosis. During this conversation the anesthesiologist discussed in detail the principles of a successful hypnosis session. At preoperative visit, all patients performed the French version of Montreal Cognitive Assessment (MoCA) test[15], and Quality of Recovery-15 questionnaire[16]. The latter consists of 15 questions, where the patient is asked to rate dimensions of his/her recovery from 0 to 10. The sum of the 15 responses results in a maximum of 150, corresponding to an ideal perspective of health status. The Quality of Recovery-15 questionnaire was repeated at postoperative day 1. At postoperative day 6 to 14, the MoCA test (a different French version to avoid a learning effect) was performed. A change in cognition was based on Z-score of the MoCA test that was calculated as follows:

$$\frac{(\text{Postoperative} - \text{Preoperative test}) - \text{Mean of } (\text{Postoperative} - \text{Preoperative test})}{\text{SD of } (\text{Postoperative} - \text{Preoperative test})}$$

Additionally, all patients were questioned about any postoperative intellectual impairment or memory loss. Exclusion criteria were: baseline glomerular filtration rate of < 30 mL min-1; mastectomy as this procedure is much more painful requiring a considerable amount of local anesthetics; preoperative psychiatric problems; patients not fluently speaking French; a baseline Kalkman score > 4/15 and thus at risk of severe postoperative pain therefore affecting total analgesic consumption being one of the secondary outcomes; any known allergy to local anesthetics, to non-steroidal anti-inflammatory drugs or to rocuronium; and patients undergoing one-day surgery in whom the NfL analysis at postoperative day one would have required additional hospital admission.

Anesthesia protocol

The anesthesia protocol and perioperative management of all patients were standardized. Patients were allowed to receive alprazolam as premedication (0.25 mg per os if weighing ≤ 60 kg; 0.5 mg if weighing between 60 - 100 kg and 1 mg if weighing > 100 kg). The NeuroSENSE® (NeuroWave Systems Inc., Cleveland Heights, OH, USA) depth-of-anesthesia monitor with bilateral frontal electroencephalogram (EEG) was used in all patients. A standard monitoring (pulse oximetry, electrocardiogram and non-invasive blood pressure measurement) was used. In patients undergoing thyroid/parathyroid surgery under GA, intraoperative neuromonitoring was used by means of Inomed C2 NerveMonitor (Emmendingen, Germany) to avoid recurrent laryngeal nerve lesion. In those who underwent thyroid/parathyroid surgery with hypnosis, direct visualization was practiced.

In the GA group, anesthesia was induced and maintained by a continuous infusion of propofol according to Schnider model and a continuous infusion of remifentanil at 0.13 to 0.50 µg Kg-1 min-1 was used as analgesic. The depth of anesthesia was guided by the NeuroSENSE® monitor with Wavelet - based Anesthesia Value for Central Nervous system between 40 and 60, avoiding any EEG burst suppression and further guided by the Density Spectral Analysis. In patients undergoing breast cancer surgery the use of an endotracheal tube or a laryngeal mask airway was at the discretion of the anesthesiologist, whereas an endotracheal tube was systematically placed for thyroid/parathyroid surgery. Rocuronium was used as a muscle relaxant to facilitate intubation conditions and Sugammadex was allowed to be used, if required.

In patients undergoing a hypnosis session, only remifentanil at a continuous infusion of 0.02 to 0.06 µg Kg-1 min-1 was allowed.

Additionally, all patients in both groups received intraoperatively IV Ketorolac (0.5 mg Kg-1) with a maximum of 30 mg and 2 g of Mg Sulphate intravenously. For breast cancer as well as thyroid/parathyroid surgery lidocaine 1% (1.5 to 2.5 mg Kg-1) and levobupivacaine 0.5 % (1 to 1.5 mg Kg-1) were administered locally by the surgeon. At the end of surgery 1 g of Acetaminophen was administered and IV piritramide could be titrated in the operating room. In the immediate postoperative period patients were allowed to receive IV piritramide. At ward, acetaminophen, ketorolac and tramadol could be administered. The total dose of all IV anesthetics and analgesics as well as local anesthetics were recorded over the entire hospitalization.

Neurofilament light analysis

Three blood samples were obtained for NfL measurements: 1) Before start of anesthesia or hypnosis 2) At day one 3) At first postoperative visit with the surgeon. The latter could vary depending on type of surgery. It occurred between day 6 and 14 postoperatively. Blood samples were collected in 4.9 mL Serum tubes (S-Monovette®, Sarstedt B.V., Nümbrecht, Germany). After centrifugation (1800 revolutions per minutes, room temperature, 10 minutes), the serum was aliquoted in 1.8 mL polypropylene microtubes (VWRTM, Leuven, Belgium) and stored at -80°C in the biobank of Cliniques universitaires Saint-Luc (Brussels, Belgium). Single-molecule array technology (Simoa®, QuanterixTM, Boston, USA) was used for NfL quantification[17]. All the samples were sent on dry ice to the Neurochemistry laboratory of the Amsterdam University Medical Centers (VUMc, Amsterdam, The Netherlands). Measurements were performed once per sample by certified technicians who were blinded to clinical information. Inter-assay coefficients of variation were 11.9% for a quality control sample with a NfL concentration of 16.4 pg mL-1 and 8.8% for a quality control sample with a concentration of 170.3 pg mL-1. Regarding the repeatability, intra-assay coefficients of variation were 4.3% for a NfL concentration of 16.4 pg mL-1 and 2.6% for a NfL concentration of 170.3 pg mL-1. Both inter- and intra-assay coefficients of variation were defined according to the work of Andreasson et al.[18]. The functional lower limit of quantification of NfL was 0.7 pg mL-1.

Statistical analysis

The primary endpoint of the study was the absolute change in NfL concentration at postoperative day 1 (postoperative day 1 minus baseline) between both groups. As the distributions of NfL concentrations are known to be highly skewed, all values were log transformed (log10 of NfL).

The size of the study was calculated based on an equivalence trial in which the 95% confidence interval (CI) on the difference in changes was expected to include a ± 15% difference between the two groups with a power of 95%. Preliminary data on 50 patients showed that serum NfL level (pg mL-1) was multiplied by 3.3 from day 0 to day 5 after surgery with conventional anesthesia. This corresponds to a mean change in log NfL from day 0 to day 5 of 1.2 with a sigma of 0.22. If the difference between the changes in log NfL in hypnosis group and GA group does not exceed 15%, that is an

absolute difference in differences lower than 0.18, the study will have a power of 95% to find a 95% CI included in the interval (- 0.18 to + 0.18) if at least 47 patients are included in each group, corresponding to a sample size of 94 patients. Considering a loss rate of about 5%, the study needed to enroll at least 99 patients and 100 subjects were thus included.

The secondary endpoints were: comparison of serum NfL in function of type of surgery; comparison of NfL between the studied groups at postoperative surgical visit; total analgesic consumption; comparison of Quality of Recovery-15 scale between the studied groups.

The normality of data was checked. Categorical data are presented as numbers and percentages. Continuous variables are presented as mean ± SD, median (P25 - P75), depending on whether they were normally distributed or not. Comparison of continuous variables between both groups was performed with an independent t-test or Mann–Whitney U test, depending on data distribution. Cohen's effect size (d) was presented for comparison between groups. A Pearson chi-square test or Fisher's exact test was used to compare categorical variables between the two groups. A univariate linear regression analysis sought the relationship between baseline log NfL and different covariates from the literature that are known to influence its values (age, gender, baseline cognitive score, renal function, breast cancer surgery)[19-22]. A linear mixed-effects model was then used to test the primary endpoint.

A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 27.

Results

Patients

A total of 100 patients were included in the study. Figure 1 shows the Strengthening the Reporting of Observational Studies in Epidemiology diagram. Fifty-two patients wished to undergo surgery under a hypnosis session and 48 patients chose GA. In three patients in the hypnosis group, conversion from hypnosis to GA was needed due to patient discomfort. Subsequently 51 patients were analyzed in the GA group and 49 patients in the hypnosis group. Thirty-nine (39%) patients underwent breast cancer surgery, and 86 (86%) patients were female. Table 1 illustrates patients' characteristics. The mean ± SD age of patients in the GA and in the hypnosis group was respectively 51 ± 17 years and 57 ± 15 years, p = 0.081. There were significantly more breast cancer surgery patients in the hypnosis arm [26/49 (53%)] compared to the GA arm [13/51 (26%)], p = 0.005. Five patients in the Hypnosis group received intraoperatively 0.2 mg of midazolam because they had not received any premedication as indicated in the study protocol. Three patients in the GA group had to undergo emergent surgery for cervical hematoma. Sevoflurane was administered during these surgeries for a duration of respectively 5, 39 and 48 minutes. Their NfL levels at postoperative day 1 were respectively 9.50, 29.90 and 12.21 pg mL-1. At postoperative surgical visit their values were respectively 13.80, 36.70 and 29.15 pg mL-1. As these NfL concentrations were not considered as outliers, it was decided not to exclude these patients from the analysis. In total 7 NfL analyses were missing (Figure 1). The intra- and postoperative data of the patients are shown in Table I. The postoperative surgical visit was performed in median at day 12 and at day 9 in respectively the GA and in the hypnosis group, p = 0.066.

The median MoCA Z-score in the GA arm and in the hypnosis arm was respectively - 0.02 (- 0.52 - 0.32) and - 0.02 (- 0.35 - 0.32), p = 0.761. Two patients in the GA group complained about postoperative memory loss. Their baseline MoCA score was 27 and 30 and their postoperative score was respectively 21 and 30. Both patients had undergone thyroid surgery. The NfL levels at baseline, at postoperative day 1 and at postoperative surgical visit were respectively 7.40 pg mL-1, 4.60 pg mL-1, and 7.10 pg mL-1 in the first patient. The NfL levels in the second patient were as follows: 5.30 pg mL-1, 4.40 pg mL-1, and 6.90 pg mL-1. These levels were as such very much comparable to the general population.

The absolute change in the Quality of Recovery-15 scale was in median - 16 (- 34 - 2) and - 7 (- 18 - 7) in respectively the GA and the hypnosis group, p = 0.017.

Neurofilament light analyses

Table II shows the absolute NfL for the entire cohort and in function of type of surgery. Figure 2 illustrates the geometric mean of NfL concentrations at different time points between the GA and the hypnosis group. As demonstrated in Table II and in Figure 2, NfL concentrations were higher in the hypnosis group at baseline and at postoperative day 1. For this reason and as significantly more breast cancer surgery was performed in the hypnosis group, data were separated in function of type of surgery.

Figure 3 illustrates the geometric mean of NfL concentration between both groups for breast cancer surgery and for thyroid/parathyroid surgery.

Considering that the primary endpoint of

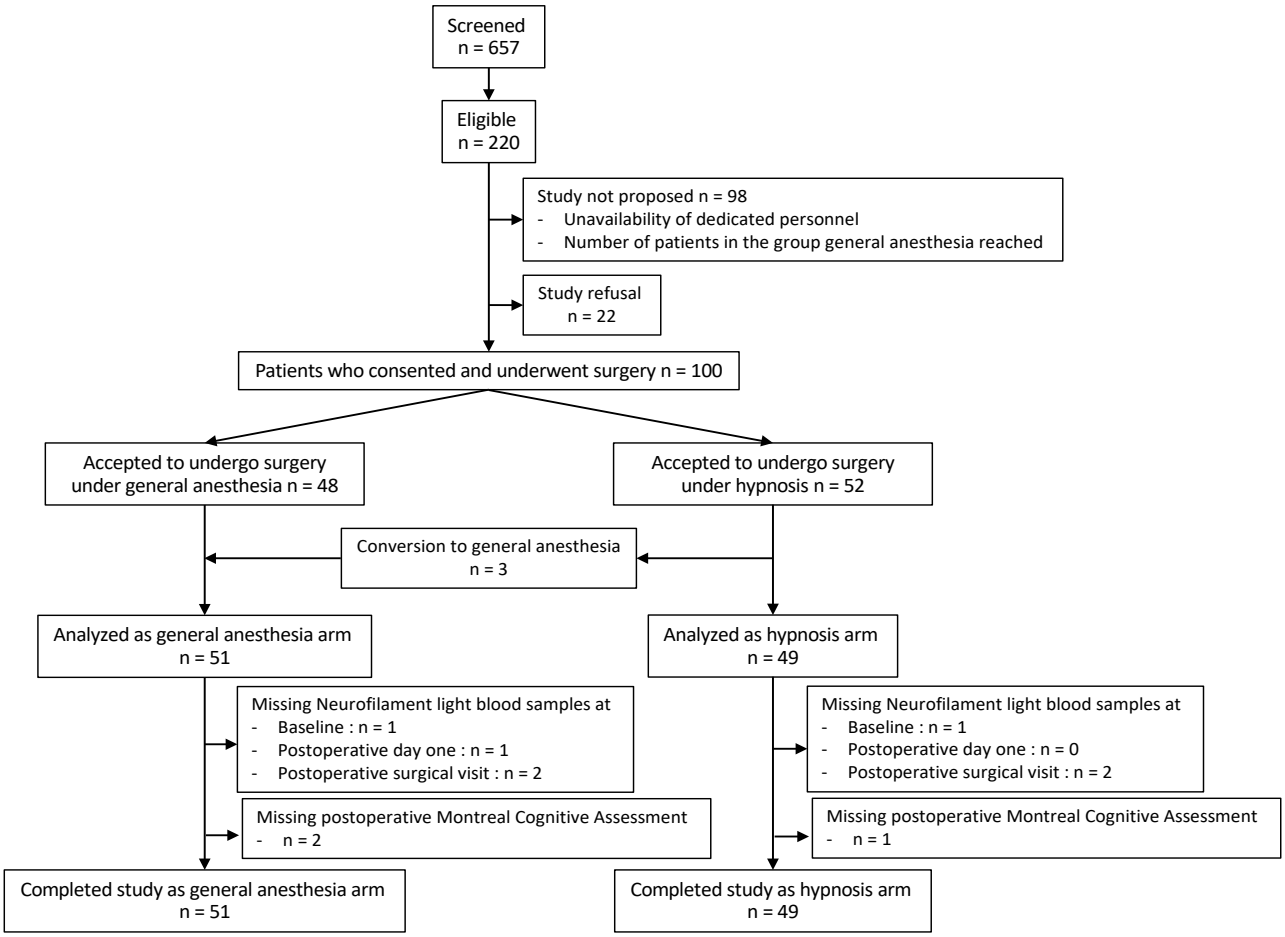


Fig. 1 — The Strengthening the Reporting of Observational Studies in Epidemiology diagram.

the study was the absolute change in NfL concentration at postoperative day 1, we subtracted the log-transformed baseline values from the log-transformed postoperative values to analyze normalized NfL values. We did the same to analyze the change in NfL concentration at postoperative surgical visit. The mean normalized NfL values for the entire group and the different surgical groups are illustrated in Table III. The mean normalized value at postoperative day 1 (primary endpoint) was 0.02 (± 0.11) in the GA group and 0.03 (± 0.20) in the hypnosis group; p = 0.979 [Cohen's effect size d = 0.16, 95 CI: -0.40 to 0.39]. When considering these normalized postoperative NfL values for the entire cohort, the time trends overlapped demonstrating that there was no difference between both groups as illustrated in Figure 4. Figure 5 and Figure 6 show the normalized postoperative NfL values for respectively the thyroid/parathyroid and breast cancer surgery patients and illustrate that there was no significant difference between both groups. Additionally, the Cohen's effect size for all analyses was very low (Table III) indicating that there was no meaningful effect of study group on postoperative NfL release.

Considering that baseline NfL concentrations were significantly higher in the hypnosis group and that significantly more patients with breast cancer had chosen for hypnosis, a univariate linear regression analysis was performed to seek the association between specific covariates and baseline NfL. As shown in Table IV, in univariate linear regression analysis, baseline log NfL was significantly higher with advanced age, with higher baseline creatinine values and in breast cancer surgery but was not influenced by gender or by baseline MoCA scores. A linear mixed-effects model was then used with absolute change in log NfL at postoperative day 1 (primary endpoint) as outcome variable. Age (in decile), type of surgery (breast cancer surgery or not), study group (GA versus hypnosis) and baseline creatinine (mg dL-1) were considered as fixed effects and patients as random effects. The model showed that the mean difference (95 % CI) in absolute change of log NfL at postoperative day 1 between the studied groups was 0.007 (- 0.04 to 0.06); p = 0.791. A linear mixed-effects model was repeated with absolute change in log NfL at postoperative surgical visit as outcome variable and did not show any statistically significant difference between both groups. Figure 7 shows the equivalency results in absolute change of log NfL at postoperative day 1 between the studied groups.

Table I. — Patients' characteristics and perioperative data.

Variables	General anesthesia (N = 51)	Hypnosis (N = 49)	p
Age, years min-max	51 ± 17 22 - 86	57 ± 15 27 - 88	0.081
Weight, kg	74 (63 - 83)	67 (58 - 83)	0.204
Baseline creatinine (mg dL^{-1})	0.79 (0.72 - 0.90)	0.81 (0.72 - 0.88)	0.947
Baseline hemoglobin (g dL^{-1})	13.3 (12.7 - 14.1)	13.9 (13.2 - 14.6)	0.020
Baseline C-reactive protein (mg L^{-1})	1.5 (1.0 - 2.5) (N = 32)	1.6 (1.0 - 3.4) (N = 32)	0.978
Female gender, n (%)	45 (88)	41 (84)	0.511
Arterial hypertension, n (%)	13 (26)	19 (39)	0.155
Diabetes mellitus, n (%)	2 (4)	3 (6)	0.614
History of alcohol consumption, n (%)	5 (10)	5 (10)	0.947
History of smoking, n (%)	3 (6)	3 (6)	0.960
Breast cancer surgery, n (%)	13 (26)	26 (53)	0.005
Type of surgery, n (%) - Thyroid surgery ▯ Total thyroidectomy ▯ Thyroid lobectomy - Parathyroid surgery ▯ Selective parathyroidectomy - Breast cancer surgery ▯ Tumorectomy ▯ Quadrantectomy	 35 (69) 25 10 3 (6) 1 13 (26) 12 1	 19 (39) 4 15 4 (8) 1 26 (53) 15 11	0.010
Baseline Montreal Cognitive Assessment Score (max 30)	27 (25 - 29)	27 (25 - 29)	0.399
Postop Montreal Cognitive Assessment Score (max 30)	27 (26 - 28) (N = 49)	28 (25 - 29) (N = 48)	0.540
Z-score Montreal Cognitive Assessment	- 0.02 (-0.52 - 0.32)	- 0.02 (-0.35 - 0.32)	0.761
Total IV propofol dose (mg kg^{-1})	15.82 (12.55 - 19.03)	0	< 0.001
Total IV remifentanil dose (mg kg^{-1})	15.84 (12.56 - 19.84)	5.29 (4.04 - 7.06)	< 0.001
Total local lidocaine dose (mg kg^{-1})	1.05 (0.73 - 1.33)	1.58 (1.03 - 2.32)	< 0.001
Total local levobupivacaine dose (mg kg^{-1})	0.57 (0.35 - 0.75)	0.75 (0.51 - 1.12)	0.008
Duration of anesthesia, minutes	86 ± 24	88 ± 29	0.773
Hospital stay, days	1 (1 - 2)	1 (1 - 2)	0.876
Day of postoperative surgical visit	12 (9 - 14)	9 (8 - 13)	0.066
Baseline Quality of Recovery-15 Scale (max 150)	127 (113 - 136)	134 (125 - 142)	0.020
Postoperative Quality of Recovery-15 Scale (max 150)	107 (85 - 123)	125 (112 - 135)	< 0.001
Absolute change in Quality of Recovery-15 scale	-16 (-34 - 2)	-7 (-18 - 7)	0.017
Piritramide administered postoperatively, n (%)	40 (78)	12 (25)	< 0.001
Total dose piritramide administered, mg	6 (2 - 8)	0 (0 - 0)	< 0.001
Data are expressed in median (P25 - P75), mean ± SD or n (%).			

Discussion

The results of this prospective study show that in patients undergoing breast cancer or thyroid/parathyroid surgery, induction, and maintenance of GA with propofol and high doses of remifentanil was not associated with a different postoperative increase in serum NfL compared to a group that only received very low doses remifentanil without any anesthetic agents.

The NfL concentrations increased in both groups after surgery but the mean absolute change from baseline, represented as normalized NfL values, was not statistically and clinically (low effect size) different between both groups at postoperative day 1 and at postoperative surgical visit.

To evaluate the eventual neurotoxicity of general anesthetics by means of serum biomarkers, one needs to consider the baseline values. In our study patients in the hypnosis group had higher NfL

concentrations at all study time points, with values that were nearly statistically significantly different at baseline. Indeed, there were significantly more breast cancer surgery patients in the hypnosis group compared to the GA group, and in both univariate and multivariate regression analysis breast cancer was significantly associated with higher baseline NfL concentrations. This is an interesting finding because NfL has been rather considered as a sensitive marker of neuro-axonal injury with serum concentrations elevated in various neurodegenerative diseases[23,24], as well as in the perioperative period[25-30]. Its increase in the context of cancer is mainly reported in cases of brain metastases[31,32], and chemotherapy-induced peripheral neuronal injury[23,33]. But recent data point out an increase in several cancers and more specifically in breast cancer[22,23]. The increase in NfL level in cancer cells has been hypothesized as an adaptive cytoprotective mechanism. When malignant transformation happens, normal tissue cells change their expression profile to adapt to the new environment[34].

To overcome the confounding effect of breast cancer surgery and the higher baseline values in patients with breast cancer, we used a linear mixed-effects model. This model showed that there was no statistically significant effect of the study group on absolute change in log NfL at postoperative day 1 which was our primary endpoint, nor at postoperative surgical visit.

Our results demonstrate that maintenance of GA with propofol and guided with a depth-of-anesthesia monitor, induces a similar extent of neuronal injury in commonly performed non-cardiac surgery compared to a group that underwent surgery without GA. Moreover, there was no significant difference in the observed median z-score of MoCA. Our observations are in line with work performed by Deiner and colleagues where anesthesia alone in 59 healthy volunteers did not result in an increase of biomarkers of neuronal injury in the absence of surgery and the ensuing inflammation[14]. However, it should be noted that in the study by Deiner et al., biomarkers of neural injury were only evaluated 5

Table II. — Neurofilament light concentrations (pg mL-1) for the entire cohort and in function of type of surgery.

	General anesthesia	Hypnosis	p*
Entire study cohort			
Baseline	8.46 (5.66 - 14.40) (n = 50) 19.97 ± 39.45 2.40 - 241.85	12.09 (7.71 - 19.01) (n = 48) 18.51 ± 20.86 0.93 - 106.56	0.050
Postoperative day 1	8.91 (5.30 - 17.40) (n = 50) 20.13 ± 36.98 3.80 - 219.22	12.67 (8.31 - 15.63) (n = 49) 18.37 ± 20.74 4.90 - 123.05	0.033
Postoperative surgical visit	13.10 (7.10 - 29.15) (n = 49) 25.39 ± 34.79 3.32 - 172.59	15.40 (10.46 - 27.56) (n = 47) 23.76 ± 24.35 4.90 - 126.77	0.430
Breast cancer surgery			
Baseline	11.70 (8.80 - 26.80) (n = 12) 43.81 ± 74.16 7.00 - 241.85	16.53 (10.46 - 33.02) (n = 25) 25.42 ± 26.56 5.62 - 106.56	0.685
Postoperative day 1	12.20 (7.83 - 20.75) (n = 12) 40.34 ± 68.90 4.30 - 219.22	14.77 (11.30 - 31.13) (n = 26) 25.21 ± 26.57 6.42 - 123.05	0.330
Postoperative surgical visit	35.90 (15.63 - 55.20) (n = 12) 53.04 ± 57.09 6.90 - 172.59	21.86 (15.40 - 45.10) (n = 26) 33.59 ± 29.04 10.21 - 126.77	0.379
Thyroid/parathyroid surgery			
Baseline	7.40 (5.12 - 13.64) (n = 38) 12.45 ± 13.54 2.40 - 71.90	9.56 (6.55 - 13.86) (n = 23) 10.99 ± 6.85 0.93 - 32.03	0.312
Postoperative day 1	8.29 (5.00 - 16.55) (n = 38) 13.75 ± 15.02 3.80 - 76.40	9.27 (7.09 - 13.26) (n = 23) 10.64 ± 4.26 4.90 – 21.46	0.384
Postoperative surgical visit	11.36 (6.90 - 17.67) (n = 37) 16.43 ± 16.75 3.32 - 88.60	10.56 (7.68 -13.32) (n = 21) 11.57 ± 5.29 4.90 - 27.27	0.614

Data are expressed in median (P25 - P75), mean ± SD, and as Min - Max; * Mann Whitney test.

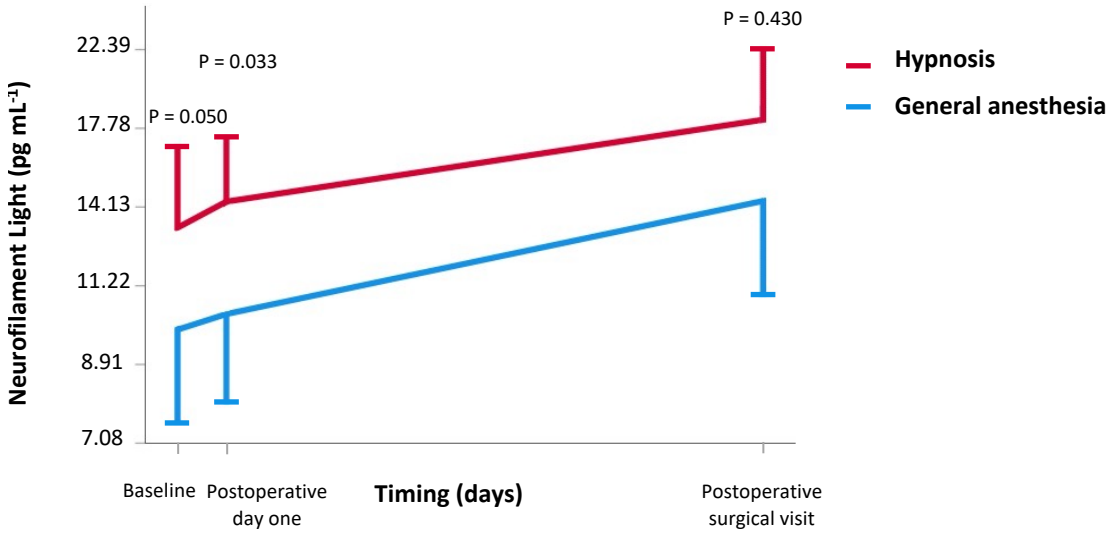
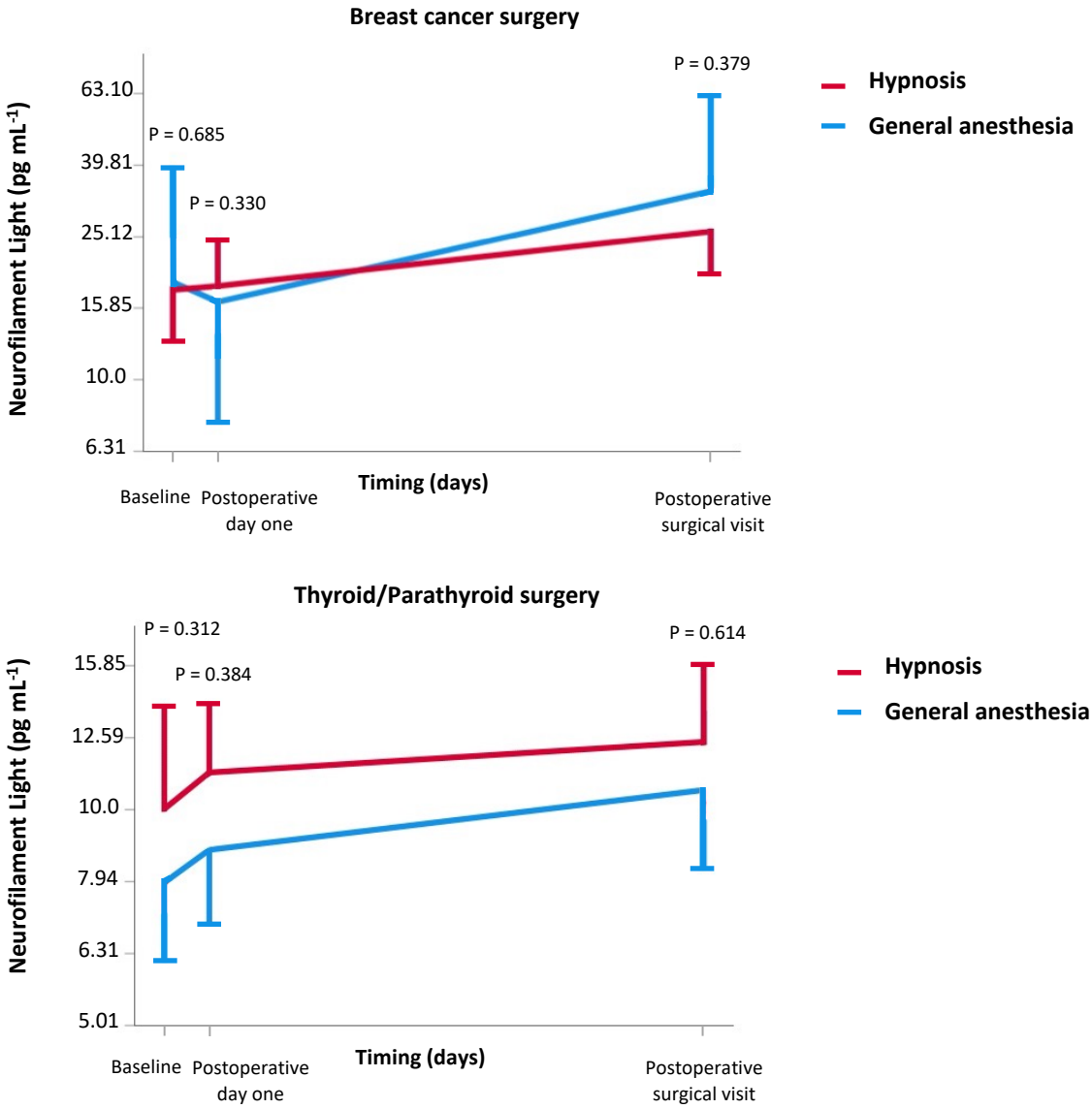


Fig. 2 — Time trends in geometric means of serum neurofilament light concentrations (pg mL-1) in the general anesthesia group (blue) and in the hypnosis group (red).
Bars indicate standard error. p-values are between-group comparison by Mann-Whitney test.

A



B

Fig. 3A — Time trends in geometric means of serum neurofilament light concentrations (pg mL-1) in the general anesthesia group (blue) and in the hypnosis group (red). Figure 3A: Breast cancer surgery. *Fig. 3B* — Thyroid/parathyroid surgery.
Bars indicate standard error. p-values are between-group comparison by Mann-Whitney test.

Table III. — Normalized Neurofilament light concentrations (log-transformed postoperative values – log-transformed baseline values) (pg mL-1) for the entire cohort and in function of type of surgery.

	General anesthesia	Hypnosis	Cohen's effect size d (95% CI)	p*
Entire study cohort				
Postoperative day 1	0.02 ± 0.11	0.03 ± 0.20	0.16 (-0.40 to 0.39)	0.979
Postoperative surgical visit	0.15 ± 0.17	0.14 ± 0.21	0.19 (-0.33 to 0.48)	0.720
Breast cancer surgery				
Postoperative day 1	-0.05 ± 0.13	0.001 ± 0.09	0.10 (-1.26 to 0.19)	0.146
Postoperative surgical visit	0.23 ± 0.23	0.16 ± 0.13	0.17 (-0.28 to 1.15)	0.234
Thyroid/parathyroid surgery				
Postoperative day 1	0.04 ± 0.09	0.06 ± 0.27	0.18 (-0.54 to 0.49)	0.933
Postoperative surgical visit	0.13 ± 0.14	0.11 ± 0.28	0.20 (-0.45 to 0.62)	0.758
Data are expressed in mean ± SD; *: Comparison by independent t-test.				

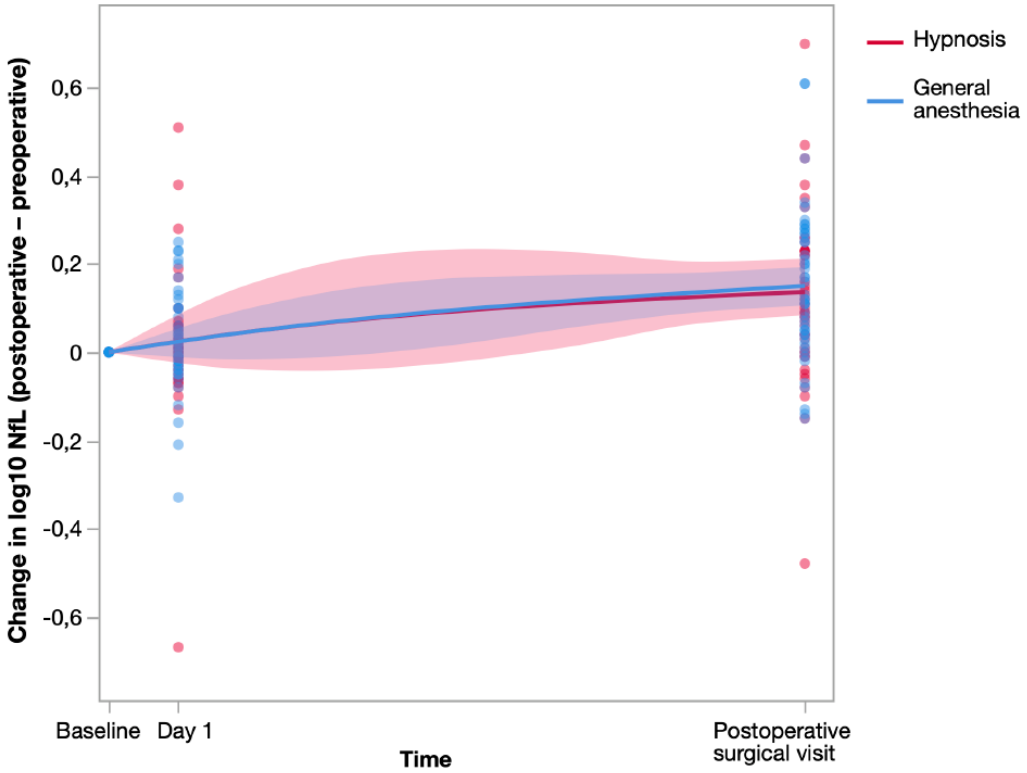


Fig. 4 — Perioperative time trends of neurofilament light concentrations, from baseline to postoperative day 1 and to postoperative surgical visit stratified by group (red line = hypnosis; blue line = general anesthesia). Neurofilament light values were normalized by log-transforming pre-and postoperative values, and then by subtracting the log-transformed baseline values from the log-transformed postoperative values.

hours after anesthesia. An eventual increase later on, cannot be excluded. Otherwise, a multicenter experiment that randomized 60 young healthy volunteers to deep GA alone or no anesthesia found that within 3 hours of emergence from anesthesia, the GA group performed overall cognitive tests with an accuracy level that was not substantially different from the control group[35].

Although in our study, surgery with hypnosis did not result in a different risk of neural injury compared to GA, it showed other beneficial effects that should not be neglected. Hypnosis resulted in significantly less opioid consumption in the immediate postoperative period. Otherwise, patients who were in the hypnosis group showed a higher satisfaction as demonstrated by the absolute change in Quality of Recovery-15 scale. These observations are in line with previous findings[36].

This study has some limitations. First, our results cannot be extrapolated to inhalational anesthetics. A recent meta-analysis showed that the incidence of cognitive dysfunction in the first 30 days postoperatively was significantly lower when total intravenous anesthesia was used compared to inhalational anesthesia[37]. Second, the NfL measurements at postoperative surgical visit were

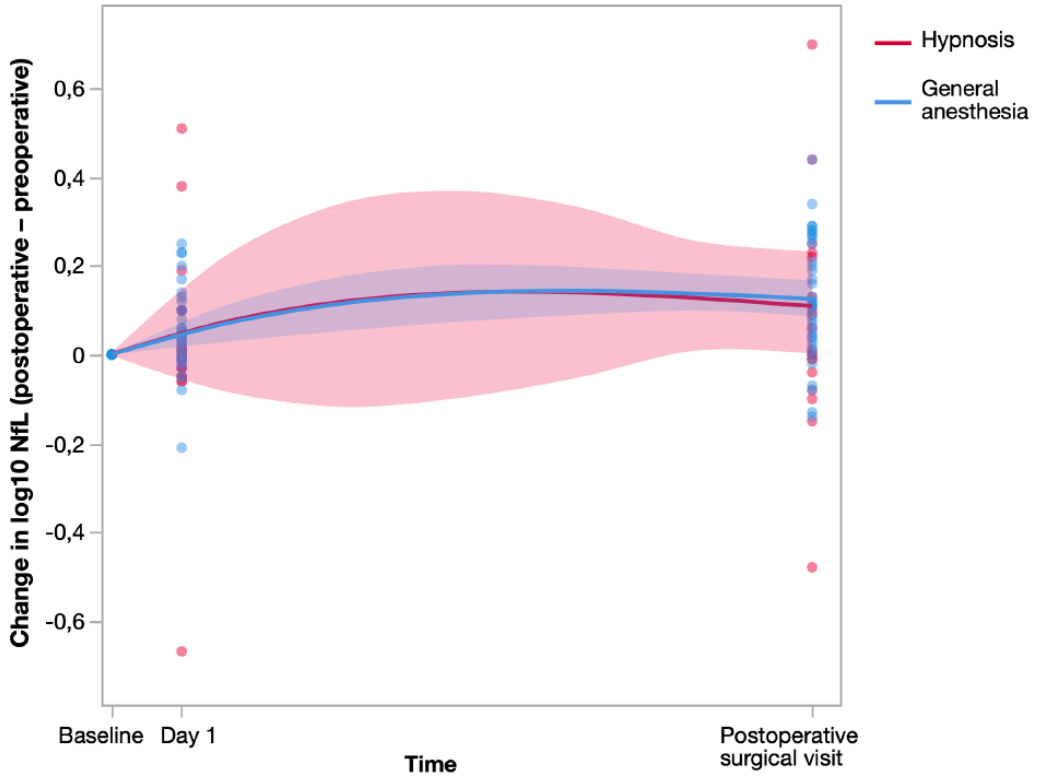


Fig. 5 — Perioperative time trends of neurofilament light concentrations of thyroid/parathyroid surgery patients, from baseline to postoperative day 1 and to postoperative surgical visit stratified by group (red line = hypnosis; blue line = general anesthesia).
Neurofilament light values were normalized by log-transforming pre-and postoperative values, and then by subtracting the log-transformed baseline values from the log-transformed postoperative values.

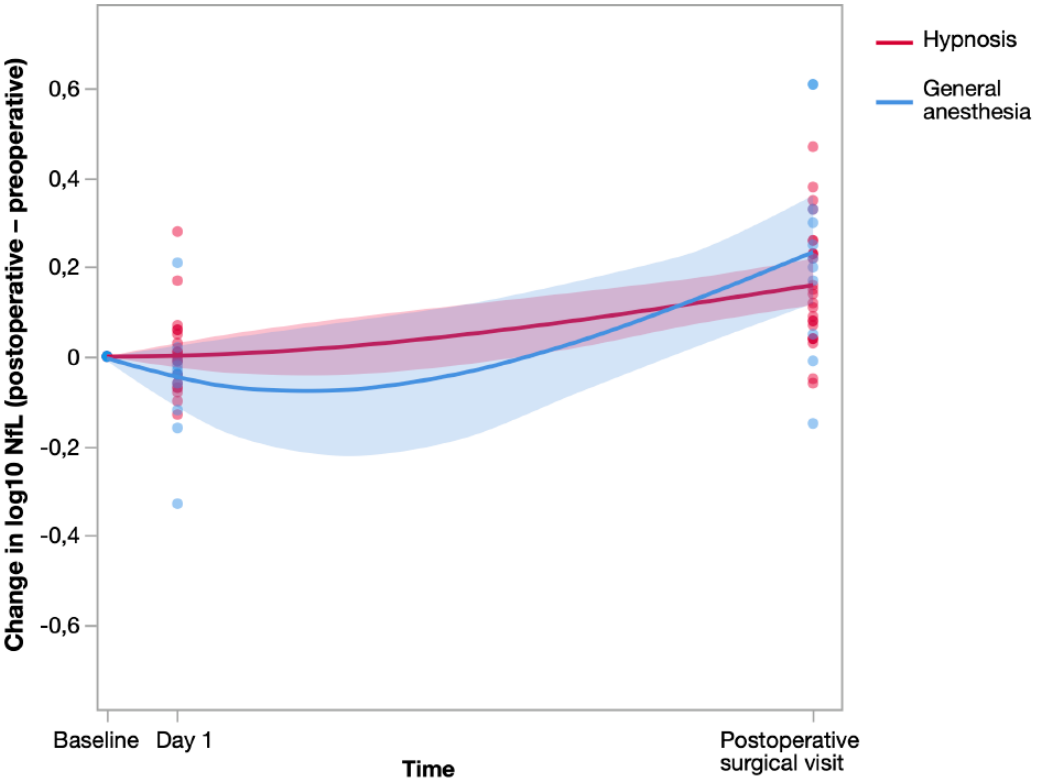
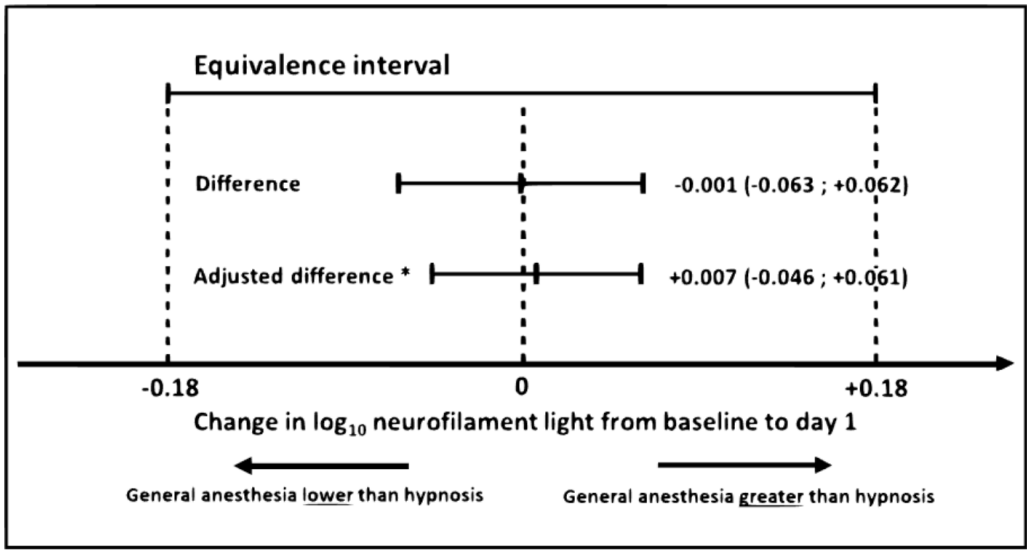


Fig. 6 — Perioperative time trends of neurofilament light concentrations of breast cancer surgery patients, from baseline to postoperative day 1 and to postoperative surgical visit stratified by group (red line = hypnosis; blue line = general anesthesia).
Neurofilament light values were normalized by log-transforming pre-and postoperative values, and then by subtracting the log-transformed baseline values from the log-transformed postoperative values.

Table IV. — Univariate linear regression analysis – Dependent variable: baseline log transformed serum neurofilament light.

	Standardized Coefficient ß	95% CI for ß	t	p-value
Age in deciles	0.573	0.098 to 0.178	6.850	< 0.001
Female gender	- 0.013	- 0.243 to 0.213	- 0.129	0.897
Baseline creatinine (mg dL^{-1})	0.214	0.02 to; 0.932	2.089	0.040
Breast cancer surgery	0.392	0.166 to 0.466	4.179	< 0.001
Baseline Montreal Cognitive Assessment Score	- 0.114	- 0.041 to 0.011	- 1.126	0.263



*** adjusted difference on age, baseline creatinine and breast cancer**

Fig. 7 — Equivalency results for absolute change in log-transformed neurofilament light concentrations between general anesthesia and hypnosis.

not performed at a fixed time point. Moreover, NfL measurements were only performed at three time points. The kinetics of NfL release after surgery are not yet completely elucidated[30]. It might be that we missed a difference between both groups because the difference occurred after postoperative day 1 but before the postoperative surgical visit. Third, we only included patients undergoing minor surgery that did not last more than two hours as hypnosis cannot be used for prolonged surgeries. Fourth, we included two different kinds of surgeries. Breast and thyroid surgery differ regarding the surgical stress response and expected NfL levels. Fifth, our findings apply only to a single anesthesia exposure. It might be that multiple anesthesia exposures could have made a difference. Lastly, the mean age of our study population was 51 years and 57 years for respectively the GA and the hypnosis group. Including older patients would have allowed to obtain more meaningful results in terms of perioperative neurocognitive disorders. Our results need therefore to be confirmed in older patients undergoing major surgery.

This study has some strengths as well. We combined a biomarker of neural injury with a well validated and easy to use screening test for detection of cognitive impairment. We evaluated the neurotoxicity of anesthetic agents in the context of surgery and the ensuing perioperative inflammation.

In summary, the results of this study show that the extent of postoperative neural injury is not different whenever minor non-cardiac surgery is performed either with a propofol/remifentanil-based anesthesia or with hypnosis.

Acknowledgments: We would like to thank Mrs. Laetitia Miltoni (Clinical Research Coordinator, Cliniques universitaires Saint-Luc, Brussels, Belgium) who assisted in data acquisition, as well as Dr. Arnaud Steyaerts (Department of Anesthesiology, Cliniques universitaires Saint-Luc, Brussels, Belgium) who assisted in the design of some figures.

Conflicts of interest: Charlotte E. Teunissen has a collaboration contract with QuanterixTM (Boston, USA). The other authors have no competing interests to declare.

Funding: This study received a research grant from Belgian Society of Anesthesiology, Resuscitation, Perioperative medicine and Pain management (BeSARPP), and a research grant from Fondation Roi Baudouin, Pink Ribbon.

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doi.org/10.56126/75.3.48