Neuron-Specific Enolase and Matrix Metalloproteinase 9 Signal Perioperative Silent Brain Infarction During or After Transcatheter Aortic Valve Implantation

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Magnetic resonance imaging (MRI) studies have consistently identified a high incidence of silent brain infarction (SBI) after cardiac intervention. The frequent occurrence, objective measurement and clinical sequelae of SBI have seen interest in their detection for both research and clinical purposes. However, MRI is expensive, time-consuming, unsafe in acutely-ill patients, and not always available, limiting its use as a routine screening tool. For this purpose, a blood biomarker of SBI would be the "Holy Grail." By performing targeted profiling of serologic biomarkers this study aimed to assess their potential as screening tools for perioperative SBI. This is a nested case-control study of 20 prospectively recruited patients undergoing transcatheter aortic valve implantation under general anesthesia. Clinical and diffusion-weighted MRI assessments were performed at baseline and on day 3 postprocedure to identify the presence (cases) or absence (controls) of new SBI. Blood was collected at baseline and 24, 48, and 72 hours postprocedure and analyzed for S100 calcium-binding protein B, neuron specific enolase (NSE), matrix metalloproteinase 9 (MMP 9), and glial fibrillary acidic protein. Best-fit polynomial curves using a smoothing model were generated for each biomarker and inferential testing at a predefined 24-hour postprocedure timepoint detected a significant difference for MMP 9 (72,435; SEM: 25,030; $p = 0.027$). Longitudinal regression revealed a statistically significant case-control difference for both NSE (mean: 10,747; SEM: 3,114) and MMP 9 (63,842; SEM: 16,173). In conclusion, NSE and MMP 9 are present in higher levels following SBI and warrant further investigation for their utility as screening tools. **(Am J Cardiol 2019;123:434−439)**

Neuroimaging studies have consistently identified new silent brain infarction (SBI) as a common finding in the early postoperative period after transcatheter aortic valve implantation (TAVI).[1,2] Concerningly, there is mounting evidence to suggest that these infarcts are not silent at all; rather, they are associated with subtle neurologic deficits, neurocognitive dysfunction, psychiatric disorders, and increased risks of overt stroke and early mortality.[3] Their frequent occurrence, objective measurement, and clinical implications have increased interest in their routine detection and resulted in consensus statements recommending SBI as a surrogate for clinically apparent ischemic stroke.[4] However, imaging is expensive, time consuming, unsafe in acutely-ill patients, and not always available, rendering it unfeasible as a routine screening tool. For this purpose, a serum biomarker of cerebral infarction (equivalent to troponin for cardiac injury) would be the "Holy Grail." This study specifically assesses 4 established biomarkers of clinically apparent ischemic stroke: (1) S100 calcium-binding protein B (S100B); (2) neuron specific enolase (NSE); (3) glial fibrillary acidic protein (GFAP); and, (4) matrix metalloproteinase 9 (MMP 9) in patients with versus without perioperative SBI to determine their potential utility detecting this subtler injury.

Methods

Between 2014 and 2016, all patients undergoing TAVI with a SAPIEN-XT valve (Edwards LifeSciences, Irvine, California) inserted through transfemoral access under general anesthesia were recruited prospectively at the Prince

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Charles Hospital, Australia, as part of a larger study.[5] The study protocol received institutional ethics review board approval (HREC/12/QPCH/291) and study methods were carried out in accordance. Informed written consent was obtained from all eligible participants who were consecutively enrolled for this nested case-control. Subsequent to the TAVI procedure, participants were grouped according to whether they had postprocedural MRI evidence of subclinical infarction (cases) or did not (controls). This process was continued until there were 10 patients with complete serum assessments in each group. Patients with clinically apparent neurologic injury, including cerebrovascular events, delirium, or cognitive impairment (both before and after the TAVI procedure), procedural complication, elevated hemolysis index, renal or liver failure, and those with chronic inflammatory disease (excluding aortic stenosis) or recent/active infection were excluded. We have previously characterized the role of immunologic/inflammatory mechanisms associated with SBI in this cohort of patients.[6]

Blood samples were collected in serum separator tubes within 24-hour before the procedure and 24-, 48-, and 72-hour postprocedure. Samples were left to clot for 30 minutes, centrifuged at 3,000g for 15 minutes at 4°C, aliquoted into separate tubes, and stored at −80°C until analysis. Serum levels of S100B and GFAP were determined using specific ELISA commercial kits (EZHS100B-33k and NS830, Merck, Darmstadt, Germany). Serum levels for NSE and MMP 9 were determined on a Millipore MAGPIX System, using human magnetic bead panels (HNS1MAG-95K and HMMPZMA-G55K01, Merck, Darmstadt, Germany). C-reactive protein with high-sensitivity determination (hsCRP) was measured as a general inflammatory marker using a commercially-available ELISA kit (DE740011, Demeditec Diagnostics GmbH, Kiel-Wellsee, Germany). All kits were used as per the manufacturer's instructions.

A 1.5 Tesla MAGNETOM Aera (Siemens Healthcare, Erlanger, Germany) was used to acquire diffusion-weighted MRI sequences at baseline (within 24-hour preprocedure) and on day 3 ± 1 postprocedure. Any new focus of restricted diffusion (high diffusion weighted imaging signal and low apparent diffusion coefficient) occurring in either the white or gray matter located in the cerebrum, cerebellum, or brainstem on the postprocedural MRI was considered a perioperative brain infarction. These infarcts were quantified on diffusion-weighted MRI by both number and volume per patient.

At baseline and day 3 and 6 weeks postprocedure, the National Institutes of Health Stroke Scale, confusion assessment method, and Montreal Cognitive Assessment tool were used to exclude clinically apparent stroke, delirium and cognitive impairment, respectively, to determine subject eligibility. Criteria for SBI included both evidence of brain infarction on MRI and absence of clinically apparent deficit.

Summary statistics are reported as group mean ± standard error of the mean for approximately normally (or Gaussian) distributed data, median ± interquartile range for non-normally distributed data, or simple percentages (%). Student's *t* test (normal distribution) or Mann-Whitney *U* test (non-normal distribution) was performed to identify any statistically significant differences between groups. For each patient, biomarker changes from baseline were plotted as concentration over time considered as a categorical variable. However, as the exact sampling time is different for each participant and we anticipated nonlinear changes in biomarker concentrations over time, primary analysis used a smoothing model to identify best-fit polynomial curves for each group. Six polynomial curves were tested for each group and the best combination chosen by selecting the model with the smallest deviance (best fit to data). Inferential testing to identify statistically-significant mean differences between the 2 curves was performed at the 24-hour postprocedure time point, and we predefined this 1 clinically meaningful time rather than test the entire area under the curve or test multiple times. Secondary analysis used longitudinal regression to test for an overall case-control difference across all time points while accounting for repeated measures over time from the same participant. A random intercept was used to account for serial results from the same patient, which is equivalent to an exchangeable correlation in a Generalized Estimating Equation model. The dependent variable was difference from baseline (time 0) to between 3 and 4 follow-up time points, with measured differences between groups the primary parameter. Pearson's correlation coefficient was used to evaluate the relation between promising biomarkers in cases and controls in a post hoc analysis. All analyses were performed using R software version 3.1.1 (www.r-project.org), Stata software version 13 (StataCorp, Texas) or DataGraph version 4.3 (Visual Data Tools Inc.).

Results

Baseline characteristics of the case and control groups are shown in Table 1. Among the 10 patients who sustained SBI, there were 45 lesions (median ± interquartile range of 3 ± 3 lesions/patient and 197 ± 245 μl/patient); over 80% of these lesions by volume occurred in cortical grey matter. No statistically significant case-control differences were detected for any serological biomarker at baseline. Sampling times between cases versus controls for each grouped timepoint (categorical) was not statistically significantly different. Per patient change from baseline for each biomarker and timepoint (categorical) are presented in Figure 1.

The best-fit polynomial curves for each neurologic biomarker over time (continuous) are presented in Figure 2. Inferential testing identified a statistically-significant case-control difference at the predefined 24-hour timepoint between the best fit polynomial curves for MMP 9 (mean 72,435 ± 32,885 pg/ml, $p = 0.027$). Such difference was not evident for S100B (mean 8 ± 9.7 pg/ml), NSE (mean 9,179 ± 6,853 pg/ml), GFAP (mean 0.2 ± 0.3 ng/ml), or the general inflammatory marker hsCRP (mean 3,261 ± 968 μg/ml).

Longitudinal regression revealed statistically significantly-greater ($p < 0.05$) change from baseline in the SBI than control group for both NSE (mean 10,747 ± 4,092) and MMP 9 (mean 63,842 ± 21,248), but not for S100B (mean 7 ± 10), GFAP (mean 0.06 ± 0.16), or hsCRP (mean: 4,067 ± 2,933). MMP 9 and NSE showed very

Table 1
Baseline characteristics of included participants

Variable	MRI-negative (n = 10)	MRI-positive (n = 10)	p value
Age (years)	83.3 ± 3.0	84.2 ± 1.0	0.78
Women	4 (40%)	5 (50%)	0.66
Body mass index (kg/m^2)	31.4 ± 2.7	27 ± 1.9	0.19
Creatinine (μmol/L)	101 ± 10.0	107.4 ± 9.0	0.64
EuroSCORE II (%)	5.9 ± 0.8	5.1 ± 1.0	0.54
Society of Thoracic Surgeons risk score (%)	6.6 ± 1.1	5.5 ± 0.9	0.45
Aortic valve area (cm^2)	0.8 ± 0.03	0.8 ± 0.03	1.00
Peak jet velocity (m/s)	4.3 ± 0.1	4.4 ± 0.2	0.66
Mean pressure gradient (mm Hg)	49.2 ± 3.6	47 ± 5.1	0.73
Pre-procedural left ventricular ejection fraction (%)	58.4 ± 2.9	51.3 ± 5.3	0.26
Device success	10 (100%)	10 (100%)	1.00
Procedure time (minutes)	93 ± 21.5	61 ± 4.1	0.16

Values are expressed as mean ± SEM or as n (%).

good correlation (Figure 3, panel A) in cases (R = 0.84) and not controls (R = 0.1). MMP 9 poorly correlated with hsCRP (Figure 3, panel B) when all measures were considered (R = 0.07) and when cases (R = 0.3) and controls (R = 0.2) were considered separately indicating that general inflammatory response did not explain the rise in this MMP 9 postprocedure.

Discussion

The limitations of perioperative radiologic assessments increase interest in identifying blood biomarkers of SBI. In this study, we tested the serum from prospectively enrolled TAVI patients with (cases) versus without (controls) SBI using a panel of promising biomarkers of neurologic injury

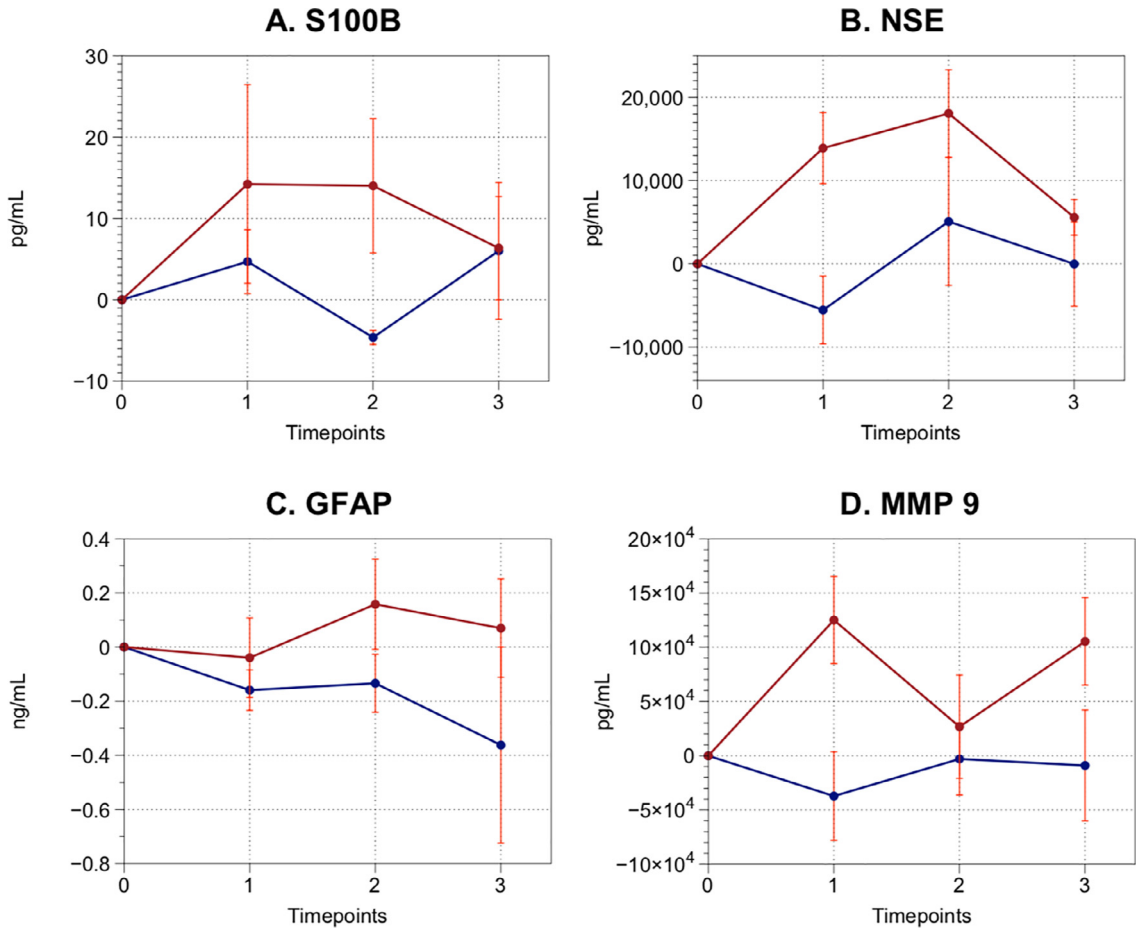

Figure 1. Group mean per patient change from baseline over time (categorical) for biomarker concentrations: S100 calcium binding protein B, or S100B (panel A); neuron specific enolase, or NSE (panel B); glial fibrillary acidic protein, or GFAP (panel C); and matrix metalloproteinase 9, or MMP 9 (panel D). Control group presented in blue and case group in red with the vertical bars representing ± SEM.

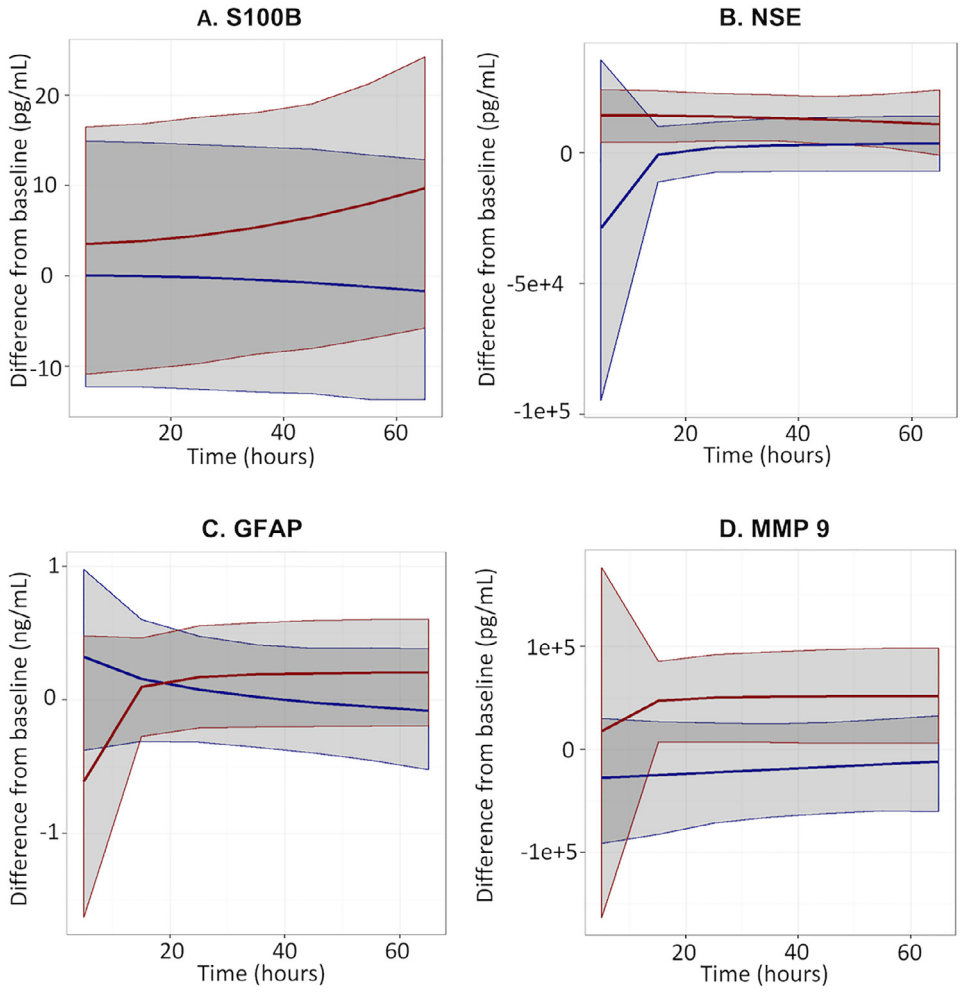

Figure 2. Best fitting polynomial curves over time for the biomarkers: S100 calcium binding protein B, or S100B (panel A); neuron specific enolase, or NSE (panel B); glial fibrillary acidic protein, or GFAP (panel C); and, matrix metalloproteinase 9, or MMP 9 (panel D). Control group presented in blue and case group in red with the shaded area representing 95% confidence intervals. Panel D is reproduced under creative commons license.[5]

including S100B, NSE, MMP 9, and GFAP. Per patient change in NSE and MMP 9 from baseline revealed a significant difference in cases versus controls when using logistic regression analysis and for MMP 9 at the predetermined 24-hour timepoint using a smoothing model to account for the anticipated nonlinear nature of the biomarker kinetics. Both MMP 9 and NSE correlated well within the case group.

Over 58 individual biomarkers have been assessed for their utility detecting clinically apparent ischemic stroke.[7] These have included proteins: (1) released from damaged neurological cells (e.g., S100B and GFAP from astroglia and NSE from neurons); (2) associated with the response to ischemia (e.g., inflammatory proteins such as MMP 9); or, (3) associated with the underlying etiology/pathophysiology (e.g., CRP, BNP, D-dimer, von Willebrand's factor, and fibrinogen). While no blood biomarker can yet be considered validated for diagnosis, management, or prognostication in ischemic stroke the biomarkers chosen for use in this study have been the most consistently favorable.[8-11]

In the setting of clinically apparent ischemic stroke a number of obstacles to peripheral blood biomarker detection and interpretation have been recognized and are also likely to have implications for both the perioperative period and SBI. These include: (1) the capacity to serve as markers for infarction as opposed to other neurological injuries; (2) the degree to which the blood-brain barrier restricts the release of biomarkers into the systemic circulation, (3) release from extra-cerebral tissue.

Cerebral ischemia is a heterogeneous disease resulting from varied etiologies (e.g., large-vessel atherosclerosis, cardioembolic, small vessel disease, and undetermined) and affecting different tissue types (cortical gray matter, subcortical gray or white matter, white matter, cerebellum, brainstem) and vascular distributions.[12] This heterogeneity is believed to be one of the obstacles to clearly validating blood biomarkers of clinically apparent ischemic stroke. However, SBI in the perioperative TAVI setting is a unique exception. Here, infarcts have a relatively distinct onset likely caused by 1 of 2 acute etiologies (cardiogenic or aortic emboli or hypoperfusion).[13,14] Additionally,

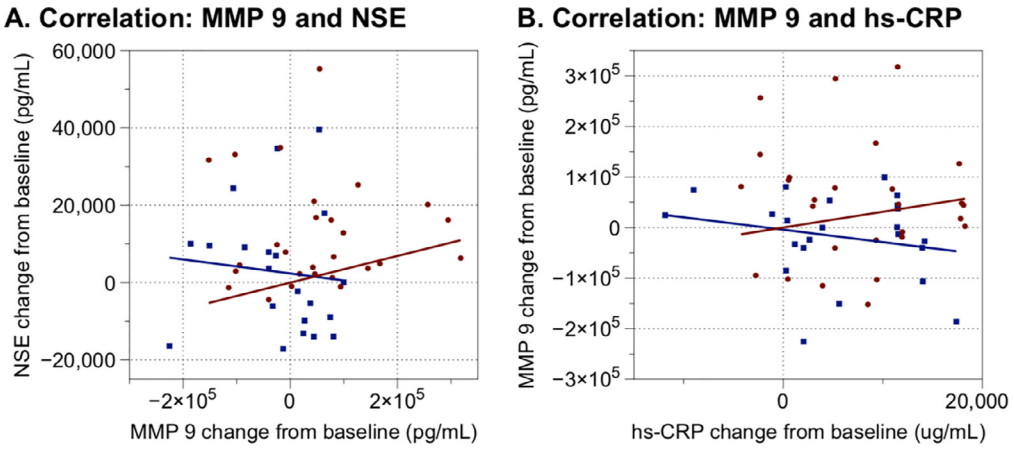

Figure 3. Linear correlation between biomarkers: matrix metalloproteinase 9, or MMP 9 and neuron specific enolase, or NSE (panel A) and MMP 9 and high sensitivity C-reactive protein, or hsCRP (panel B). Control group presented in blue and case group in red with lines representing best linear fits by group.

topographical analysis shows that 80% of SBI in this setting affect the cortical gray matter consistent with our imaging findings.[15] Therefore, our cohort offers a homogenous disease when attempting to identify peripheral blood biomarker signals.

The presence of S100B, NSE, and GFAP in extra-cerebral cells may result in their release after surgical trauma.[16,17] However, the negligible change in neurologic biomarker levels from baseline in our control group suggests minimal extra-cerebral release of biomarkers in the TAVI setting, likely due to the minimally invasive nature of the procedure. Furthermore, surgery is associated with tissue injury, organ ischemia, neurohumoral stress responses, and exposure of blood to foreign surfaces, all potentially causing a systemic inflammatory response and elevated MMP 9 levels. The negligible change in hsCRP from baseline and poor correlation between hsCRP and MMP 9 support that the elevated levels of MMP 9 were not due to a systemic inflammatory response.

Biomarker kinetics profiled in clinically apparent ischemic stroke patients have variably reported that levels of S100B and MMP 9 peak at 24 hours, GFAP at 48 hours and NSE at 72 hours.[9,18,19] Few studies, with conflicting conclusions, have assessed biomarkers of SBI specifically post TAVI. Ghanem et al was unable to identify significant changes in NSE from baseline, despite a 73% incidence of new cerebral lesions on day 3 post-TAVI.[20] In a subsequent study, the same investigators found that NSE increased significantly in 29 of 61 patients postoperatively; but this was not associated with SBI burden suggesting other causes for the increase.[21] However, the timing of serum collection (baseline, 3 days, 3 months post-TAVI) in both studies makes it unlikely that peak NSE levels were measured. Reinsfelt et al, detected significantly elevated S100B in all patients who underwent TAVI, peaking 1-hour postprocedure and returning to baseline within 4 hours.[22] The 24-hour AUC correlated to the number of micro-embolic events on transcranial Doppler; but they did not perform neuroimaging to assess for SBI.[22] No preceding data exists for GFAP or MMP 9 in the TAVI setting. Figures 1 and 2 suggest a similar timing for peak biomarker levels for perioperative SBI as compared with clinically apparent ischemic stroke for all but NSE which peaked earlier (peak 24 to 48 hours).

Although we were able to detect some notable biomarker signals associated with SBI it is important to recognize a number of limitations to our study. First, the small sample size might have diminished statistical power to detect subtle differences in baseline variables and biomarker levels between groups. Second, as discussed, the optimal timepoints for assessing neurological injury biomarkers perioperatively have not yet been determined. As such, the 24-hour sampling intervals and approximately 3-day follow-up might not coincide with the kinetic profiles of these biomarkers in serum. Moreover, while it is assumed that these SBIs occur intraprocedurally, the exact timing remains unknown and may have occurred at any time between the baseline and day 3 ± 1 MRI scan. Finally, our study recruited a highly selected group of homogenous participants and as such, results should not be extrapolated to other settings, including TAVI using different access approaches.

Differences in neurologic biomarkers were evident in the peripherally-sampled blood of our patients with versus without SBI following TAVI. Our targeted profiling identified per-patient change in NSE and MMP 9 from baseline as significantly different in cases versus controls. While no such difference was evident for S100B or GFAP separation between cases and controls when modeling the data using best-fit polynomial curves suggests potential differences between groups with longer follow-up. Future studies are needed to build the evidence base of blood-based biomarkers for detection of perioperative SBI. Specifically, further investigation is warranted to assess the discriminative ability (especially sensitivity) of NSE and MMP 9 in larger cohorts of postprocedural patients.

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Disclosures

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