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NMDA Receptor Antibodies Predict Adverse Neurological Outcome After Cardiac Surgery in High-Risk Patients

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Background and Purpose—The goal of this study was to compare the predictive ability of S100B, *N*-methyl-D-aspartate (NMDA) receptor antibodies (NR2Ab) and C-reactive protein (CRP) for neurological deficits after cardiac surgery with cardiopulmonary bypass (CPB).

Methods—We investigated 557 high-risk adult patients who underwent coronary artery or valve replacement surgery using CPB as a substudy of a prospective, blinded, multicenter clinical trial. Serum concentrations of S100B (n=513 patients), NR2Ab (n=398) and CRP (n=510) were measured preoperatively, 24 and 48 hours after CPB. Neurological adverse events were assessed at baseline and postoperative days 1 and 2; neurocognitive function (mini-mental status examination) was assessed at baseline and on postoperative days 1, 7 and 28.

Results—Fifty-five (9.9%) patients had moderate or severe neurological adverse events (confusion/delirium, transient ischemic attack, or stroke) within 48 hours of CPB. Women had significantly more neurological complications than men (15.5% versus 7.8%; $P=0.007$). Ninety-six percent (24/25) of patients with NR2Ab concentrations ≥2.0 ng/mL preoperatively had neurological complications within 48 hours post-CPB, versus only 5.4% (20/373) of patients with NR2Ab concentrations <2.0 ng/mL, resulting in a 17.9-fold increase (95% CI, 11.6 to 27.6) in postoperative neurological complications for patients with high levels of NR2A antibodies. Preoperative serum S100B and CRP did not predict neurological complications from CPB. Decreased mini-mental status examination scores for orientation, attention and recall were associated with neurological adverse events early after CPB.

Conclusions—Preoperative serum concentrations of NR2Ab, but not S100B or CRP, are predictive of severe neurological adverse events after CPB. Patients with a positive NR2Ab test (≥2.0 ng/mL) preoperatively were nearly 18 times more likely to experience a postoperative neurological event than patients with a negative test (<2.0 ng/mL). **(*Stroke*. 2006; 37:1432-1436.)**

Key Words: brain ischemia ■ cardiac surgery ■ cardiopulmonary bypass

Neurological complications after cardiac surgery are now recognized as a serious and costly healthcare problem. The incidence of stroke ranges between 1% and 5.2% during the perioperative period,[1–5] postoperative delirium occurs in 10% to 30%,[6] and cognitive changes are reported in 33% to 83% of patients.[3,4] The impact on the patient's family, postoperative care, prolonged hospitalization and costs are enormous. Roach et al[6] reported a 6.1% incidence of serious adverse neurological events in a survey of 2108 patients undergoing coronary artery bypass surgery (CABG) at 24 US hospitals. Patients experiencing neurological complications had significantly prolonged hospitalization and 47% were transferred to a chronic care facility. A blood test that can reliably predict who is at risk for neurological complications from heart surgery is presently not available.

During the last decade several biomarkers to predict and diagnose brain injury have been proposed. Serum S100B, a calcium-regulating protein found primarily in glia and Schwann cells, correlates with brain damage after stroke, traumatic brain injury, and cardiac arrest.[7–13] C-reactive protein (CRP), an acute-phase reactant and indicator of underlying systemic inflammation, is a novel plasma marker for atherothrombotic disease and predictor of cerebrovascular disease.[14] Recently, *N*-methyl-D-aspartate (NMDA) receptor peptides and their antibodies have been proposed as biomarkers of neurotoxicity underlying cerebral ischemia and stroke.[15,16] With neuronal

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death or ischemia, subunits (NR2) of the NMDA receptor are degraded, and proteolytic fragments appear in the bloodstream. Many subjects generate an antibody response to the NR2A subunit fragments (NR2Ab) that can be assayed in blood samples.[15] Adult patients who experience acute ischemic stroke have elevated blood levels of NR2Ab that correlate with the amount of brain damage on brain scans (MRI) and neurocognitive status.[16]

The routine application of specific biomarkers with predictive, diagnostic and prognostic capabilities could reduce morbidity and mortality and improve management of cardio- and cerebrovascular patients. The goal of the present study was to investigate the predictive ability of preoperative blood levels of S100B, CRP and NR2Ab for postoperative neurological complications after cardiac surgery using cardiopulmonary bypass (CPB).

Materials and Methods

This was a substudy of a previously reported, prospective, blinded, clinical trial at 29 centers in the US.[17] Approval was obtained from the Institutional Review Boards of all centers, and written informed consent was obtained from all patients. Inclusion criteria were high-risk adult patients (>18 years) undergoing cardiac surgery on CPB such as repeat sternotomy, ejection fraction ≤30%, combined CABG and valve surgery, or urgent CABG or valve surgery. Patients were excluded if they were not expected to survive 7 days after surgery, had renal or hepatic failure, showed inability to cooperate or communicate with the investigator, or a history of noncompliance.

Patients had preoperative testing within 2 days of surgery that included medical history, physical examination, CRP, ECG, and mini-mental status examination (MMSE). On the day of surgery blood was collected before anesthesia for subsequent biomarker analysis. Blood was collected again 24 and 48 hours after termination of CPB for future analysis.

All patients had CPB that was conducted using moderate hypothermia (32°C to 36°C), hemodilution, and cardioplegia as per practice standard at each institution. No attempt was made to alter or standardize anesthetic or surgical techniques. All patients were followed for 30 days after surgery for adverse events and serious adverse events.

Neurological Adverse Events and Testing

Neurological adverse events were assessed from the case report forms at baseline and on postoperative days 1 and 2 at each study site. An adverse event was any unfavorable or abnormal neurological finding, sign or symptom recorded in the patient's case report form that: (1) required treatment or therapeutic intervention; (2) required further diagnostic evaluation; or (3) was associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact. Severe adverse neurological events were those resulting in death, prolonged hospitalization or incapacitation. Only moderately severe and severe adverse neurological events that occurred within 48 hours of surgery were included in this analysis. The assessment of neurological adverse events is expressed as scores of 0, 1 or 9, where 0 indicates no adverse neurological event, 1 indicates anxiety or agitation, and 9 indicates moderate or severe neurological adverse event such as confusion/delirium, transient ischemic attack (TIA), or stroke.

The MMSE was performed at each study site by a qualified neuropsychologist or designee. The preoperative examination was done within 2 days of surgery. The MMSE was repeated at 24 hours, 7 days (or day of discharge) and 28 days after surgery. Scores for all patients were compiled at a core center.

Biological Assays

Blood samples were immediately centrifuged, and serum and plasma were stored at −80°C until assayed. Patients were assigned a study site and enrollment number. There were no patient identifiers on any samples. Serum S100B concentrations were determined at a core laboratory using a monoclonal 2-site immunoradiometric assay (LIA-MAJ Sangtec 100, AB Sangtec Medical) according to the manufacturer's directions. All samples were assayed in duplicate. The precision was <10.0% coefficient of variation. Lower level of detection was 0.1 μg/L, and the upper level was 20.0 μg/L. Serum NR2Ab concentrations were determined by ELISA (CIS-Biotech, Inc) as previously described.[16] Lower level of detection was 0.5 μg/L. Serum CRP was measured at each study site hospital laboratory.

Statistical Analyses

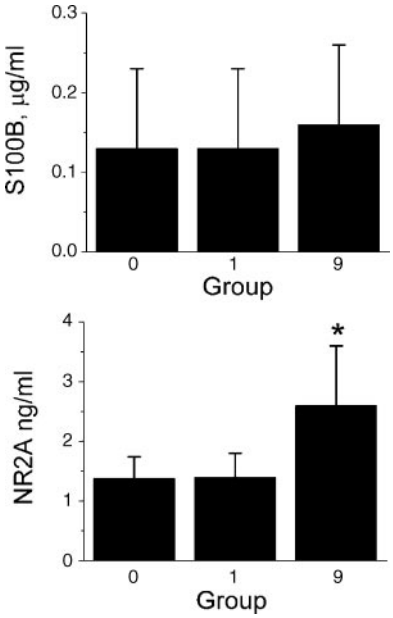
Data were analyzed using SAS version 8.2 by an independent investigator. All patients who had preoperative assay results and neurocognitive examination data were included in the primary analysis. Preoperative S100B test results were classified as positive (≥0.2 μg/mL) or negative (<0.2 μg/mL) based on normal values reported in adults.[18] Preoperative CRP were classified as positive (≥0.8 μg/mL) or negative (<0.8 μg/mL). Preoperative NR2Ab test results were classified as positive (≥2 ng/mL) or negative (<2 ng/mL). The percent of patients within each of these groups who had a postprocedure adverse neurological event within 48 hours of the end of their heart surgery was calculated. The ratio of this risk among patients with a positive preoperative test to the risk among patients with a negative preoperative test defined the risk ratio of the test. This risk ratio represents the increase in predictive ability of a postprocedure adverse neurological outcome as a result of a positive preoperative test result. These results are summarized with the relative risk estimate and its 95% confidence interval. Mean preoperative serum concentrations of S100B and NR2Ab by postoperative neurological status were compared by 1-way analysis of variance. Statistical tests were 2-sided. A Bonferroni adjustment ($P<0.0167$) was used for the 3 pairwise comparisons performed between the 3 types of postoperative neurological response (no events, anxiety or agitation, and confusion/delirium or TIA/stroke). Mean baseline MMSE scores were compared between patients with and without postoperative neurological events by *t* tests.

Results

Most of the 557 high-risk patients were male (74.1%). Median age was 66.8±10.9 years; 22% were diabetics.[17] Fifty-five (9.9%) patients had moderate or severe neurological adverse events associated with brain ischemia (confusion/delirium, TIA or stroke) within 48 hours of CPB. Of these patients, 4 had stroke, 2 had TIA and the remainder were moderate or severe confusion/delirium. Another 41 patients had postoperative anxiety requiring medication. There was no correlation of CPB time, crossclamp time or type of surgery with adverse neurological events.

Results were obtained for S100B in 513 patients, NR2Ab in 398 patients and CRP in 510. There was no significant difference in preoperative mean serum S100B concentrations in patients with and without neurological adverse events (Group 0, 1 and 9; Figure). 17.3% (18/104) of patients with S100B concentrations ≥0.2 μg/mL preoperatively had neurological complications within 48 hours after cardiac surgery with CPB, versus 8.1% of patients with S100B concentrations ≤0.2 μg/mL (Table 1). Therefore, S100B concentration ≥0.2 μg/mL results in a 2.1-fold increase (95% CI, 1.3 to 3.6) of the predictive ability for postoperative neurological adverse events (Table 1).

Preoperative CRP concentrations in patients with and without neurological adverse events demonstrated a 1.2 risk ratio (95% CI, 0.7 to 1.9) showing a low predictive ability of



Preoperative serum S100B and NR2Ab and postoperative neurological events. 0 indicates no neurological event; 1, anxiety or agitation; 9, confusion/delirium, TIA or stroke. Patients in group 9 had significantly higher preoperative serum NR2Ab than groups 0 or 1 ($P=0.0004$). S100B was slightly elevated in group 9 patients, but did not achieve statistical significance (mean±SD: 0.16±0.1 versus 0.13±0.1 for group 0; $P=0.11$).

preoperative CRP for postoperative neurological adverse events (Table 1). This biomarker demonstrated the worst performance characteristics to predict neurological complications after heart CPB.

Ninety-six percent (24/25) of patients with NR2Ab concentrations ≥2.0 ng/mL preoperatively had serious neurological complications (confusion/delirium, TIA, or stroke) within 48 hours post-CPB, versus only 5.4% (19/373) of patients with NR2Ab concentrations <2.0 ng/mL, resulting in a 17.9-fold increase (95% CI, 11.6 to 27.6; Table 1) in the predictive ability of a postoperative neurological complication. Patients with elevated NR2Ab preoperatively were significantly older, 76.0±4 years ($P<0.5$). The incidence of diabetes among patients with NR2Ab ≥2.0 preoperatively was 24% compared with 22% of patients with NR2Ab <2.0. Preoperative NR2Ab levels were significantly ($P=0.0004$) elevated in patients with postoperative serious adverse neurological events but not in patients without neurological events or with agitation/anxiety. The positive and negative predictive values for the NR2Ab marker based on the cutoff point of 2.0 ng/mL were 96.0% (24/25) and 94.6% (353/373) respectively. Therefore, the accuracy of the preoperative NR2Ab marker is high for this particular clinical population of high-risk adults.

TABLE 1. Preoperative S100B, CRP and NR2Ab Concentrations and Postoperative Neurological Events

Preoperative	Neuro Event n/N (%)	No Neuro Event n/N (%)	Risk-Ratio* (95% CI)†
S100B: <0.2 µg/ml	37/456 (8.1%)	419/456 (91.9%)	2.1
S100B: ≥0.2 µg/ml	18/104 (17.3%)	86/104 (82.7%)	(1.3–3.6)
CRP: <0.8	27/240 (11.3%)	213/240 (88.7%)	1.2
CRP: ≥0.8	26/270 (9.6%)	244/270 (90.4%)	(0.7–1.9)
NR2Ab: <2 ng/ml	20/373 (5.4%)	353/373 (94.6%)	17.9
NR2Ab: ≥2 ng/ml	24/25 (96.0%)	1/25 (4.0%)	(11.6–27.6)

*Ratio of event rate among patients with positive preoperative S100B, CRP or NR2Ab divided by event rate among patients with negative preoperative S100B, CRP or NR2Ab, respectively.

†95% CI on the risk ratio.

TABLE 2. Mean Baseline MMSE Scores and Presence of Postoperative Neurological Event (Confusion, TIA or Stroke)

Event 9 Present	n	Total Score	Attent	Lang	Orient	Recall	Regist
Yes	54	26.8	4.3	8.2	9.3	2.0	2.9
No	497	28.3	4.4	8.7	9.6	2.5	3.0
P value		<0.001	0.65	<0.001	0.016	<0.001	0.31

Attent indicates attention; Lang, language; Orient, orientation; Regist, registration.

Women had significantly more neurological complications than men (15.5% versus 7.8%; $P=0.007$). Although the adverse neurological event rate in women was significantly higher than that in men, the predictive ability of the test was somewhat better in men than women because of the higher event rate among women with NR2Ab <2.0 (8.9%) than among men (4.1%). The analysis of risk ratio by age using NR2Ab showed that neurological adverse events occurred in 100% of patients >70 years who had preoperative NR2Ab ≥2.0 ng/mL.

Mean baseline MMSE results were significantly ($P<0.001$) decreased in patients who had subsequent adverse neurological events, in particular, the language, recall and orientation component scores (Table 2). However, MMSE scores alone were not predictive of postoperative adverse neurological events.

Discussion

Cerebral complications are the leading cause of morbidity and disability after cardiac surgery with CPB. This study provides several important findings. First, patients with elevated blood levels of NR2Ab ≥2.0 ng/mL before heart surgery are at very high risk for debilitating neurological complications (confusion/delirium, TIA or stroke) after heart surgery. Second, preoperative serum S100B and CRP are not predictive for adverse neurological complications after heart surgery.

The Ca^{++} binding protein S100B is located predominantly in astoglial cells that make up 50% to 60% of the central nervous system. Astroglia together with endothelial cells act as a supply and protective barrier between the cerebral microvasculature and the neurons.[19] The biological function of S100B is largely unknown, but its release into the blood stream appears to be associated with functional disturbance and increased permeability of the blood–brain barrier (BBB).[20] Seizures, stroke, head trauma, inflammation and CPB have all been shown to cause dysfunction of the BBB accompanied by increased serum S100B concentrations.[7–13,20] Serum concentrations of S100B have been found to correlate with brain damage after cardiac surgery but with significant

limitations. In particular, extracerebral contamination from fat and tissue trauma falsely elevates serum S100B levels.[20] The present study indicates that elevated preoperative serum S100B is weakly predictive of postoperative neurological adverse events. Patients with preoperative S100B >0.2 μg/mL had 2.1 times the risk for confusion/delirium, TIA or stroke after CPB.

Acute phase reactants, especially CRP, are considered risk factors for cardiovascular disease, including myocardial infarction, stroke, and vascular mortality.[21–23] Independent of other cardiovascular risk factors and age, Rost et al[22] reported elevated plasma CRP levels significantly predict the risk of future ischemic stroke and TIA. In another study of ischemic stroke patients, plasma CRP levels at admission were predictors of new vascular events or death at 1 year.[23] These findings are consistent with the hypothesis that elevated CRP may predict future cardiovascular events or death.[23] In the present study, however, elevated plasma CRP preoperatively did not predict ischemic neurological events after heart surgery.

Recently, NMDA receptors and their antibodies have been proposed as biomarkers of neurotoxicity underlying cerebral ischemia and stroke.[18] A sensitivity of 95% (97%) and specificity of 98% (98%) at cut-off values of 2.0 ng/mL were shown in clinical studies for TIA (stroke) respectively.[16] In the present study, patients with serum NR2Ab ≥2.0 ng/mL preoperatively were nearly 18 times more likely to experience a postoperative neurological event than patients with a negative test (<2.0 ng/mL; Table 1). This study also found a higher incidence of adverse neurological events in women than in men (15.5% versus 7.8%), although the predictive ability of the test was somewhat better in men than women attributable to the higher event rate among women with NR2Ab <2.0 preoperatively. All patients >70 years of age with NR2Ab ≥2.0 ng/mL preoperatively had neurological complications.

Whereas NR2Ab may be a marker of cerebral ischemia, S100B is considered a marker of BBB dysfunction. Frequently, the 2 coexist. Ischemic stroke is usually accompanied by BBB dysfunction. However, BBB dysfunction is not necessarily indicative of cerebral ischemia. In the present study, preoperative serum S100B levels were increased but did not achieve statistical significance in the patients who manifested adverse neurological complications after CPB (Figure).

Izykenova et al reported that rats with induced cerebral ischemia exhibited increased plasma concentrations of NMDA receptor peptide fragments within 2 hours of reperfusion and antibody to these receptor fragments within 72 hours.[24] Dambinova et al reported a dramatic increase in NR2Ab within 3 hours of onset of ischemic stroke or TIA in humans.[16] Autoantibodies to the glutamate receptor can rapidly increase in the blood during acute ischemia with dysfunction of BBB.[25] The abrupt increase in NR2Ab within 3 hours in ischemic stroke is analogous to the immune response in patients with allergies. In the present study, elevated NR2Ab in patients before surgery implies existing ongoing silent cerebral ischemia or possibly a remote silent neurological event. Goto et al reported that 56 of 111 neurologically asymptomatic patients over the age of 65 scheduled for coronary artery bypass surgery had preoperative infarctions on cerebral MRI.[26] Furthermore, Rolfson et al reported that patients who have delirium after coronary artery bypass surgery are more likely to have a history of stroke.[27]

This study has several limitations. Validated, standardized tests to diagnose delirium and confusion prospectively were not used. Furthermore, patients with delirium and confusion were identified retrospectively from the list of adverse events in the case report forms. Although we can speculate that patients with elevated NR2Ab preoperatively may have had a previous stroke, there is no supporting radiologic evidence or patient history in the Case Report forms.

The etiology of neurological complications from heart surgery is multifactorial, including intraoperative micro- and macroemboli, abnormal cerebral perfusion, reperfusion injury, inflammatory and neurohumoral responses.[28–30] All patients who undergo CPB are exposed to these factors. Although most patients may have subtle neurocognitive dysfunction after surgery, short-term memory deficits, stroke and acute confusional state (delirium), in particular, occur less frequently. The present study identifies patients preoperatively who may have cerebral ischemic events after cardiac surgery. These results suggest that some patients are more vulnerable to emboli and the rigors of CPB, probably from an already compromised cerebrovascular circulation. Patients known to be at increased risk preoperatively may benefit from medical management, interventions or alternative therapy.

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