# Genomic expression pathways associated with brain injury after cardiopulmonary bypass

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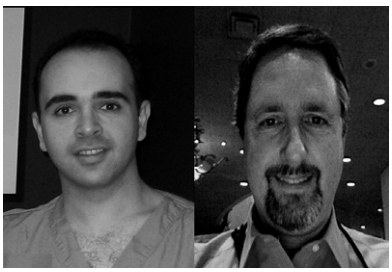

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**Objectives:** Neurologic injury after cardiac surgery, often manifested as neurocognitive decline, is a common postoperative complication without clear cause. We studied acute variations in gene-expression profiles of patients with neurocognitive decline (NCD group) compared with those without neurocognitive decline (NORM group) after cardiopulmonary bypass.

**Methods:** Forty-two patients undergoing coronary artery bypass grafting, valve procedures, or both by using cardiopulmonary bypass were administered a validated neurocognitive battery preoperatively and postoperatively at day 4. Neurocognitive decline was defined as 1 standard deviation from baseline on 25% or greater of tasks. Whole-blood mRNA was isolated preoperatively and at 6 hours after surgical intervention for fold-change calculation. Relative gene expression in the NCD versus the NORM group was assessed by using Affymetrix GeneChip U133 Plus 2.0 (>40,000 genes) from mRNA samples collected. Differential expression, clustering, gene ontology, and canonical pathway analysis were performed. Validation of microarray gene expression was performed with SYBR Green real-time polymerase chain reaction.

**Results:** Patients with neurocognitive decline (17/42 [40.5%] patients) were associated with a significantly different gene-expression response compared with that of healthy patients. Compared with preoperative samples, 6-hour samples had 531 upregulated and 670 downregulated genes uniquely in the NCD group compared with 2214 upregulated and 558 downregulated genes uniquely in the NORM group ($P < .001$; lower confidence bound, $\geq 1.2$). Compared with patients in the NORM group, patients with neurocognitive decline had significantly different gene-expression pathways involving inflammation (including *FAS*, *IL2RB*, and *CD59*), antigen presentation (including *HLA-DQ1*, *TAP1*, and *TAP2*), and cellular adhesion (including *ICAM2*, *ICAM3*, and *CAD7*) among others.

**Conclusions:** Patients with neurocognitive decline have inherently different genetic responses to cardiopulmonary bypass compared with those of patients without neurocognitive decline Genetic variations in inflammatory, cell adhesion, and apoptotic pathways might be important contributors to the pathophysiology of neurologic injury after cardiopulmonary bypass and could become a target for prevention and risk stratification.

Brain injury after cardiopulmonary bypass (CPB) remains a common and serious complication that is often misdiagnosed.[1,2] Brain-protective strategies include improved operative approach selection (eg, "no-touch" technique for the calcified aorta), preoperative investigation (eg, carotid duplex scan-

**Abbreviations and Acronyms**

BP = biologic process
CNS = central neurologic system
CPB = cardiopulmonary bypass
FC = fold change
GO = gene ontology
LCB = lower confidence bound
NCD = neurocognitive decline
NORM = without neurocognitive decline
PRE = preoperatively after induction of anesthesia and before skin incision
SD = standard deviation
6h = 6 hours postoperatively in the cardiovascular intensive care unit

ning), and intraoperative measures (eg, hypothermia and CPB filters). Despite these advances in surgical technique, CPB, and anesthesia, central neurologic system (CNS) injury remains an important complication for patients. The American College of Cardiology/American Heart Association guidelines for coronary artery bypass graft surgery divide postoperative neurologic deficits into 2 categories.[3] Type 1 deficits (incidence of 1%-5%) include major focal neurologic events, stupor, and coma. Type 2 deficits, with an incidence as high as 65% in some studies,[4,5] describe more global cognitive deficits, such as deterioration in intellectual function, memory, and confusion without evidence of focal injury. Type 1 deficits are usually caused by identifiable sources of cerebral hypoxia caused by intraoperative hypoperfusion or embolic phenomena. In contrast, the cause of type 2 deficits is unclear and likely multifactorial, where factors such as hypoxia, time on CPB, age, type of procedure, preoperative creatinine levels, and perioperative inflammatory response have been implicated in its pathophysiology.[6]

The remarkably high incidence of type 2 brain injury (measured at 1 week postoperatively), usually improves and decreases to around 10% to 30% at 1 year. Interestingly, Newman and colleagues[7] have reported that the occurrence of early neurocognitive decline (NCD) in cardiac surgical patients is predictive of long-term decline. One of the reasons why the incidence of type 2 CNS injury remains so high in spite of recent advances might be a fundamental lack in the understanding of the pathophysiology of this type of injury.[1,6]

The inflammatory response and associated oxidative stress have been implicated in the development of postoperative CPB-associated complications.[8-12] These are triggered by the activation of blood components on the artificial surface of the extracorporeal circuit, such as the activation of leukocytes, complement, expression of adhesion molecules, cytokine release, and an increase in reactive oxygen species, such as peroxides, that mediate oxidative stress. It is increasingly recognized that there exists a certain amount of variability in the magnitude and duration of response to CPB between patients, which has been implicated in transient and permanent end-organ damage. Our group, among others, has reported on the strong association between NCD and the magnitude of the inflammatory response after CPB.[13,14]

Transcriptional profiling with high-density microarrays provides unique data about disease mechanisms, drug responses, regulatory pathways, and gene function by comparing the level of mRNA transcribed in cells in a given pathologic state versus a control. This technology can potentially elucidate complex pathophysiologic association mechanisms directly at the gene-expression level. The present study was conducted to examine the differences in gene-expression profiles of patients who have NCD after cardiac surgery compared with those who do not have this complication in an effort to improve our understanding of type 2 brain injury and provide perioperative strategies aimed at prevention and treatment.

## Materials and Methods

### Patient Enrollment

We carried out a single-institution, prospective cohort study that was approved by the Beth Israel Deaconess Medical Center Institutional Review Board/Committee on Clinical Investigations. Forty-three patients scheduled for elective or urgent primary coronary artery bypass grafting, valvular surgery (aortic or mitral), or a combination of the 2 with CPB provided informed written consent and were enrolled. Exclusion criteria included the following: patients undergoing aortic arch/root procedures and those with calcified aorta, recent stroke, severe preoperative neurologic deficits, known high-grade carotid stenosis, advanced hepatic disease (cirrhosis), and chronic renal failure (serum creatinine, >2.0 mg/dL). Patients who were unable to complete the baseline neuropsychological battery because of severe cognitive impairment, psychiatric disease, substance abuse, blindness, or poor English were also excluded. One patient was excluded from the analysis because of inability to complete the neuropsychological assessment before discharge. Thus 42 patients were included in the analysis.

### Anesthetic and Surgical Techniques

The conventional operative approach at our institution was followed, including induction of general anesthesia, invasive monitoring, midline sternotomy, and systemic heparinization. CPB was initiated through cannulation of the right atrium and ascending aorta with a nonpulsatile system, membrane oxygenator, and 40-$\mu$m arterial filter. Crystalloid pump prime was used. For all patients, mild hypothermic CPB (minimum temperature, 32°C-34°C) with intermittent cold blood hyperkalemic (25 mmol/L) cardioplegia was used. Serum glucose levels were monitored, and we attempted to maintain a value of less than 130 mg/dL by means of intermittent intravenous insulin injections or insulin infusion. During CPB, pump blood flow was maintained at 2 to 2.4 $L \cdot min^{-1} \cdot m^{-2}$ body surface area. Arterial partial oxygen

CSP