

Platelet PI^{A2} Polymorphism Enhances Risk of Neurocognitive Decline After Cardiopulmonary Bypass

Joseph P. Mathew, MD, Christine S. Rinder, MD, J. Greg Howe, PhD, Manuel Fontes, MD, Jill Crouch, MHS, Mark F. Newman, MD, Barbara Phillips-Bute, PhD, Brian R. Smith, MD and the Multicenter Study of Perioperative Ischemia (McSPI) Research Group

Departments of Laboratory Medicine and Anesthesiology, Yale University School of Medicine, New Haven, Connecticut, and Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina

Background. Neurocognitive decline, often produced by atherosclerotic plaque embolization, remains a frequent complication of cardiopulmonary bypass. Plaque fragments may initiate local thrombosis, which, in turn, aggravates the embolic insult. Prothrombotic genetic factors may exacerbate this process. We investigated whether the PI^{A2} polymorphism of platelet GPIIIa, a prothrombotic risk factor in other cardiovascular settings, is associated with early neurocognitive decline after cardiopulmonary bypass.

Methods. Neurocognitive changes were evaluated by the Mini-Mental State Examination administered preoperatively and on postoperative day 4 and the PI^A genotype determined in 70 patients undergoing cardiopulmonary bypass.

Results. Forty-nine patients were $PI^{A1/A1}$, and 21 were $PI^{A1/A2}$ or $PI^{A2/A2}$. Fifty-two patients (74%) demonstrated

post-cardiopulmonary bypass neurocognitive decline, of which 34 were $PI^{A1/A1}$ and 18 were $PI^{A1/A2}$ or $PI^{A2/A2}$. Multivariate analysis revealed that the PI^{A2} genotype and baseline Mini-Mental State Examination were significantly associated with greater neurocognitive decline (decreased Mini-Mental State Examination scores, $p = 0.036$ and 0.024 , respectively).

Conclusions. This study demonstrates a link between the PI^{A2} allele of platelet GPIIIa and more severe neurocognitive decline after cardiopulmonary bypass. Although the mechanism is unknown, it could represent exacerbation of platelet-dependent thrombotic processes associated with plaque embolism.

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Neurologic complications, most commonly resulting from atherosclerotic plaque embolism [1], contribute significantly to the morbidity of cardiopulmonary bypass (CPB) surgical procedures. Major neurologic deficits are found in 6% of patients who, in turn, experience a 10-fold greater mortality compared with neurologically intact patients undergoing CPB [2]. In addition to major deficits, up to 69% of patients exhibit subtle neurocognitive changes [3] that, although less catastrophic, can nevertheless be devastating to their quality of life.

The variability in CPB-induced neurologic dysfunction mandates the use of comprehensive standardized neuropsychologic tests for neurologic assessment [4]. The Mini-Mental State Examination (MMSE) is the most widely accepted psychometric test of cognitive performance [5] for assessing neurologic disease prevalence and tracking subtle neurocognitive decline in progressive disorders, eg, Alzheimer's disease. Its ease of administration, high sensitivity to cognitive dys-

function, and correlation with functions central to activities of daily living make the MMSE suitable for evaluating early post-CPB neurobehavioral changes [5].

Alterations in platelet glycoprotein (GP) receptors may contribute to the pathophysiology of vascular events. PI^{A2} , a polymorphism of the GPIIIa constituent of the platelet integrin receptor, GPIIb/IIIa, has been proposed as one risk factor for myocardial infarction [6]. Although still controversial [7], studies of PI^A allelic frequencies in patients with coronary artery disease with and without coronary thrombosis [8] suggest that the PI^{A2} polymorphism may predispose to increased thrombogenicity. A recent study demonstrating increased PI^{A2} prevalence in young atherosclerotic stroke patients now suggests an enhanced risk for cerebrovascular thrombosis in PI^{A2} positive individuals [9]. We hypothesize that the cerebrovascular insult of CPB may be exacerbated by platelet-dependent factors, such as the PI^{A2} polymorphism. The current study examined whether the PI^{A2} polymorphism correlates with increased risk of early neurocognitive decline after CPB.

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Address reprint requests to Dr. Rinder, Department of Anesthesiology, Yale School of Medicine, PO Box 208051, 333 Cedar St, New Haven, CT 06520-8051; e-mail: christine.rinderyale.edu.

Material and Methods

Patient Selection and Conduct of Cardiopulmonary Bypass

After Human Investigation Committee approval and informed consent, 70 consecutive adults undergoing CPB at Yale-New Haven Hospital who were enrolled in the Multicenter Study of Perioperative Ischemia Research Group's prospective study of post-CPB outcomes were studied. All patients underwent CPB using a membrane oxygenator, roller pumps, and cardiotomy suction. Transesophageal echocardiography examination was performed after anesthetic induction using a Hewlett-Packard Multiplane transesophageal echocardiography probe to grade aortic pathology [10].

Neuropsychological Testing

The MMSE was chosen for this multicenter study because of its ease of administration, brief test duration, and reliability in different ethnic groups [5], making it suitable for a multicenter study of early neurocognitive changes. Although a battery of neurocognitive tests may give a more comprehensive assessment of the neural injury [11], the investigators elected to restrict the testing to facilitate comparisons between different centers. At Yale-New Haven Hospital, the MMSE was administered preoperatively and again on postoperative day 4 by the same individual to minimize any variability attributable to the test-giver, and to minimize any effects of sedation or perioperative medications. All MMSE changes (improvements and decrements) were analyzed, as early retesting may produce a training effect [11].

Determination of PI^A Genotypes

Blood drawn into tubes containing 5 mmol/L ethylenediaminetetraacetic acid was spotted onto sterile filter paper and dried [12]. Disks cut out of the blood spot were placed in polymerase chain reaction tubes with 20 μ L of methanol. After drying overnight, two separate polymerase chain reaction reactions were performed for PI^{A1} and PI^{A2} as reported by Skogen and associates [13]. Amplification products were electrophoresed onto a 2:1 Nusieve: Seakem agarose (FMC Bioproducts, Rockland, ME) gel in TBE (Tris, Boric acid, EDTA [ethylenediaminetetraacetic acid]) buffer. Primers for β -actin were included as a control [14].

Platelet and Leukocyte Activation

Four blood samples were drawn into fixative (1% paraformaldehyde) (1) at the start of surgery, (2) before and (3) after aortic cross-clamp release, and (4) on arrival in the intensive care unit. Platelet and leukocyte activation were examined by flow cytometry exactly as previously reported [15] using monoclonal antibodies to CD62P and CD11b, respectively.

Statistics

Genotype was categorized by the presence or absence of the PI^{A2} allele as in previous studies [6, 8]. PI^{A1} homozygotes were compared by unpaired two-sided Student's *t* test to PI^{A2} heterozygotes or homozygotes for character-

Table 1. Baseline Patient Demographics

Variable	PI ^{A1/A2} and PI ^{A2/A2} (n = 21)	PI ^{A1/A1} (n = 49)	<i>p</i> Value
Mean age (years)	69.0 \pm 10.0 (SD)	64.7 \pm 10.7	0.13
Women (%)	28.5%	18.4%	0.17
Patients with \geq grade III AS ^a	2/17 (12%)	3/44 (7%)	0.54
Diabetes mellitus (%)	40.9%	31.4%	0.44
Previous stroke (%)	22.7%	11.8%	0.24
Basal MMSE score (maximum of 30)	26.6 \pm 2.9	26.2 \pm 1.8	0.57
Bypass time (min)	96 \pm 25	101 \pm 29	0.47
Cross-clamp time (min)	67 \pm 24	68 \pm 24	0.92
Lowest temperature during CPB	30 \pm 2	30 \pm 2	0.47
Percentage undergoing valvular surgery	9.1%	9.8%	0.92
Number of patients receiving aprotinin	1	1	NA
Years of education ^b	13.5 \pm 4.0	14.2 \pm 3.1	0.51

^a Atherosclerosis of ascending aorta; transesophageal echocardiographic examination not diagnostic on 9 patients. ^b Data available on 20 of 21 PI^{A1/A2} and PI^{A2/A2} patients and 45 of 49 PI^{A1/A1} patients.

CPB = cardiopulmonary bypass; MMSE = Mini-Mental State Examination; NA = not applicable.

istics identified as preoperative neurocognitive risk factors [16]. The change in MMSE scores from the preoperative to the postoperative test (Δ MMSE) was analyzed as a continuous variable. Multiple linear regression using SAS software (SAS Institute, Cary, NC) evaluated the effect of PI^{A2} and circulating activated platelets and leukocytes on the Δ MMSE, with baseline MMSE performance and age as covariates.

Results

The prevalence of preoperative risk factors for post-CPB cognitive decline was comparable for PI^{A1} homozygotes versus PI^{A1/A2} and PI^{A2/A2} patients (Table 1). Patients were largely white (93%), and the PI^A genotype distribution, 49 PI^{A1/A1} (70%), 18 PI^{A1/A2} (26%), and 3 PI^{A2/A2} (4%) was comparable to previously reported studies [9].

Follow-up neurologic testing on postoperative day 4 determined that the average change in MMSE scores (Δ MMSE) was -3.8 ± 5.3 for all patients. Fifty-two patients (74%) demonstrated some degree of neurocognitive decline (range, -1 to -29). There was a significant univariate relationship between Δ MMSE and PI^{A2} genotype by multiple linear regression analysis ($p = 0.029$). Multivariate analysis similarly demonstrated that both PI^{A2} genotype and baseline MMSE were significantly associated with the Δ MMSE ($p = 0.024$ and 0.037 , respectively); age was not predictive of Δ MMSE. Figure 1 shows all 70 patients separated into four quartiles on the basis of Δ MMSE and demonstrates the proportion of PI^{A1} homozygotes versus PI^{A2} heterozygotes and homozygotes in each quartile. First quartile patients demonstrated either no change in MMSE or slightly improved scores (range 0 to +6), as sometimes noted with repeat MMSE

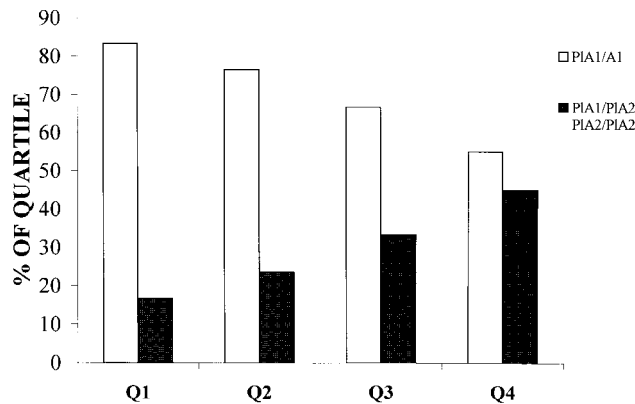


Fig 1. Association of PI^A genotypes with the change in Mini-Mental State Examination scores from the preoperative to the postoperative test (Δ MMSE). Patients were divided into quartiles by Δ MMSE. For each quartile, PI^{A1/A1} are represented by the open bars, and PI^{A1/A2} and PI^{A2/A2} by solid bars. (Q1 = improved or unchanged MMSE [range, 0 to +6; n = 18]; Q2 = slightly worse Δ MMSE [range, -1 to -3; n = 17]; Q3 = moderately worse Δ MMSE [range, -4 to -5; n = 15]; Q4 = most severe Δ MMSE [range, -6 to -29; n = 20].)

(training effect) [11]. Δ MMSE scores of second quartile patients were -1 to -3. PI^{A1/A2} and PI^{A2/A2} patients represented 17% and 24% of the first and second quartiles, respectively. Δ MMSE scores for third and fourth quartile patients were -4 to -5 and -6 to -29, respectively; 33% of third quartile and 45% of fourth quartile patients were PI^{A1/A2} and PI^{A2/A2}, confirming progressive enrichment for the presence of PI^{A2} as the post-CPB Δ MMSE worsened.

The percentage of circulating activated platelets increased over the course of CPB as previously detailed [15]. Univariate analysis revealed a significant association between the change in platelet activation and Δ MMSE ($p = 0.003$); this association, however, was not statistically significant by multivariate analysis ($p = 0.13$). The changes in platelet activation over time are shown in Figure 2, expressed as a percentage of their baselines, with patients divided according to their PI^A genotype. Both groups demonstrated a comparable activation response to the stimulus of CPB, and overall the two groups were not statistically different. On the first postoperative day, however, the percentage of circulating activated platelets in the PI^{A1} homozygotes approached baseline, whereas in the PI^{A1/A2} and PI^{A2/A2} patients, circulating activated platelets persisted, suggesting either an ongoing prothrombotic process or decreased clearance of the already activated platelets. Circulating monocytes and neutrophils demonstrated increased surface expression of the β 2 integrin, CD11b, over time, but these were not significantly associated with the Δ MMSE in either univariate or multivariate analysis ($p > 0.1$).

Comment

Neurocognitive decline is frequent after CPB, with alterations present in nearly 70% of patients soon after the operation [3]. This pilot study demonstrated that patients who carry the PI^{A2} allele demonstrated more severe early

neurocognitive decline compared with PI^{A1} homozygotes. Whether this allele predisposes to plaque embolization, thrombotic processes aggravating the initial injury, or other factors contributing to neurocognitive decline is uncertain. GPIIIa is the β_3 subunit common to the platelet fibrinogen receptor, GPIIb/IIIa ($\alpha_{IIb}\beta_3$ integrin), and the platelet and endothelial vitronectin receptor ($\alpha_v\beta_3$ integrin). The PI^{A2} polymorphism results from a Leu³³→Pro substitution in the GPIIIa amino terminus [17], and a recent study [18] demonstrating a lower activation threshold for PI^{A2} platelets suggests one mechanism for the allele's prothrombotic effects. In the present study, increased platelet activation correlated with a greater decline in neurocognitive outcome in the univariate analysis suggesting that this may represent part of the PI^{A2} association with adverse neurologic outcome, but this did not hold up in the multivariate analysis. The greater percentage of activated platelets on postoperative day 1 in the PI^{A2+} patients suggests that postoperative events and processes may account for part of the enhanced risk conferred by this polymorphism, and the postoperative period deserves critical attention in further follow-up studies.

Weiss and colleagues [6] first demonstrated a greater prevalence of the PI^{A2} allele in coronary thrombosis patients, and others [8, 19] have supported this finding. However, the association of PI^{A2} and non-CPB stroke risk remains uncertain, with Carter and coworkers [9] demonstrating increased PI^{A2} prevalence in stroke patients, whereas Carlsson and associates [20] found no allelic enrichment of PI^{A2} among patients with stroke. Larger patient numbers and restriction to patients with ischemic stroke by Carter and coworkers [9] may explain this discrepancy in findings.

Neurobehavioral change after cardiac surgical proce-

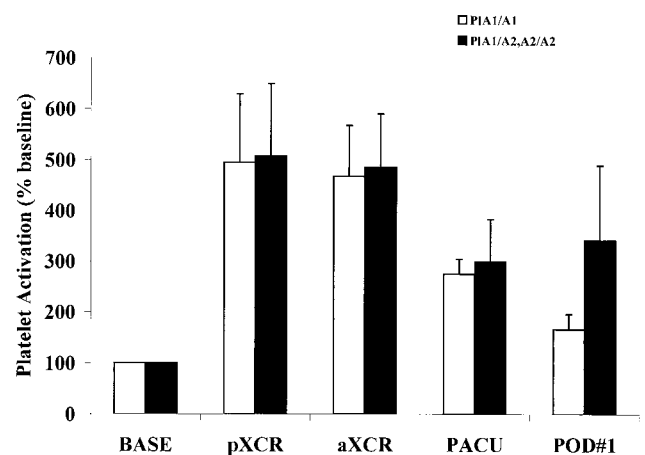


Fig 2. Platelet activation during cardiopulmonary bypass in patients according to PI^A genotype. The circulating activated platelets (CD62P+ platelets) expressed as a percentage of baseline value are shown at the following time points: baseline (BASE), immediately before cross-clamp release (pXCR), 5 minutes after cross-clamp release (aXCR), immediately postoperatively (PACU), and on the morning of the first postoperative day (POD#1). For each time point, PI^{A1/A1} patients are represented by the open bars, and PI^{A1/A2} and PI^{A2/A2} by solid bars.

dures represents a major complication of CPB, despite changes in the conduct of bypass aimed at reducing its frequency [21]. Pharmacologic interventions have been investigated, with some investigators asserting that CPB represents an ideal testing ground for neuroprotective agents given its high frequency of adverse outcomes and the ability to medicate prophylactically [22]. These and other similar studies have given investigators an appreciation of the subtlety and complexity of the neurologic changes produced by CPB, and a recent consensus conference published considerations that should be entertained when selecting neuropsychologic tests in this setting [11]. The Multicenter Study of Perioperative Ischemia Research Group investigators were cognizant of these recommendations, but were constrained by needs unique to a multicenter study design. The MMSE is somewhat restricted in its range, with greater sensitivity to moderate-to-severe cognitive impairment, particularly in the verbal domain [5]. However, the proven applicability of the MMSE in many nationalities, its 25-year track record in evaluating progressive neurocognitive decline stemming from multiple causes, the minimal training needed for the test-giver, and its brevity [5] made it the best choice for an international study of neurocognitive changes early after CPB. As demonstrated graphically in Figure 1, fully 25% of patients had no detectable worsening in their neurologic status by MMSE. Another 25% had only a modest change in MMSE scores, typical for the average change found over more than 28 months time in an epidemiological study of a population aged 75 years and older [23]. However, the next lower 25% of patients demonstrated even more severe changes, and the worst 25% showed a decline in scores in the range associated with major neurobehavioral deterioration in Alzheimer's disease [24]. Follow-up testing at a later date will be performed to determine what proportion of these changes are transient.

Our study has demonstrated a link between the PI^{A2} allele and neurocognitive decline early after CPB, suggesting that the PI^{A2} polymorphism may exacerbate pre-existing vascular pathologies. Larger studies will be needed to confirm this association and to determine whether PI^{A2} predicts a higher incidence of permanent neurocognitive deficits.

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