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Atherosclerosis is not a risk factor for anti-platelet factor 4/heparin antibody formation after cardiopulmonary bypass surgery

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Heparin-induced thrombocytopenia (HIT) is a prothrombotic disorder mediated by antibodies against complexes of platelet factor 4 (PF4) and heparin (1). HIT affects 0.5-1.4% of patients following cardiac surgery (2-4) and is associated with poor outcomes (5). Anti-PF4/heparin antibody formation is common after cardiopulmonary bypass (CPB), occurring in 22-51% at 5 days, 53-75% at 10 days, and 52% at 6 weeks (2-4,6,7). Although the frequency of seropositivity greatly exceeds the prevalence of clinical disease, PF4/heparin antibody formation is a prerequisite for development of HIT. An understanding of risk factors for seroconversion might allow for prospective identification of individuals at highest risk and implementation of targeted monitoring and prevention strategies.

Anti-PF4/heparin IgG is detected in previously heparin-naïve patients as early as 4 days after CPB, often without antecedent IgM (8). This early IgG response suggests preimmunization to antigenic epitopes on PF4. Recent work has focused on the potential exposure of such epitopes upon binding of PF4 to gram-negative bacteria (9). Immune sensitization could also occur as a result of platelet activation and PF4 release at sites where antigen presenting cells are present, such as atherosclerotic plaques. We previously identified PF4 in carotid endarterectomy specimens and demonstrated an association between PF4 deposition and severity of atherosclerosis (10). We hypothesized that atherosclerosis may sensitize the immune system to PF4 and predict anti-PF4/heparin seroconversion after CPB. We tested this hypothesis in a prospective cohort study.

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Disclosure of Conflicts of Interest

The authors state that they have no conflict of interest.

Consecutive adults scheduled for elective CPB surgery at Penn-Presbyterian Medical Center were enrolled. Exclusion criteria included a history of HIT, circulating anti-PF4/heparin antibodies prior to surgery, or ongoing treatment with heparin. The protocol received ethics board approval. All subjects provided written informed consent. Demographic and clinical information was collected before surgery, during hospitalization, at a telephone interview on post-CPB day 15, and at a post-CPB day 35 study visit. Patients had the option of participating in the day 35 visit in person or by telephone. All subjects received unfractionated heparin (UFH) during CPB surgery per institutional protocol. All treatment decisions, including postoperative heparin use, were made by the treating clinicians.

Anti-PF4/heparin antibodies were measured preoperatively and on post-CPB days 1, 5, and 35 using a polyspecific (detects IgG, IgA, and IgM) and IgG-specific ELISA (Hologic Gen-Probe GTI Diagnostics, Waukesha, WI). An optical density (OD) 0.4 (the manufacturer-recommended cut-off) was considered positive for both assays. The primary endpoint was anti-PF4/heparin seroconversion on post-CPB day 5, defined as a negative polyspecific ELISA prior to surgery and a positive polyspecific ELISA on post-CPB day 5. Seroconversion by IgG-specific ELISA on post-CPB day 5 and by polyspecific and IgG-specific ELISA on post-CPB day 35 were secondary endpoints. Because clinical HIT occurs almost exclusively in patients with a strongly positive ELISA, we also specified "high" seroconversion (OD 1.0) on post-CPB days 5 and 35 as secondary endpoints.

All subjects underwent preoperative coronary angiography. Angiograms were adjudicated by an experienced interventional cardiologist and scored as follows: 0 (no atherosclerosis), 1 (<20% stenosis), 2 (20-50% stenosis), 3 (>50% stenosis). The adjudicator was blinded to ELISA results and the clinical course.

Eighty-six subjects enrolled. Nineteen were excluded because the preoperative ELISA was positive (n=11), surgery was performed off-pump (n=1), or a post-CPB day 5 blood sample was not collected due to refusal (n=2) or hospital discharge (n=5). The remaining 67 subjects were included. A post-CPB day 35 blood sample was collected from 48 subjects, who presented for the optional in-person day 35 study visit. The remaining 19 subjects completed day 35 follow-up by telephone.

The mean age was 62 years. Subjects were predominantly male (62.7%) and Caucasian (92.5%). Cardiovascular risk factors including diabetes mellitus (23.9%), hypertension (62.7%), dyslipidemia (64.2%), and smoking history (46.3%) were prevalent. Preoperative coronary angiography showed grade 0, 1, 2, and 3 atherosclerosis in 17 (25.4%), 16 (23.9%), 19 (28.4%), and 15 (22.4%) subjects, respectively. Eight (11.9%) subjects received UFH during angiography. Sixty (89.5%) subjects underwent valve surgery, 4 (6.0%) coronary artery bypass grafting (CABG), and 3 (4.5%) combined CABG and valve surgery. Forty-two (62.7%) subjects received postoperative UFH between post-CPB days 1 and 4.

Twenty-six (38.8%) subjects met the primary endpoint. Plasma from 6 (9.0%) of these subjects exhibited high polyspecific seroconversion. IgG seroconversion and high seroconversion were observed in 9 (13.4%) and 2 (3.0%) subjects at day 5, respectively. Of the 48 subjects who provided a post-CPB day 35 sample, 29 (60.4%) were seropositive by

polyspecific and 19 (39.6%) by IgG-specific ELISA. Nine (18.8%) exhibited high seroconversion by polyspecific and 8 (16.7%) by IgG-specific ELISA.

Table 1 shows baseline patient and treatment characteristics and clinical outcomes, stratified by the primary endpoint. None of these variables was predictive of the primary or secondary (data not shown) endpoints. The prevalence of any coronary atherosclerosis was 69.2% in subjects who met and 78.1% in subjects who did not meet the primary endpoint ($p=0.57$). Severity of atherosclerosis was also similar between groups ($p=0.66$). Neither the presence nor grade of atherosclerosis was predictive of IgG seroconversion or polyspecific or IgG-specific high seroconversion at post-CPB day 5 or seroconversion at day 35. None of the study cohort was diagnosed with HIT after surgery. Clinical outcomes were similar among patients who did and did not meet the primary endpoint.

To our knowledge, this is the first study to assess the contribution of atherosclerosis to anti-PF4/heparin antibody formation after CPB. Neither the presence nor severity of atherosclerosis predicted postoperative seroconversion. This observation is consistent with findings from the pediatric literature. In a study of 75 children with congenital heart disease undergoing reoperation on CPB, a population with a presumably very low prevalence of atherosclerosis, the rate of anti-PF4/heparin seroconversion at post-CPB day 10 was 52% (11). These results and our findings suggest that factors other than atherosclerosis are likely to be the major drivers of the PF4/heparin immune response after CPB. Rates of seroconversion at post-CPB days 5 and 35 in our study were similar to those reported by other investigators (2-4,6,7). Limitations of our study include a relatively small study population recruited from a single institution. At α 0.05 and β 0.2, our study was powered to detect a 33% greater seroconversion rate in the atherosclerosis group. We cannot exclude a smaller effect size of atherosclerosis on seroconversion. Patient characteristics, type of surgery, and intra- and postoperative heparin use vary among centers and may influence the likelihood of seroconversion.

We conclude that atherosclerosis is not a major risk factor for anti-PF4/heparin seroconversion after CPB surgery. PF4 deposited in atherosclerotic plaques may not undergo the conformational alterations and exposure of antigenic epitopes necessary for immune sensitization.

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Table 1
Patient and treatment characteristics and clinical outcomes stratified by anti-PF4/heparin seroconversion at 5 days after cardiopulmonary bypass (CPB) surgery

Characteristic	Anti-PF4/heparin seroconversion at post-CPB day 5		P
	No (n=41)	Yes (n=26)	
Baseline demographic and clinical characteristics			
Age (years) ^a	64.4 ± 13.5	58.7 ± 13.1	0.89
Female ^b	13 (31.7%)	12 (46.2%)	0.30
BMI (kg/m ²) ^c	27.1 (25.1, 29.7)	27.1 (23.8, 31.4)	0.95
Diabetes mellitus ^b	11 (26.8%)	5 (19.2%)	0.57
Hypertension ^b	27 (65.9%)	15 (57.7%)	0.61
Dyslipidemi ^b	28 (68.3%)	15 (57.7%)	0.44
Ever smoker ^b	16 (39.0%)	15 (57.7%)	0.21
History of myocardial infarction ^b	5 (12.2%)	1 (3.8%)	0.39
History of ischemic stroke ^b	3 (7.3%)	0	0.28
History of peripheral arterial disease ^b	1 (2.4%)	2 (7.7%)	0.56
Preoperative platelet count (× 10 ⁹ /L) ^a	220 ± 50	214 ± 52	0.66
Preoperative polyspecific ELISA (optical density units) ^c	0.21 (0.17, 0.26)	0.26 (0.19, 0.33)	0.14
Preoperative IgG-specific ELISA (optical density units) ^c	0.13 (0.11, 0.19)	0.12 (0.10, 0.15)	0.22
Atherosclerosis			
Any atherosclerosis ^b	32 (78.1%)	18 (69.2%)	0.57
Grade ^d			0.66
0	9 (22.0%)	8 (30.8%)	
1	9 (22.0%)	7 (26.9%)	
2	12 (29.3%)	7 (26.9%)	

Characteristic	Anti-PF4/heparin seroconversion at post-CPB day 5		P
	No (n=41)	Yes (n=26)	
3	11 (26.8%)	4 (15.4%)	
<i>Treatment characteristics</i>			
UHF during cardiac catheterization ^b	7 (17.1%)	1 (3.8%)	0.14
Type of surgery ^d			0.08
CABG	4 (9.8%)	0	
Valve surgery	34 (82.9%)	26 (100%)	
CABG + valve surgery	3 (7.3%)	0	
Cardiopulmonary bypass time (mins) ^c	121 (88, 144)	130 (99, 171)	0.35
Cross-clamp time (mins) ^c	85 (60, 107)	90 (64, 128)	0.30
Intraoperative UFH dose (units) ^c	30000 (25000, 35500)	30000 (24750, 36500)	0.93
Any post-CPB UFH exposure ^{b,e}	24 (58.5%)	18 (69.2%)	0.44
Days of post-CPB UFH exposure ^{c,e}	2.0 (0, 3.0)	2.5 (0, 3.0)	0.79
<i>Clinical outcomes</i>			
Days on ventilator ^c	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	0.44
Days in intensive care ^c	2.0 (1.0, 2.0)	2.0 (1.0, 2.3)	0.99
Length of stay after surgery ^c	7.0 (5.0, 9.0)	7.0 (6.0, 8.3)	0.61
Venous thromboembolism ^{b,f}	1 (2.4%)	1 (3.9%)	1.00
Myocardial infarction ^{b,f}	0	0	-
Stroke ^{b,f}	0	1 (3.9%)	0.39
Sternal wound infection ^{b,f}	1 (2.4%)	0	1.00
Renal replacement therapy ^{b,f}	1 (2.4%)	0	1.00
Reoperation ^{b,f}	4 (9.8%)	3 (11.5%)	1.00

Characteristic	Anti-PF4/heparin seroconversion at post-CPB day 5		<i>P</i>
	No (n=41)	Yes (n=26)	
Readmission ^{bf}	7 (17.1%)	2 (7.7%)	0.46

BMI, body mass index; CABG, coronary artery bypass grafting; UFH, unfractionated heparin

^a Mean $\pm$ SD (p-values are from a 2-tailed *t* test)

^b p-values are from a 2-tailed Fisher's exact test

^c Median $\pm$ interquartile ranges (p-values are from a 2-tailed Mann-Whitney *U* test)

^d p-values are from a 2-tailed chi-square test

^e During post-CPB days 1 to 4 (inclusive)

^f By post-CPB day 35