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Risk Factor Analysis for Positive Postoperative Anti-platelet Factor 4/heparin Antibody in Cardiac Surgery

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Heparin-induced thrombocytopenia (HIT) is a serious, immune system-mediated complication of heparin therapy. Antibody to the platelet factor 4/heparin complex is linked to the pathogenesis of HIT. However, there is insufficient information on anti-PF4/heparin antibody (HIT antibody) in cardiac surgery. In this study, we examined the occurrence of HIT and HIT antibody, seroconversion and the risk factors. We selected 28 patients who underwent cardiac procedure following catheterization within 3 months. An enzyme-linked immunosorbent assay (ELISA) for the antibody was measured using preoperative and postoperative serum samples. We could not identify patients with clinical HIT. The incidence of preoperative HIT antibody was 4%. Six of the 28 (21%) patients changed to positive postoperative assay from negative preoperative assay. Comparison of preoperative laboratory data and surgical details did not show significant differences between the patients with positive assays and those with negative assays on postoperative day 10, except total globulin. Preoperative total globulin of positive antibody assay was higher than that of negative antibody assay (2.89 ± 0.33 g/dl versus 2.42 ± 0.50 g/dl, p=0.030) and was related to positive postoperative antibody (p=0.048; odds ratio, 12.09; 95% confidence interval, 1.02-144.06). The rate of seroconversion was low, and elevated level of preoperative total globulin may be the risk factor for positive postoperative HIT antibody.

Key words: heparin-induced thrombocytopenia,
anti-PF4/heparin antibody, total globulin, cardiac surgery, seroconversion

Introduction

Unfractionated heparin (UFH) is the anticoagulant of choice in cardiac surgery requiring cardiopulmonary bypass (CPB) because of its efficacy in preventing thrombosis, ease of monitoring using activated clotting time (ACT), reversibility with protamine and accumulated clinical experience. However, UFH is quite immunogenic. Pouplard et al measured anti-platelet factor 4 (PF4)/heparin antibody (heparin-induced thrombocytopenia antibody; HIT antibody) by enzyme-linked immunosorbent assay (ELISA) before surgery and 3 times after surgery in 328 patients with cardiopulmonary bypass[1)2)]. The observed prevalence of HIT antibodies was 25.3% after eight days after surgery. In most of these cases the antibody appears to be nonpathogenic, however this antibody strongly activates platelets in a minority of cases (1 to 3% overall). This activation causes thrombocytopenia, formation of procoagulant platelet microparticles, and massive thrombin generation, resulting in limb and life-threatening complications. This is HIT. Therefore, HIT is an immune system-mediated reaction in which pathologic antibody develops to a complex composed of heparin and the platelet-derived alpha granule protein, namely PF4. The first step in the management of patients with HIT is to detect HIT antibody and eliminate all exposure to heparin. This is important because HIT carries both significant thrombotic morbidity (38 to 81%) and mortality (28%) in patients with the use of CPB[3)4)]. Moreover, seroconversion of the antibody is also important for morbidity. As in a previous study, seroconversion may cause a more rapid onset of thrombocytope-

Table 1 Patient characteristics

Total	28
M/F	19/9
Age (months)	351 ± 319
Cyanosis	6
Diagnosis	
VSD	5
ASD	2
SRV	1
DORV	2
TOF	2
PAIVS	1
TA	1
AS	3
ASR	1
AR	1
TR	2
MR	2
AP	3
MI	2

M: male, F: female, VSD: ventricular septal defect, ASD: atrial septal defect, SRV: single right ventricle, DORV: double outlet right ventricle, TOF: tetralogy of Fallot, PAIVS: pulmonary atresia with intact ventricular septum, TA: tricuspid atresia, AS: aortic stenosis, ASR: AS with regurgitation, AR: aortic regurgitation, TR: tricuspid regurgitation, MR: mitral regurgitation, AP: angina pectoris, MI: myocardial infarction.

Table 2 Surgical procedures

Procedure	No.
VSD patch closure	5
Glenn	3
Lateral tunnel	1
TCPC	1
PS release	1
Ross	2
TOF ICR	1
ASD direct closure	1
PAPVR ICR	1
TVP	1
TVR	1
AVR	3
MVP	2
OPCAB	5

TCPC: total cavopulmonary connection, PS: pulmonary stenosis, ICR: intracardiac repair, TVP: tricuspid valve plasty, TVR: tricuspid valve replacement, AVR: aortic valve replacement, MVP: mitral valve plasty, OPCAB: off-pump coronary artery bypass.

nia[5], and may play a major role in determining the outcome[6] and postoperative recovery without HIT. However, there have been few studies on HIT antibody. In this study, we measured HIT antibody and examined the occurrence of HIT and HIT antibody, seroconversion and risk factors for positive postoperative antibody in cardiac surgery.

Patients and Methods

Twenty-eight patients ranging in age from 3 months to 72 years old (19 men and 9 women) following catheterization within 3 months underwent cardiac surgery. Informed consent was systematically obtained according to the principles of the Declaration of Helsinki. The adult patients had been preoperatively exposed to a dose of 3,000 IU of UFH and the child patients also had been exposed to 100 IU/kg during catheterization. Anticoagulation for CPB was achieved with sodium UFH, with CPB priming and an intravenous (IV) bolus of UFH, corresponding to 200 IU/kg before aortic cannulation. Additional heparin was administered to maintain ACT for over 450 seconds. We dispensed 100 IU/kg of UFH in off-pump coronary artery bypass grafting (CABG) before anastomoses and retained ACT for over 200 seconds. Patients who underwent valve replacement, repair or total cavopulmonary connection (TCPC) received continuous IV infusion of sodium UFH at a dosage of 200 IU/kg/day for at least 3 postoperative days. The pathologies of patients were follows: ventricular septal defect (VSD, n=5); atrial septal defect (ASD, n=2); single right ventricle (SRV, n=1); double outlet right ventricle (DORV, n=2); tetralogy of Fallot (TOF, n=2); pulmonary atresia with intact ventricular septum (PAIVS, n=1); tricuspid atresia (TA, n=1); aortic stenosis (AS, n=3); AS with regurgitation (ASR, n=1); aortic regurgitation (AR, n=1); tricuspid regurgitation (TR, n=2); mitral regurgitation (MR, n=2); angina pectoris (AP, n=3); myocardial infarction (MI, n=2) (Table 1). The surgical procedures are listed in Table 2.

1. ELISA for HIT antibody

The patients were screened for presence of HIT antibody before each procedure and on POD 10 by ELISA according to the manufacturer's instructions (Asserachrom HPIA; Diagnostica Stago, France). A positive antibody screen was defined by the manufacturer as an absorbance value of greater than 0.5

optical density (OD) units.

2. Diagnosis of heparin-induced thrombocytopenia

HIT was diagnosed by positive antibody and the pretest probability score including thrombocytopenia, timing of platelet count fall, thrombosis and other causes of thrombocytopenia[4].

3. Measurement of outcome

We examined seroconversion of HIT antibody and compared preoperative laboratory data and surgical details of two groups. Group 1 consisted of subjects with negative assays on POD 10, and Group 2 consisted of patients with positive postoperative assays. The analyzed variables for risk factor included age, sex, cyanosis, total protein (TP), albumin, total globulin (= total protein-albumin), white blood cell (WBC), hematocrit (Ht), platelet (Plt), C-reactive protein (CRP), prothrombin time international normalized ratio (PTINR), activated partial thromboplastin time (APTT), ADP-induced aggregation, cross clamp time, units of allogenic packed red blood cells (pRBCs) during operations, units of fresh frozen plasma (FFP) during operations, units of platelets during operations, and heparin during operations. In order to standardize the platelet aggregation test, we used the grading curve produced by plotting four concentrations of adenosine diphosphate (ADP).

4. Statistical analysis

Cumulative data were expressed as mean ± standard deviation, and the unpaired t test was used for comparisons between groups. Univariate logistic regression analysis was performed to estimate the risk factor of positive postoperative HIT antibody. A significant odds ratio was obtained if the 95% confidence interval (CI) exceeded 1 and the P value was less than 0.05. $p<0.05$ was considered statistically significant.

Results

1. Immediate and long-term clinical events

We could not identify patients with clinically significant HIT. There was no occurrence of thrombotic events at 1, 6 months, and 1 year follow-up in all patients.

Table 3 Seroconversion

	Preoperative assays	Postoperative assays	No.
HIT antibody	positive	positive	1
	negative	positive	6
	negative	negative	21

HIT: heparin-induced thrombocytopenia.

2. Incidence and seroconversion of anti-platelet factor 4/heparin antibody

One of the 28 patients (4%) had a positive preoperative HIT antibody assay. After cardiac surgery, 6 of the 28 (21%) patients had changed to positive postoperative assay from negative preoperative assay, namely seroconversion (Table 3). The cases included VSD, ASD, ASD with PAPVR, TOF, MR and unstable angina.

3. Comparison of preoperative laboratory data and surgical details

Table 4 shows preoperative laboratory data of two groups. Group 1 consisted of subjects with negative assays on POD 10, and Group 2 consisted of patients with positive postoperative assays. WBC, CRP and coagulation data including platelet count and aggregation did not differ between the two groups. Total globulin was higher in Group 2 than in Group 1 (2.89 ± 0.33 g/dl versus 2.42 ± 0.50 g/dl, $p=0.030$). The results of surgical details are compared in Table 5. There was no significant difference in CPB time and amount of UFH.

4. Risk factor for positive postoperative anti-platelet factor 4/heparin antibody

Univariate analysis revealed that elevated level of preoperative total globulin was related to positive postoperative HIT antibody (Table 6) ($p=0.048$; odds ratio, 12.09; 95% confidence interval, 1.02-144.06).

Discussion

The major findings of our study are that the rate of seroconversion was low and the preoperative total globulin value may be a risk factor for positive postoperative HIT antibody. Our study focused on information about HIT antibody besides the clinical event of HIT because we analyzed preoperative and postoperative samples from patients with prior

Table 4 Preoperative laboratory data

Variable	Negative assays	Positive assays	p value
No.	21	7	
Age (months)	297 ± 320	387 ± 298	0.542
M/F	14/7	5/2	
Preoperative			
Cyanosis	5	1	
Total protein (g/dl)	6.58 ± 0.64	7.1 ± 0.41	0.053
Albumin (g/dl)	4.16 ± 0.53	4.21 ± 0.41	0.796
Total globulin (g/dl)	2.42 ± 0.50	2.89 ± 0.33	0.030
WBC (/mm^3)	7,559 ± 3,988	6,784 ± 1,857	0.623
Ht (%)	40.4 ± 9.4	40.4 ± 7.4	0.989
Plt (×10^4/mm^3)	26.2 ± 11.3	26.5 ± 7.3	0.939
CRP (mg/dl)	0.51 ± 1.68	0.21 ± 0.24	0.649
PTINR	1.11 ± 0.11	1.12 ± 0.83	0.922
APTT (second)	45.8 ± 24.1	64.0 ± 32.0	0.181
ADP-induced aggregation (μM)	2.94 ± 2.44	1.38 ± 0.44	0.223

Data are presented as mean ± SD.
M: male, F: female, WBC: white blood cell, Ht: hematocrit, Plt: platelet, CRP: C-reactive protein, PTINR: prothrombin time international normalized ratio, APTT: activated partial thromboplastin time, ADP: adenosine diphosphate.

Table 5 Surgical details

Variable	Negative assays	Positive assays	p value
CPB time (min)	127 ± 82	108 ± 68	0.589
Cross clamp time (min)	53 ± 55	66 ± 43	0.564
Operation time (min)	397 ± 113	329 ± 78	0.156
Units of pRBCs during operations	11 ± 11	4 ± 6	0.103
Units of FFP during operations	13 ± 13	5 ± 11	0.155
Units of platelets during operations	12 ± 12	6 ± 15	0.279
Heparin during operations (ml/kg)	0.29 ± 0.08	0.31 ± 0.08	0.526

Data are presented as mean ± SD.
CPB: cardiopulmonary bypass, pRBCs: packed red blood cells, FFP: fresh frozen plasma.

heparin exposure.

Clinical HIT was not identified and there was no occurrence of thrombotic events at follow-up in all patients. HIT is a clinical diagnosis based on a substantial fall in platelet count (usually by more than 50%), development of thrombosis and skin lesions by the pretest probability of HIT[7]. The clinical diagnosis can be confirmed by a positive HIT antibody value, most frequently immunoglobulin G. A 58-year-old patient who underwent aortic valve replacement had positive preoperative and postoperative assays in this study. However, he was not eventually diagnosed with HIT because he did not have venous or arterial thromboses and his score of the pretest probability was intermediate. As in a previous study[8], HIT is a rare (2.4% of all patients) though severe complication after cardiac surgery. It may not be possible to confirm the incidence because there are some limitations due to small numbers and the antibody type may be associated with differential platelet activation. We also think there was a small possibility of activating vascular endothelium and platelet which may induce thrombosis, because 3 of 7 patients with positive postoperative HIT antibodies were children and their endothelium is less injured than that of adults, and their plasma level of clotting factor might be lower than that of adults.

The prevalence of postoperative HIT antibodies identified in our investigation was 7 of 28 (25%) and the ratio of seroconversion was 6 of 28 (21%). No patients were diagnosed with thrombosis and HIT caused by the first infusion of heparin. If patients had been diagnosed with them, the prevalence of HIT antibody and HIT might have been higher than in this study. Several studies have assessed

Table 6 Risk factor analysis for positive postoperative HIT antibody

Predictor	Odd ratio [95%CI]		p value
Age (months)	1.00	[0.99-1.00]	0.53
M/F	1.25	[0.19-8.14]	0.82
Preoperative			
Cyanosis	0.53	[0.05-5.56]	0.59
Total protein (g/dl)	7.66	[0.85-69.1]	0.06
Albumin (g/dl)	1.30	[0.19-8.93]	0.79
Total globulin (g/dl)	12.1	[1.02-144.06]	0.04
WBC (/mm^3)	1.00	[1.00-1.00]	0.61
Ht (%)	0.99	[0.91-1.10]	0.99
Plt ($\times 10^4$/mm^3)	1.00	[0.92-1.09]	0.93
CRP (mg/dl)	0.76	[0.25-2.44]	0.68
PTINR	1.63	[1.51-17,558.15]	0.92
APTT (sec)	1.02	[0.99-1.06]	0.21
ADP-induced agreggation (μM)	0.39	[0.08-2.01]	0.26
Cross clamp time (min)	0.99	[0.97-1.03]	0.77
Units of pRBCs during operations	0.78	[0.49-1.23]	0.28
Units of FFP during operations	0.65	[0.30-1.38]	0.26
Units of platelets during operations	0.23	[1.95-2.72]	0.99
Heparin during operations (ml/kg)	0.11	[1.83-623,294.57]	0.78

Data are presented as odd ratio: 95% confidence interval (CI) and p value.
M: male, F: female, WBC: white blood cell, Ht: hematocrit, Plt: platelet, CRP: C-reactive protein, PTINR: prothrombin time international normalized ratio, APTT: activated partial thromboplastin time, ADP: adenosine diphosphate, pRBCs: packed red blood cells, FFP: fresh frozen plasma.

the incidence of HIT antibody after cardiac surgery and seroconversion[9]. Bauer et al measured anti-PF4/heparin antibody by ELISA before surgery and 5 days after surgery in 111 patients undergoing cardiac surgery with CPB[10]. The observed prevalence of the antibody was 51% after surgery versus 19% before surgery. They also measured the antibody by 14C-serotonin-release assay and the prevalence was lower than that by ELISA. Seroconversion to positive HIT antibody 5 to 10 days after adult cardiac surgery occurs in 27 to 50% of patients undergoing cardiac operation[11]. The ratio of positive preoperative HIT antibody assay and seroconversion in this study was lower than that of previously published reports. We included 16 children besides adults in the study population and HIT antibody includes IgG, IgM and IgA against heparin. Therefore, the children's immature immune system might decrease the ratio. Moreover, ELISA assays for HIT showed high false positive rates, especially in the acute cardiac care setting. The present study included only 3 patients with AP and 2 patients with MI, which may be why the ratio was lower than that of previous reports.

Our study is the first to demonstrate the risk factor for positive postoperative HIT antibody in cardiac surgery. Previous studies showed the clinical course for HIT and patients with HIT antibody, but did not adequately assess the relationship between antibodies and laboratory data including platelet count and aggregation. In contrast, our study assessed the risk factor for positive postoperative HIT antibody based on data and surgical details. Preoperative total globulin levels were higher in patients who subsequently developed heparin-dependent antibody because anamnestic response to heparin was likely to cause the seroconversion. Total globulin includes α1, α2, β and γ globulin. There was a smaller possibility of high α1, α2, and β globulin because the patients did not have thyroid disease, nephrosis, abnormality of metabolism, or inflammation. Therefore, elevated level of γ globulin with anamnestic response might cause elevated level of total globulin. The preoperative value of total globulin may be variable, especially in infectious cases. This study showed no significant difference in WBC and CRP, so we think there is a possibility that the high preoperative total globulin was

caused by heparin exposure. We also showed that there was no statistically significant difference in CPB time, although platelet activation enhancing the production of heparin-dependent antibody may have been more intense as a result of longer extracorporeal circulation. Although this report is based on a small number of cases, insufficient clinical details were provided to be able to determine the risk factor. We need to conduct further studies with a larger cohort of patients.

Conclusion

There was no occurrence of clinical HIT and thrombotic events at follow-up in this study. The ratio of positive preoperative HIT antibody assay and seroconversion was lower than that of previously published reports. Elevated level of preoperative total globulin may be a risk factor for positive postoperative HIT antibody in cardiac surgery.

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心臓手術における抗 PF4・ヘパリン複合体抗体に対する危険因子の検討

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ヘパリン起因性血小板減少症（HIT）は，免疫機序で発生するヘパリンの重篤な合併症である．抗 PF4・ヘパリン複合体抗体（HIT 抗体）は HIT の病因となるが，心臓外科領域において，その詳細は明らかではない．今回，カテーテル検査後 3 ヵ月以内に心臓手術を施行された計 28 例を対象とし，HIT・HIT 抗体の発生率，HIT 抗体の seroconversion・危険因子について検討した．HIT 抗体は術前と術後に ELISA を用いて測定した．全症例において臨床上明らかな HIT は認めなかった．術前 HIT 抗体は 28 例中 1 例（4%）にみられ，28 例中 6 例（21%）で術前 HIT 抗体陰性から術後陽性となった（seroconversion）．術後 HIT 抗体陽性と陰性患者において術前検査結果と人工心肺時間などを比較検討した結果，総グロブリンのみが有意差を示した．術前総グロブリンは術後 HIT 抗体陽性群（2.89±0.33 g/dl）において陰性群（2.42±0.50 g/dl）より高値であった（p=0.030）．また，術前総グロブリン値は術後 HIT 抗体陽性と関連性があった（p=0.048；オッズ比，12.09；95% 信頼区間，1.02-144.06）．Seroconversion の発生率は低く，術前総グロブリン高値は術後 HIT 抗体陽性の危険因子となる可能性があると考えられた．