ORIGINAL ARTICLE

The frequency of heparin-induced thrombocytopenia in Taiwanese patients undergoing cardiopulmonary bypass surgery

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Background/Purpose: There are few studies on heparin-induced thrombocytopenia (HIT) reported from Taiwan and Asian countries. We conducted a prospective study to investigate the frequency of HIT in patients undergoing cardiopulmonary bypass surgeries.
Methods: A cohort of 54 patients was enrolled from January 01, 2010 to October 31, 2011. Patients' clinical information was obtained for 4T score classification. Plasma (2—4 mL) was also collected before surgery and on Days 5 and 10 following heparin administration during the bypass procedure. This was tested for anti-heparin/PF4 antibodies and functional assay using flow cytometry (FC).
Results: The mean platelet count for this cohort followed the expected pattern in the postoperative setting. Seven of the 54 (13%) patients had positive antibodies assays before bypass surgery. This increased to 32% on Day 5 and was markedly elevated to 63% on Day 10 after surgery. Only one of the 54 patients (1.8%) was found to have both positive antibody assay and platelet activation, but no clinical HIT/thrombosis developed.
Conclusion: Our study is the first report on the rates of HIT in the setting of cardiopulmonary bypass surgery in Taiwan and demonstrated no clinical HIT occurrence, despite the high frequency of HIT antibody in our cohort.
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Introduction

Heparin-induced thrombocytopenia (HIT) is an acquired immunologic and thrombotic disorder characterized by thrombocytopenia with/without arterial or venous thrombosis following heparin exposure. Devastating complications such as limb gangrene and even fatality may occur if it is not recognized and treated in a timely fashion.[1] Early identification of HIT is therefore essential for the appropriate clinical management of affected patients. In the typical presentation of HIT, thrombocytopenia with or without vascular thrombosis usually develops on Days 5—12 after heparin exposure.[2] The reported incidence of HIT is 1—5%, depending on the clinical situation and the type of heparin used.[3]

Patients undergoing cardiovascular or orthopedic surgeries have a higher probability of developing HIT antibodies as compared to medical patients exposed to heparin.[4,5] However, many patients with detectable anti-heparin/PF4 antibodies may not develop clinical HIT. In fact, only 5—50% of these patients will develop HIT, depending on the patient population.[6] The relationship between the clinical presentation of HIT, the presence of HIT antibodies, and the type of assay used can be conceptualized as an iceberg model, as proposed by Warkentin.[7]

HIT has been well recognized in Western countries, but there are few studies reported from Taiwan and other Asian countries and the Taiwanese have been considered ethnically as a low-risk population for developing thrombosis.[8] Our colleagues have previously reported a female patient with breast cancer who developed progressive thrombocytopenia and venous thrombosis of axillary vessels with impending digit and limb gangrene after Port-A catheter insertion with minimal heparin exposure.[9] She was diagnosed as having HIT by positive enzyme-linked immunosorbent assay (ELISA), and was successfully treated with heparin cessation and subsequent thrombolytic therapy. Systematic studies of the frequency of HIT in various subsets of patients in Taiwan have so far been lacking. Therefore, we consider HIT to be a disease needing further exploration in the Taiwanese population to better understand the epidemiologic and clinical spectrum.

In this study, we focus on patients undergoing cardiopulmonary bypass surgery with unfractionated heparin (UFH) exposure, because these patients have been identified as having a high risk of developing HIT antibodies. The aims of this study were: (1) to determine the frequency of anti-heparin/platelet factor 4 antibody formation in Taiwanese patients undergoing cardiopulmonary bypass surgeries; (2) to estimate how many of these patients have a positive functional assay for HIT antibodies; and (3) to determine the overall frequency of clinical HITT in this cohort of patients.

Patients and methods

Patients

We conducted a prospective study involving a cohort of patients who had undergone cardiac surgeries with UFH use for cardiopulmonary bypass at our institution from January 01, 2010 to October 31, 2011. This study was approved by our institutional review board and signed informed consent was obtained from each patient before their enrollment. Each patient's medical history including age, sex, disease and type of operation were recorded. Additionally, a series of platelet counts before surgery and Days 0, 2, 5, 8, and 10 after surgery were collected for analysis.

None of the 54 patients in this cohort was given either UFH or low molecular weight heparin for deep venous thrombosis (DVT)/pulmonary embolism (PE) prophylaxis in the intensive care unit after surgery.

Inclusion criteria
Patients who were given UFH for extracorporeal anticoagulation while undergoing cardiopulmonary bypass surgery were included.

Exclusion criteria
Patients with known thrombocytopenia (platelet count $< 100 \times 10^3/mm^3$) from causes such as infection, sepsis, liver disease, or acute disseminated intravascular coagulopathy before cardiac surgery were excluded.

Heparin use
UFH (Agglutex, China Chemical and Pharmaceutical Corporation, Taipei, Taiwan), a porcine heparin sodium, was given by intravenous bolus of 300 units/kg with additional dosing to get activated clotting time >400 seconds before cardiopulmonary bypass surgery, followed by repeated UFH boluses as needed to keep the activated clotting time >400 seconds continually during cardiopulmonary bypass.

Platelet transfusion
One unit of platelet pheresis product was transfused for each patient after surgery, which is a standard practice for patients who receive cardiac surgery at our institution.

Patient's plasma
Each patient's plasma (2—4 mL) was collected in a vacuum tube containing 3.2% sodium citrate as an anticoagulant before surgery and on Days 5 and 10 after heparin administration. The samples were centrifuged, aliquoted, and stored at $-80°C$. One stored frozen sample from each patient was thawed to test for anti-heparin/PF4 antibodies by ELISA. If the result of the ELISA was positive, the other one was thawed and subjected to functional assay using the flow cytometry (FC) method.

Methods

ELISA testing for detection of HIT antibodies
ELISA (Asserachrom HPIA, Stago, Asnières, France) was used according to the manufacturer's guidelines. This assay determined the amount of antibodies (IgG, IgM, and IgA) against heparin-PF4 complexes in a patient's plasma. The absorbance value of each tested sample was measured at 450 nm. The HIT ELISA assay was classified as positive if the absorbance value, optical density (OD), of the tested sample was greater than the absorbance value of the reference reagent multiplied by a fraction number (0.27 or

0.29) accessed in the reagent package insert, according to the manufacturer's recommendations.

FC assay for detection of HIT antibodies

The FC assay was performed as reported by Tomer,[10] with some modifications as follows: (1) the use of normal donor platelets regardless of blood group type; (2) the use of CD61 (glycoprotein IIIa) and CD62p (p-selectin) as markers of platelet identification and activation, respectively; (3) the use of adenosine diphosphate to confirm normal platelet activation; (4) all procedures were performed at room temperature (20—25 °C); (5) use of phosphate-buffered saline as the buffer solution for the entire procedure; and (6) analysis of 10,000 platelets per sample.

The plasma of patients whose ELISA was positive was subjected to the FC assay to assess platelet activation. The patient's plasma was incubated with no heparin, 0.1 IU/mL, 0.3 IU/mL, and 100 IU/mL UFH. The samples without UFH and those with 100 IU/mL heparin were used as internal negative controls. The definition of a positive FC assay was adopted from two previous studies.[11,12] The proportion of activated platelets was at least >11% in the presence of heparin (0.1 or 0.3 IU/mL) compared with baseline (no heparin) and the activation could be suppressed by a high dose of heparin (100 IU/mL) evidenced by a ratio of more than two between platelet activation percentage at 0.3 and 100 IU/mL heparin.

Clinical HIT

A diagnosis of clinical HIT was established if the following three criteria were fulfilled: (1) intermediate or high probability group by Warkentin pretest scoring system (4T scores).[13] In brief, the 4Ts refer to four items including thrombocytopenia, time to platelet count decrease, thrombosis, and the presence of other causes for thrombocytopenia. Each item has 0—2 points and the total score from these four items is used to classify patients into three probability categories: high = 6—8, intermediate = 4—5, and low = 0—3; (2) positive ELISA assay; and (3) positive functional assay of platelet activation.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 for Windows, version 5.01 (La Jolla, CA, USA). The independent t test was used for calculation and comparison of the continuous data including patients' series of average platelet count and the absorbance values (OD unit) of anti-heparin/PF4 antibodies titer before and on Days 5 and 10 after UFH exposure assayed by ELISA. A p value < 0.05 was considered statistically significant.

Results

There were 54 consecutive patients who had clinical evaluation with a full history and physical, and a series of platelet counts and plasma samples for analysis before and after cardiopulmonary bypass surgery and on Days 5 and 10 thereafter. There were 34 male and 20 female patients enrolled in this study and their median age was 72 years with a range of 38—93 years. The demography, cardiac disease, and types of surgery for this cohort of 54 patients are listed in Table 1. The total dose of UFH ranged from 14,000 U to 33,000 U, with a median of 19,000 U. None of the patients developed thromboembolic events postoperatively.

Platelet count trajectory

All but five patients were given one unit of pheresis platelets after cardiopulmonary bypass surgery on the operative day. Five patients did not receive platelet transfusion because of low perioperative bleeding or unavailability of the platelet pheresis units. The change in platelet count before and after operation with UFH exposure is depicted in Fig. 1. The mean platelet count decreased from $186.53 \pm 73.81 \times 10^3/\mu L$ preoperatively, to the lowest value of $106.62 \pm 38.75 \times 10^3/\mu L$ on Day 2 after surgery ($p < 0.001$). The mean platelet count then increased up to $127.08 \pm 57.66 \times 10^3/\mu L$ on Day 5 and $239.17 \pm 107.42 \times 10^3/\mu L$ on Day 10 ($p < 0.001$ as compared to that on Day 2).

The average platelet count was compared between patients with and without positive anti-heparin/PF4 antibodies at various time points before and after surgery, as shown in Fig. 2. Although those patients whose ELISA tests were positive (36 patients) had higher mean platelet counts compared to those with a negative ELISA test (18 patients) at all six time points, there was no statistical difference in the mean platelet count between these two groups.

The probability of clinical HIT

By the 4Ts classification, none of the 54 patients had a high probability of HIT. There were 18 patients with an intermediate probability, including two with five points and 16 with four points. The other 36 patients all had a low probability.

Absorbance value of the ELISA assay

Seven of the 54 (13%) patients had positive anti-heparin/PF4 antibodies before the cardiac surgery. The percentage

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Table 1 Clinical information on 54 patients undergoing cardiopulmonary bypass surgeries.

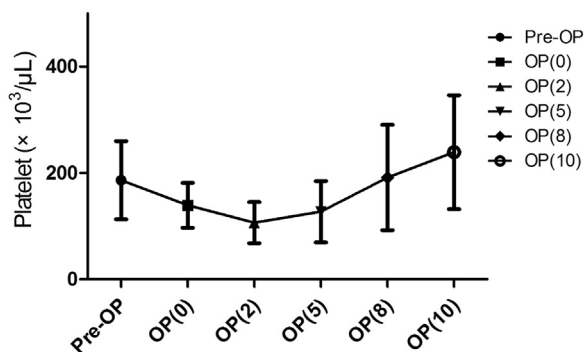| Disease	Total no. of patients	Sex (M/F)	Age, median (range)	Surgery	Duration of cardiopulmonary bypass (h)
Coronary artery disease	31	23/8	71 (44—93)	Coronary arterial bypass grafts	1—2
Valvular heart disease	19	8/11	76 (52—86)	Valves repair or replacement	1—2
Miscellaneous	4	2/2	59 (38—75)	Septal myomectomy, removal of a cardiac tumor, etc.	1

Figure 1 The change in perioperative platelet count. The error bar represents mean ± standard deviation.

of positive anti-heparin/PF4 antibodies increased to 32% (17 patients) on Day 5 after surgery, and was markedly elevated to 63% (34 patients) on Day 10 after surgery. The average antibody titer expressed by OD value in patients with positive anti-heparin/PF4 antibodies is shown in Fig. 3. The OD value was 0.79 ± 0.45 (mean ± standard deviation) before surgery, which increased to the highest level of 1.70 ± 1.08 on Day 5 after surgery ($p < 0.05$ as compared to that before surgery). The OD value on Day 10 after surgery was 1.64 ± 0.99, which was not statistically different in comparison to the absorbance value on Day 5.

Platelet activation by FC assay

Only one patient, who had undergone mitral valve repair and tricuspid valve annuloplasty, was found to have positive platelet activation by the FC assay, as shown in Fig. 4. Anti-heparin/PF4 antibodies were first detected by ELISA in this 65-year-old female patient on Day 10 after surgery, but she had no clinical persistent thrombocytopenia or thromboembolic events. Her clinical HIT score was 2 according to the Warkentin 4Ts scoring system, which classified her
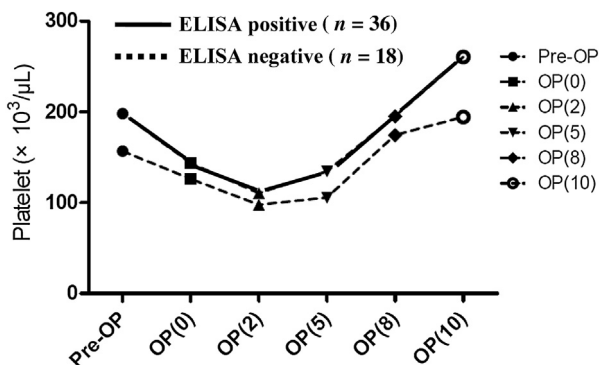

Figure 2 Comparison of the platelet count change over the time from preoperation to Day 10 after surgery. The solid line (upper) represents the platelet count in patients with anti-heparin/PF4 antibodies and the dotted line (lower) represents the platelet count in patients without these antibodies. There was no statistical difference in platelet counts at the stated time points on preoperation (op), op(0), op(2), op(5), op(8), and op(10) between the two groups of patients.
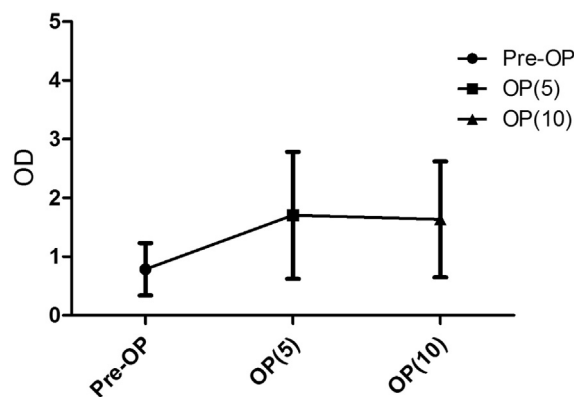

Figure 3 The change in the mean titer of positive anti-HIT antibodies. The error bar represents mean ± standard deviation.

probability as low risk; we therefore chose not to treat her with a direct thrombin inhibitor. Thrombocytopenia and/or thrombotic events were not experienced during 6 months of follow up after surgery.

Discussion

Over the past decade, HIT has been increasingly recognized worldwide as a clinically important entity, with the potential for serious and even fatal consequences if it is not recognized and treated appropriately. This awareness has been lacking in Taiwan outside the setting of large academic referral centers, largely due to a perception that the incidence is very low. The absence of studies defining the true incidence has further hindered educational efforts and needs to be addressed. Our study represents the first step in that direction and looks at a specific subset of patients exposed to heparin in the context of cardiac surgery.

Several laboratory tests have been used to diagnose HIT. These include antigen assays such as ELISA, and functional assays including the platelet aggregation test, serotonin release assay,[14] the heparin-induced platelet activation test,[15] and an assay using FC that detects platelet activation.[11–13] Although all of these tests are aimed at detecting antibodies implicated in HIT, none are entirely satisfactory. ELISA is the most commonly used test for the detection of HIT antibodies, because it is sensitive, technically simple, and has commercially available reagents. However, this assay is not specific for HIT antibodies and can detect antibodies which do not result in clinical HIT, as evidenced in our study. Hence, for patients who have positive ELISA results, further testing with a functional assay plus clinical assessment by 4Ts is highly recommended to confirm the HIT diagnosis.[7] Although the serotonin release assay is considered as a gold standard functional assay for HIT, it is difficult to set up in a general coagulation laboratory and has the risk of radiation exposure.[14] As reported in our previous study, the combination of ELISA and the FC assay can provide both high sensitivity and specificity for diagnosis of clinical HIT.[16]

In this study, 13% of patients were found to have anti-heparin/PF4 antibodies before surgery, which is suspected

No heparin

0.1U/mL heparin

0.3U/mL heparin
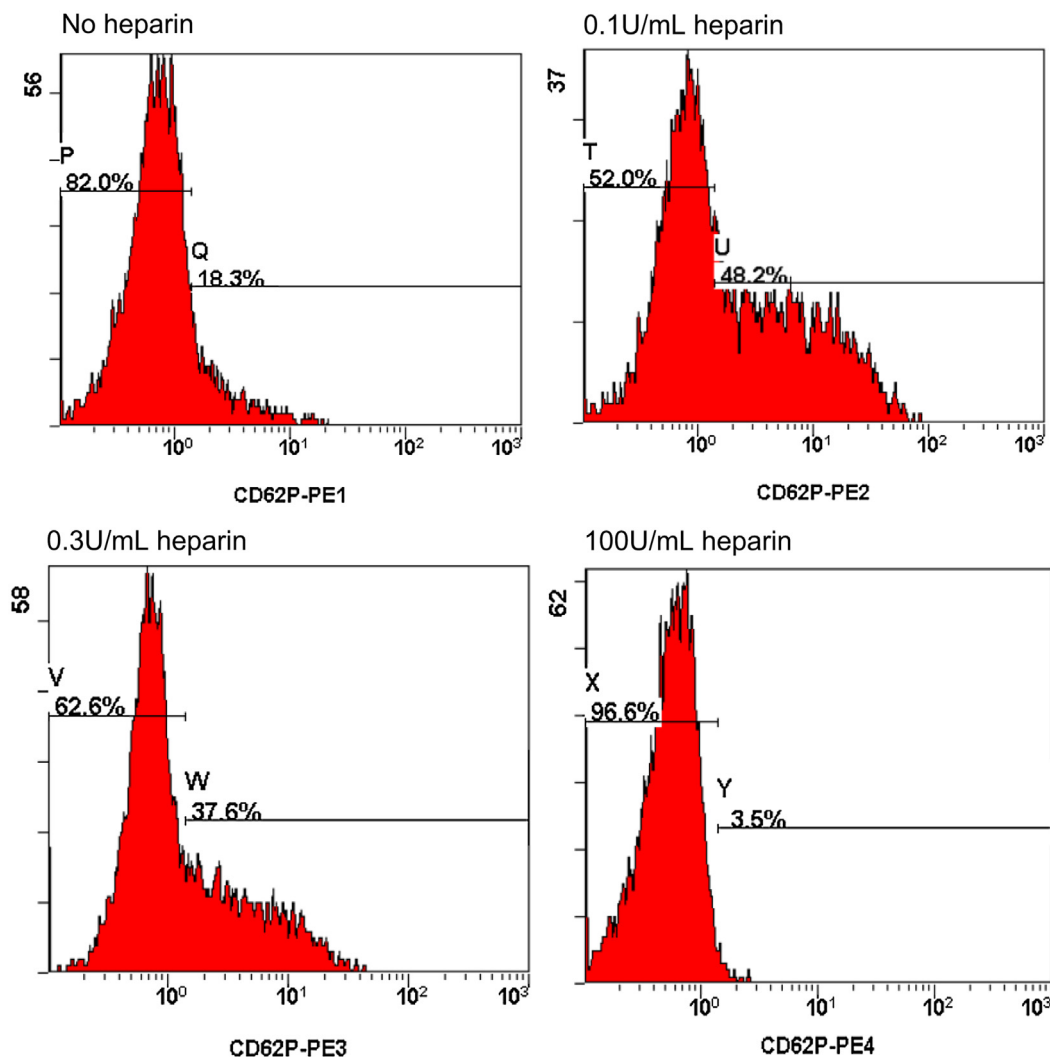
100U/mL heparin

Figure 4 The only patient with positive platelet activation assayed by flow cytometry is depicted here. As compared to no heparin, the proportion of activated platelets was markedly increased to 48.2% and 37.6% in the presence of 0.1 U/mL and 0.3 U/mL unfractionated heparin, respectively, and was suppressed to 3.5% in the presence of 100 U/mL unfractionated heparin.

to be due to previous heparin exposure during cardiac catheterization, or treatment for ischemic heart disease. This figure increased to as high as 63% on Day 10 post-operatively. Table 2 lists the rate of positive heparin/PF4 antibodies, positive platelet activation tested by functional assays, and the corresponding rates of clinical HIT as reported in six prospective studies in the literature.[5,17—21] We had a higher proportion of positive antibodies as compared with those in other series, which could be attributed to the type of ELISA assay used in the present study, which detected IgM and IgA, in addition to IgG antibodies. In contrast, the rate of positive platelet activation assay was 1.8% in our study, which is similar to 0.5~3.0% reported in the literature. No clinical case of HIT was identified in this study as compared to 0.5~3.2% incidence of clinical HIT reported in the literature. This may be due to the short duration of heparin use in cardiopulmonary bypass surgery and the lack of subsequent use in the intensive care unit for DVT/PE prophylaxis after surgery.

Thromboembolic diseases such as DVT/PE are not as prevalent in Taiwan as in Western countries[9] and Asian

ancestry populations carry a lower incidence of venous thromboembolism (VTE) risk in comparison with African, European, and Hispanic populations.[22] However, the true incidence of VTE, including HIT, may be underestimated or underdiagnosed in Taiwan because of low clinical suspicion and awareness. Recently, in a Japanese prospective multicenter study on HIT incidence in 172 acute ischemic stroke patients treated with UFH, the definite HIT incidence was 1.7%.[23] In another study reported from India, as high as eight out of 100 (8%) patients undergoing cardiac surgery were diagnosed as having confirmed HIT by the pre-test clinical scoring and antibody assay.[24] Therefore, we believe that HIT deserves clinical attention in Asian and Taiwanese populations, and more clinical and epidemiological studies are needed to get a better understanding of this disease in Asian countries.

The platelet count change seen in our cohort of patients, as depicted in Figs. 1 and 2, is known to be the typical pattern for patients who undergo cardiopulmonary bypass surgery, or any other major surgical intervention for that matter. The platelet count usually falls rapidly after

Table 2 Comparison of frequency of preoperative and postoperative heparin-induced thrombocytopenia (HIT) antibody and functional assays and clinical HIT in the literature.

Authors	Published year	No. of patients	Cardiac surgery	Positive HIT antibody assay (%)		Positive HIT functional assay (%)		Clinical HIT (%)
				Pre-OP	Post-OP	Pre-OP	Post-OP	
Visentin et al[17]	1996	51	CABG, valve replacement	22	72	None	None	0
Bauer et al[18]	1997	111	Cardiopulmonary bypass	19	51	5[a]	13[a]	0
Pouplard et al[19]	1999	328	CABG, valve replacement or repair	0.9	25.3	None	2.4[a]	1.8
Everett et al[5]	2007	299	CABG, valve replacement or repair	4.3	22.4	None	none	0.7
Kress et al[20]	2007	1114	CABG, valve surgery	5.4	7.9	None	3.0[b]	3.2
Selleng et al[21]	2010	591	CABG, valve surgery, both	21.7	None	1.0[c]	0.5[c]	0.5
This study	2012	54	CABG, valve surgery, others	13	63	0	1.8[d]	0

CABG = coronary artery bypass graft; HIT = heparin-induced thrombocytopenia; OP = operation.
[a] Serotonin release assay.
[b] Heparin antibody aggregation assay.
[c] Heparin-induced platelet activation assay.
[d] Flow cytometry assay.

surgery to the lowest level on the second or third postoperative day, followed by a rise and a return back to the normal range and even a brief rebound above the normal range around 1 week later.[25] We further explored and compared the series of platelet count changes in patients with and without anti-heparin/PF4 antibodies after cardiac surgery. There were higher platelet counts in patients with anti-heparin/PF4 antibodies as compared to those in patients without antibodies at each time point from before surgery, to Day 10 after surgery, but no statistical difference was noted. Therefore, it appears that the emergent anti-heparin/PF4 antibodies during cardiac surgery did not have a significant effect on platelet consumption or destruction.

In conclusion, our study demonstrates that around 70% patients developed anti-heparin/PF4 antibodies in the context of cardiopulmonary bypass surgery and about 2% patients had a positive functional assay for platelet activation. There was no clinical HIT, which may be attributed to short heparin exposure. We did not use any pharmacologic thromboprophylaxis, including heparin, in the postoperative setting. These figures are similar to those reported in Western studies and in general, the rate of clinical events appears to be very low, despite the high rate of antibody detection in this subset of patients. To the best of our knowledge, this is the first study to explore HIT incidence in the setting of cardiopulmonary bypass surgery in the Taiwanese population.

Acknowledgments

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