

# Utility of ELISA Optical Density Values and Clinical Scores for the Diagnosis of and Thrombosis Prediction in Heparin-induced Thrombocytopenia

Seon Young Kim, M.D.<sup>1</sup>, Hyun Kyung Kim, M.D.<sup>1</sup>, Kyou Sup Han, M.D.<sup>1</sup>, Inho Kim, M.D.<sup>2</sup>, Sung-Soo Yoon, M.D.<sup>2</sup>, Seonyang Park, M.D.<sup>2</sup>, and Byoung Kook Kim, M.D.<sup>2</sup>

Departments of Laboratory Medicine<sup>1</sup> and Internal Medicine<sup>2</sup>, Seoul National University College of Medicine, Seoul, Korea

**Background:** Heparin-induced thrombocytopenia (HIT) is an adverse drug reaction caused by antibodies to the heparin/platelet factor 4 (PF4) complex, resulting in thrombocytopenia and prothrombotic state. HIT diagnosis is challenging and depends on clinical presentation and laboratory tests. We investigated the usefulness of clinical scores and heparin/PF4 ELISA optical density (OD) as a diagnostic marker and thrombosis predictor in HIT.

**Methods:** We analyzed 92 patients with suspected HIT. The heparin/PF4 antibody was measured using a commercial ELISA kit (GTI, USA). For each patient, the 4 T's score and Chong's score were calculated.

**Results:** Of the 92 patients, 28 were anti-heparin/PF4-seropositive. The 4 T's score and Chong's score showed good correlation ( $r = 0.874$ ). The 4 T's score and OD values showed good performance for diagnosis of the definite and unlikely HIT groups; however, OD levels showed better sensitivity (93.8%) than the 4 T's score used alone (62.5%). Of the 92 patients, 26 developed thrombosis. The OD values were significantly higher in patients with thrombosis than in those without thrombosis (0.52 vs. 0.22,  $P < 0.001$ ). Patients with high OD values ( $OD > 0.4$ ) had an increased risk of thrombosis (adjusted odds ratio 9.44 [3.35–26.6],  $P < 0.001$ ) and a shorter 250-day thrombosis-free survival (32.1% vs. 54.7%,  $P = 0.012$ ).

**Conclusions:** ELISA OD values in combination with clinical scoring can improve the diagnosis of and thrombosis prediction in HIT. More attention should be paid to the use of clinical scores and OD values as thrombosis predictors in HIT.

**Key Words:** Heparin-induced thrombocytopenia, Heparin-platelet factor 4 antibody, Clinical scoring system, Optical density value

## INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is a distinct clinicopathologic syndrome caused by platelet-activating antibodies that recognize heparin/platelet factor 4 (PF4) complexes. HIT is a potentially serious hypercoagulable state and is associated with a wide spectrum of venous and arterial thrombosis. The early recognition of HIT is critical

because thrombotic complication can be life-threatening, and failure to discontinue heparin and initiate an alternative anticoagulant can result in increased morbidity and mortality [1]. The diagnosis of HIT is difficult and primarily relies on clinical criteria and serologic testing for heparin/PF4 antibodies. Various scoring systems have been developed to estimate the probability of HIT based on clinical information. These previously developed clinical scoring systems are useful only for retrospective analysis because these scores use criteria such as response to heparin withdrawal or re-exposure to heparin, which limit their applicability for prospective diagnosis [2]. Recently, Warkentin and Heddle [3] developed a new scoring system for the pretest probability of HIT, the 4 T's scoring system (Table 1), which takes advantage of new information regarding the clinical features of HIT and is simple to apply prospectively. The 4 T's scoring system was demonstrated to be accurate when used in conjunction with the serotonin release assay (SRA), and a low score had a high negative predictive value [4]. Labora-

Received: July 9, 2010

Manuscript No: KJLM-10-115

Revision received: October 12, 2010

Accepted: November 16, 2010

Corresponding author: Hyun Kyung Kim, M.D.

Department of Laboratory Medicine, Seoul National University College of Medicine, 101 Daehang-no, Jongno-gu, Seoul 110-744, Korea  
Tel: +82-2-2072-0853, Fax: +82-2-747-0359, E-mail: lukekhk@snu.ac.kr

ISSN 1598-6535 © The Korean Society for Laboratory Medicine.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Table 1. The 4T's scoring system

Characteristics	2 points	1 point	0 point
Thrombocytopenia	> 50% platelet fall (nadir $\geq 20 \times 10^9/L$ )	30–50% platelet fall or nadir $10–19 \times 10^9/L$	< 30% platelet fall or nadir $\leq 10 \times 10^9/L$
Timing of platelet count fall	Clear onset between days 5–10 or $\leq 1$ day (if heparin exposure within past 30 days)	Consistent with days 5–10 fall, but not clear (e.g., missing platelet counts) or $\leq 1$ day (heparin exposure within past 31–100 days)	Platelet count fall $\leq 4$ days without recent heparin exposure
Thrombosis or other sequelae	New thrombosis; skin necrosis; acute systemic reaction after intravenous heparin bolus	Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis (not yet proven)	None
Other cause of thrombocytopenia	None evident	Possible	Definite

Pretest probability score: 6–8 = high; 4–5 = moderate; 0–3 = low. Modified from Greinacher and Warkentin [1].

tory documentation of HIT antibodies has been crucial in determining the clinical scope of HIT syndrome. The SRA is considered the gold standard for HIT diagnosis; however, the SRA cannot be performed in most clinical laboratories. Alternatively, the commercial heparin/PF4 ELISA, which detects heparin/PF4 antibodies, can be easily performed on standard platforms in clinical laboratories and is more widely available. The heparin/PF4 ELISA is known to be very sensitive to the presence of anti-heparin/PF4 antibody [5]; however, it is less specific for the diagnosis of HIT, partly because it can also detect non-pathologic antibodies [6]. Not all patients with anti-heparin/PF4 antibodies develop HIT, and, conversely, the absence of detectable anti-heparin/PF4 does not completely exclude HIT [1]. Although the heparin/PF4 ELISA is designed to report dichotomous results (positive/negative), recent studies have shown that absolute optical density (OD) values have diagnostic and prognostic utility. A previous study reported that increasing OD was associated with an increasing probability of HIT and that higher OD values were associated with an increasing risk of subsequent thrombosis in HIT [7]. We performed a retrospective review of all patients with suspected HIT who underwent heparin/PF4 ELISA at a tertiary care hospital. The aim of this study was to evaluate the clinical utility of clinical scoring systems and the heparin/PF4 ELISA for diagnosing HIT and to investigate whether higher OD values correlate with a higher HIT pretest probability score and higher risk of thrombotic complication and mortality.

## MATERIALS AND METHODS

### 1. Patients and methods

Ninety-two patients who were requested to undergo anti-heparin/PF4 antibody test from October 2007 to July 2009 were the subjects of the present study. The anti-heparin/PF4 antibody level was measured using a commercial ELISA kit (PF4 Enhanced; GTI, Waukesha, WI, USA). Briefly, the

Table 2. Chong's scoring system

Criteria	Point
Onset of thrombocytopenia* (or a substantial decrease in platelets, by > 50% of baseline) 4–14 days after starting heparin	3
Onset of thrombocytopenia outside above time interval	1
Exclusion of other causes of thrombocytopenia	2
Thrombocytopenia resolves after stopping heparin	2
Thrombocytopenia recurs on heparin rechallenge	1
Thrombosis associated with thrombocytopenia	1
Laboratory tests	
Immunoassay positive	2
Functional assays: two-point system	3
Functional assays: non-two-point system	2

Modified from Chong and Chong [2].

\* The presence of thrombocytopenia is mandatory. Thrombocytopenia is defined as a platelet count below  $150 \times 10^9/L$ . If the total point is > 7, 5–6, 3–4, and < 3, the diagnosis of HIT is considered definite, probable, possible, and unlikely, respectively. Abbreviation: HIT, heparin-induced thrombocytopenia.

solid-phase ELISA microwells provide immobilized PF4: polyvinyl sulfonate (PVS) complexes as a target for the detection of antibodies, because antibodies associated with HIT recognize sites on platelets that are created when PF4 is complexed with heparin or another linear polyanionic compound such as PVS. After ensuring the binding of antibodies recognizing a site on PF4:PVS, the unbound antibodies are washed away. An alkaline phosphatase labeled anti-human globulin reagent (anti-IgG/A/M) is added to the wells and incubated. The unbound anti-IgG/A/M is washed away, and the substrate p-nitrophenyl phosphate is added. After a 30 min incubation period, the reaction is stopped by a sodium hydroxide solution, and the OD at 405 nm is measured in a spectrophotometer. A result was considered positive if the OD value was greater than 0.40, in accordance with both the manufacturer's instructions and the FDA-approved cut-off value. All the procedures were performed according to the manufacturer's guidelines. Then

data were reviewed from patients' electronic clinical, laboratory, and radiographic records from initial hospital visits and a follow-up period of up to 10 months. Using the 4 T's score, the pre-test probability of HIT was retrospectively calculated for each patient on the basis of the clinical records at the time the test was ordered by one of the investigators (Table 1) [6]. Retrospective probability scores were calculated using Chong's scoring system (Table 2) [2].

## 2. Statistical analysis

Data were compared using the Mann-Whitney *U* test and Kruskal-Wallis ANOVA for continuous variables and the chi-square test for categorical variables. The association of clinical scores and OD values was analyzed using Spearman's correlation coefficient. Sensitivity and specificity were calculated for the heparin/PF4 ELISA and 4 T's scoring sys-

tem for the definite and unlikely HIT groups by using Chong's scoring system. Thrombosis-free survival and overall survival were estimated using the Kaplan-Meier method, and the difference between curves was determined by using the log-rank test. Heparin/PF4 ELISA OD level and 4 T's score cut-offs for predicting thrombotic complication were determined using ROC curve analysis.

## RESULTS

### 1. Patient characteristics

The median age was 69-yr-old (range, 1–92 yr), and 44 patients (47.8%) were female (Table 3). A majority of patients had undergone cardiovascular surgery (62 [67.4%]) or were managed by internal medicine for underlying malignancy (17 [18.5%]). Forty-four patients (47.8%) had re-

**Table 3.** Characteristics of 92 patients who underwent the anti-heparin/PF4 antibody ELISA

	Total (N = 92)	Heparin/PF4 Ab+ (N = 28)	Heparin/PF4 Ab- (N = 64)	*P value
Age (yr)	69.1 (1.4–91.8)	71.5 (10.0–82.1)	68.4 (1.4–91.8)	0.717
Female sex (%)	44 (47.8)	16 (57.1)	28 (43.8)	0.264
Platelet count, nadir ( $\times 10^9/L$ )	29 (4–266)	24 (6–69)	33 (4–266)	0.053
% Decrease platelet count from baseline	81.4 (11.5–3.2)	88.2 (35.1–97.9)	78.8 (11.5–98.4)	0.019
Type of anticoagulation, N (%)				
UFH only	44 (47.8)	16 (57.1)	28 (43.8)	0.481
UFH + LMWH	43 (46.7)	11 (39.3)	32 (50.0)	
LMWH only	5 (5.4)	1 (3.6)	4 (6.2)	
Patient type, N (%)				
Cardiothoracic surgery	62 (67.4)	24 (85.7)	38 (59.4)	0.045
General surgery	4 (4.3)	0	4 (6.2)	
Cardiac medical	9 (9.8)	0	9 (14.1)	
Other medical	17 (18.5)	4 (14.3)	13 (20.3)	
OD value	0.256 (0.015–3.242)	0.665 (0.420–3.242)	0.207 (0.015–0.371)	< 0.001
4 T's score	3 (0–8)	5 (0–8)	3 (0–6)	< 0.001
Low probability	48 (52.2)	6 (21.4)	42 (65.6)	< 0.001
Intermediate probability	33 (35.9)	13 (46.4)	20 (31.3)	
High probability	11 (11.9)	9 (32.1)	2 (3.1)	
Chong's score	3.5 (0–11)	7 (2–11)	3 (0–7)	< 0.001
Unlikely HIT	29 (31.5)	1 (3.6)	28 (43.8)	< 0.001
Possible HIT	28 (30.4)	4 (14.3)	24 (37.5)	
Probable HIT	19 (20.7)	8 (28.6)	11 (17.2)	
Definite HIT	16 (17.4)	15 (53.6)	1 (1.6)	
Direct thrombin inhibitor therapy	14 (15.2)	8 (28.6)	6 (9.4)	0.018
Thromboembolic events	26 (28.3)	17 (60.7)	9 (14.1)	< 0.001
Mortality	41 (44.6)	10 (35.7)	31 (48.4)	0.259

\*P value: chi-square test for categorical variables and Man-Whitney *U* test for continuous variables.

Data are shown as the median (range) for continuous variables or the number (percentage) for categorical variables unless otherwise indicated. Heparin/PF4 Ab was measured by a commercial ELISA kit, and positive results were defined as optical density (OD) value  $\geq 0.4$ .

Abbreviations: PF4, platelet factor 4; Ab, antibody; UFH, unfractionated heparin; LMWH, low molecular weight heparin; OD, optical density; HIT, heparin-induced thrombocytopenia.

ceived only unfractionated heparin (UFH), 43 (46.7%) both UFH and low molecular weight heparin (LMWH), and 5 (5.4%) LMWH only. Twenty-eight (30.4%) patients were identified as anti-heparin/PF4-seropositive. According to Chong's scoring system, 16 (17.4%) of 92 patients were defined as having definite HIT, 19 (20.7%) as having probable HIT, 28 (30.4%) as having possible HIT, and 29 (31.5%) as unlikely to have HIT. According to the 4 T's scoring system, there were 11 patients (12.0%) with high pretest probability, 33 (35.9%) with intermediate pretest probability, and 48 (52.2%) with low pretest probability. There was good correlation (Spearman correlation,  $r = 0.874$ ,  $P < 0.001$ ) between 4 T's scores and Chong's scores. Heparin was discontinued for all patients after the clinical suspicion of HIT, regardless of the anti-heparin/PF4 test results. Fourteen patients underwent further anticoagulation with direct thrombin inhibitors (11 patients with lepirudin, 2 patients with argatroban, and 1 patient with both lepirudin and argatroban).

## 2. Diagnostic performance of HIT scoring systems and heparin/PF4 antibody ELISA OD values

Heparin/PF4 antibody ELISA OD values according to HIT scoring systems are shown in Fig. 1. The OD was significantly different between the 4 T's category of high, intermediate, and low probability ( $P < 0.001$ ). There was a significant difference between the categories of high versus low and intermediate ( $P < 0.001$ ,  $P = 0.001$ , respectively: Dunn test) but not between the categories of intermediate and low ( $P = 0.118$ ). The OD was significantly different between

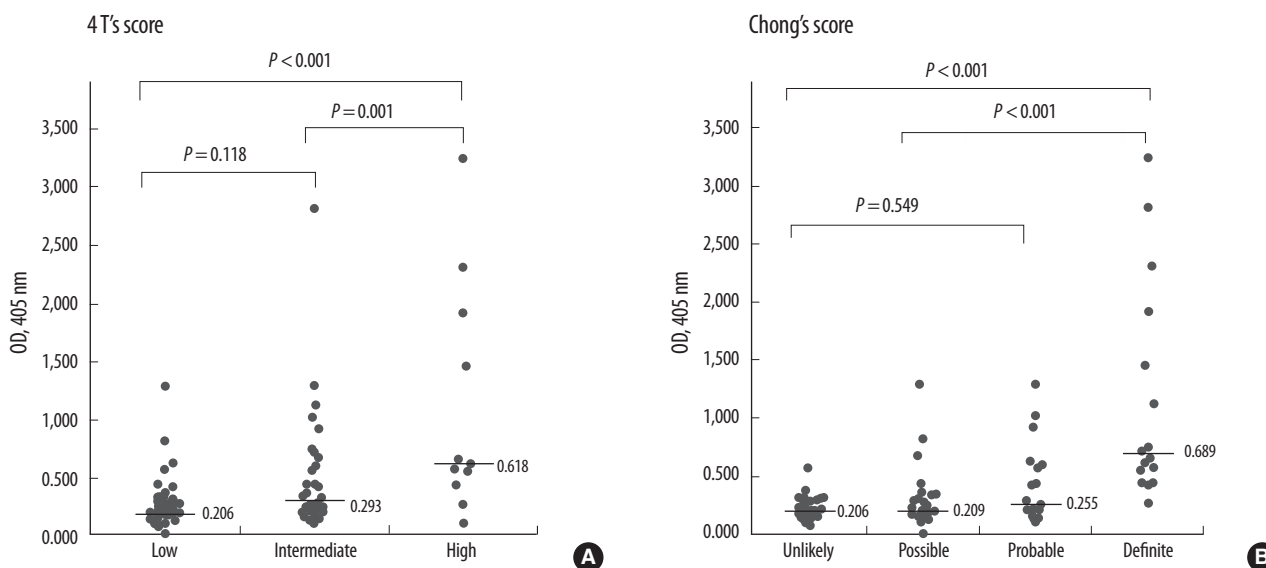
Chong's scoring category of definite, probable, possible, and unlikely for HIT ( $P < 0.001$ ). There was significant difference between the categories of definite vs. unlikely, definite vs. possible, and definite vs. probable ( $P < 0.001$ ) but not between the categories of probable vs. possible and possible vs. unlikely ( $P = 1.0$ ).

The diagnostic characteristics of the 4 T's score and heparin/PF4 antibody test were compared with Chong's scoring system (Table 4). Using the manufacturer's threshold for a positive test at  $OD > 0.4$ , only 1 patient was negative for heparin/PF4 antibody in the definite HIT group, and 1 pa-

**Table 4.** The 4 T's score and heparin/PF4 ELISA positivity according to Chong's category

Chong's category	N of patients	OD > 0.4	4 T's pretest probability	4 T's High + OD > 0.4
Definite	16 (17.4)	15/16 (93.8)	High: 10/16 (62.5) Low: 0/19 (0)	9/16 (56.3)
Probable	19 (20.7)	8/19 (42.1)	High: 1/19 (5.3) Low: 2/19 (10.5)	0/19 (0)
Possible	28 (30.4)	4/28 (14.3)	High: 0/28 (0) Low: 19/28 (67.7)	0/28 (0)
Unlikely	29 (31.5)	1/29 (3.5)	High: 0/29 (0) Low: 27/29 (93.1)	29/29 (100)
		*Sensitivity 93.8%	†Sensitivity 62.5%	Sensitivity 56.2%
		Specificity 96.6%	*Specificity 93.1%	Specificity 100%

\*Sensitivity and specificity were defined for the 45 patients in the Definite and Unlikely HIT categories by Chong's scoring system; †Sensitivity of high pretest probability was defined for the patients in the Definite HIT category by Chong's scoring system; \*Specificity of low pretest probability was defined for the patients in the Unlikely HIT category by Chong's scoring system. Data are shown as the number (percentage) unless otherwise indicated. Abbreviations: PF4, platelet factor 4; OD, optical density.



**Fig. 1.** Optical density (OD) values according to (A) the 4 T's and (B) Chong's scoring system. Bars represent median OD level. Uppermost  $P$  values were calculated using the Kruskal–Wallis test, and pairwise comparisons were performed using the Dunn test.

tient was positive for heparin/PF4 antibody in the unlikely HIT group. Therefore, considering the definite and unlikely HIT patients only, the sensitivity and specificity of the heparin/PF4 ELISA with an OD cut-off of 0.4 was 93.8% and 96.6%, respectively. Ten among 11 patients with high pretest probability according to the 4 T's score were classified as definite HIT by Chong's scoring system. None of the patients with high pretest probability was assessed as unlikely HIT by Chong's scoring system. Therefore, the heparin/PF4 antibody test showed better sensitivity (93.8%) than the 4 T's score used alone (62.5%). When heparin/PF4 antibody was combined with 4 T's high pretest probability, the specificity was 100%, but the sensitivity was only 56.3%.

### 3. Thrombosis and mortality

Thromboembolic events were observed in 26 patients (28.3%). In 17 patients (65.4%), arterial thrombosis occurred (6 had thrombosis involving peripheral arteries, 5 intra-atrial or intraventricular thrombi, 4 brain infarction, and 2 splenic infarction), and 9 patients developed venous thrombosis (4 experienced new or progressive pulmonary embolism, 4 peripheral venous stasis or venous gangrene, and 1 recurrent extracorporeal circuit thrombosis). The clinical and laboratory characteristics of all patients who experienced thromboembolic complications were summarized in Table 5. Of the 26 patients, 17 patients (65.4%) tested positive for

heparin/PF4 antibody (OD value >0.4), while 9 patients (34.6%) tested negative for heparin/PF4 antibody (OD ≤ 0.4). Thrombocytopenia was more severe in patients who developed thrombosis (platelet nadir  $21 \times 10^9/L$ ,  $P=0.015$ ). OD values were significantly higher in patients with thrombosis (median, 0.52), compared to those without thrombosis (0.22,  $P<0.001$ ). More patients in the 4 T's category of intermediate or high pretest probability experienced thromboembolic complication (Table 5). As the OD value increased, the frequency of thromboembolic complication tended to increase (Fig. 2). When the OD cut-offs of 0.4 and 1.0 were considered, 60.7% (17/28) patients with OD > 0.4 subsequently developed thromboembolic complications, while 14.1% (9/64) of patients with OD ≤ 0.4 developed thrombosis. Among patients with OD > 1.0, 77.8% (7/9) developed thrombosis, and 22.9% (19/83) of patients with OD ≤ 1.0 developed thrombosis. Thus, differences of the thrombosis risk between patients with OD > 1.0 and those who with OD ≤ 1.0 were greater, compared with differences between patients with OD > 0.4 and those who with OD ≤ 0.4. When the combination of OD values and 4 T's scores were considered, among 22 patients with 4 T's score ≥ 4 and OD > 0.4, 11 patients (68.2%) developed thrombosis, a higher frequency than when 4 T's score ≥ 4 alone (22/44, 50.0%), or OD > 0.4 alone (17/28, 60.7%) were considered. Meanwhile, among 4 patients with 4 T's score ≥ 6 (high probability) and OD > 1.0, 3 patients (75.0%) developed thrombotic complication, the frequency for which was between 72.7% (8/11) when high probability patients were considered alone and 77.8% (7/9) when patients with OD > 1.0 considered alone. ROC curves were constructed to determine the best cut-offs for the OD value and 4 T's score for the prediction of thrombosis (Fig. 3). The OD value of 0.427 and 4 T's score of 4 had the best performance as cut-off values. At the OD

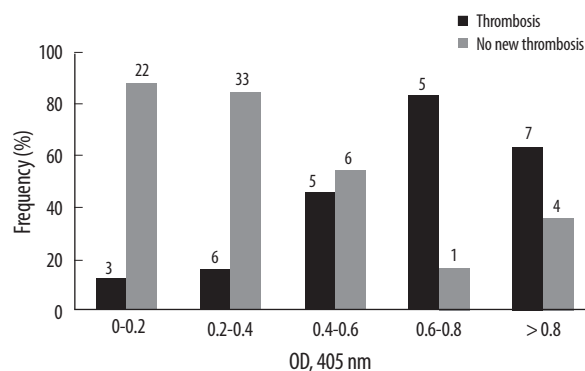
**Table 5.** Characteristics of patients with and without thromboembolic complication

	Thrombosis (N = 26)	No thrombosis (N = 66)	P value*
Age (yr)	63.3 (10.0–83.8)	69.2 (1.4–91.8)	0.776
Female sex (%)	15 (57.7)	29 (43.9)	0.234
Platelet count, nadir ( $\times 10^9/L$ )	21 (7–67)	33 (4–266)	0.015
% Decrease platelet count from baseline	89.5 (48.5–97.9)	79.3 (11.5–98.4)	0.019
LMWH only	1 (3.9)	4 (6.1)	0.673
Cardiopulmonary bypass surgery	19 (73.1)	73 (65.2)	0.622
ELISA optical density	0.52 (0.11–2.81)	0.22 (0.02–3.24)	< 0.001
OD > 0.4	17 (65.4)	11 (16.7)	< 0.001
OD > 1.0	7 (26.9)	2 (3.0)	0.001
4 T's score	5 (1–8)	3 (0–6)	< 0.001
4 T's score ≥ 4	22 (84.6)	22 (33.3)	< 0.001
4 T's score ≥ 6	8 (30.8)	3 (4.6)	0.001
Direct thrombin inhibitor therapy	8 (30.8)	6 (9.1)	0.009

\*P value: chi-square test for categorical variables and Mann-Whitney U test for continuous variables.

Data are shown as the median (range) for continuous variables or the number (percentage) for categorical variables unless otherwise indicated.

Abbreviations: LMWH, low molecular weight heparin; OD, optical density.



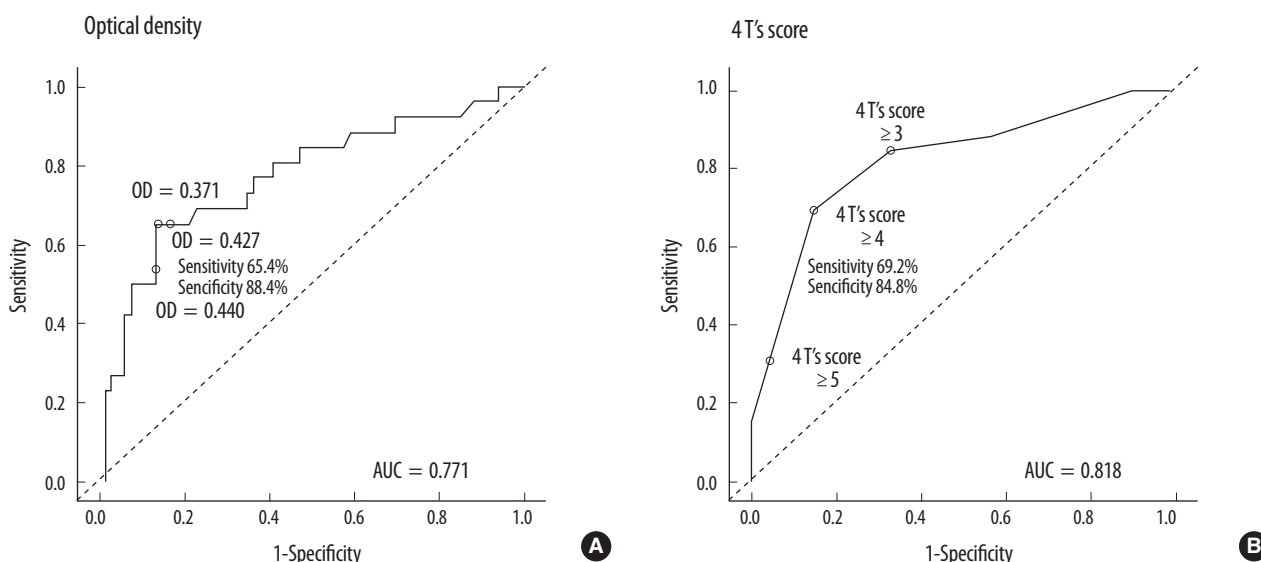
**Fig. 2.** Frequency of thromboembolic complications according to heparin/PF4 ELISA optical density (OD) level increment. The numbers on each bar indicate the number of patients.

value of 0.427, the sensitivity to predict thrombosis was 65.4%, and specificity was 88.4%.

We compared the overall survival and thrombosis-free survival for all the patients according to the OD cut-offs of 0.4 and 1.0 (Fig. 4). Overall mortality of the patients was 44.6%. The overall survivals (OS) were not different between patients with high OD values (OD > 0.4 or OD > 1.0) and patients with lower OD values (250-day OS rate for patients with OD > 0.4 vs. OD ≤ 0.4: 67.9% vs. 56.3%,  $P=0.246$ ; OD > 1.0 vs. OD ≤ 1.0: 55.6% vs. 60.2,  $P=0.908$ ). The 250-day

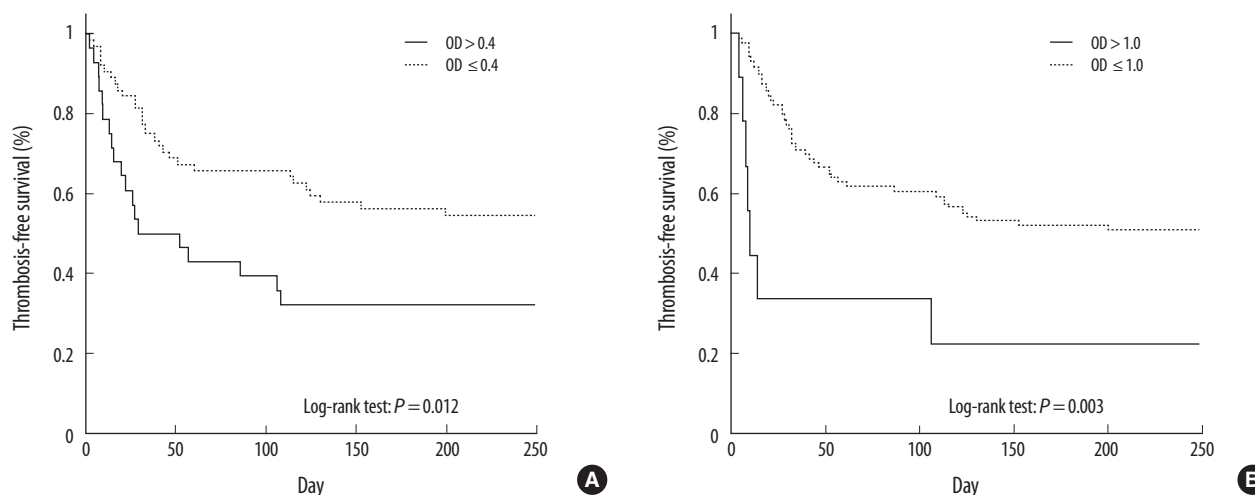
thrombosis-free survival rate of patients with OD > 0.4 was 32.1%, and that of patients with OD ≤ 0.4 was 54.7% ( $P=0.012$ ) (Fig. 4A). The 250-day thrombosis-free survival rate of patients with OD > 1.0 was also significantly lower than that of the patients with lower OD values (22.2% vs. 50.6%,  $P=0.003$ ) (Fig. 4B).

Patients with OD > 0.4 had increased risk of thrombosis, as shown by the odds ratio (OR) for thromboembolic events of 9.44 (95% confidence interval (CI), 3.35–26.6;  $P<0.001$ ). The OR for patients with OD > 1.0 was 11.79 (95% CI, 2.26–



**Fig. 3.** ROC curve relating heparin/PF4 ELISA (A) optical density (OD) value and (B) 4 T's scores to occurrence of thromboembolic complication. (A) When the OD cut-off is 0.427, the sensitivity is 65.4%, and the specificity is 88.4%. (B) When the 4 T's score is greater than or equal to 4, the sensitivity is 69.2%, and the specificity is 84.8%.

Abbreviation: AUC, area under curve.



**Fig. 4.** Thrombosis-free survival for patients who underwent heparin/PF4 ELISA for detecting the presence of the heparin/PF4 antibody based on the optical density (OD) cut-off of 0.4 (A) and 1.0 (B).

61.55;  $P=0.003$ ). When OD values  $>1.0$  were compared with those with lower positive OD values between 0.4 and 1.0, those with higher OD values tended to have increased risk; however, the increase was not statistically significant (OR, 3.85; 95% CI, 0.6–23.05;  $P=0.139$ ). With multiple logistic regression analysis, higher OD value was an independent predictor of thromboembolic event. The OR of patients with OD  $>0.4$ —after adjusting for age, gender, cardiovascular bypass, and baseline platelet count—was 10.01 (95% CI, 3.26–30.73;  $P<0.001$ ), and the adjusted OR of patients with OD  $>1.0$  was 12.10 (95% CI, 2.17–67.55;  $P=0.004$ ). Overall, for every increase of 0.2 OD values in the heparin/PF4 ELISA, the risk of the thromboembolic events increased by OR of 1.39 (95% CI, 1.09–1.78;  $P=0.007$ ).

## DISCUSSION

HIT is a serious complication of heparin treatment, which often leads to life-threatening thrombotic events. Although HIT is a relatively well-known complication, its diagnosis is still challenging because of its variable presentation and the difficulty of proving heparin's effects in patients with many other risk factors [8]. The gold standard for HIT diagnosis is the SRA; however, because this test is technically difficult, time-consuming, and involves the use of radioactive materials, it is usually only performed by a few reference laboratories. Therefore, obtaining SRA results in a timely manner is almost impossible in real practice. Rapid diagnosis of HIT is critical, because timely discontinuation of heparin and use of alternative anticoagulants, such as direct thrombin inhibitors, is known to reduce both morbidity and mortality [8]. Recently, several reports have suggested the usefulness of the clinical pretest probability scoring system, the 4 T's score, in combination with the more rapid and simple enzyme-immunoassay (EIA) for heparin/PF4 antibodies to assess the probability of HIT [9, 10]. Patients with HIT typically have thrombocytopenia 5–10 days after exposure to heparin. HIT-IgG antibodies generally are not detectable before day 5 of heparin treatment, but are readily detectable using sensitive assays when the platelet count first begins to fall because of HIT. EIA for heparin/PF4 antibodies is known to be less specific because it detects all antibodies, including those that do not activate platelets. However, in combination with clinical scoring systems, interpretation of the test results could become more reliable [10, 11]. Although the turnaround time of the heparin/PF4 ELISA may vary depending on the conditions of each laboratory, it can usually be performed far more rapidly and easily than the SRA. Furthermore, another useful aspect of the EIA for heparin/PF4

antibody is that the results can be interpreted quantitatively. Several reports have shown that higher OD values are related to either the higher diagnostic probability of HIT [12–14] or the risk of thrombosis [7, 15]. Other studies have demonstrated that the greater the magnitude of a positive EIA, the greater the likelihood that the patient has heparin-dependent platelet-activating antibodies and, hence, clinical HIT [6, 7, 14, 15]. Zwicker et al. [7] reported that the risk of thrombosis in patients with higher OD values (OD  $>1.0$ ) was approximately sixfold higher than that in patients with weak-positive results (0.4–1.0 OD units), and Warkentin et al. [14] found that strong-positive results of heparin/PF4 ELISA were associated with stronger SRA results. The results of our study support the findings of previous studies in that patients with increasing OD values also had a greater risk of thromboembolic complication. Thrombotic events of the arterial system were more frequently observed in this study. Previous prospective studies on HIT showed that HIT patients with cardiovascular disease were more likely to develop arterial thrombosis, whereas venous and arterial thrombotic events occur in approximately equal numbers in medical patients, and venous thrombosis was strongly associated with the postoperative state [16]. The pattern of thrombosis in this study could be thought of as reflecting the characteristics of the study population, with its predominance of cardiovascular surgery patients. In contrast to thrombosis risk, mortality did not differ according to the OD values. One possible explanation for this is that clinicians requested HIT testing for those patients with suspected HIT, with the result that most suspected patients underwent proper HIT management, namely discontinuation of heparin and administration of alternative anticoagulants. With the ROC analysis, the best cut-off for the prediction of thrombosis was an OD value of 0.427 and a 4 T's score of 4. These are close to the currently used cut-offs of OD value 0.4 and 4 T's category of intermediate probability. This finding implies that the current cut-offs can be used as predictors of thrombosis.

Our study had some limitations. First, it was a retrospective study, and hence, an exact assessment of the prevalence and incidence of HIT and its complications was not possible. Second, it was a single-center study of a tertiary university hospital, and cardiovascular surgical patients were predominant; therefore, the study population did not reflect the characteristics of the entire Korean population. Finally, the gold standard laboratory test, the SRA, was not performed; hence, interpretation of the data was limited.

This is the first report of the clinical and laboratory characterization of HIT in the Korean population. Our results

were consistent with those of previous studies, and we were able to confirm the usefulness of the heparin/PF4 ELISA and the clinical scoring system for the diagnosis of and thrombosis prediction in HIT in Korean patients. More attention must be paid to the use of clinical scores and OD values as thrombosis predictors in HIT.

### Authors' Disclosures of Potential Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

### Acknowledgements

This work was supported by a grant from the SNUH Research Fund (04-2010-017).

## REFERENCES

- Greinacher A and Warkentin TE. Recognition, treatment, and prevention of heparin-induced thrombocytopenia: review and update. *Thromb Res* 2006;118:165-76.
- Chong BH and Chong JJ. Heparin-induced thrombocytopenia. *Expert Rev Cardiovasc Ther* 2004;2:547-59.
- Warkentin TE and Heddle NM. Laboratory diagnosis of immune heparin-induced thrombocytopenia. *Curr Hematol Rep* 2003;2:148-57.
- Lo GK, Juhl D, Warkentin TE, Sigouin CS, Eichler P, Greinacher A. Evaluation of pretest clinical score (4 T's) for the diagnosis of heparin-induced thrombocytopenia in two clinical settings. *J Thromb Haemost* 2006;4:759-65.
- Greinacher A, Juhl D, Strobel U, Wessel A, Lubenow N, Selleng K, et al. Heparin-induced thrombocytopenia: a prospective study on the incidence, platelet-activating capacity and clinical significance of antiplatelet factor 4/heparin antibodies of the IgG, IgM, and IgA classes. *J Thromb Haemost* 2007;5:1666-73.
- Warkentin TE, Sheppard JA, Moore JC, Moore KM, Sigouin CS, Kelton JG. Laboratory testing for the antibodies that cause heparin-induced thrombocytopenia: how much class do we need? *J Lab Clin Med* 2005;146:341-6.
- Zwicker JI, Uhl L, Huang WY, Shaz BH, Bauer KA. Thrombosis and ELISA optical density values in hospitalized patients with heparin-induced thrombocytopenia. *J Thromb Haemost* 2004;2:2133-7.
- Warkentin TE and Greinacher A. Heparin-induced thrombocytopenia: recognition, treatment, and prevention: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004;126:311S-37S.
- Bakchoul T, Giptner A, Najaoui A, Bein G, Santoso S, Sachs UJ. Prospective evaluation of PF4/heparin immunoassays for the diagnosis of heparin-induced thrombocytopenia. *J Thromb Haemost* 2009;7:1260-5.
- Bryant A, Low J, Austin S, Joseph JE. Timely diagnosis and management of heparin-induced thrombocytopenia in a frequent request, low incidence single centre using clinical 4T's score and particle gel immunoassay. *Br J Haematol* 2008;143:721-6.
- Janatpour KA, Gosselin RC, Dager WE, Lee A, Owings JT, Zhou J, et al. Usefulness of optical density values from heparin-platelet factor 4 antibody testing and probability scoring models to diagnose heparin-induced thrombocytopenia. *Am J Clin Pathol* 2007;127:429-33.
- Whitlatch NL, Perry SL, Ortel TL. Anti-heparin/platelet factor 4 antibody optical density values and the confirmatory procedure in the diagnosis of heparin-induced thrombocytopenia. *Thromb Haemost* 2008;100:678-84.
- Weiss BM, Shumway NM, Howard RS, Ketchum LK, Reid TJ. Optical density values correlate with the clinical probability of heparin induced thrombocytopenia. *J Thromb Thrombolysis* 2008;26:243-7.
- Warkentin TE, Sheppard JI, Moore JC, Sigouin CS, Kelton JG. Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. *J Thromb Haemost* 2008;6:1304-12.
- Mattioli AV, Bonetti L, Carletti U, Ambrosio G, Mattioli G. Thrombotic events in patients with antiplatelet factor 4/heparin antibodies. *Heart* 2009;95:1350-4.
- Warkentin TE, Sheppard JA, Horsewood P, Simpson PJ, Moore JC, Kelton JG. Impact of the patient population on the risk for heparin-induced thrombocytopenia. *Blood* 2000;96:1703-8.