Original Article

Relationship between the 4Ts scoring system and the antiplatelet factor 4/heparin antibodies test in critically ill patients

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Aim: Heparin-induced thrombocytopenia (HIT) is an adverse drug reaction and potentially progresses to fatal thrombosis. The 4Ts scoring system has been reported as a clinical pretest for HIT. However, its usefulness in critically ill patients has not yet been thoroughly examined. Thus, we evaluated the clinical usefulness of the 4Ts score in the diagnosis of HIT in critically ill patients.

Methods: One hundred and four critically ill patients who were admitted to our intensive care unit and who underwent the antiplatelet factor 4/heparin complex antibodies (PF4/heparin Ab) test with suspected HIT were enrolled in the study. The primary endpoint variable was the 4Ts score. The secondary endpoint variables were laboratory data, length of stay, and mortality, compared between thePF4/heparin Ab positive and negative groups.

Results: There was no significant difference in the 4Ts scores between the PF4/heparin Ab positive and negative groups. The positive predictive value (HIT patients/4T high score patients) was 15.4% (2/13), the negative predictive value (non-HIT patients/4T low score patients) was 87.5% (42/48), and the false-negative rate for the 4Ts score (4T low score patients/HIT patients) was as high as 54.5% (6/11). The PF4/heparin Ab positive patients had longer stay in intensive care compared to the PF4/heparin Ab negative patients ($P = 0.035$).

Conclusions: The present study showed the discrepancy between the 4Ts score and PF4/heparin Ab. When HIT is suspected in critically ill patients, an immediate HIT antibody test and initiation of therapeutic management of HIT are required regardless of the 4Ts score.

Key words: 4Ts score, critically ill patients, heparin-induced thrombocytopenia, ICU length of stay

INTRODUCTION

CRITICALLY ILL PATIENTS frequently develop thrombocytopenia.[1–4] The cause of thrombocytopenia involves the following four mechanisms: increased destruction or consumption (e.g., idiopathic thrombocytopenic purpura, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, heparin-induced thrombocytomenia); decreased production (e.g., aplasctic anemia, bone marrow suppression); dilution (e.g., massive hemorrhage); sequestration (e.g., splenomegally). The major etiologies of thrombocytopenia encountered in intensive care units (ICUs) are sepsis, trauma, disseminated intravascular coagulation, and drugs including heparin.[2]

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Heparin is used in critically ill patients in ICUs for various purposes, such as treatment for thrombotic diseases, as an anticoagulant in arterial lines and extracorporeal circuits, and for prevention of deep venous thrombosis. Thus, heparin is widely used in critically ill patients. Heparin-induced thrombocytopenia (HIT) is an adverse drug reaction associated with heparin exposure and occurring at a low incidence. Immune-mediated HIT may progress to fatal arterial and venous thrombosis.[5] In particular, thrombosis is associated with HIT in critically ill patients at a high incidence,[6] which makes establishment of a method for exact diagnosis of HIT an important and urgent study subject.

When HIT is suspected, it is recommended in standard treatment guidelines that heparin should be stopped and switched to an alternative anticoagulant, with an HIT antibody test carried out as a serological diagnosis.[5,7] Methods for serological diagnosis of HIT can be divided into two major categories, antigen assays and functional assays. An antigen assay detecting antiplatelet factor 4/heparin complex antibodies (PF4/heparin Ab) by enzyme-linked immunosorbent assay (ELISA) is widely used in clinical settings, but the detection

Table 1. Pretest clinical scoring system (the 4Ts) for suspected heparin-induced thrombocytopenia in critically ill patients

4Ts	2 points	1 point	0 points
Thrombocytopenia	Platelet count fall >50% or platelet nadir ≥20	Platelet count fall 30–50% or platelet nadir 10–19	Platelet count fall <30% or platelet nadir <10
Timing of platelet count fall	Clear onset between days 5–10 or platelet fall ≤1 day (prior heparin exposure within 30 days)	Consistent with days 5–10 fall, but not clear (e.g., missing platelet counts); onset after day 10; or fall <1 day (prior heparin exposure 30–100 days ago)	Platelet count fall <4 days without recent exposure
Thrombosis or other sequelae	New thrombosis (confirmed); skin necrosis, acute systemic reaction post-intravenous unfractionated heparin bolus	Progressive or recurrent thrombosis; non-necrotizing (erythematous) skin lesions; suspected thrombosis (not proven)	None
Other cause for thrombocytopenia	None apparent	Possible	Definite

From: Crowther MA, Cook DJ, Albert M *et al*. The 4Ts scoring system for heparin-induced thrombocytopenia in medical–surgical intensive care unit patients. J. Crit. Care 2010; 25: 287–93, with permission. See references 8 and 9 for the further detail.

of PF4/heparin Ab cannot be a definitive diagnosis of HIT. In contrast, functional assays examining the biological function of PF4/heparin Ab to activate platelets and induce HIT are generally accepted as the reference standard assays for HIT.[5] Whereas antigen assays are easy to carry out and widely used to detect HIT antibodies, functional assays are only available at a few centers because they are technically difficult.[5] In addition to serological diagnosis by an HIT antibody test, a pretest clinical scoring system involving evaluation of the following four scores (4Ts score) has been reported as a clinical pretest for HIT (Table 1):[8,9] (i) thrombocytopenia, (ii) timing of platelet count fall, (iii) thrombosis or other sequelae, (iv) other cause for thrombocytopenia. Validation of the clinical usefulness of the 4Ts score has been reported for cardiac surgery patients[10–12] and hemodialysis patients.[13,14] Although the 4Ts score has an advantage in that it eliminates HIT without carrying out an HIT antibody test due to its high negative predictive values,[7] some patients are reported to develop HIT even with a low 4Ts score.[15] Some patients with positivity for anti-PF4/heparin antibodies proven by ELISA have been reported to be negative for platelet activation when tested using a functional assay, indicating that they do not develop clinical HIT.[5] We have experienced the discrepancy between the 4Ts score and PF4/heparin Ab, especially in critically ill patients. Therefore, we assessed the clinical usefulness of the 4Ts score in the diagnosis of HIT in critically ill patients who developed thrombocytopenia and thrombosis after exposure to heparin and underwent a PF4/heparin Ab test with suspected HIT. Furthermore, two patient groups, PF4/heparin Ab positive and negative were compared for hematological parameters related to coagulation and inflammation after suspicion of HIT, treatment duration including length of ICU stay, and survival outcome.

MATERIALS AND METHODS

Patients

THE SUBJECTS OF the study consisted of 104 critically ill patients who were admitted to the medical–surgical ICU (18 beds) of Chiba University Hospital (Chiba, Japan) between January 2006 and September 2012 and underwent PF4/heparin Ab test with suspected HIT. Platelet counts were obtained every day during the ICU stay. Suspicion of HIT was determined by the attending critical care physician based on the following criteria: (i) development of thrombocytopenia (i.e., a drop in platelet count) or thrombosis after heparin administration, (ii) the absence of any other obvious clinical explanation for thrombocytopenia.[16] In all patients with suspected HIT, a PF4/heparin Ab test was carried out for serological diagnosis with immediate discontinuation of heparin and commencement of argatroban, an antithrombin agent. We diagnosed HIT in all patients by both PF4/heparin Ab and the normalization of the platelet count within 10 days after heparin discontinuation.[16]

The Institutional Review Board at Chiba University approved the study.

Measurements

For serological diagnosis of HIT, PF4/heparin Ab was detected by ELISA using a commercial assay kit (HPIA-IgG;

Diagnostica Stago, Parsippany, NJ, USA). The positivity for PF4/heparin Ab was determined using a cut-off value for optical density (0.41–0.50) specified by the kit manufacturer for each lot of assay kits and indicated in the kit protocol. The patients positive and negative for PF4/heparin Ab were classified into PF4/heparin Ab positive and PF4/heparin Ab negative groups, respectively. The 4Ts score was calculated for individual patients to further divide them into three HIT probability categories: low score (0–3 points); intermediate score (4–5 points); and high score (6–8 points).[9] The incidence of thrombosis was calculated based on the definition of thrombosis used in the 4Ts scoring system: new thrombosis (confirmed) (including cerebral infarction, pulmonary embolism, deep venous thrombosis, and clotting in the extracorporeal circuit); skin necrosis; and acute systemic reaction post-intravenous unfractionated heparin bolus. When HIT was suspected, additional laboratory data related to blood coagulation including prothrombin time-international normalized ratio (PT-INR), fibrin/fibrinogen degradation products (FDP), D-dimer, as well as those related to inflammatory reaction including white blood cells, C-reactive protein (CRP) and blood interleukin-6 (IL-6) levels, were also collected.

Statistical analysis

The primary endpoint variable was the 4Ts score. As a secondary analysis, laboratory data related to blood coagulation and inflammatory reactions collected upon suspicion of HIT were compared between PF4/heparin Ab positive and negative groups to explore laboratory data helpful in diagnosis of HIT. Furthermore, we tested for differences in incidence of thrombosis, length of ICU and hospital stay, and survival prognosis between the PF4/heparin Ab positive and negative groups in the secondary analysis.

All data are presented as median and interquartile range. The statistical analysis was carried out using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). We tested for differences in baseline characteristics using Fisher's exact test for categorical data and a Mann–Whitney *U*-test for continuous data. Differences were considered significant using a two-tailed $P < 0.05$.

RESULTS

DURING THE PERIOD between January 2006 and September 2012, a total of 5,095 patients were admitted to the ICU of Chiba University Hospital for critical care, including 104 patients (2.0%) with suspected HIT. When the HIT antibody test was carried out in all of the 104 suspected patients, 18 patients (17.3%) were positive for PF4/heparin Ab. We diagnosed 11 patients as HIT. Thus, the incidence of HIT in our ICU was 0.22% (11/5,095).

No significant difference in age, gender, severity score, or blood IL-6 level at ICU admission was observed between the PF4/heparin Ab positive and negative groups (Table 2). No significant difference in the 4Ts total score or any of its four subscores was observed between the PF4/heparin Ab positive and negative groups (Table 3). The positive predictive value (defined as the percentage of HIT patients in the "4T high score" patient category) was 15.4% (2/13), whereas the negative predictive value (defined as the percentage of non-HIT patients in the "4T low score" patient category) was 87.5% (42/48) (Table 4). Six HIT patients were in the "4T

Table 2. Baseline characteristics of 104 critically ill patients who underwent antiplatelet factor 4/heparin complex antibodies (PF4/heparin Ab) test for suspected heparin-induced thrombocytopenia

	PF4/heparin Ab positive	PF4/heparin Ab negative	*P*-value
	(*n* = 18)	(*n* = 86)	
Age, years	70 (57.8–74.8)	63.0 (49.0–71.0)	0.123
Gender – male, *n* (%)	10 (55.6)	58 (67.4)	0.416
APACHE II	32.5 (18.3–40)	24 (17–32)	0.073
SOFA	9.5 (8–12)	10 (7–13)	0.981
Surgical, *n* (%)	3 (16.7)	21 (24.4)	0.759
Cardiovascular, *n* (%)	1 (5.6)	13 (15.1)	0.455
Sepsis, *n* (%)	3 (16.7)	23 (26.7)	0.551
Blood IL-6 level, pg/mL	682 (204–2,698)	211 (73–771)	0.145

Data are median (25th–75th percentile). *P*-values were calculated with the use of Fisher's exact test or a Mann–Whitney *U*-test. APACHE, Acute Physiology and Chronic Health Evaluation score on intensive care unit (ICU) admission; IL-6, interleukin-6 on ICU admission; SOFA, sequential organ failure assessment score on ICU admission.

Table 3. Comparison of 4Ts pretest clinical scoring system between heparin-induced thrombocytopenia-antibody positive and negative patients

4Ts pretest clinical score	PF4/heparin Ab positive (n = 18)	PF4/heparin Ab negative (n = 86)	P-value
Thrombocytopenia	1 (1–2)	1 (0–2)	0.758
Timing of platelet count fall	1 (1–2)	1 (1–2)	0.443
Thrombosis or other sequelae	0 (0–2)	0 (0–2)	0.771
Other causes for thrombocytopenia	1 (0–1)	1 (1–1)	0.091
Total score	3.5 (2.3–4.8)	4.0 (3.0–5.0)	0.988

Data are median (25th–75th percentile). P-values were calculated with the use of a Mann–Whitney U-test. PF4/heparin Ab, antiplatelet factor 4/heparin complex antibodies.

Table 4. Diagnostic ability of 4Ts pretest clinical scoring system in critically ill patients (n = 104)

4Ts score	PF4/heparin Ab positive (HIT patients)	PF4/heparin Ab negative	P-value
Low, n	9 (6)	39	0.261
Intermediate, n	5 (3)	38	
High, n	4 (2)	9	

Categories of 4Ts score: 0–3, Low; 4–5, intermediate; 6–8, high. P-values were calculated with the use of a χ^2-test. HIT, heparin-induced thrombocytopenia; PF4/heparin Ab, antiplatelet factor 4/heparin complex antibodies.

Table 5. Laboratory data of critically ill patients with suspected heparin-induced thrombocytopenia (n = 104)

	PF4/heparin Ab positive (n = 18)	PF4/heparin Ab negative (n = 86)	P-value
Coagulation			
Platelet (10^3/μL)	32.5 (19.3–67)	35 (23.3–87.3)	0.537
PT-INR	1.23 (1.14–1.39)	1.24 (1.08–1.45)	0.887
FDP (μg/mL)	27.7 (12.9–50.7)	27.8 (14.3–52.0)	0.998
D-dimer (μg/mL)	24.0 (11.1–36.6)	21.4 (9.8–37.0)	0.702
Inflammation			
WBC (10^3/μL)	9.4 (5.4–13.6)	9.4 (6.0–14.2)	0.968
CRP (mg/dL)	7.1 (2.9–12.4)	5.7 (1.7–11.9)	0.486
IL-6 (pg/mL)	140 (67.5–316)	87 (28–314)	0.278

Data are median (25th–75th percentile) for continuous variables. P-values were calculated with the use of a Mann–Whitney U-test. CRP, C-reactive protein; FDP, fibrin/fibrinogen degradation products; WBC, white blood cells; IL-6, interleukin-6; PF4/heparin Ab, antiplatelet factor 4/heparin complex antibodies; PT-INR, prothrombin time–international normalized ratio.

low score" category, indicating a false-negative rate for the 4Ts score of 54.5% (6/11) (Table 4).

No significant difference in any of the laboratory parameters obtained at the time point when HIT was suspected (platelet count, PT-INR, FDP, D-dimer, white blood cells, C-reactive protein, and blood IL-6) was observed between the PF4/heparin Ab positive and negative groups (Table 5). The incidence of thrombotic events in the PF4/heparin Ab

Table 6. Comparison of clinical outcome between heparin-induced thrombocytopenia antibody positive and negative patients (*n* = 104)

	PF4/heparin Ab positive (*n* = 18)	PF4/heparin Ab negative (*n* = 86)	*P*-value
Heparin exposure duration, days	12 (10.3–17.3)	9.5 (5–19.8)	0.382
Thrombotic event, *n* (%)	5 (27.8)	22 (25.6)	1.000
ICU length of stay, days	31.5 (24–52.5)	20 (9–41.8)	0.035
Hospital length of stay, days	49 (34–94)	76 (36.5–117.5)	0.436
28-day mortality, *n* (%)	2 (11.1)	14 (16.3)	0.732
90-day mortality, *n* (%)	7 (38.9)	30 (34.9)	0.790
Hospital mortality, *n* (%)	9 (50)	45 (52.3)	1.000

Data are median (25th–75th percentile) for continuous variables. *P*-values were calculated with the use of Fisher's exact test and Mann–Whitney *U*-test. PF4/heparin Ab, antiplatelet factor 4/heparin complex antibodies.

positive group was 27.8% (5/18), and that in the negative group was 25.6% (22/86). The classification and number of thrombotic events were: cerebral infarction, 3; myocardial infarction (coronary thrombosis), 1; portal thrombosis, 1 in PF4/heparin Ab positive group; pulmonary embolism/deep venous thrombosis, 7; myocardial infarction (coronary thrombosis), 4; frequent thrombi formation in extracorporeal circuit, 3; mesenteric arterial thrombosis, 2; portal thrombosis, 1; shunt thrombosis, 1; and pulmonary venous thrombosis, jugular venous thrombi, 1 in PF4/heparin Ab negative group. There was no HIT-associated death. The length of ICU stay was significantly longer in the PF4/heparin Ab positive group than in the PF4/heparin Ab negative group ($P = 0.035$) (Table 6). No significant difference in length of hospital stay or mortalities was observed between these two groups.

DISCUSSION

THE PRESENT STUDY indicated no significant difference in 4Ts score between two groups of critically ill patients with suspected HIT, PF4/heparin Ab positive and PF4/heparin Ab negative. The positive predictive value (the percentage of HIT patients in the "4T high score" patient category) was low, whereas the negative predictive value (the percentage of non-HIT patients in the "4T low score" patient category) was high. Positivity for PF4/heparin Ab and diagnosis of HIT was shown even in the "4T low score" patient category, indicating that a 4T low score per se could not rule out the possibility of HIT. The length of ICU stay was significantly longer in the PF4/heparin-Ab positive group than in the PF4/heparin-Ab negative group. However, there was no significant difference in length of hospital stay or survival prognosis.

In patients with HIT, platelet factor 4 (PF4) is released from platelets after heparin exposure and forms a complex with heparin. Conformational changes in the PF4 molecule induced by complex formation with heparin generate new antigenicity of this protein to induce production of anti-PF4/heparin antibodies directed against newly generated antigenic determinants.[5,7] Previous studies have reported the existence of genetic predisposition to production of HIT antibodies[17] and thrombotic complications.[18] As the frequency distribution of disease-associated single nucleotide polymorphisms depends on ethnicity, the incidence of HIT may vary in different ethnic groups and different geographic areas. The present study showed that the incidence of HIT in critically ill Japanese patients was 0.22% (11/5,095), a value within the range 0–0.47% reported in European and North American ICUs.[4,12,19] The present study also showed that HIT was diagnosed in 10.6% (11/104) of our patients with suspected HIT, whereas the reported prevalence of HIT in 13 studies selected for a recent meta-analysis to estimate the predictive value of the 4Ts scoring system in a heterogeneous group of patients with suspected HIT ranged from 4% to 42%.[20]

The 4Ts score was first proposed by Warkentin and Heddle in 2003[21] and the first assessment of its diagnostic utility was reported by Lo *et al*. in 2006.[9] In the latter study, the 4Ts score was used in two different centers (a Canadian general hospital and a German HIT testing laboratory) to assess patients with suspected HIT mainly derived from the patient categories of cardiovascular surgery, internal medicine, and intensive care. The negative predictive values of the 4Ts score in Canada and Germany were calculated to be 98.4% and 100%, respectively.[9] High negative predictive values ranging from 91.4% to 100% have been reported in subsequent studies carried out in medical–surgical ICU

patients and cardiothoracic surgical patients.[8–10,15,22–26] In the present study, a high negative predictive value of 4Ts score, 87.5%, was obtained for HIT, similarly to the previous studies mentioned above. This value was still lower than the values ranging from 91.4% to 100% in previous reports. This is because there were six patients whose PF4/heparin Ab was positive but 4Ts score was low. The characteristics of these patients were: (i) timing of platelet count fall was after day10 of heparin exposure, (ii) there was no thrombosis, (iii) there were other possible (not definite) causes for thrombocytopenia (e.g., disseminated intravascular coagulation, bone marrow suppression). In line with previous reports that the etiology of thrombocytopenia is multifactorial,[1,2] there were still many patients for whom we could not fully explain their etiologies. However, the reported prevalence of HIT is extremely low, less than 0.2–5% in patients receiving heparin[16,27,28] and less than 0.5% in ICU patients,[4,12,19,29] and it should be noted that the negative predictive value of a diagnostic test is higher when the prevalence of the target disease in the population to be tested is lower.[30] The positive predictive value of the 4Ts score was as low as 15.4%, a value less than the previously reported range from 21.4% to 100%.[8–10,15,22–26] The false-positive rate of the 4Ts score (percentage of "4T high score" patients in the non-HIT patients) was 11.8%, a value comparable to those previously reported (0–15.3%).[8–10,15,22–26] In contrast, the false-negative rate of the 4Ts scores (percentage of "4T low score" patients in HIT patients) was 54.5% (6/11), a value considerably higher than those previously reported (0–25%).[8–10,15,22–26] These findings suggested that many individuals might develop HIT even with a low 4Ts score.

The present study showed that the underlying disease of HIT was sepsis in 25% (26/104) of the patients studied. Spontaneous formation of HIT antibodies has been observed in 3–8% of healthy individuals.[31–33] Production of HIT antibodies is reportedly associated with periodontal disease[34] and induced by complex formation between PF4 generated after bacterial infection and invading bacterial cells.[32] Cases of "spontaneous HIT," characterized by manifestation of clinical symptoms of HIT and positivity for HIT antibodies without prior heparin exposure, have also been reported.[35] Platelet factor 4/heparin Ab positive individuals may include those developing spontaneous HIT.

In addition to the 4Ts score, the present study investigated the clinical usefulness of indicators of blood coagulation and inflammatory reaction in the prediction of positivity for PF4/heparin Ab. No significant difference in these hematological parameters was observed between the two patient groups, PF4/heparin Ab positive and negative, and it was impossible to predict positivity for PF4/heparin Ab based on laboratory testing other than serological detection of HIT antibodies.

The length of ICU stay was significantly longer in the PF4/heparin Ab positive group than in the PF4/heparin Ab negative group, consistent with Gettings *et al*. who reported that the length of ICU stay in the PF4/heparin Ab positive group (mean, 20 days) was longer than that in the PF4/heparin Ab negative group (mean, 10 days).[36] The definite causes of difference in length of ICU stay remain obscure. There were more HIT patients in the PF4/heparin Ab positive group, and they might need longer duration to treat HIT with argatroban or warfarin. Although the average length of hospital stay was reported to be longer in HIT patients (26.3 days) than in non-HIT patients (10.5 days),[37] the present study identified no significant difference in length of hospital stay or mortality rates between HIT and non-HIT patients. This lack of difference in length of hospital stay and survival outcome may be ascribable to immediate heparin discontinuation on suspicion of HIT regardless of the value of the 4Ts score, the use of argatroban as an alternative anticoagulant until establishment of positivity for the HIT antibody test, and early initiation of appropriate therapeutic management in the PF4/heparin-Ab positive group.

The present study has two limitations: (i) this was a retrospective study in a single center, (ii) we diagnosed eleven patients as HIT, but they were not assessed for platelet activation by a functional assay.

CONCLUSION

THE PRESENT STUDY assessed the clinical usefulness of the 4Ts score in critically ill patients with suspected HIT and showed the discrepancy between 4Ts score and PF4/heparin Ab. The results indicated that there was no difference in 4Ts score between the PF4/heparin Ab positive and negative groups, and that the false-negative rate of the 4Ts score was high, indicating that a low 4Ts score alone could not completely eliminate HIT. When HIT is suspected in critically ill patients, immediate HIT antibody test and initiation of therapeutic management of HIT are required regardless of 4Ts score.

CONFLICT OF INTEREST

NONE.

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