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SHORT COMMUNICATION

Evaluation of STic Expert® HIT Kit and Its Comparison with ID-PaGIA™ Test in Suspected Heparin-Induced Thrombocytopenia

Narender Kumar[1] · Varun Uppal[1] · Jasmina Ahluwalia[1] · Pankaj Malhotra[2] · Neelam Varma[1] · Ashish Jain[3]

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Abstract Heparin-induced thrombocytopenia (HIT) is an adverse drug reaction caused by heparin. HIT occurs due to IgG antibodies directed against heparin-bound platelet factor 4 (PF4). The aim of this study was to evaluate the STic® Expert HIT for detection of anti-PF4/heparin antibodies in comparison with ID-PaGIA™ kit. The results were further confirmed by Heparin-induced platelet aggregation test (HIPA). A total of 17 patients with a suspected diagnosis of HIT were enrolled. The 4 T scoring of each case was performed. Testing for HIT was carried out by ID-PaGIA™, STic® Expert HIT, and HIPA. Testing by STic® Expert HIT test demonstrated positivity in three cases while testing by ID-PaGIA™ test kit revealed four positive cases. Two of these cases were confirmed as HIT by HIPA. The study suggests that STic® Expert HIT an equally effective test, in combination with the 4T scoring system for detecting/excluding the diagnosis of HIT. A large number of cases are needed for validation.

Keywords ID-PaGIA™ · Heparin-induced platelet aggregation · Heparin-induced thrombocytopenia · STic® Expert HIT

✉ Narender Kumar
narenderkalson@gmail.com

[1] Department of Hematology, Level 5, Research Block A, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India

[2] Department of Internal Medicine, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India

[3] Department of Transfusion Medicine, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India

Introduction

Heparin-induced thrombocytopenia (HIT) is an immune-mediated, prothrombotic condition that happens after exposure to unfractionated heparin (UFH), low molecular weight heparin (LMWH), or other polyanions [1]. HIT occurs due to the formation of IgG antibodies against the complex formed between heparin and platelet factor 4 (PF4) leading to platelet/endothelial cell activation followed by thrombocytopenia [2]. The incidence of HIT ranges from 3 to 5% in UFH and 0.2–0.6% with LMWH therapy [3]. HIT usually occurs within 4–15 days after heparin infusion but may occur as early as 1 day in cases with a prior history of heparin exposure. The disorder is associated with venous thromboembolic events—heparin-induced thrombocytopenia-thrombosis (HITT) and a mortality rate up to 30% [4]. The diagnosis of HIT is based on the 4T pre-test probability score and laboratory documentation of heparin-dependent antibodies [5, 6]. The laboratory gold-standard for the diagnosis of HIT is the demonstration of in vitro platelet-activating HIT antibodies. The functional assays are time-consuming and not widely available. The rapid laboratory evidence of anti-PF4/heparin antibodies can be achieved by immunoassays, especially enzyme-linked immunosorbent assays (ELISA) and particle gel immune assays (PaGIA) [7, 8]. Recently a new diagnostic test (STic® Expert HIT) has been introduced for fast detection of HIT [9].

Since HIT is associated with a risk of thrombosis and mortality; a rapid laboratory diagnosis is necessary to guide treatment decisions. The aim of the study was to assess the ability of two commercially available immunoassays for detection of anti-PF4/heparin antibodies and their confirmation by Heparin Induced platelet aggregation test

(HIPA). The results of the testing were evaluated with the 4T score to predict the pretest clinical probability for HIT.

Materials and Methods

This was a prospective study carried out in the coagulation laboratory of Hematology Department over a period of 2 years (2014–2016). The study was approved by the ethics committee of the Institute (Reference No. NK/1446/RES/292). A total of 17 patients with a suspected diagnosis of the HIT were enrolled from Medicine unit. All these patients had received either prophylactic or therapeutic dose of unfractionated heparin (UFH) or low molecular weight heparin (LMWH) and developed thrombocytopenia. The clinical and initial laboratory details (including platelet count) were noted to calculate 4 T score.

Blood samples were collected in sterile (tube without anticoagulant) vacutainers from the above patients. Serum samples were stored at − 20 °C for the PF4-heparin antibody Tests. A total of three laboratory assays were used for the detection of HIT antibody. Particle gel immunoassay was performed using Gel Card kit [ID-PaGIA Heparin/PF4 Antibody Test (ID-PaGIA^{TM}) Bio-Rad-DiaMed GmbH, Switzerland]. STic® Expert HIT kit (Diagnostica Stago, Asnieres, France) uses lateral flow immunoassay to detect IgG antibodies against PF4/polyanion complexes contained in an evaluation card. The Heparin-induced platelet aggregation (HIPA) test was performed using a single donor with O blood group. The Heparin was used in two different concentrations (low and high; working concentrations of 10 and 100 IU/ml). The testing followed the protocol described in the literature [10].

Results

Data collection for the study was done over a period of 2 years. During this time, samples from 17 patients (male: female; 10: 7) with clinical suspicion of the HIT were collected. The patients were from both ICU (9) and ward (8). None of the patients was from OPD. Among these nine patients were on UFH while eight were receiving LMWH. The patients who were on UFH, four were receiving therapeutic doses and five cases were on prophylactic doses. The clinical details, admitting units, type of heparin and doses of heparin of each case are mentioned in Table 1. The 4T pre-test probability score of each case was calculated (Table 1). According to the scoring system, 6 (35%) had a high probability, 8 (47%) had intermediate probability, and 3 (18%) had a low probability for the diagnosis of HIT.

STic® Expert HIT, ID-PaGIA^{TM} and HIPA were performed in all cases. Testing by STic® Expert HIT test showed positivity in three cases (3/17; 17%) while testing by ID-PaGIA^{TM} revealed four positive cases (4/17; 23%) (Figs. 1, 2). None of the cases with low probability 4 T score (0/3) showed positivity by these tests. The 25% (2/8) of cases with intermediate probability and 33% (2/6) with high probability showed positivity by these tests. Two out of these four cases showed HIPA positivity. All of the cases with negative results by ID-PaGIA^{TM} and STic® Expert HIT were also negative by HIPA. The probability of development of HIT among different subgroups and the results of screening tests are tabulated (Table 2). The specificity and sensitivity, positive predictive value (PPV), and negative predictive value (NPV) of STic® Expert HIT and ID-PaGIA^{TM} against the HIPA are as follows: for STic® Expert HIT (sensitivity: 100%; specificity: 93.33%; PPV: 66.67%; and NPV: 100%) and for ID-PaGIA^{TM}(sensitivity: 100%; specificity: 86.67%; PPV: 50%; and NPV: 100%). Positivity of at least one immunological assay and the functional assay is usually considered as confirmatory of HIT. In the present study, we had 2 cases of confirmed HIT (11%) in 17 patients.

Discussion

Heparin-induced thrombocytopenia usually manifests 4–15 days after heparin exposure. The diagnosis of HIT must be made as early as possible, because the lack of appropriate and early treatment may expose to a risk of dramatic complications which can be life-threatening. The clinical criteria (The 4T score) alone is not sufficient for the diagnosis of HIT and laboratory assays for a demonstration of heparin-dependent antibodies are essential. The present study was carried out on 17 patients who developed thrombocytopenia following heparin exposure. The incidence of positivity of anti-PF4/heparin antibodies using STic® Expert HIT and ID-PaGIA^{TM} test for HIT in the present study was 17 and 23% respectively.

The incidence of PaGIA reported in India ranges from 1.4 to 23% [11–14] and the data from different studies from India are compared in Table 3. Kapadia et al. [11] had reported PaGIA positivity 1.4%. In this study 3 out of 207 ICU cases were positive. The study by Kotwal et al. [12] had 125 patients who underwent cardiac surgery and received UFH. Among these 9 cases (7.8%) showed positivity by PaGIA. Sachan et al. [13] showed positivity in 10% (10/100) of the cases with open cardiac surgery. All these studies included patients from either ICU or from cardiac surgery unit. All the patients included in these studies were on UFH. In the present study, nine patients were from ICU and 8 were from medical ward. Among

Table 1 The demographic details of patients

Serial no.	Age	Sex	Ward/ICU	Clinical diagnosis	Type of heparin	4-T score
1	63	F	ICU	Pulmonary thromboembolism	LMWH	5
2	77	M	ICU	Chronic obstructive airways disease with pseudoaneurysm of aorta	LMWH	5
3	52	M	WARD	Deep vein thrombosis with the previous history of HIT	UFH	1
4	47	F	ICU	Chronic kidney disease with atrial septal defect closure	UFH	5
5	51	M	ICU	Multiple Myeloma with chronic kidney disease and on dialysis	UFH	5
6	85	M	WARD	Bilateral wet gangrene	UFH	3
7	48	F	ICU	Road traffic accident with left DVT	LMWH	6
8	70	M	ICU	Atrioventricular block	UFH	3
9	45	M	WARD	Catastrophic antiphospholipid antibody syndrome	LMWH	5
10	28	M	WARD	Wet gangrene	LMWH	4
11	28	M	WARD	Known case of lymphoma with deep vein thrombosis	LMWH	6
12	55	F	WARD	Diffuse large B-cell lymphoma with spinal cord compression	UFH	7
13	46	F	WARD	Budd–Chiari syndrome	LMWH	6
14	44	F	WARD	Uremic encephalopathy	UFH	5
15	11	M	WARD	Pulmonary thromboembolism with cortical vein thrombosis	LMWH	7
16	54	M	ICU	Road traffic accident with subdural hemorrhage	UFH	6
17	63	F	ICU	Stroke (Right middle cerebral artery)	UFH	5

Fig. 1 Negative (single line) (case 1) and positive (case 14) result (two coloured line) by STic® Expert HIT (color figure online)

these 8 patients received UFH while 9 were receiving LMWH. The overall incidence of PaGIA positivity in the present study was 23% (4/17). Among these cases, 33% (3/9) patients were receiving UFH and 12.5% (1/8) patients were on LMWH. The details of each study, the probability of HIT and results of PaGIA are compared in Table 3. The reason for high incidence of PaGIA positive cases in the present study is a higher number of cases with a high and intermediate probability 4 T score. If we compare the cases with intermediate and high 4T probability score among different Indian studies than the incidence of positivity by Kapadia et al. [11] is 6.1% (3/49), by Kotwal et al. [12] is 25% (9/36) and by Sachan et al. [13] is 23.8%. The incidence of intermediate and high 4T probability score cases in our study 28.6% (4/14) and this is similar to these studies.

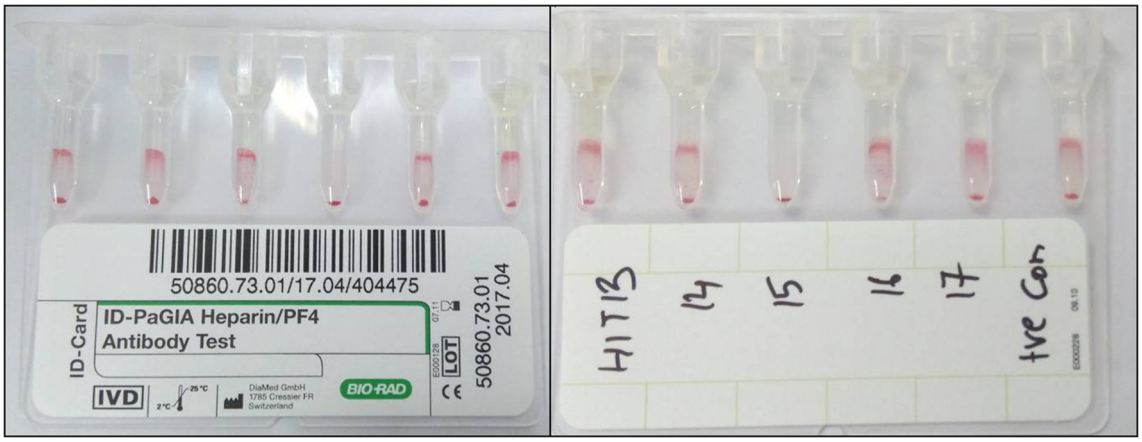

Fig. 2 Negative (case 15) and positive (cases 13, 14, 16 and 17) result by ID-PaGIA™

Table 2 Probability of HIT among different subgroups

Groups	Subgroup	No. of patients	Mean 4 T score (range)	Probability of HIT based on 4 T score (high/intermediate/low)
Ward/ICU	ICU	8	5 (3–6)	2/5/1
	WARD	9	4.8 (1–7)	4/3/2
Type of heparin	UFH	9	4.8 (1–7)	2/4/3
	LMWH	8	5.5 (4–7)	4/4/0
UFH dose	Therapeutic	4	5.25 (3–7)	2/1/1
	Prophylactic	5	3.8 (1–7)	0/3/2
Results of screening tests	Positive	4	6.25 (6–7)	2/2/0
	Negative	13	4.7 (1–7)	4/6/3

There are a few studies available worldwide on STic® Expert HIT [14–16] and none from India. The comparison of worldwide studies on STic® Expert HIT is tabulated (Table 3). The positive cases of STic® Expert HIT reported by Berroëta et al. [14] were 26%. In this study, a total of 90 cases were recruited. The majority of these cases underwent cardiac surgery and were on UFH. Among these 24 cases (26%) showed positivity by STic® Expert HIT and 18 cases (75%) were finally confirmed as HIT by serotonin release assay(SRA). Another study by Leroux et al. [15] showed positivity by STic® Expert HIT in 27% (71/256) cases. The patients included in this study were from the medical unit (104 cases), surgery unit (120 cases) and thrombosis cases (101). Majority of them received UFH (189 cases) followed by LMWH (125 cases). Thirty-nine cases (44%) were further confirmed as HIT by SRA. Vianello et al. [16] detected STic® Expert HIT positivity in 31.5% (36/114) cases. Among 114 cases 45 were from medical unit, 23 cases were from cardiovascular surgery unit and 19 cases from ICU. HIPA was performed as a confirmatory test and 75% (29/36) of these were confirmed as HIT. In the present study, 9 patients were from medical units and 8 were from ICU. Eight patients had received UFH while 9 were on LMWH. The STic® Expert HIT test showed positivity in 17% (3/17) cases and 66% (2) of the cases were confirmed as HIT.

The comparison with previous studies from India on PaGIA in cases with high and intermediate probability T score showed that the sensitivity and NPV of PaGIA were almost similar (Table 3). The specificity of the present study was lower while PPV was in-between. Similarly, the sensitivity, specificity, PPV and NPV of STic® Expert HIT among worldwide studies were compared (Table 3). The sensitivity, specificity and NPV were within the range of the earlier published literature. In the present study, the PPV is lower as compared to other studies. The comparison of these parameters showed that both PaGIA and STic® Expert HIT are equally sensitive (High sensitivity and NPV) for detection of a positive case. These are the requirement for a good screening test and hence both the tests are equally effective for detection and exclusion of HIT. The STic® Expert HIT has high specificity, however, the kit needs to be further validated with more number of cases to reach any conclusion. One important point to highlight about STic® Expert HIT is that the test is much easy to perform and does not require any specialized instrument. The test results can be obtained within 1 h.

There are few limitations of the study. The important one is of small sample size. The patient cohort was generalised and not against a specific disease/intervention. The number of screening tests for comparison is less. Future studies with a large number of cases with a specific disease on a specific causative drug (UFH/LMWH) and the

Table 3 Comparison of different studies on HIT from India on PaGIA and worldwide on STic® Expert HIT and comparison of sensitivity, specificity, PPV and NPV in high and intermediate probability 4 T score cases

	No. of patients	Unit		Type of heparin (UFH/ LMWH)	Probability of HIT based on 4 T score (high/ intermediate/ low)	No. of positive cases among different probability groups	Sensitivity	Specificity	PPV	NPV
PaGIA										
Kapadia et al. [11]	207	Medical-surgical ICU		UFH	1/48/168	1/2/0	100	96	33	100
Kotwal et al. [12]	125	Cardiac surgery		UFH	11/25/89	8/1/0	87	92.8	77.8	96.3
Sachan et al. [13]	100	Cardiac surgery		UFH	8/34/58	6/4/0	–	–	–	–
Present study	17	Medical	ICU (8)	UFH (4), LMWH (4)	2/5/1	1/1/0	100	83.3	50	100
			Ward (9)	UFH (4), LMWH (5)	4/3/2	1/1/0				
STic®Expert HIT										
Berroëta et al. [14]	90	Cardiac surgery-69, non-cardiac surgery-6, and medicine unit-15		74 UFH/8 LMWH/8 both	12/37/41	24/90	95	92	75	98
Leroux et al. [15]	334	Medicine unit-104, surgery-120 (66 cardiovascular surgery), thrombosis cases-101 and others-9		UFH (n = 189), LMWH (n = 125) or both (n = 18)	32/206/96	71/256 (only 256 serum sample tested with STic® Expert HIT)	100	82.2	100	43.7
Vianello et al. [16]	114	Medicine unit-45, cardiovascular surgery-23, ICU-19 and others-27		–	–	36/114	89	93	75	97
Present study	17	Medicine unit-9 and ICU-8		UFH (8), LMWH (9)	6/8/3	3/17	100	93.3	66.6	100

addition of more screening test (ELISA, Chemiluminescence assay or flow cytometry) and confirmatory test (SRA) will be helpful.

In conclusion, The study suggests that STic® Expert HIT an equally effective test, in combination with the 4T scoring system for detecting/excluding the diagnosis of HIT. A large number of cases are needed for validation.

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Compliance with Ethical Standards

Conflict of interest There is no conflict of interest amongst any of the authors.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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