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RAPID COMMUNICATION

Comparison of HIT Tests in Patients with COVID-19 and Thrombocytopenia

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Abstract: Thrombosis and coagulopathy have been found to be the most prevalent complications in patients with COVID-19. Thromboprophylaxis to prevent thromboembolic events is recommended for hospitalized COVID-19 patients. Heparin-induced thrombocytopenia (HIT) is a known complication of heparin use. This study aimed to determine the incidence of HIT among admitted patients with confirmed COVID-19 by PCR. In this study, two different HIT assays, rapid immunoassay (STic Expert HIT, Stago) and H-PF4 specific enzyme-linked immunosorbent assay (Asserachrom® HPIA – IgG), were performed. Of 200 patients with confirmed COVID-19, we identified 49 patients who met the possibility of HIT (low platelet count and high D-Dimer level). Only five (10.2%) had a positive HIT rapid test. However, none of the tested samples tested positive by ELISA. Thrombosis was reported in two of five (40%) patients. Further extensive studies are required to determine the prevalence and clinical significance of a positive HIT test among patients with COVID-19.

Keywords: COVID-19, thrombosis, coagulopathy

Introduction

COVID-19 has a systemic effect on the respiratory and cardiovascular systems. The correlation between thrombosis and COVID-19 is clinically important. Venous thromboembolism (VTE) has been reported in approximately 20–30% of patients, especially in critical cases.[1] Heparin-induced thrombocytopenia (HIT) is a known complication of heparin therapy, which changes the characteristics of heparin from anticoagulant to procoagulant.[2] The incidence of HIT in COVID-19 patients has been reported to be 0.8%.[3] A meta-analysis of seven studies revealed that the combined incidence of HIT was higher in patients with severe COVID-19 who received heparin (2.2%) than those without severe symptoms (0.1%).[3] HIT is caused by produced antibodies directed against a complex of platelet factor 4 (PF4) and polyanions such as heparin. Pathogenic immunoglobulin G (IgG) binds to PF4/heparin and activates cellular human immunoglobulin receptor IIa (FcRIIA) in platelets and monocytes, causing hypercoagulability and potentially fatal thrombosis.[4]

Materials and Methods

This study was conducted on 200 patients with COVID-19 who were admitted to our institute, between 1 March and 17 June 2021. Any patient who has confirmed COVID-19 via PCR and meets any of the following criteria should be admitted (clinical or radiological evidence of pneumonia; age >65 years; low oxygen saturation SpO2 <94% on room air; Acute respiratory distress syndrome (ARDS), chronic pulmonary disease;, chronic kidney disease history of comorbidities; diabetes Mellitus or/and hypertension; history of cardiovascular disease; obesity (BMI ≥40); use of immunosuppressant medications; history of organ transplant or another immunosuppression disease; history of active malignancy; and other Co-illness). Plasma samples were collected (in 3.8% sodium citrate) from patients (age range: 17–86 years old; median: 59 years old) with

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confirmed COVID-19. All samples were centrifuged for 15 minutes at 2000–2500 RCF within two hours of sample collection, divided into aliquots, and stored at −80°C until testing.

Patient Sample Size and Clinical Presentation

Out of 200 patients with confirmed COVID-19, 49 (24.5%) met the inclusion criteria (platelet count less than 140×10^9/L; D-dimer more than 1 ng/mL). These patients had thrombocytopenia (median platelet count 83×10^9/L; range: 8 to 138×10^9/L) and had high D-dimer levels (median: 2.87 ng/mL; range: 1.12 to >20). All patients had received an anticoagulant (enoxaparin or unfractionated heparin) on the same day of hospital admission or two days later, according to the hospital guidelines for the management of COVID-19 patients.

Laboratory Assay

Plasma samples from these patients were tested for HIT by two different tests: rapid immunoassay and H-PF4 specific ELISA.

Rapid Immunoassay (STic Expert HIT, Stago)

STic Expert HIT is a qualitative detection of IgG antibodies against the PF4/polyanion complex in human plasma. It is a lateral-flow immunoassay that is based on the capillary action that induces a flow of the test sample along a test strip. A conjugate, which is a gold nanoparticle coated with an anti-ligand, is included in the test strip. The plasma was pipetted into a sample pad, and then drops of buffer that contained biotinylated human PF4/polyanion (PA) complex were added.

Human anti-PF4/PA antibodies in the plasma bind to the PF4/PA complexes. When the fluid passes through the test line on the strip, complexes develop. A biotinylated PF4/PA system, biotin coated gold nanoparticles and human antibodies are fixed in place by an immobilized goat antibody. This goat antibody was chosen for its ability to immobilize human IgG antibodies in a specific manner (which serve as the capture antibody printed onto the membrane). A strongly colored line can be interpreted visually to indicate a positive reaction (test line). A second line is included on the test strip (the control line). The presence of the control line indicates that the test was carried out correctly.[5]

H-PF4 Specific ELISA (Asserachrom® HPIA–IgG)

HPIA–IgG is a highly sensitive and specific ELISA for the detection of H-PF4-specific IgG antibodies. It is a quantitative assay based on the ability of the H-PF4 antibody to activate platelets in the presence of heparin, whereas immunoassays confirm the presence of the antibody without considering its ability to cause platelet activation. Heparin–PF4 complexes were coated on the inside of the ELISA. Anti-human IgG antibodies (from each plasma sample), combined with peroxidase, bound to any antigenic determinants of immobilized antibodies that were available. The action of the attached enzyme, peroxidase, on the Tetramethylbenzidine substrate reveals. A strong acid was used to terminate this reaction. The plate reader was set to read absorbance values at 450 nm, and the intensity of the color produced was proportional to the number of antibodies present in the sample. As a result, optical density units (cut-off OD $\leq$ 0.39) values are used to detect the actual concentration computed for each sample against a standard via interpolation in the standard curve.

Results

Clinical Results

The 4Ts is an HIT pre-score method designed to improve and standardize clinical diagnosis. It includes four common aspects of HIT: (1) the severity of thrombocytopenia; (2) the timing of thrombocytopenia in relation to heparin exposure; (3) thrombosis or other HIT complications; and (4) the likelihood of additional thrombocytopenia causes. The algorithm generates an integer score ranging from 0 to 8, with 0–3, 4–5, and 6–8 indicating low, intermediate, and high HIT pretest probability, respectively.[6] For all patients who met the required criteria for possible HIT, the 4T clinical probability score was calculated. The median HIT 4T score was 5 (range: 2–8).

Table 1 Laboratory and Clinical Results of Five Suspected Cases of HIT with COVID-19

Patient	Platelets/ 10^9L	D-Dimer	HIT 4T Score	VTE Score	STic Expert HIT	H-PF4 ELISA OD (≤0.39)	Asserachrom® HPIA -IGg	Thrombosis	Type of Anticoagulant	Dose
1	139	1.17	5	14	Weak positive	0.09	Negative	PE	Heparin	5000, 0.2 mL q8hr
2	101	2.9	5	9	Weak positive	0.178	Negative	NO	Enoxaparin	0.4 mL of 40 mg q12hr
3	119	1.52	2	13	Weak positive	0.103	Negative	NO	Enoxaparin	0.4 mL of 40 mg q24hr
4	84	>20	8	9	Positive	0.142	Negative	STROKE	Enoxaparin	0.4 mL of 40 mg q24hr
5	98	4.06	5	11	Positive	0.112	Negative	NO	Heparin	2500 units q8hr

Abbreviations: HIT, heparin-induced thrombocytopenia; VTE, venous thromboembolism; OD, optical density; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G.

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The risk of developing VTE was assessed for all cases using the value of a simple risk assessment model proposed by the Padua Prediction Score.[7] The Padua Prediction Score was created to predict the risk of venous thromboembolism (VTE) in medical patients who were hospitalized. Very low risk (score 0), low risk (1–2), moderate risk (3–4), or high risk (score 5) are the five risk levels assigned to patients.[7] The median VTE score was 9 (range, 5–16). Thrombosis was reported in two (40%) out of five patients.

Laboratory Results

Rapid Immunoassays (STic Expert HIT, Stago)

The results showed that only five (10.2%) of the 49 patients were positive for the rapid test. Strong positive reactions were recorded in two (4.08%) patients, weakly positive in three (6.12%) patients, while all other patients (44; 89%) had a negative result. Remarkably, one patient who had a positive STic expert HIT suffered a stroke. It developed on the same day as enoxaparin treatment (0.4 mL of 40 mg q24h). Interestingly, this patient had elevated D-dimer levels (>20 ng/mL) and thrombocytopenia (84×10^9/L). Pulmonary embolism (PE) was reported in another patient (20%) who had a weakly positive STic Expert HIT assay. PE started five days after heparin administration (5000 iu sc, q8h). This patient had a mildly elevated D-dimer level (1.17 ng/mL) and platelet count of 139×10^9/L (Table 1).

H-PF4 Specific ELISA (Asserachrom® HPIA–IgG)

No positive results were detected in all 49 suspected cases of HIT.

Discussion

In this study, we report a higher incidence of HIT in patients with COVID-19, as detected by a screening method, while a more specific test (ELISA) was negative in all cases. In addition, we report the development of VTE in two patients. The first patient who developed a stroke one day after starting heparin treatment was probably not related to HIT positivity but rather to COVID-19 infection severity. However, the second case was probably due to HIT, although the ELISA test result was negative.

Conclusions

HIT can occur in COVID-19 patients receiving heparin and should be recognized as a possible cause of thrombocytopenia in these individuals. Overall, our results provide further support to show that rapid HIT immunoassays are more sensitive and less specific compared to PF4-specific enzyme-linked immunosorbent assays. Further investigations with a larger sample size are warranted to confirm this finding and to determine the incidence of HIT among COVID-19 patients.

Abbreviations

HIT, heparin-induced thrombocytopenia; VTE, venous thromboembolism; PF4, platelet factor 4; IgG, immunoglobulin G; FcRIIA, F cellular human immunoglobulin receptor IIa; ARDS, acute respiratory distress syndrome; OD, optical density; PA, polyanion.

Data Sharing Statement

Data are available upon request to the corresponding author.

Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of King Faisal Hospital and Research Center (KFSH&RC), Riyadh, Saudi Arabia. Informed consent was obtained from the study participants prior to the commencement of the study. This study was conducted in accordance with the Declaration of Helsinki.

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Disclosure

The authors report no conflict of interest in this work.

References

1. Schulman S, Hu Y, Konstantinides S. Venous thromboembolism in COVID-19. *J Thromb Haemost*. 2020;120:1642–1653. doi:10.1055/s-0040-1718532
2. Lingamaneni P, Gonakoti S, Moturi K, et al. Heparin-induced thrombocytopenia in COVID-19. *J Investig Med High Impact Case Rep*. 2020;8:2324709620944091. doi:10.1177/2324709620944091
3. Uaprasert N, Tangcheewinsirikul N, Rojnuckarin P, et al. Heparin-induced thrombocytopenia in patients with COVID-19: a systematic review and meta-analysis. *Blood Adv*. 2021;5:4521–4534. doi:10.1182/bloodadvances.2021005314
4. Arepally GM. Heparin-induced thrombocytopenia. *Blood*. 2017;129:2864–2872. doi:10.1182/blood-2016-11-709873
5. Sachs UJ, von Hesberg J, Santoso S, Bein G, Bakchoul T. Evaluation of a new nanoparticle-based lateral-flow immunoassay for the exclusion of heparin-induced thrombocytopenia (HIT). *Thromb Haemost*. 2011;106(6):1197–1202. doi:10.1160/TH11-06-0390
6. Cuker A, Gimotty PA, Crowther MA, et al. Predictive value of the 4Ts scoring system for heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood*. 2012;120:4160–4167. doi:10.1182/blood-2012-07-443051
7. Barbar S, Noventa F, Rossetto V, et al. A risk assessment model for the identification of hospitalized medical patients at risk for venous thromboembolism: the Padua Prediction score. *J Thromb Haemost*. 2010;8:2450–2457. doi:10.1111/j.1538-7836.2010.04044.x