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P-selectin expression assay in a repeatedly serotonin-release assay-negative patient with heparin-induced thrombocytopenia

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Heparin-induced thrombocytopenia (HIT) is an immune complication of heparin therapy caused by antibodies to complexes of platelet factor 4 (PF4) and heparin. Pathogenic antibodies to PF4/heparin bind and activate platelets to propagate a hypercoagulable state culminating in life-threatening thrombosis. The serotonin-release assay (SRA) is considered the gold-standard test to diagnose HIT. However, the sensitivity of the SRA was questioned with reported cases of clinical diagnosis of HIT and negative SRA. Herein, we present the utility of platelet factor 4-dependent P-selectin expression assay (PEA) in diagnosing HIT in a patient with thrombocytopenia and recurrent thrombosis who repeatedly tested negative with SRA. ***Blood Coagul Fibrinolysis*** **30:000–000 Copyright © 2021 Wolters Kluwer Health, Inc. All rights reserved.**

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Introduction

Heparin-induced thrombocytopenia (HIT), with an incidence of 0.1–5% of patients receiving heparin therapy [1,2], is a thromboembolic immune-mediated disorder caused by autoantibody production to platelet factor 4 (PF4) and heparin complexes [3]. These pathogenic antibodies to PF4/heparin complexes activate platelets, which can progress to life-threatening thrombosis [4]. The serotonin-release assay (SRA) is considered the gold standard diagnostic test for HIT. However, the sensitivity of the SRA was questioned by some researchers who were confronted with cases of clinical diagnosis of HIT and negative SRA testing [5,6]. Recently, the PF4-dependent P-selection Expression Assay (PEA) was found to rapidly detect HIT antibodies in SRA-negative patients [7–9]. In this report, we discuss the utility of the PF4-dependent PEA in diagnosing HIT in a patient with thrombocytopenia and recurrent thrombosis who repeatedly tested negative with SRA.

Case history

A 66-year-old Caucasian nonsmoker male patient with past medical history of hypertension and hyperlipidemia presented to an outside hospital with vague neurological symptoms, right arm pain, and chest pain. He was managed for acute coronary syndrome in the setting of high troponins and was placed on heparin drip (day 0 is the time when heparin was started); however, few days later he had worsening neurological symptoms while on heparin. MRI revealed multiple embolic strokes in both anterior and posterior cerebral circulations, bilateral cerebellar areas, and frontal and parietal lobes. Heparin was continued during that hospital stay, and the patient was eventually discharged on clopidogrel and aspirin.

One week later, he presented with worsening neurological symptoms and radiologic evidence of new ischemic changes and was found to be mildly thrombocytopenic with a platelet count of 79×10^9/l (baseline platelet count 164×10^9/l); however, no work up was initiated for the thrombocytopenia. Patient was started on enoxaparin (low-molecular-weight heparin) as inpatient and was later switched to apixaban on discharge. He presented again 12 days after the initial presentation with right subclavian vein thrombosis while on apixaban, and platelets were noted to be still low, so he was transferred to our hospital for escalation of care. Upon presentation, he was noted to have right lower extremity swelling; Doppler ultrasound showed distal acute deep venous thrombosis (DVT) and left superficial vein thrombosis. A subsequent computed tomography (CT) scan of the chest with contrast showed bilateral pulmonary embolism along with renal and splenic infarcts. Echocardiogram showed no evidence of patent foramen ovale or cardiac thrombus. At this time (day 12), his 4T score [10] was high with a score of 6, and his PF4 ELISA (polyspecific assay) was noted to be positive [optical density (OD) = 1.986] (Fig. 1). Patient was started on argatroban because of high concern for HIT, and confirmatory SRA was sent; however, it

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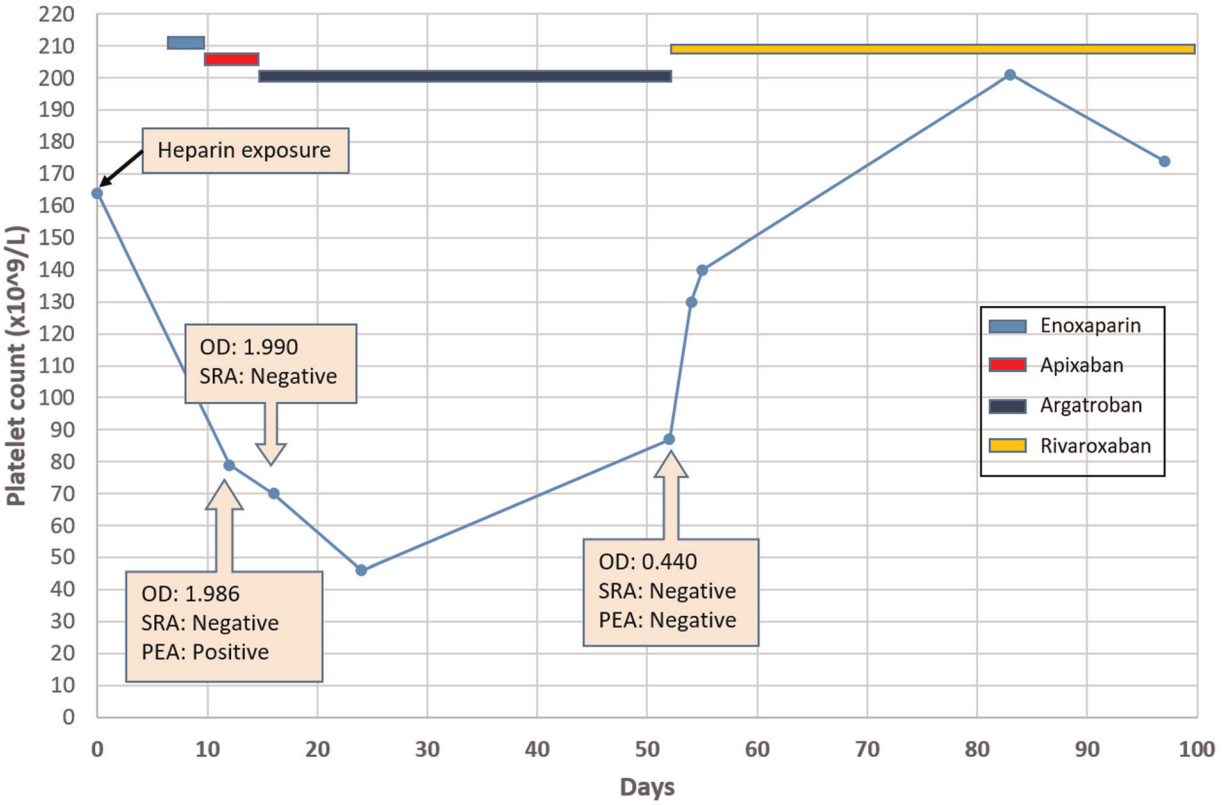
Fig. 1



Trend of platelet count (×1000) along with heparin-induced thrombocytopenia antibody testing represented by optical density, serotonin release assay and PF4-dependent P-selectin expression assay results. Timeline of anticoagulation is also illustrated. OD, optical density; PEA, P-selectin expression assay; SRA, serotonin-release assay.

resulted as negative (0% serotonin release). HIT testing with ELISA and SRA were repeated 4 days later (day 16) and gave the same negative results (OD = 1.990 and 6% serotonin release). Repeat HIT testing 1 month later gave a positive ELISA (OD = 0.440) and again negative SRA (2% serotonin release). Extensive vasculitis work up was unremarkable. Antiphospholipid syndrome was considered unlikely. Thrombophilia work up was unremarkable including antiphospholipid antibodies, proteins C and S, antithrombin III, and paroxysmal nocturnal hemoglobinuria screen.

The high clinical suspicion for HIT prompted further investigation using the novel PEA that utilizes PF4-treated platelets for HIT diagnosis [11]. Platelet-activating HIT antibodies were detected in the PEA assay from the specimen at initial time of presentation at 42% (a positive result is ≥24%) and was confirmed by inhibition in the presence of high-dose heparin. Patient's specimen collected 6 weeks after initial presentation was negative (0%) likely because of the decrease in antibody titers. Patient was eventually maintained on rivaroxaban with improvement in platelet count and no further episodes of thrombosis 9 months from initial presentation.

Discussion

HIT is an immune-mediated life-threatening disorder caused by antibodies to complexes of PF4 and heparin following administration of heparin [12]. These pathogenic PF4/heparin antibodies (HIT antibodies) activate platelets; therefore, triggering serotonin release and propagating a hypercoagulable state culminating in life-threatening thrombosis with associated severe thrombocytopenia [13–15]. Despite the advances in diagnosis and management, HIT is still a major cause of thrombosis with associated morbidity and mortality [16].

The prelaboratory prediction of HIT utilizes a validated scoring system to assess the risk factors to the development of HIT called 4T score [10,17,18]. This is based on the level of thrombocytopenia, presence of thrombosis, duration of heparin exposure, and other contributing factors to development of thrombocytopenia. The risk factors are summed up yielding integer scores ranging from 0 to 8. The probability to develop HIT is classified as low (≤3 points), intermediate (4–5 points), and high (≥6 points) [10]. Anti-PF4/heparin antibodies can be detected using immunologic assays, such as the ELISA (PF4 ELISA), with a high sensitivity and low specificity

[18]. A negative PF4 ELISA would argue against HIT diagnosis. The SRA has been generally considered the gold standard test for HIT diagnosis with high level of specificity (>90%) [19]. However, this is a technically complex and time-consuming assay to perform and requires the use of radioactive material for most of the available assays. In addition, it is not available except in a few reference laboratories. Moreover, recent reports from multiple groups have confirmed the presence of a recently recognized entity, 'SRA-negative HIT' [5,8,20].

Due to the technical challenges in running the SRA and because of its low sensitivity in 'SRA-negative HIT' patients, there was a need for faster and safer assay for HIT diagnosis. PF4-dependent P-selectin Expression Assay (PEA) was recently found to rapidly detect HIT antibodies with better accuracy. Padmanabhan *et al.* [7] demonstrated that PEA had greater sensitivity than SRA and could detect HIT antibodies in SRA-negative patients. They compared the diagnostic performance of PEA to that of SRA; PEA was positive in 11 out of 16 patients (69%) who tested negative with SRA and had clinical HIT diagnosis. Additional reports supported the use of assays using PF4-treated platelets in identifying HIT early in the course of disease when the SRA is negative [9,21]. A more recent prospective study also confirmed the high accuracy of the PEA [22]. The PEA is relatively simpler than the SRA, and it is based on detecting platelet-activating HIT antibodies based on the flow cytometric expression of P-selectin (CD62p) by donor platelets treated with PF4 and patient serum. The assay does not require the use of radioisotopes and uses ~20-fold fewer platelets than the SRA.

Our patient was treated with heparin for coronary syndrome after which he presented with thrombosis and thrombocytopenia. He had a high pretesting prediction of HIT based on the 4Ts score. His clinical course was highly suggestive of HIT, and he had recurrent thrombosis affecting multiple organs. Although his PF4 ELISA was strongly positive, his SRA was negative on two occasions during thrombosis. We decided to further investigate the case with additional testing using the PEA, which came back positive confirming the diagnosis of HIT. This case highlights the limitation of the SRA confirming previous reports that questioned the diagnostic sensitivity of this assay [6,7,9]. We are not sure why the PFA was positive when the SRA was negative in our patient; however, we speculate that this case is a good example of the utility of PFA in detecting HIT early in the disease course when the HIT antibody titers are still lower than the threshold for platelet activation and detection by the SRA.

In as much as the PEA was helpful in identifying SRA-negative HIT cases, an equal number of cases of SRA-positive HIT had negative PEA. In their most recent prospective study evaluating the accuracy of PEA in detecting HIT, Samuelson Bannow *et al.* reported on 10 SRA-negative/PEA-positive patients (5 patients were deemed to have HIT) and 8 SRA-positive/PEA-negative patients (3 patients had HIT) [7]. The SRA and PEA are confirmatory tests for HIT; however, their sensitivities are 86 and 88%, respectively. It remains important to review the clinical and HIT serologic (HIT ELISA) profiles of patients with discordant SRA and PEA results.

Conclusion

The diagnosis of HIT is a challenging venture even in the setting of high clinical suspicion. Our case reconfirms the limitation of the gold standard SRA in HIT testing. On the basis of the recent evidence, PEA has higher diagnostic accuracy than SRA and is technically less demanding. PEA was instrumental in making an accurate diagnosis of HIT in our patient. In addition, our case demonstrates the fact that HIT is a clinical diagnosis; our patient was treated appropriately for HIT based on clinical suspicion despite the negative SRA results. The high clinical suspicion for HIT was what drove repeat SRA testing and finally PEA testing.

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Conflicts of interest

There are no conflicts of interest.

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