

False-positive heparin-PF4 latex immunoturbidimetric assay due to lupus anti-coagulant interference: a case report

The prothrombotic, antibody-mediated reaction to heparin therapy known as heparin-induced thrombocytopenia (HIT) requires both laboratory testing and clinical evaluation for diagnosis, though the latter is subjective and can be challenging in certain clinical scenarios. However, the use of pre-test probability scoring systems (i.e. 4Ts score) can decrease this subjectivity. Numerous laboratory HIT tests are available to assist with diagnosis, including enzyme-linked immunosorbent assays (ELISA), ¹⁴C-serotonin release assay (SRA), heparin-induced platelet activation (HIPA), and recently, a fully automated latex immunoturbidimetric assay (HIT-LIA) (HemosIL[®] HIT-Ab(PF4-H); Instrumentation Laboratory, Bedford, MA, USA). Automated HIT-LIAs have the advantage of quick turnaround and good sensitivity and specificity, and are increasingly in use.^{1,2}

Anti-phospholipid syndrome (APS) is an acquired immune-mediated syndrome primarily associated with thrombosis and pregnancy morbidity, though it can present with a range of clinical manifestations, including thrombocytopenia, with a reported incidence of 22–42%.^{3–7} APS is characterized by autoantibodies including lupus anti-coagulant (LA), anti-cardiolipin (aCL), and beta-2 glycoprotein (B2GP-1) antibodies, which target phospholipids or phospholipid-bound proteins.⁸ Concurrent thrombocytopenia and thrombosis in APS patients receiving heparin can pose diagnostic challenges, as these are symptoms of both APS and HIT. Laboratory testing plays an important role in distinguishing between these diagnoses. The interference of LA with coagulation-based assays is well established;⁸ however, there are fewer studies describing the effect of anti-phospholipid (aPL) antibodies on HIT assays. We report a case of false-positive automated HIT-LIA tests related to high LA titres, which appear to interfere with test results in a concentration-dependent fashion. This is an unreported phenomenon which clinical and laboratory personnel should be aware of when interpreting the results of this test.

Case presentation

A 61-year-old white male presented to the emergency department with weakness, involuntary movements of the right arm, and confusion. Physical examination was notable for right gaze deviation, left homonymous hemianopsia, lip and right arm automatism, and aphasia. His medical history included three prior cerebrovascular accidents (CVA) and

mildly elevated B2GP-1 IgG titres not meeting the threshold for APS diagnosis. Five days prior to admission, he had a positive LA test (HemosIL[®] dRVVT, Instrumentation Laboratory, Bedford, MA, USA) with screening and confirmatory titres of 2.48 and 1.99, respectively (cut-off ≤ 1.16). Of note, the dRVVT screen was calculated from the ratio of the patient's value to a known standard (normal pooled plasma); since this ratio was >1.16 , the confirmatory test was automatically performed. The confirmatory test ratio was calculated similarly but with the addition of a high phospholipid concentration reagent; values > 1.16 were reported positive for LA. The patient had no allergies or history of autoimmune disease. A computed tomography angiogram of the brain revealed acute right-pericallosal artery occlusion. His overall presentation was consistent with focal status epilepticus secondary to acute ischaemic stroke and he was admitted to the neuro-medicine intensive care unit for further care. He was not a candidate for tissue plasminogen activator or embolectomy.

He was started on subcutaneous unfractionated heparin (5000 units/8 h) for thromboembolism prophylaxis and thereafter developed thrombocytopenia. By day 5, his platelet count had fallen from 150 to $53 \times 10^9/l$ (Fig 1); heparin was discontinued and HIT-LIA was negative at <0.6 (cut-off < 1.0). His previous heparin exposure was limited to enoxaparin for a total of 17 non-consecutive days over the last year. However on day 9, his platelets decreased to $42 \times 10^9/l$ and surprisingly, HIT-LIA was strongly positive at 15.9 (upper detection limit 16.0). The 4Ts score was 4, indicating an intermediate clinical probability of HIT.

On day 9 LA was also found to be strongly positive with screening and confirmatory titres of 3.09 and 2.43, respectively. aCL and B2GP-1 (IgG and IgM) were negative. APS was strongly suspected given the patient's history of recurrent CVA and positive LA testing on two occasions; though not separated by 12 weeks as specified by the Sydney classification criteria.⁹ However, HIT could not be ruled out due to persistent thrombocytopenia and strongly positive HIT-LIA. Administration of full dose anti-coagulation was deemed unsafe due to recent stroke and severe thrombocytopenia; low-dose fondaparinux (2.5 mg daily) was thus started on day 10. Platelets continued to fall despite avoidance of heparin, which was incongruous with a diagnosis of HIT. Immune thrombocytopenic purpura (ITP) was increasingly suspected and a five-day course of dexamethasone (40 mg

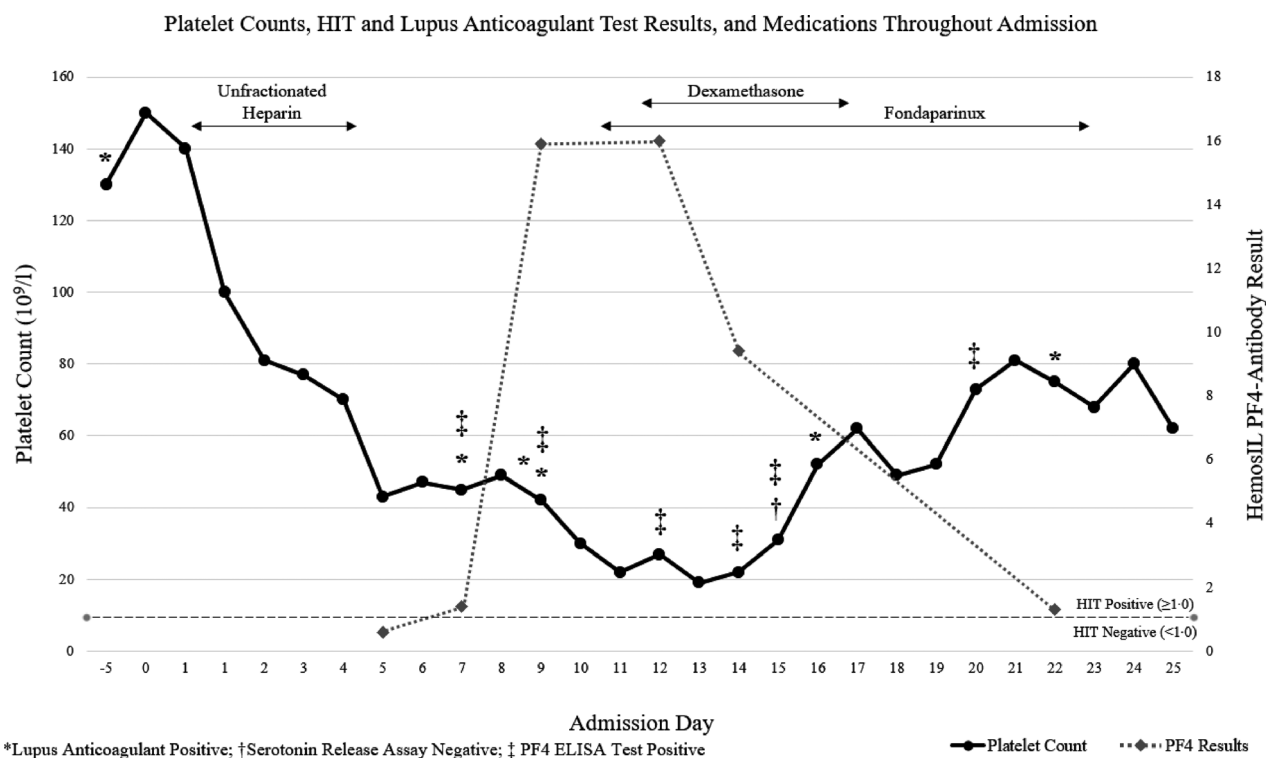


Fig 1. Platelet counts, heparin-induced thrombocytopenia-latex immunoturbidimetric assay (HIT-LIA), ^{14}C -serotonin release assay (SRA), PF-4 enzyme-linked immunosorbent assays (ELISA), lupus anti-coagulant results, and medications throughout admission. In order of occurrence, respective detailed laboratory results as follows: *, Lupus anti-coagulant screen and confirm titres: day -5: 2.48, 1.99; day 7: 3.09, 2.43; day 8: 3.36, 2.49; day 9: 3.19, 2.49; day 16: 2.64, 1.94; day 22: 2.22, 1.63; †, SRA on day 15: negative with 0% platelet serotonin release in the presence of both high- and low-dose heparin; ‡, PF-4 ELISA results: day 7: 0.446 (borderline positive); day 9: 1.841 (positive); day 12: 2.077 (positive); day 14: 1.894 (positive); day 15: 1.639 (positive); day 20: 0.751 (borderline positive). Platelet counts and HIT-LIA results shown along the primary and secondary y-axes, respectively.

daily) was started on day 12. On day 13, the platelet count reached a nadir of $19 \times 10^9/l$ and a repeat HIT-LIA was again strongly positive at >16.0 . The dexamethasone course was completed on day 16 by which time the platelet count had recovered to $62 \times 10^9/l$. LA titres remained positive at 2.64 and 2.94 and the HIT-LIA was still positive at 9.4. Fondaparinux was titrated up to the full therapeutic dose given the improvement in platelet count.

On day 15, SRA testing was performed at a reference laboratory and resulted negative with 0% platelet serotonin release in the presence of both high- and low-dose heparin (negative $\leq 20\%$). Frozen plasma samples from days 7, 9, 12, 14, 15, and 21, corresponding to dates of the previous HIT-LIA and SRA testing, were sent for PF4-ELISA testing (GTI[®] PF4 Enhanced[®] ELISA; Immucor, Norcross, GA, USA). ELISA testing resulted positive, with samples from days 7 and 21 in the borderline positive range [borderline optical density (OD) 0.400–1.00; positive OD >1.00] (Fig 1). By day 22, the platelets recovered to $80 \times 10^9/l$, LA was still positive at 2.22 and 1.63, and HIT-LIA was weakly positive at 1.3. A bone marrow biopsy revealed increased megakaryocytes without evidence of any underlying bone marrow disorder.

Discussion

Our patient's response to dexamethasone and bone marrow biopsy findings support the hypothesis that his thrombocytopenia was caused by ITP or LA. Given the low clinical suspicion for HIT and a negative SRA, we concluded that the HIT-LIA and PF-4 ELISA results were false positives presumably due to high titres of aPL. The initial negative HIT-LIA test could be explained by *in vivo* heparin binding to aPL;^{10,11} given that the initial HIT-LIA was performed 5 h after heparin discontinuation, the presence of heparin could account for the lack of interference of aPL with the HIT-LIA. We postulate that as heparin was cleared and the patient's LA titres increased, there was increasing interference with HIT-LIA leading to high false positivity. There have been rare reports of patients with simultaneous HIT and APS.^{12–15} More alarming are the reports of false-positive HIT tests in thrombocytopenic patients with aPL, resulting in unwarranted changes in anticoagulation and increased risk of thrombosis. This phenomenon has previously been reported with ELISA and particle gel immunoassay in patients with APS and SLE.^{16–18} These findings explain our positive PF4-ELISA results. We posit that similar interference led to our positive HIT-LIA.

To our knowledge, this phenomenon has not been reported with automated HIT-LIA. In fact the HIT-LIA reagent package insert states that aPL have been shown not to interfere with this assay.¹⁹ However, our data suggests a concentration-dependent aPL interference with HIT-LIA; the strongly positive HIT-LIA results correlated with higher LA titres. This case serves as a reminder that automated HIT-LIA should be interpreted with caution in the presence of aPL.

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Author contributions

Hannah L. McRae: Case report conception, analysis of laboratory data, compiled medical and laboratory data, wrote manuscript, approved final version. Jozal W. Moore: Case report conception, clinical management of patient and analysis of clinical data, obtained patient consent for publication, revised manuscript, approved final version. Jainulabdeen J. Iftikharuddin: Case report conception, clinical management of patient and analysis of clinical data, final manuscript review, approved final version. Majed A. Refaai: Case report conception, analysis of laboratory data, oversaw manuscript preparation, final manuscript review, approved final version.

Conflict of interest

Dr. Majed Refaai has received research support from CSL Behring, Octapharma, Bayer, Instrumentation Laboratory, Diagnostica Stago, and iLine microsystems. All other authors have no conflicts of interest to report.

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