

CASE REPORT

Falsely positive heparin-induced thrombocytopenia antibody testing in severe hyperbilirubinemia

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Abstract

Heparin-induced thrombocytopenia (HIT) is a life-threatening pathologic reaction to heparin-based products. Diagnosis of this condition can be confounded by other comorbidities or by acute illness—oftentimes presenting challenging clinical dilemmas, particularly in critically ill patients. A 67-year-old woman was admitted with liver failure and severe hyperbilirubinemia. She developed thrombocytopenia after prophylactic heparin exposure. Subsequent quantitative latex immunoturbidimetric assay (LIA) HIT antibody testing was intermediately positive. Confirmatory serotonin release assay testing subsequently returned negative. Platelet factor4-dependent P-selectin expression assay also returned negative, suggesting false positivity of the initial LIA tests. Concern was raised that hyperbilirubinemia (total bilirubin, 55.5 mg/dL) interfered with the original assay. Further testing with a separate HIT ELISA assay, which includes multiple washes and dilutions of the serum in order to effectively remove bilirubin, returned negative. Medical providers must consider the possibility of false-positive LIA testing when evaluating for HIT in the setting of severe hyperbilirubinemia.

Essentials

- Heparin-induced thrombocytopenia (HIT) is a life-threatening condition that causes blood clots.
- This case describes a woman with liver failure and high bilirubin, who tested positive for HIT.
- More accurate testing was negative; high bilirubin likely caused this false-positive result.
- Medical practitioners should be wary of HIT screening tests in patients with high bilirubin.

1 | INTRODUCTION

Thrombocytopenia in the hospitalized patient can present a challenging clinical scenario—namely, differentiating between the benign or chronic causes of low platelets and the more life-threatening complications. One such potentially lethal pathology is heparin-induced thrombocytopenia (HIT), which results from the production of IgG autoantibodies to a complex of heparin and platelet factor 4

(PF4), resulting in the activation of platelets and a profoundly prothrombotic state.¹ Treatment involves withdrawal of heparin and replacement with an alternative anticoagulant.

Diagnosis of HIT rests on clinical assessment and laboratory testing. Laboratory tests for HIT include antigen assays, which tend to have high sensitivity but limited specificity, and functional assays, which tend to have higher specificity. We present a case of a false-positive antigen assay test due to severe hyperbilirubinemia.

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2 | CASE PRESENTATION

The patient was a 67-year-old woman with a past medical history of nonalcoholic steatohepatitis without known cirrhosis, gastroesophageal reflux disease, vertigo, and kidney stones, who presented to an outside hospital with dizziness. During hospitalization, she was noted to have an elevated total bilirubin of 7.0 mg/dL (reference range, 0.2-1.2) with concern for choledocholithiasis on imaging. Endoscopic retrograde cholangiopancreatography revealed a stone in the common bile duct, requiring sphincterotomy and percutaneous cholecystostomy. Also noted were esophageal varices and portal hypertensive gastropathy. Subsequently, the patient underwent liver biopsy confirming cirrhosis. She was transferred to our institution for consideration of liver transplant due to acute liver failure.

On admission to our center, the patient had a markedly elevated direct and total bilirubin of 38.6 and 63.4 mg/dL, respectively (reference ranges, 0.1-0.5; 0.2-1.2). Other liver function tests demonstrated an alkaline phosphatase of 176 IU/L (reference range, 40-150), aspartate aminotransferase of 123 IU/L (reference range, 5-34), and alanine aminotransferase of 64 IU/L (reference range, 0-55). A complete blood cell count on admission revealed a macrocytic anemia with a hemoglobin of 8.4 g/dL (reference range, 12.0-16.0) and a mean corpuscular volume of 109.5 fL (reference range, 81.0-96.0) and a platelet count of $67 \times 10^3/\mu\text{L}$ (reference range, 140-400). The patient was continued on subcutaneous unfractionated heparin upon transfer, which she had been receiving at the outside hospital for thromboprophylaxis (started 16 days prior). While undergoing liver transplant evaluation, the patient's platelet count declined to a nadir of $25 \times 10^3/\mu\text{L}$, with a decline of >30% within 1 day. The 4Ts (thrombocytopenia, timing of platelet count fall, thrombosis or other sequelae, and other causes of thrombocytopenia) score was calculated to be 4, representing an intermediate probability ($\approx 14\%$) for HIT.² HIT antibody testing with the HemosIL HIT-Ab (PF4-H) (Instrumentation Laboratory, Bedford, MA, USA) was moderately positive with a value of 5.4 U/mL (reference ranges: weak positive, 1.0-4.9; intermediate positive, 5.0-15.9; strong positive, ≥ 16). This particular assay is a latex immunoturbidimetric assay (LIA), found to have a sensitivity of 97.4% and specificity of 94.0% in a collective analysis of a prospective cohort study ($n = 429$) and a case study of HIT-positive patients ($n = 129$).^{3,4} The hematology service was consulted to assist with management of suspected HIT. A serotonin release assay (SRA) was ordered, heparin was stopped, and an argatroban infusion (AuroMedics Pharma, East Windsor, NJ, USA), dose-reduced for hepatic dysfunction, was initiated.⁵ Repeat HIT LIA testing on two separate blood draws returned positive twice more (values of 5.7 and 4.9 U/mL, respectively) over the next 3 days. The patient's platelet count fluctuated but did not normalize after discontinuation of heparin. Ultrasound of the upper and lower extremities was negative for deep vein thrombosis. Subsequently, the result of the SRA returned negative, arguing against a diagnosis of HIT.⁶

To reconcile the discrepant LIA and SRA results, we requested a third HIT assay, the PF4-dependent P-selectin expression assay (PEA), a flow-based assay that assesses for P-selectin expression as

a marker of HIT antibody-mediated platelet activation. This test has been shown to be noninferior to the SRA.^{7,8} Like the SRA, the PEA returned negative, indicating that the patient likely did not have HIT and suggesting that the initial HIT antibody LIA was falsely positive, possibly due to severely elevated bilirubin.

To evaluate for possible interference by hyperbilirubinemia, a second and distinct immunoassay was performed at a reference laboratory (Versiti) using heparin-dependent platelet antibody PF4 ELISA monospecific IgG, IgA, and IgM tests (Versiti Wisconsin, Inc., Milwaukee, WI, USA). Unlike the HemosIL PF4-H LIA, the Versiti PF4 ELISA involves dilution with multiple washes, which should eliminate bilirubin interference. The results of the Versiti assays were universally negative. The IgM-, IgG-, and IgA-specific assays demonstrated an optical density (OD) of 0.169 (reference range ≤ 0.57), 0.078 (reference range, ≤ 0.40), and 0.117 (reference range, ≤ 0.40), respectively.

3 | DISCUSSION

We postulate that severely elevated bilirubin was the etiology for the multiple initial false-positive HIT LIA tests. The etiology for this discrepant testing is likely related to procedural aspects of LIA testing that differ from ELISA.

The LIA indirectly evaluates HIT antibody concentration by measuring the degree of particle agglutination between PF4/polyvinyl-sulfonate (PVS) complexes and HIT-like monoclonal antibodies on the surface of latex particles. The presence of positive HIT plasma reduces agglutination of monoclonal antibody-coated latex particles with PF4/PVS complexes, resulting in reduced light absorbance and a positive test. ELISA tests use light spectroscopy to evaluate for serum antibodies; increased concentration of antibody is denoted by higher OD values, correlating to more light absorbed at a certain wavelength.⁹ Both ELISA and LIA have demonstrated >95% concordance in testing with the confirmed presence or absence of HIT antibody.¹⁰ Known confounding factors that can cause false-positive HIT testing include hypertriglyceridemia and polycythemia, in addition to diseases such as antiphospholipid antibody syndrome and systemic lupus erythematosus.¹¹

Bilirubin is known to absorb light at a peak wavelength of 450 to 490 nm, establishing the basis of blue light therapy for neonatal icterus (blue light at wavelength 460-490 causes conjugation of indirect bilirubin into a water-soluble and nonneurotoxic compound).¹² Blue light absorption also forms the basis for traditional HIT antibody ELISA tests, when a blue dye is formed secondary to an agglutination reaction prompted by a patient's HIT antibody. Procedural differences between the LIA and ELISA in this case differ in the presence or removal of bilirubin during the preparation steps. In the LIA, all of the components of the assay are mixed simultaneously in the same cuvette without any washing or alteration steps. In contrast, the ELISA test includes washing steps that are designed remove contaminants such as bilirubin. Contrasting these two procedures, in the context of their discrepant results, suggests

that contaminants present in the LIA may have interfered with its accuracy. Notably, on the package insert for the HemosIL PF4-H LIA assay, the Interferences section notes "HIT-Ab results ... are not affected by ... bilirubin up to 18 mg/dL."¹³ In this clinical scenario with severely elevated bilirubin concentration well above 18 mg/dL and bilirubin's known propensity to absorb light, it is likely that hyperbilirubinemia led to falsely elevated results in the LIA testing.

Critically ill patients, such as those with severe liver disease as noted in this case, often require the use of heparin or heparin products. Accurate diagnosis of HIT in any hospitalized patient is crucial, as delays in diagnosis and initiation of treatment are associated with an initial daily 6% risk of thromboembolism, amputation, or death.¹⁴ At the same time, misdiagnosis may expose patients without HIT to costly nonheparin anticoagulants and their attendant bleeding risk. Knowledge that significantly elevated bilirubin may interfere with the diagnosis of HIT for certain immunoassays may aid diagnostic accuracy in these challenging patients.

RELATIONSHIP DISCLOSURE

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AUTHOR CONTRIBUTIONS

Each author listed on this manuscript contributed equally to the content of this manuscript including background research, writing, editing, and formatting for submission. DE compiled relevant data from the patient's admission, wrote the draft of the manuscript, edited the manuscript for accuracy and clarity, assessed and compiled background information and sources, and completed revisions of the manuscript. He served as corresponding author for submission. VJ, AC, and GV edited the manuscript for accuracy and clarity and assessed and compiled background information and sources.

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