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The Role for Optical Density in Heparin-Induced Thrombocytopenia
A Cohort Study

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BACKGROUND: Heparin-induced thrombocytopenia (HIT) is a serious complication of heparin utilization. An enzyme-linked immunosorbent assay (ELISA) is usually performed to assist in the diagnosis of HIT. ELISAs tend to be sensitive but lack specificity. We sought to use a new cutoff to define a positive HIT ELISA.

METHODS: We conducted a prospective observational study of hospitalized patients undergoing ELISA testing. All patients who underwent ELISA testing were eligible for inclusion (n = 496). Irrespective of the results, all subjects had confirmatory testing with a serotonin release assay (SRA). We compared a threshold optical density (OD) > 1.00 to the current definition of a positive ELISA (OD > 0.40) as a screening test for a positive SRA. We used sensitivity, specificity, and area under the receiver operating curve to determine whether an OD > 1.00 would improve diagnostic accuracy for HIT.

RESULTS: The SRA was positive in 10 patients (prevalence, 2.0%). Adjusting the definition of a positive HIT ELISA to > 1.00 maintained the sensitivity and negative predictive value at 100% in the cohort. The positive predictive value of the higher cutoff OD was more than triple the positive predictive value of an OD > 0.40 (41.7% vs 13.3%). No patient with a positive SRA had an OD measurement ≤ 1.00.

CONCLUSIONS: Increasing the OD threshold enhances specificity without noticeably compromising sensitivity. Altering the definition of the HIT ELISA could prevent unnecessary testing and/or treatment with non-heparin-based anticoagulants in patients with possible HIT.

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ABBREVIATIONS: AUROC = area under the receiver operating curve; CABG = coronary artery bypass grafting; ELISA = enzyme-linked immunosorbent assay; HIT = heparin-induced thrombocytopenia; LMWH = low-molecular-weight heparin; MCS = major cardiac surgery; NPV = negative predictive value; OD = optical density; PPV = positive predictive value; ROC = receiver operating curve; SRA = serotonin release assay; UFH = unfractionated heparin

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Heparin-induced thrombocytopenia (HIT) is a serious complication of heparin utilization that is strongly associated with venous or arterial thrombosis.[1] Despite leading to thrombocytopenia, the generation of heparin-dependent platelet-activating antibodies is the hallmark of HIT.[1-3] Certain types of patients, such as those undergoing major cardiac surgery (MCS) who receive postoperative anticoagulation treatment, face an increased risk for HIT.[1-4]

Although multiple guidelines recommend treatment approaches for HIT, the diagnosis remains clinically challenging.[5,6] Several risk stratification schemes exist to aid clinicians in evaluating patients for HIT.[6,7] Irrespective of the implementation of clinical risk stratification, diagnosing HIT usually requires confirmatory testing to document the presence of heparin-dependent, platelet-activating antibodies.[3,8] Two "functional" washed platelet assays exist, the serotonin release assay (SRA) and the heparin-induced platelet activation assay. Each represents a "gold standard" for diagnosing HIT, as washed platelets enhance diagnostic sensitivity and specificity for the detection of pathogenic HIT antibodies.

In the United States, the SRA is available only at a handful of reference laboratories. Most institutions use an "antigen" test such as a commercial enzyme-linked immunosorbent assay (ELISA), which can be readily performed but lacks diagnostic specificity because of its frequent detection of nonpathogenic, non-platelet-activating antiplatelet factor 4/heparin antibodies.[9] The prevalence of SRA positivity among those with positive ELISAs ranges from 10% to 50%.[1-3] The risks of clinical decision-making based on a test with limited specificity are not insignificant. Current guidelines recommend initiating a nonheparin anticoagulant if there is clinical suspicion for HIT and a positive ELISA.[5,6]

FOR EDITORIAL COMMENT SEE PAGE 1

Thus, patients with thrombocytopenia are exposed to the risk of full-strength anticoagulation treatment with an expensive alternative to heparin.

Altering the definition of a positive ELISA represents one potential paradigm for addressing the limitations of this assay. Historically, the ELISA is defined as positive when the optical density (OD) exceeds 0.40.[1-3] This threshold was chosen based on control groups composed mainly of healthy blood donors who do not reflect the appropriate comparator population for hospitalized patients. Bakchoul et al[10] demonstrated that altering the breakpoint for the ELISA to an OD $\geq$ 1.00 improved the specificity of the test by nearly 20%. Warkentin et al[11] showed that the prevalence of a positive SRA in people with ODs $<$ 1.00 was rare.

Most studies exploring alternative definitions of a positive ELISA are limited because they are either retrospective, focus on a narrow cohort of patients, or include few critically ill subjects, therefore, limiting generalizability. Additionally, the paucity of prospective data are concerning. Therefore, we conducted a prospective observational study of consecutive patients being evaluated for HIT, including those in the ICU and those on the general floors, to assess the utility of adopting a new cutoff for the HIT ELISA.

Materials and Methods

Study Overview and Subjects

We conducted a prospective observational study of patients (NCT00946400) undergoing HIT ELISA testing at our institution between August 2009 and April 2012. The decision to order the ELISA was determined by the treating clinicians. Documentation of any formal risk stratification prior to ordering the ELISA was not required as this is not done routinely at the host institution. All adult subjects (age $\geq$ 18 years) were eligible. We excluded subjects with a known prior history of HIT. Either subjects or their surrogates provided written informed consent. The MedStar Washington Hospital Center Human Use Committee approved the protocol (Institutional Review Board No. 2009-202).

Study Objective and Assays

The primary objective was to determine the performance characteristics of an OD $>$ 1.00 for the diagnosis of HIT. We sought to compare a threshold OD $>$ 1.00 to the current definition of a positive ELISA (OD $>$ 0.40, per manufacturer's recommendation). The diagnosis of HIT was based on confirmatory SRA. All patients underwent SRA testing, irrespective of the results of the ELISA. The HIT ELISA (Genetics Testing Institute Inc) represented a commercially available test performed routinely in the study institution. This assay is a polyspecific assay that detects IgA, IgG, and IgM antibodies. SRAs were conducted by one investigator at a central laboratory (Platelet Immunology Laboratory, McMaster University) with specialized expertise in HIT testing. For the SRA, a $<$ 20% release was classified as a negative result, a 20% to 49.9% serotonin release as a "weak-positive," and $\geq$ 50% serotonin release as a "strong-positive." Testing was performed at two pharmacologic concentrations of heparin (0.1 and 0.3 International Units/mL) and one high dose (100 International Units/mL) in both patient samples and using known strong, weak, and negative controls. SRA results were reported as a positive or negative result with a mean percentage release (at 0.1 and 0.3 Interational Units/mL heparin). For the purposes of analysis, we categorized both the weak and strong release amounts as "positive." The laboratory technician conducting the SRAs was blinded to the results of each patient's clinical scenario.

Covariates and Subgroup Analysis

We collected information regarding patient demographics, comorbid illnesses, and acute disease processes. Patients were dichotomized as hospitalized for a medical or surgical reason, and we recorded if there was a history of coronary artery disease, congestive heart failure, hypertension, diabetes mellitus, stroke, or VTE. We also assessed whether the patient had his/her ELISA collected while in an ICU or a general floor. To determine the influence of severity of illness on sensitivity, we repeated our