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Editorial

Demand on-demand testing for the diagnosis of heparin-induced thrombocytopenia

In theory, most laboratory tests for immune heparin-induced thrombocytopenia (HIT) can be performed within a few hours of blood sample acquisition. But for the most common type of test performed – the platelet factor 4 (PF4)-dependent enzyme-linked immunosorbent assay (ELISA) – the blood samples are almost always tested in batches, at most once-daily (and often not on weekends), and so results are frequently not available to the clinician until 1–4 days later. More definitive tests for detecting HIT antibodies (with greater diagnostic specificity), such as certain platelet activation assays, are performed by relatively few centers, and thus there is an additional time delay that reflects sample delivery to a different facility that could even be in another state or province. Even at my own medical center, with an excellent assay for HIT (the platelet serotonin-release assay), testing is performed just twice-weekly (Tuesdays and Thursdays, with results reported Wednesdays and Fridays, respectively), meaning that a turnaround of up to several days is common [1].

But some PF4-dependent immunoassays have been specifically designed for rapid turnaround, defined as 30 min or less [2]. Moreover, such "on-demand" tests are engineered for evaluation of single blood specimens. Thus, if such an assay is available on-site at a laboratory located within (or near) a clinical institution, there is the prospect for a speedy test result within a relatively short period of time. Rapid, on-demand assays for HIT that have been developed include automated tests requiring proprietary machines, such as the HemosIL® HIT-Ab$_{(PF4-H)}$ (performed using an ACL TOP® hemostasis analyzer; Instrumentation Laboratory, Bedford, MA) [3,4], the HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ and HemosIL AcuStar HIT-Ab$_{(PF4-H)}$ (IgG-specific and polyspecific fully-automated chemiluminescence assays, respectively, using the ACL AcuStar™ hemostasis testing system) [4,5], the particle gel immunoassay (PaGIA; H/PF4-PaGIA®, Bio—Rad, Marne La Coquette, France), using standard blood bank centrifugation equipment [6,7], and the lateral flow immunoassay (STic Expert® HIT, Diagnostica Stago, Asnières sur Seine, France) [8,9]. (Although another on-demand assay – the particle immunofiltration assay (PIFA®; Akers Biosciences, Thorofare, NJ) – is marketed, this test has poor operating characteristics [10], and its use for HIT diagnosis is problematic [11].)

In this issue of *Thrombosis Research*, Caton and colleagues [12] have performed a literature review and conducted semi-structured interviews and surveys evaluating various diagnostic and treatment strategies for different HIT laboratory tests. They then modeled the frequency and overall costs associated with various adverse HIT-related outcomes, such as bleeding and thrombosis, for different test approaches. The authors concluded that "modeling estimated more HIT-related [adverse] outcomes for patients maintained on heparin whilst awaiting test results and patients switched onto replacement anticoagulant therapy awaiting test results, compared with on-demand testing

and treatment based on these results." The authors further noted that a "budget impact model estimated that on-demand testing reduced replacement anticoagulant therapy costs from $39,616 to $12,799 per patient." Do these claims withstand scrutiny?

Consider some truisms regarding HIT:

(a) only 5% to 10% of patients investigated for HIT with laboratory testing ultimately are shown to have this diagnosis, using a washed platelet activation test as the reference standard [13,14];

(b) anticoagulants widely approved for treatment of HIT, such as argatroban and danaparoid, are relatively expensive (note: danaparoid is not approved for treatment of HIT in the U.S.), especially compared to the cost of maintaining (or switching back to) unfractionated heparin, a very inexpensive medication;

(c) the frequency of major hemorrhage using the direct thrombin inhibitors approved for HIT (argatroban, lepirudin) are approximately 1% per treatment-day [15] (note: lepirudin is no longer marketed and thus is not available for treating HIT);

(d) the thrombosis rate is approximately 5–10% per day, for at least the first 2 or 3 days, if heparin is held in a patient who is subsequently confirmed to have HIT by a positive washed platelet activation assay [16].

Given that there are significant costs associated with bleeding and/or thrombotic events, not to mention the human cost of related morbidities and mortality, it would seem that the conclusions of Caton and colleagues are likely to be correct. Based upon evolving concepts in HIT, the following statements should reflect the potential clinical value of on-demand testing for HIT antibodies:

(a) a negative result using an on-demand assay would avoid the expense and bleeding risks associated with initiating therapy with an alternative non-heparin anticoagulant, provided that the diagnostic sensitivity of the on-demand assay is sufficiently good to ensure a high negative-predictive value.

(b) a positive result using an on-demand assay would justify the expense and bleeding risks associated with initiating therapy with an alternative non-heparin anticoagulant, provided that the false-positive rate is not excessively high.

(c) assays that provide *semi-quantitative* results, in other words, weak-, moderate- or strong-positive results that correspond to comparatively greater probability of (platelet-activating) HIT antibodies being detectable, can provide graded likelihood ratios, which are useful for refining the clinician's post-test estimation of a patient having (or not having) HIT. For example, a patient judged to have an intermediate or high probability of HIT can

have a near-certain diagnosis of HIT if an ELISA (or another semi-quantitative immunoassay) yields a result that is strongly positive. To express this in Bayesian post-test probability terms, a blood sample obtained from a patient with clinically-suspected HIT who has a positive polyspecific anti-PF4/polyanion ELISA of greater than 2.0 optical density units has a probability of confirmed HIT that ranges from 90% (with an intermediate pre-test probability) to 99% (based on a high pre-test probability) [17,18].

How can these concepts apply to on-demand testing? The HemosIL automated assays routinely provide results expressed quantitatively (i.e., units per milliliter), and it therefore will be important to examine the positive predictive values of different strengths of positive results (in comparison with the negative predictive values of negative test results), at varying degrees of pre-test probability (e.g., as predicted by a clinical scoring system for HIT such as the 4Ts [7,19]). The three HemosIL assays' outputs are termed "semi-quantitative", as there is no absolute linear correlation between the degree of positive test results and differing levels of HIT antibodies. Nevertheless, it is known that other semi-quantitative assays, such as the PF4-dependent ELISAs, provide enormously valuable information beyond merely providing a "positive" or "negative" result. For example, if an ELISA is "positive" but only weakly so (i.e., 0.40 to 0.99 OD units), the patient has less than a 5% chance of having HIT; conversely, if the ELISA result is greater than 2.0 units, the probability of the patient having HIT is at least 90%. The PaGIA test, by way of contrast, provides only a dichotomous result (positive or negative); however, even the PaGIA can be made semi-quantitative by performing the assay using various blood sample dilutions; here, a positive result using patient plasma diluted 1/4 or greater predicts more strongly for HIT than a test that is positive using only neat (undiluted) plasma [20].

The study by Caton and colleagues indicates that on-demand testing is likely to hasten diagnostic evaluation of patients with suspected HIT, contributing to substantial cost savings and possibly also to improved outcomes. Future research that examines also the impact of interpreting the specific numerical results of the semi-quantitative on-demand assays should demonstrate more accurate assessment of the post-test probability of HIT. This will improve clinical decision-making, for example, in cases of relatively high probability clearly justifying the switch from heparin to a different anticoagulant, while at the same time identifying low-probability situations that will avoid unnecessary use of expensive and risky anticoagulants with a high chance of causing bleeding. Before long, clinicians will demand on-demand testing for their patients.

Conflict of interest statement

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