

Evaluation of the Particle Immunofiltration Anti-platelet factor 4 (PIFA) Rapid Assay in MICU Patients with Thrombocytopenia

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ABSTRACT*

Introduction: Heparin-induced thrombocytopenia (HIT) is a life and limb threatening, immune-mediated, pro-thrombotic disease resulting from an interaction between heparin and platelet factor 4 (PF4). Due to the many causes of thrombocytopenia in critically ill patients the diagnosis of HIT is difficult, requiring both a high clinical suspicion and confirmatory testing. The ELISA test is the most commonly performed method to detect anti-PF4 antibodies; however the ELISA results generally take one or more days to report. The Particle Immunofiltration Assay (PIFA®) test has the advantage of being done rapidly; with results generally available within 1 hour.

Methods: Starting in July 2009, patients in our MICU were screened daily for thrombocytopenia; defined as either a platelet count that decreased by at least 30% from baseline or an absolute platelet count less than 150,000. Patients with suspected HIT (thrombocytopenia and heparin exposure) underwent both PIFA (Akers Biosciences, Inc.) and GTI ELISA (Genetics Testing Institute) testing for anti-PF4 antibodies. PIFA is a qualitative test reporting results as positive or negative. The GTI ELISA test reports an optical density (OD), with an OD greater than or equal to 0.40 considered positive. Patients were followed through hospitalization.

Results: 166 patients were admitted to the MICU, with 76 developing thrombocytopenia. 17 patients had no recent (confirmed or suspected) exposure to unfractionated or low molecular weight heparin. In 2 patients the PIFA test was inconclusive. The remaining 57 patients make up the population reported in this analysis.

In these 57 patients, the PIFA results were negative in 36 and positive in 21. In the 36 patients with a negative PIFA result, the GTI ELISA test was negative in 35 (97.2%) and positive in 1 (5.6%). In the 21 patients with PIFA positive results, 2 GTI ELISA tests were positive (10%) and 19 (90%) tests were negative. For clinical reasons, serotonin release assay (SRA) was done in 13 patients with discordant PIFA and GTI ELISA tests, of which 1 SRA result was positive. Of the 57 patients only 6 (10.5%) developed thrombotic complications.

Conclusions: Our analysis suggests that a negative PIFA test can quickly exclude the presence of anti-PF4 antibodies and therefore HIT as the cause of the thrombocytopenia. The PIFA test can be used as a rapid screening procedure for ICU patients with possible HIT, and when negative, decrease the exposure of thrombocytopenic MICU patients to alternative anticoagulation. The clinical significance of a positive PIFA test requires further study and must be evaluated in conjunction with clinical judgment and, as appropriate, additional laboratory testing for anti-PF4 antibodies.

- Please note that these numbers reflect updated data on patients with suspected HIT and both a PIFA GTI ELISA sent on or before February 14, 2010. Length of stay and outcome data was censored on March 1, 2010.

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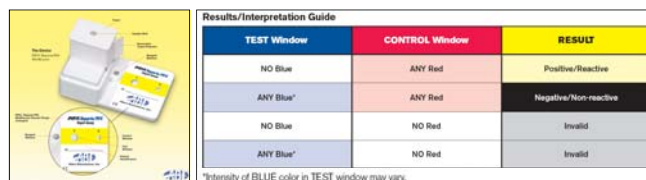
METHODS

Methods: Patients in the MICU were screened daily for thrombocytopenia (defined for this study as the platelet count that has decreased by at least 30% or is less than 150,000). If patients had risk factors for HIT, serum was sent to the University of Miami Special Coagulation Laboratory (www.med.miami.edu/pathology/x57.xml) for PIFA and GTI ELISA testing. Based on these results and clinical suspicion of HIT, the serum could undergo SRA analysis for anti-PF4 antibodies.

Concurrent with clinical care, and before the results of the PIFA and GTI ELISA tests were reported, the pre-test probability for HIT will be assessed using the Warkentin's 4Ts score (Thrombocytopenia, Timing of thrombocytopenia, Thrombosis, and oTher reason)².

Heparin/Anti-platelet Factor 4 testing:

PIFA: The PIFA assay is based on the interaction of serum anti-PF4 antibody (IgG/A/M) with Platelet Factor 4 – coated microparticles. The microparticles are dyed blue. The assay provides a qualitative result, either negative (blue color) or positive (no color change/white) in the test window of the device. Patients with anti-PF4 antibodies recognize and aggregate the blue dyed microparticles, preventing their passage through a permeable membrane. This appears as no color in the TEST result window and is considered a POSITIVE test. Patients lacking anti-PF4 antibody do not cause aggregation of the blue dyed microparticles and a blue color appears in the TEST window, which is considered a NEGATIVE test. A control window reveals a red color, indicating a satisfactory test.



ELISA: The GTI test is an ELISA based solid phase assay that produces an optical density readout. Patient sera containing antibody (IgG/A/M) react with platelet factor 4 that is complexed to the plastic plate. Thus the target antigen is a complex between the polyanionic compound Polyvinyl Sulfonate (PVS) and PF4. In this assay, PVS serves in the solid phase as a surrogate for heparin. A positive result is equal or greater to an Optical Density of 0.40.

Serotonin Release Assay: Sent to a reference laboratory – Quest Diagnostics.

RESULTS

Table 1: Anti-PF4 Test Results

	PIFA Negative	PIFA Positive	GTI ELISA Totals
GTI ELISA Negative	35	19	54
GTI ELISA Positive	1	2	3
PIFA Totals	36	21	57

RESULTS Continued

Table 2: Demographics and Baseline Characteristics

	PIFA		ELISA	
	Negative (n=36)	Positive (n= 21)	Negative (n=54)	Positive (n=3)
Age, mean (SD)	57±14	57±15	57±14	56±12
Sex (Male, %)	21 (58%)	11 (52%)	30 (56%)	2 (67%)
Platelet count	101±47	99±46	101±42	74±40
4Ts score, mean (SD)	4.0±1.1	3.9±1.1	4.0±1.1	4.1±1.0

Table 3: Outcomes

	PIFA		ELISA	
	Negative (n=36)	Positive (n=21)	Negative (n=54)	Positive (n=3)
Any VTE (n, %)	4 (11%)	1 (5%)	6 (11)	0 (0%)
LOS ICU (days)*	15±19	14±20	15±18	11±14
LOS Hospital (days)*	25±26	26±27	24±26	22±20
Hospital Mortality	11 (31%)	1 (5%)	13 (24%)	1 (33%)

* Length of stay days calculated from time of anti-PF4 antibody studies sent

- The one patient with a negative PIFA, had a positive GTI ELISA optical density of 0.517. The Warkentin's 4Ts score of 3 (low pretest probability for HIT). The patient did not have any clinically identified arterial or venous thrombotic event.
- The average optical density in the GTI ELISA negative group was 0.16±1.1.
- The average optical density in the GTI ELISA positive group was 0.45±0.3.
- Serotonin release assay (SRA) was performed on 13 patient specimens with discordant PIFA and GTI ELISA results. 1 SRA result was positive. This specimen was reported as positive by PIFA and negative by GTI.
- Only 6 of the 57 patients with thrombocytopenia and heparin exposure (10.5%) developed thrombotic complications, all in patients with PIFA and GTI ELISA negative results.

CONCLUSIONS

- A negative PIFA test can quickly exclude the presence of anti-PF4 antibodies and therefore essentially rule out HIT as the cause of the thrombocytopenia.
- The PIFA test can be used as a rapid screening procedure for patients with possible HIT, and when negative, decrease the exposure of thrombocytopenic MICU patients to alternative anticoagulation.
- The clinical significance of a positive PIFA test requires further study and must be evaluated in conjunction with clinical judgment and, as appropriate, additional laboratory testing.

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