Transfusion and Apheresis Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

# Absolute immature platelet counts in the setting of suspected heparin-induced thrombocytopenia may predict anti-PF4-heparin immunoassay testing results

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ARTICLE INFO

*Keywords:*
anti-PF4
anti-PF4-heparin
Heparin-Induced thrombocytopenia
HIT
Absolute immature platelet count

ABSTRACT

*Background:* Heparin-induced-thrombocytopenia (HIT) is a disease mediated by antibodies to platelet factor 4 (PF4)-heparin complexes. Immature platelet fraction (%-IPF) and absolute immature platelet count (A-IPC) measure newly-released platelets into circulation and can prove useful in differentiating patients with thrombocytopenic presentations due to consumptive or hypoproduction processes. Therefore, we evaluated utility of A-IPC in a cohort of thrombocytopenic patients suspected of HIT.

*Patients and Methods:* Twenty-six thrombocytopenic patients ($< 150 \times 10^9$/L) tested for anti-PF4-heparin and 36 non-thrombocytopenic controls were included. Platelet count, %-IPF, and A-IPC were determined at time of anti-PF4-heparin testing.

*Results:* Sixteen patients tested anti-PF4-heparin negative and 10 tested positive. Patients with positive anti-PF4-heparin did not differ in A-IPC from normal range ($7.2 \pm 2.9 \times 10^9$/L vs. $7.1 \pm 3.2 \times 10^9$/L respectively; $p = 0.97$). However, there was a significant A-IPC decrease in patients negative for anti-PF4-heparin compared to normal range and those testing anti-PF4-heparin positive ($4.2 \pm 3.1 \times 10^9$/L vs. $7.1 \pm 3.2 \times 10^9$/L vs. $7.2 \pm 2.9 \times 10^9$/L respectively, $p < 0.01$). An A-IPC of greater than $5 \times 10^9$/L characterized 80% of anti-PF4-heparin positive cases.

*Conclusion:* A-IPC measurements can complement anti-PF4-heparin testing of patients suspected of HIT while potentially predicting anti-PF4-heparin immunoassay results.

## 1. Introduction

Heparin-induced thrombocytopenia (HIT) is a life-threatening complication of heparin therapy that affects a significant number of patients. It occurs in two distinct forms: type I and type II. While type I is defined as a mild thrombocytopenia which does not require heparin discontinuation for platelet count normalization; type II, on the other hand, is associated with high morbidity and mortality secondary to a significant decrease in platelet count, an extensive pro-coagulant state with incidence of venous and arterial thrombosis, limb loss, and even death [1,2]. Type II HIT is an immune-mediated disorder caused by the formation of antibodies to platelet factor 4 (PF4)-heparin complexes that leads to over consumption of platelets in peripheral blood. Suspicion of type II HIT requires a timely diagnostic work-up and clinical management which includes heparin discontinuation to prevent severe complications [3].

Immunoassays are essential in the diagnostic workup of patients with suspected HIT [4]. Currently, anti-PF4-heparin antibodies, including IgG, IgM, and IgA can be assayed using commercially-available enzyme-linked immunosorbent assays (ELISA) [4]. These assays are sensitive, have a high negative predictive value and moderate specificity. However, anti-PF4-heparin assays take hours to report and do not represent a real-time test of the thrombopoietic state of the patient.

Circulating immature platelet fraction (%-IPF) is a measure of newly released platelets from the bone marrow which contain a higher RNA concentration, are significantly larger than mature platelets, and can be rapidly measured by automated hematology analyzers equipped with reticulocyte detection channels [5]. A high %-IPF suggests the presence of rapid platelet consumption [6], while a low %-IPF is characteristic of bone marrow suppression states [7]. However, the absolute immature platelet count (A-IPC), calculated from %-IPF in circulation, has been reported to be a better real-time measurement of the thrombopoietic

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https://doi.org/10.1016/j.transci.2018.04.001
Received 2 January 2018; Received in revised form 30 March 2018; Accepted 1 April 2018

rate to predict marrow recovery post-chemotherapy in children [8], and in diseases such as immune thrombocytopenic purpura (ITP) [9] and thrombotic thrombocytopenic purpura (TTP) which can be used to differentiate it from other microangiopathic hemolytic anemia (MAHA) presentations [10]. In light of the potential usefulness of A-IPC in the setting of other thrombocytopenic presentations, we sought to evaluate the feasibility of using %-IPF and A-IPC to complement anti-PF4-heparin testing in a cohort of thrombocytopenic patients suspected of HIT.

## 2. Methods

### 2.1. Patients and normal controls

In order to guide study design the checklist of the STROBE (Strengthening the reporting of observational studies in epidemiology) statement was followed [11]. Study period corresponded to the time designated to carry out a pilot study for introduction of %-IPF testing at the institution which included engagement with clinical departments about test availability beginning on 02/01/2013 after completion of all validation studies. Review of %-IPF orders in the setting of concurrent anti-PF4-heparin testing indicated that no %-IPF testing orders were received beyond 08/31/2013 for this clinical indication. Inclusion criteria was defined as thrombocytopenic patients suspected of HIT, as indicated in the medical record, tested for anti-PF4-heparin who also had a platelet count and %-IPF obtained within 24 h of sampling for anti-PF4-heparin testing. For all patients included in the study the decision to test was made by the primary clinical service requesting testing as indicated in patients' medical records. The investigators of this study had no role in the clinical decision or how these patients were risk-stratified in order to test for anti-PF4-heparin. Twenty-six patients clinically suspected of HIT (platelet counts $< 150 \times 10^9$/L with history of heparin exposure) met inclusion criteria (7% of all patients tested for anti-PF4-heparin). Review of medical records indicated that no prior testing for anti-PF4-heparin existed for the cohort and there was no mention of a prior HIT diagnosis. Only one sample per patient was included in the study. Specimens from 36 subjects without thrombocytopenia were obtained to generate a reference range. Data gathered included demographic information, platelet counts and %-IPF analysis. Study was approved by the UHCMC Institutional Review Board.

### 2.2. Anti-PF4-heparin ELISA assay

Anti-PF4-heparin testing was done at UHCMC exclusively using GTI Incorporated PF4 Enhanced kit X-HAT45 designed to detect IgG, IgA, and IgM heparin-associated PF4 antibodies according to manufacturer's protocols (Lifecodes GTI Diagnostics, Waukesha, WI). All samples for anti-PF4-heparin testing were collected in red top tubes with serum clot activator and gel separator (VWR, Radnor, PA). Plates were read using the ELx800 microplate reader set at wavelength of 405/490 (BioTek Instruments, Inc. Winooski, VT). All patients with positive anti-PF4-heparin test results had OD $> 1.0$, while patients with anti-PF4-heparin negative results had OD $< 0.3$.

### 2.3. $^{14}$C-Serotonin release assay (SRA)

All 10 patients with a positive anti-PF4-heparin test were sent out to the Blood Center of Wisconsin to undergo SRA as previously described [12]. Reference ranges were defined as follows: positive result requires $> 20\%$ release of serotonin with low dose heparin and $< 20\%$ release in the presence of a high concentration of heparin.

### 2.4. Platelet counts, %-IPF analysis and A-IPC calculations

Peripheral blood samples were collected into K2 EDTA tubes (Becton Dickinson, Franklin Lakes, NJ) and analyzed for complete blood count (CBC) at the UHCMC Hematology laboratory. Patient samples corresponded to the same draw used for anti-PF4-heparin testing or obtained within 24 h of this sampling. All patient and control samples were tested for optical platelet (fluorescent) count and %-IPF using the Model XE-5000 automated hematology analyzer according to manufacturer's protocols (Sysmex America Inc., Mundelein, IL) and as previously described [10]. No other analyzer was used in this study. To determine accuracy of measurements, controls from the manufacturer (Sysmed e-Check, XE) were measured after analysis of 50 samples. A control program was also applied to reassure the analyzer's quality control as recommended by the manufacturer (XbarM, Sysmex America, Inc.) [10]. All samples were run twice with similar results. A-IPC counts were obtained by multiplying the %-IPF times the corresponding optical platelet count [5].

### 2.5. Statistical analysis

Statistics were performed using Prism 6 (GraphPad Software Inc., La Jolla, CA) unless otherwise specified. Results are presented as mean ± SD. All statistical results were further analyzed for statistical power taking into account statistical result, sample size, error and/or confidence, standard deviation, and sample mean [13]. Intergroup data comparisons were performed using one-way ANOVA, and where applicable a Student $t$ test. Receiver Operating Characteristic (ROC) curve was generated using SPSS 16.0 (SPSS Inc., Chicago, IL). A $p$ value of $< 0.05$ was set for significance.

## 3. Results

### 3.1. Clinical characteristics of thrombocytopenic patients

Twenty-six thrombocytopenic patients with clinical suspicion for HIT were tested by anti-PF4-heparin ELISA (Table 1A) and had a concurrent %-IPF ordered which corresponded to 7% of total anti-PF4-heparin tests performed. Ten of these patients (10/26) tested positive by anti-PF4-heparin and 16/26 tested negative. All patients with

**Table 1**
Demographic information of study cohort with sensitivity and specificity of A-IPC measurements.

A. Demographic of patients included in study

| Characteristic | Thrombocytopenic patients (n = 26) | | Controls (n = 36) |
|---|---|---|---|
| | Anti-PF4-heparin positive (n = 10) | Anti-PF4-heparin negative (n = 16) | |
| Age (mean, range) | 60.1 (43-81) | 58.5 (32-85) | 54.8 (17-87) |
| Gender (female/male) | 2/8 | 9/7 | 22/14 |
| Platelet (x $10^9$/L) | 99.6 ± 42.2* | 70.5 ± 30.5* | 260.2 ± 61.9 |
| %-IPF | 8.5 ± 5.4** | 5.9 ± 3.8** | 2.9 ± 1.4 |
| A-IPC (x $10^9$/L) | 7.2 ± 2.9 | 4.2 ± 3.1*** | 7.1 ± 3.2 |

B. Sensitivity, specificity, PPV and NPV of a cutoff value of A-IPC of greater than $5 \times 10^9$/L in patients suspected of HIT

| A-IPC $> 5 \times 10^9$/L | HIT-suspected patients (n = 26) | |
|---|---|---|
| | HIT | No HIT |
| Yes | 8 | 4 |
| No | 2 | 12 |

*$p < 0.01$, **$p < 0.01$ compared to controls; ***$p < 0.01$, compared to controls and anti-PF4-heparin positive group. Data are reported as Mean ± SD. Statistically significant results were confirmed by high power results. Patients testing anti-PF4-heparin positive by ELISA were reflexively tested by SRA which confirmed these results. Sensitivity: 8/10 = 80.0%; Specificity: 12/16 = 75%; PPV: 8/12 = 67%, NPV: 12/14 = 86%.