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Use of a whole-cell ELISA to detect additional antibodies in setting of suspected heparin-induced thrombocytopenia

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Abstract

Objectives: Type II heparin-induced thrombocytopenia (HIT) is mediated by formation of antibodies to platelet factor 4 (PF4)-heparin complexes. We evaluated anti-PF4-heparin negative samples for presence of additional anti-platelet and anti-red blood cell (RBC) antibodies using whole-cell platelet/ RBC ELISAs we developed.

Methods: Seventy-three samples tested for anti-PF4-heparin by ELISA were included: 62 tested negative, 9 tested positive, and 2 had equivocal results. Plasma specimens from healthy donors were used as controls.

Results: 100% (9/9) anti-PF4 positive samples had anti-platelet antibodies detected by whole-cell platelet ELISA. 42.2% (27/64) anti-PF4-heparin negative samples were negative for anti-platelet and anti-RBC antibodies. 32.8% (21/64) negative samples showed reactivity to both platelets and RBC; 12.5% (8/64) negative samples were each reactive with either platelet or RBC ELISA respectively. Additionally, two samples that tested equivocal by anti-PF4-heparin ELISA had antibodies to both platelets and RBC by whole-cell ELISA.

Conclusions: —Our study suggests that patients with thrombocytopenia testing negative for anti-PF4-heparin may still harbor antibodies to platelets. However, additional research is needed to determine the significance of these antibodies. Nevertheless, these findings may encourage clinicians to further investigate patients with possible immune-mediated etiologies of thrombocytopenia and anemia.

Keywords

anti-PF4-heparin; whole-cell ELISA; platelet; red cells; heparin-induced thrombocytopenia; antibodies

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Conflict of Interest:

The authors declare no competing financial interests in the submission of this article.

Introduction

Heparin-induced thrombocytopenia (HIT) is an adverse effect of heparin therapy that leads to thrombocytopenia and a higher risk of both arterial and venous thrombosis. Physiologically, two types of HIT have been recognized based on apparently distinct etiologies. Type I is defined as having early onset of mild thrombocytopenia presenting within the first two days after heparin exposure that does not require heparin therapy discontinuation for platelet count normalization.(1) Its etiology is not precisely known but it may result from a direct effect of heparin on platelet activity that leads to agglutination and does not involve an immune response.(1)

Type II HIT normally takes 5–14 days to develop after heparin administration.(2) Contrary to type I, type II HIT is an immune-mediated disorder caused by antibody formation (usually IgG) to platelet factor 4-heparin complexes (anti-PF4-heparin) which subsequently bind to either FcγRIIA receptor on platelets leading to their activation or on monocytes leading to tissue factor expression which facilitates platelet activation by thrombin, or to the glycosaminoglycan (GAG) molecules on the surface of platelets and endothelial cells.(1–5) Immunologically, type II HIT represents an atypical response with both T-cell dependent features represented by formation of antibodies to PF4-heparin complexes.(6) and a T-cell independent mechanism as suggested by lack of a memory response upon heparin re-exposure.(7) For the remainder of the text, type II HIT will be referred to as HIT.

Current evidence suggests that antibodies with PF4-heparin complex specificity form when cationic PF4 released from platelets' alpha granules interacts with anionic heparin leading to charge neutralization.(8) This leads to availability of a conformationally-dependent site on PF4 that is recognized by the newly formed antibody,(9) which then binds and crosslinks platelet/monocyte FcγRIIA receptors resulting in platelet activation and thrombosis.(10, 11) These events cause a decrease in platelet count (nadir less than 150×10^9/L) often greater than 50% from baseline, however, HIT-associated thrombosis can occur with a less pronounced platelet count decline.(12)

HIT diagnosis requires a correlation between clinical findings and laboratory results such that a patient with new onset thrombocytopenia or thrombotic clinical events temporally associated with heparin administration show appropriate antibody formation.(13) To increase diagnostic accuracy, the 4T probability score in the setting of an antibody-detection test followed by a confirmatory test such as heparin-induced platelet antibody (HIPA) and the serotonin release assay (SRA) is desirable for diagnosis. Antibodies to the PF4-heparin complex can be detected by enzyme-linked immunosorbent assay (ELISA),(14) and this methodology is more sensitive than SRA.(15) However, a patient's plasma may also contain antibodies that aggregate platelets in the presence of heparin that are not detected by anti-PF4-heparin ELISA.(16) Further support for the presence of these antibodies is given by results indicating that HIPA assays appear to be more sensitive for the diagnosis of HIT than anti-PF4-heparin ELISA.(17)

There are reports in the literature of thrombotic disorders resembling HIT that appear in patients who have not been exposed to heparin,(18–20) as well as examples of a delayed-

onset HIT in which symptoms persist despite heparin cessation.(21, 22) Recently, it was shown that platelet activation can occur with antibodies that were cross-reactive with PF4-nucleic acid complexes.(23) There are potentially far reaching implications of this work, as nucleic acid levels, while generally low in healthy individuals, are significantly increased in disease states such as multiple organ injuries, surgery, infection, and malignancies.(24) Consequently, the possibility exists that antibodies causing thrombocytopenia may not be detected by anti-PF4-heparin ELISA. Along these lines, despite high sensitivity of commercially available anti-PF4 ELISAs a significant proportion of patient samples test negative.

In this study we evaluated anti-PF4-heparin negative samples from patients suspected of having HIT for the presence of additional anti-platelet antibodies using a whole-cell platelet ELISA we developed. In addition, samples were analyzed for the presence of antibodies to red blood cells (RBC) using a similar whole-cell RBC ELISA to detect potentially cross-reactive antibodies.

Materials and Methods

Patients

Samples from patients with suspected HIT that were tested for anti-PF4-heparin antibodies during a one month period at University Hospitals Cleveland Medical Center (UHCMC), a (>1,000 bed) tertiary academic teaching hospital, were included in our study cohort. Seventy-three patients tested during this period had the following results on anti-PF4-heparin testing that were scored as recommended by the manufacturer: 9 tested positive (OD > 0.4 and >50% inhibition), 2 tested equivocal (OD > 0.4 with < 50%), and 62 tested negative (OD < 0.4 and no inhibition). Only one sample per patient was included in our study. For all patients in the study the decision to test was made by the primary clinical service requesting testing as indicated in patients' medical records; study investigators had no role in the decision to test for anti-PF4-heparin or how these patients were risk-stratified by clinicians to pursue testing. Study was approved by our Institutional Review Board.

Complete blood counts and differential

All patients had complete blood counts measured at UHCMC using the automated Sysmex hematology analyzer XE-5000 according to manufacturer's protocols as previously described (Sysmex America Inc., Mundelein, IL).(25) Platelet count from day of anti-PF4-heparin testing was used for comparison.

Anti-PF4-heparin ELISA

Anti-PF4-heparin testing was performed at UHCMC using GTI Incorporated PF4 Enhanced kit X-HAT45 assay in which PF4 is complexed to polyvinyl sulfonate (heparin cross-reactive). This assay is designed to detect IgG, IgA, and IgM heparin-associated PF4 antibodies which increases probability of a positive test result (26) and was performed 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 separation (VWR, Radnor, PA). Plates were read using the ELx800 microplate reader set at wavelength of 405/490 (BioTek Instruments, Inc. Winooski, VT).

^{14}C-Serotonin release assay (SRA)

All patients with a positive anti-PF4-heparin test were reflexively sent out to the Blood Center of Wisconsin to undergo SRA testing.(27) For equivocal anti-PF4 test results our institution makes a recommendation to clinicians to order SRA. 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.

Whole-cell platelet ELISA protocol

Single-donor platelet (SDP) specimens were obtained from local blood suppliers: American Red Cross (Cleveland, OH) and LifeShare Community Blood Services (Lorain, OH). SDP units were type O, leukoreduced, anticoagulated with ACD-A (10 – 15%), and had similar platelet counts (3×10^{11}/unit) as established by the Food and Drug Administration's Code of Federal Regulations Title 21. A 2 mL aliquot from SDP was centrifuged at 2,000 RPM using the Sorvall RT6000D high capacity centrifuge (DuPont, Wilmington, DE) for 5 min at 10°C. Platelet pellet was resuspended in 15 mL of Phosphate Buffered Saline solution (PBS) (Ca^{2+}- and Mg^{2+}-free) (HyClone Laboratories, Logan, UT) and centrifuged at 1,800 RPM for 10 min at 10°C. Platelets were resuspended in 15 mL and a 1.5 mL aliquot was diluted with PBS to a final volume of 50 mL and centrifuged as before. 1 mL from the prior suspension was diluted 1:10 in PBS and 100 μL of the diluted platelet suspension plated to each ELISA microtiter well (Corning, Corning, NY), followed by centrifugation at 1,800 RPM and discard of supernatant.(28–30) Platelet adherence to microtiter wells was confirmed using a tissue culture microscope (not shown). Plates were washed three times using a model 1575 ImmunoWash microplate washer (BioRad Laboratories, Inc., Hercules, CA) with 250 μL/ well of PBS buffer with 0.05% Tween. 200 μL of 1% Bovine serum albumin BSA (BSA) (HyClone Laboratories) was added to wells and stored overnight at 4°C. ELISA plates were washed as before prior to being used and assay performed using 40 μL of patient sample (or specimens from non-thrombocytopenic [> $150{,}000 \times 10^9$/L] patients used as controls) in a 1:10 dilution with 1% BSA (other dilutions 1:50 and 1:100 were also performed but resulted in higher non-specific background binding with lower sample signal); 100 μL of each sample suspension was plated in duplicate and incubated for 1 hour at 37°C. ELISA plates were washed and incubated with 100 μL/well of a 1:2000 of mouse anti-human IgG FC (secondary) conjugated to alkaline phosphatase (AP) (Abcam, Cambridge, MA). Secondary antibody alone in the absence of plasma sample and an isotype control antibody (mouse monoclonal IgG2b) (R&D Systems Inc. Minneapolis, MN) at the same dilution of secondary antibody were used as controls. Plates were incubated for 1 hour at 37°C followed by washing as before. Reaction was detected using 100 μL of *p*-nitrophenyl phosphate according to manufacturer's protocol (Sigma-Aldrich, St. Louis, MO). Plates were incubated as before and binding determined using a model 680 microplate reader (Bio-Rad Laboratories) at a wavelength of 415 nm. Alternatively, platelets were washed and treated with porcine heparin (Sigma) or with chondroitinase ABC (Sigma) as previously described (31, 32) before plating.

Whole-cell RBC ELISA protocol

RBC specimens were obtained as before. RBC specimens were type O and leukoreduced, anticoagulated with ACD-A (10 – 15%), and had a hematocrit of 60%. A 3 mL RBC aliquot was obtained and centrifuged at 2,000 RPM as before for 5 min at 10°C. The RBC pellet was resuspended in 15 mL and re-centrifuged at 1,800 RPM for 10 min at 10°C. Pellet was diluted 1:50 with PBS and re-centrifuged. Cells were re-suspended a second time in 50 mL of PBS and 100 μL of this dilution used per well and centrifuged as before. Adherence of cells was confirmed using a tissue culture microscope. ELISA plates were washed and incubated with 200 μL of 1% BSA and stored at 4°C until the following day. Plasma samples from patients and control subjects were diluted as before 1:10 and 100 μL of each specimen plated in duplicate, and incubated for 1 hour at 37°C. Plates were washed and incubated with either 100 μL of 1:2,000 dilution of mouse anti-human IgG FC-AP conjugated or with a similar dilution of isotype control for 1 hour at 37°C. Secondary antibody in the absence of plasma sample was also used as control. Plates were washed and reaction detected with 100 μL of *p*-nitrophenyl phosphate as before. Antibody binding was measured at a wavelength of 415 nm.

Statistical analysis

All statistics were performed using Prism 6 (GraphPad Software Inc., La Jolla, CA). Results are presented as mean ± SD. The intergroup data comparisons were performed using the unpaired Student *t* test. All reported *p* values are two-tailed with a type I error rate of 5% and a $p<0.05$ set for significance.

Results

Clinical characteristics of patient cohort

A total of 73 patients consisting of 43 males (59%) and 30 females (41%) were included in the study (Table 1). Mean age for the cohort was 66 years. Of the patients tested by anti-PF4-heparin ELISA during this time period, 9 patients tested positive (PF4+), 2 had equivocal results and 62 patients tested negative (PF4-). In the patient cohort, 6 had a history of autoimmune disease, 36 had a history of cardiac disease, 15 had a history of malignancy, and 2 had a history of pro-thrombotic mutation. All patients had heparin exposure prior to testing and a decrease in platelet count from baseline. None of the patients had clinician requests for platelet-specific or antibodies to human leukocyte antigens (HLA) testing.

Anti-PF4-heparin positive patients

Results from whole-cell ELISA are shown in Figure 1. Of the patients included in the study all PF4+ (9/9) showed reactivity by whole-cell platelet ELISA (Figure 1A). The reactivity was detectable even after diluting the samples up to 1:100; however, higher dilutions resulted in increased non-specific background (data not shown). One of these patients (1/9) also had reactivity by whole-cell RBC ELISA suggesting the presence of additional antibodies in the patient's sample (Figure 2A). PF4+ patients were reflexively tested by SRA and results were confirmed by this methodology. Two tests that gave ODs suggestive of positive binding but lacked appropriate inhibition were resulted as equivocal and are shown

in a separate section. Of note, treatment of platelets with heparin and chondroitinase ABC eliminated reactivity of positive samples (data not shown). Samples from non-thrombocytopenic samples (10/experiment) failed to show reactivity by either whole-cell ELISA.

Anti-PF4-heparin negative patients

Of the PF4 -patients, whole-cell ELISA testing allowed for the segregation of patients into distinct groups: 42.2% (27/64) patient samples showed no detectable reactivity by platelet or RBC whole-cell ELISA (Figure 2B); 12.5% (8/64) samples had antibodies to platelets by whole-cell ELISA (Figure 1A); 12.5% (8/64) were reactive with RBC ELISA (Figures 1B and 2B); and 32.8% (21/64) of anti-PF4 negative samples showed reactivity to both platelets and RBC by whole-cell ELISA (Figure 2B). Statistical comparison of whole-cell platelet ELISA results indicated no significant difference between the ODs of PF4- and PF4+ (Figure 1A). However, there was a significant difference in the ODs of PF4+ and PF4- compared to controls (p=0.0007 and p<0.0001 respectively). Similar analysis, of whole-cell RBC ELISA results found that the ODs of the PF4- group were significantly higher than those testing PF4+ and controls (p<0.0001). Treatment of platelets with chondroitinase ABC resulted in marked decreases or abrogation of binding by patient samples using whole-cell ELISA except for two patient samples in the platelet-binding only group which were moderately decreased (data not shown). As shown in Table 1, of the patients with antibodies to platelets, 13% (4/31) had an underlying autoimmune disease, 55% (17/31) had cardiac disease history, 26% (8/31) had a prior malignancy and none had prothrombotic mutations. These numbers were similar to those without platelet antibodies detected except for patients with prothrombotic mutations in the platelet antibody negative group (6%, n=2) (Table 1). Regardless of whole-cell reactivity patients had a similar decrease in platelet count (+platelet antibodies 75% and -platelet antibodies 73%). A review of the medical record of those patients testing positive by whole-cell platelet ELISA did not show they had been tested for the presence of platelet-specific antibodies, 2 received single platelet transfusions post-anti-PF4-heparin testing, and none had history of chronic platelet transfusions or of anti-HLA antibodies. Additionally, 3/64 patients, two from the group reacting to RBC (weakly reactive [OD: 0.27–0.34]) and platelets (strongly reactive [OD: 0.7–0.84]), and one from the group negative in both ELISA had direct antiglobulin testing performed at this time which were negative.

Anti-PF4-heparin equivocal patients

In our cohort two samples tested equivocal by anti-PF4-heparin ELISA. These two patients had OD >0.5 with one showing 35% inhibition and the other no inhibition (data not shown). These two samples were eventually scored as equivocal based on inhibition results. In these equivocal cases a recommendation is made to clinicians to order the SRA to confirm presence/absence of antibodies. In both of these patients SRA results were negative. Notably, these samples had reactivity in both whole-cell ELISAs (shown in Figure 2B in the group of samples positive to both platelets and RBC).

Comparison according to whole-cell platelet reactivity

To further understand if the reactivity seen in the whole-cell ELISA differentiates patients who tested positive or negative in comparison to the anti-PF4-heparin ELISA results, a separate analysis of platelet counts was undertaken (Table 2). A comparison of the mean platelet count between those who tested positive vs. those testing negative on whole-cell platelet ELISA failed to show a significant difference (105 ± 13 vs. 145 ± 18 respectively, p=0.0841). Among those patients who tested positive by whole-cell platelet ELISA, subdivision according to anti-PF4-heparin results showed that those patients who tested positive by whole-cell platelet ELISA and negative by anti-PF4-heparin testing had a significantly lower mean platelet count ($PF4^+$ 168 ± 43 vs. $PF4^-$ 95 ± 13, p=0.0384). When a similar comparison was performed taking into account the reactivity obtained in the whole-cell RBC ELISA no statistical difference was found.

Discussion

Heparin-induced thrombocytopenia represents a common complication in the treatment of patients requiring anti-coagulation. The discovery that heparin induces the production of IgG antibodies that bind PF4-heparin complexes has proven essential to understanding thrombocytopenia associated with heparin use.(2) In this report we show that patients in our cohort who tested negative by anti-PF4-heparin harbored antibodies not detected by this methodology but were identified in our whole-cell ELISA. Though additional approaches may identify patients having HIT from others, (33) the possibility that antibodies may still be present in patients testing negative by anti-PF4 is of interest. Nevertheless, it has to be emphasized that though it was unclear from our assay results the antigenic specificities of these antibodies, use of treated platelets with chondroitinase ABC led to abrogation or decreases in binding of most patient samples. Therefore, our results suggest that looking at sample reactivity using whole platelets with antigens in their native conformation identifies additional antibodies.

Considering that the identification of the etiology leading to thrombocytopenia can be made more difficult when different presentations lead to an overlapping clinical picture,(34) additional diagnostic tools are desirable. In this regard, using platelets in whole-cell ELISA to detect additional antibodies has been previously reported to be a highly sensitive assay to find anti-platelet antibodies,(28) as well as a logical first step when the antigen suspected of eliciting production of anti-platelet antibodies is not known.(29, 35, 36) This methodology has led to further research which resulted in the identification of specific antibodies in cases of post-transfusion purpura,(36, 37) and has aided diagnostically in idiopathic thrombocytopenic purpura to detect a greater number of antibodies.(30) Additionally, in some settings variations of this whole-cell ELISA approach can detect activated platelets which can be important in some disease states.(38) In our assay, special care was undertaken to minimize platelet activation prior to coating the plates which included keeping centrifugation at a setting shown to minimize premature activation.(39) However, if some degree of activation occurred, results were reproducible in independent experiments using different SDPs to coat plates which may indicate that binding occurred onto platelets and not

just to mediators released as a result of activation. Nevertheless, this is an area that will require future investigation.

Higher sample dilutions to that which is used in anti-PF4-heparin testing resulted in increased background signal and lower antibody detection by whole-cell ELISA. Looking at our results closely, our assay could have led to the detection of antibodies that were in low titers and were missed by commercial kits with specificity for the antigen in its native conformation. However, enzyme treatment of platelets indicated that most of these antibodies were to GAG and likely missed by commercial ELISA, but two patients still had antibodies to platelets of unknown specificity. Interestingly, we also detected antibodies that recognized RBCs, however, we could not do a full correlation with clinical tests since only 3 cohort patients had a direct antiglobulin test performed but these were negative. In 2 of these patients with positive whole-cell platelet and RBC ELISA reactivity, RBC binding may have been non-specific since OD readings were among the lowest of patient samples tested.

Our assay correlated well with the anti-PF4-heparin ELISA results since specimens from patients who tested positive by this methodology (also positive by SRA) were also positive by whole-cell ELISA. Notably, those testing equivocal by anti-PF4-herparin gave positive results in both of our ELISAs and this reactivity could have been to a stromal or cytoplasmic antigenic molecule and likely not specific to platelets. Furthermore, our assay confirmed that 42.2% of patients testing negative by anti-PF4 ELISA were negative for the presence of anti-platelet antibodies and found no reactivity in control group samples. Therefore, since anti-PF4-heparin ELISA testing is still done even in the presence of low pre-test probability,(40, 41) our approach may have been justified.

Attempts have been made to further characterize the antigenic epitopes of the PF4-heparin complex that mediate immunogenicity.(42) At the immune cell level, $CD4^+$ T-helper cells play a critical role in the production of HIT antibodies while marginal zone B cells produce the relevant antibodies.(43) However, heparin exposure is not always necessary for some anti-PF4 antibody formation.(32) These results suggest that antibodies generated in the presence of heparin may be distinct from those generated in its absence, leading to the possibility that distinct or different immunologic determinants on platelets may lead to a HIT-like clinical presentation. This highlights the potential to miss potentially relevant antibodies in patients testing negative by anti-PF4-heparin ELISA.

PF4 can also bind to lipid A on the lipopolysaccharide of gram negative bacteria, this binding exposes epitopes that cross react with PF4-heparin complexes, and these antibodies may constitute an evolutionary conserved mechanism that contributes to a host's antibacterial defense; additionally, antibodies to PF4-heparin complexes can be either IgG or IgM and are not uncommon among the normal population.(44) As a result, in patients with minimal to no exposure to heparin, infection with gram negative bacteria may lead to antigenic sensitization through antigenic mimicry that lead to the formation of HIT-like antibodies.(45) Indeed, patients with gram negative bacteremia have been described as having high titers of antibodies to PF4-heparin complexes compared to controls.(46) Bacteremia leads to cell damage and release of nucleic acids into the circulation, which can then trigger conformational changes in PF4 leading to antibody formation.(23) These

additional antibodies provide a possible mechanism for HIT-like episodes that persist despite heparin cessation without prior heparin exposure. Furthermore, it does emphasize that in some patients these cross-reactive antibodies may be part of a broader immune response that is evolutionarily conserved in response to other antigens.

In our study we looked at patients suspected of having HIT and tested them using a whole-cell ELISA protocol for the presence of additional antibodies to platelets and found that some patients who tested negative for antibodies to PF4-heparin complex did express antibodies to platelets. Further research to characterize the antigenic determinants that may lead to antibody formation in the setting of heparin exposure requires scrutiny. In light of these results we propose that in the setting of suspected HIT, unlike those testing positive by anti-PF4-heparin who undergo confirmatory tests such as the SRA, those who test negative undergo a more extensive evaluation. Furthermore, whole-cell ELISA may prove useful when there is clinical suspicion for immunological etiologies causing unexplained thrombocytopenia.

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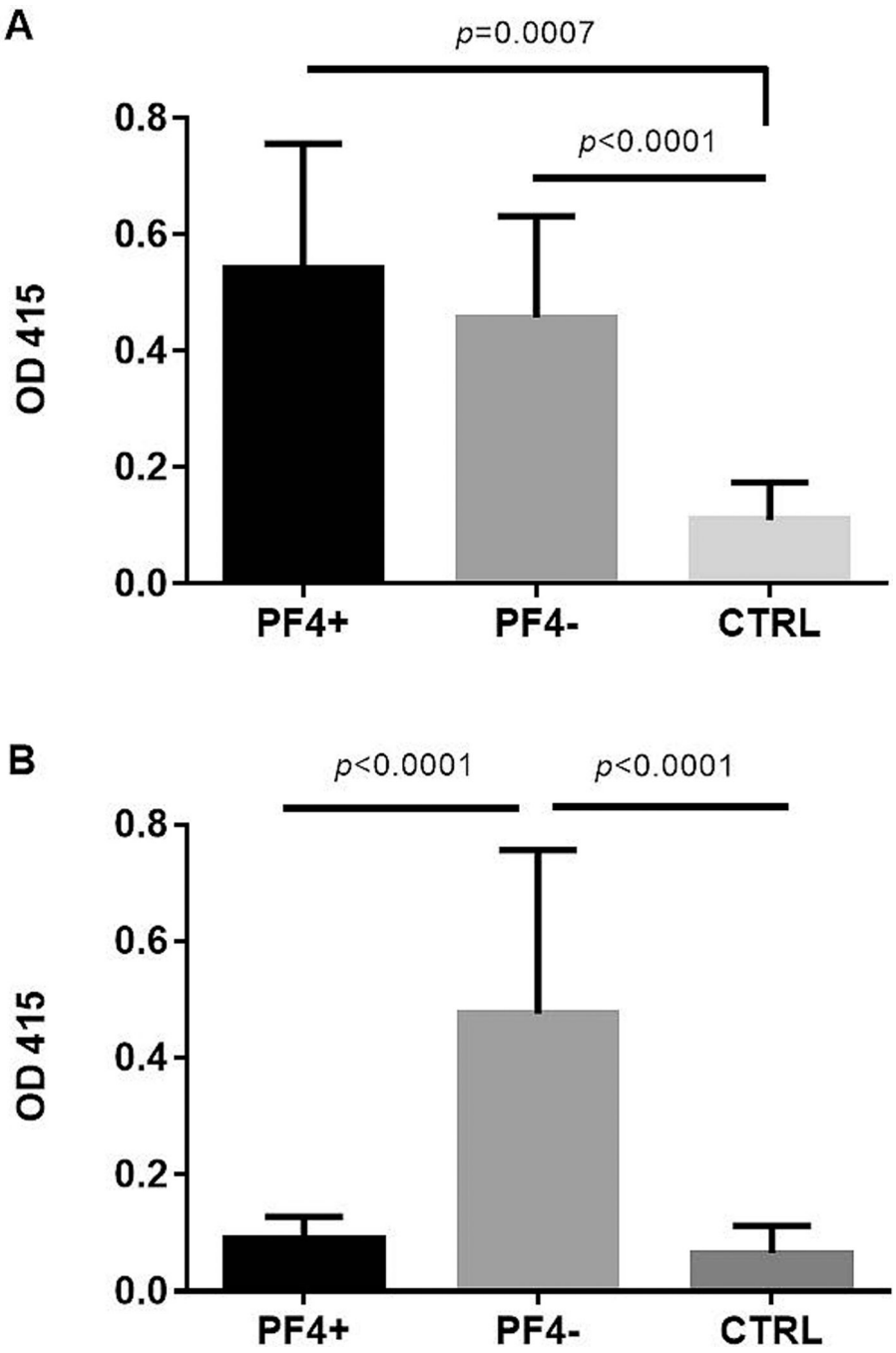

Figure 1.

Optical density (OD) of patient and control samples on whole-cell platelet ELISA (A), and whole-cell RBC ELISA (B). Samples of all patients testing positive by anti-PF4-heparin ELISA also tested positive by whole-cell ELISA. Those patients testing negative by anti-PF4-heparin ELISA and testing positive by whole-cell platelet ELISA had ODs similar by this methodology to those who tested anti-PF4-heparin positive without statistically significant difference. Plasma samples from non-thrombocytopenic subjects failed to show reactivity by whole-cell ELISA. ODs of samples testing positive by RBC ELISA were

similar to whole-cell platelet ELISA positive results. Results are representative of three independent experiments. Statistically significant differences are shown.

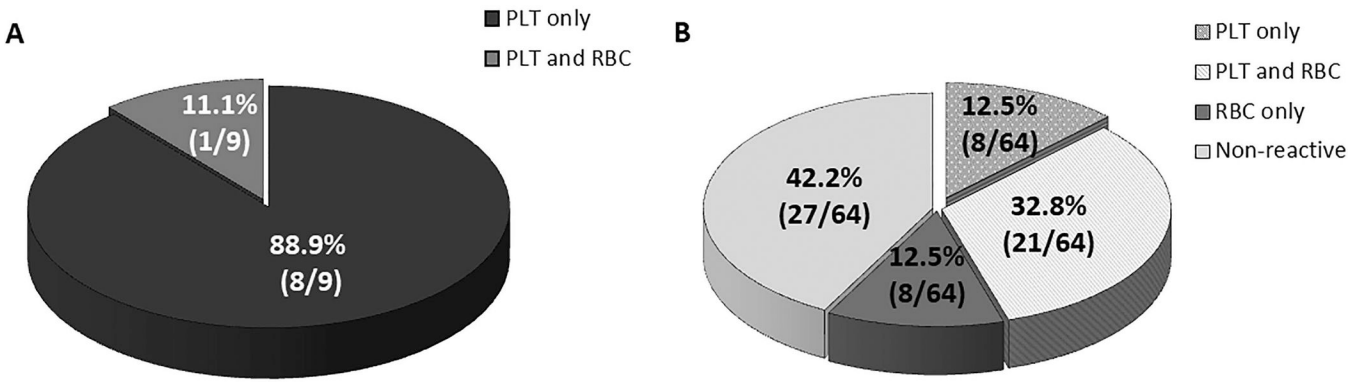

Figure 2.

Pie chart representation of anti-PF4-heparin positive (A), negative and equivocal samples (B) reactivity on whole-cell ELISA. All patients who tested anti-PF4-heparin positive also tested positive by whole-cell platelet (PLT) ELISA. Only one of these patients had reactivity by whole-cell RBC ELISA. Twenty seven patients who tested anti-PF4-heparin negative were also negative by whole-cell ELISA. Twenty-nine patients tested positive by whole-cell platelet ELISA. Two samples that tested equivocal by anti-PF4-heparin ELISA are represented in the group testing positive for platelet and RBC whole-cell ELISA. Results are representative of three independent experiments.

Table 1.

Demographic data for patient cohort

	+Platelet antibodies	−Platelet antibodies
Number	38	35
Males	26	17
Females	12	18
Age (years): Mean (Range)	66 (28–91)	64.6 (30–96)
Autoimmune disease history	13% (4/31 *)	6% (2/32 *)
Cardiac disease history	55% (17/31)	59% (19/32)
Malignancy history	26% (8/31)	22% (7/32)
Pro-thrombotic mutation **	0% (0/31)	6% (2/32)
Plt% ***	75%	73%

* Percentages are calculated based on patients with available clinical history. 31 and 32 patients who tested positive and negative respectively for anti-platelet antibodies by whole-cell ELISA had complete medical history available at time of testing.

** Pro S deficiency/Factor V Leiden mutation

*** Mean platelet (Plt) count percent is defined as change from baseline obtained 7–14 days prior to and within a day of anti-PF4 testing. Percent decrease and platelet count change is based on the analysis of 28 patients in each group for whom there was sufficient platelet count data.

Table 2.

Mean platelet counts of patients with and without anti-platelet antibodies by whole-cell ELISA

	+Plt antibodies	−Plt antibodies	*P* value
Mean platelet (× 10^9/L)	105 ± 13	145 ± 18	0.0841

Mean platelet (× 10^9/L)	**$PF4^+$**	**$PF4^-$**	***P* value**
	168 ± 43	95 ± 13	0.0384 *

Mean platelet (× 10^9/L)	**Plt^+/RBC^+**	**Plt^+/RBC^-**	***P* value**	**Plt^-/RBC^+**	**Plt^-/RBC^-**	***P* value**
	88 ± 11	134 ± 30	0.0886	130 ± 39	149 ± 20	0.0682

* Comparison among samples that tested positive by whole-cell ELISA differentiated according to anti-PF4 test result. Data are reported as Mean ± SEM.