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Platelet factor 4/heparin antibody (IgG/M/A) in healthy subjects: a literature analysis of commercial immunoassay results

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Abstract

Purpose—We determined the seroprevalence of platelet factor 4 (PF4)/heparin antibodies in healthy subjects.

Methods—A literature search identified studies in which healthy subjects were evaluated using commercial immunoassays for PF4/heparin antibody (IgG/M/A). Proportions of test-positive subjects were calculated, by assay.

Results—Across 11 eligible studies, 860 healthy subjects were tested using the Stago enzyme-linked immunosorbent assay (ELISA) (nine studies), GTI ELISA (three studies), and/or DiaMed particle gel immunoassay (PGIA) (three studies). Seropositivity occurred in 17 of 790 (2.2%, 95% CI, 1.1–3.2%) subjects by Stago ELISA, one of 100 (1.0%, 95% CI, 0–3.0%) subjects by GTI ELISA, and three of 70 (4.3%, 95% CI, 0–9.0%) subjects by PGIA ($P > 0.20$). Of seven seropositive subjects tested further, none had platelet-activating antibodies.

Conclusion—Commercial immunoassays detect PF4/heparin antibody in 1.0–4.3% of healthy subjects. Because this “background” prevalence overlaps seropositivity rates in heparin-treated patients in various clinical settings, normality cut-offs may require refinement.

Keywords

Immunoassay; Platelet factor 4; Heparin; Antibody; Healthy volunteers; Heparin-induced thrombocytopenia

Introduction

Heparin induces a conformational change in platelet factor 4 (PF4) and exposes epitopes capable of inducing PF4/heparin antibodies [1]. PF4/heparin antibodies, particularly of the IgG isotype, mediate heparin-induced thrombocytopenia (HIT), a serious prothrombotic disorder associated with thrombocytopenia and thrombosis [2, 3]. PF4/heparin antibodies, however, also frequently develop in heparin-exposed patients in the absence of clinical HIT. Asymptomatic seroconversion is greatest in patients administered unfractionated heparin in

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various clinical settings (~8–50%) and is lesser with exposure to low molecular-weight heparins (~1–8%) or the synthetic pentasaccharide, fondaparinux (~1–3%) [4, 5]. Although recent studies suggest that isolated PF4/heparin antibodies confer an increased risk of thrombosis and/or mortality [6, 7], it remains uncertain if these antibodies serve as nonspecific inflammatory markers or exert pathogenic effects.

PF4/heparin antibodies have been described almost exclusively in the setting of heparin exposure. By possible exception, their presence was reported in a few patients with acute coronary syndrome without documented previous heparin exposure [8]; however, undocumented heparin exposure such as via line flushes could not be excluded. Also preliminary data are available for four patients who, in the absence of preceding heparin therapy, had PF4/heparin antibodies and a HIT-like illness [9]. While a few case reports suggest that crossreactive antibodies may occur in other autoimmune diseases, such as antiphospholipid antibody syndrome [10, 11], to date, there have been no other descriptions of “naturally occurring” PF4/heparin antibodies in the absence of heparin exposure.

The background prevalence of PF4/heparin antibodies in healthy subjects is presumed to be extremely low due to normality ranges established by the manufacturers of the commercial immunoassays. At least four commercial immunoassays have been developed for detecting human antibodies (IgG, IgA or IgM) to PF4/heparin. Two enzyme-linked immunosorbent assays (ELISAs), initially described, respectively, by Amiral and colleagues [12, 13] and by Visentin and colleagues [14, 15], differ most notably in the antigen coated on the microtiter plate, i.e., PF4 with heparin (Asserachrom® HPIA, Diagnostica Stago, Asnieres, France) or PF4 with polyvinyl sulfonate (PVS) (PF4 Enhanced®, GTI, Waukesha, WI, USA). The upper cut-off value for normality, as established by the manufacturer, is an optical density (OD) at 492 nm of ≥0.5 in the PF4/heparin ELISA and an OD at 405 nm of ≥0.4 in the PF4/PVS ELISA. Even with these normal ranges, positivity rates as high as 22% [16] and 30% [17] in healthy subjects have been reported using commercial ELISAs, suggesting that the recommended cut-offs may be too low. Other commercial immunoassays include a particle gel immunoassay (PGIA) (ID-PGIA heparin/PF4, DiaMed, Cressier, Switzerland), initially described by Meyer and colleagues [18], and a particle immunofiltration assay (PIFA) (PIFA Heparin/PF4 Rapid Assay, Akers Bioscience, Thorofare, NJ, USA). These rapid assays employ dyed particles coupled with PF4/heparin or PF4, respectively, and test positivity is based on visual detection of a color pattern consistent with particle agglutination by specific antibodies.

The purpose of this study was to estimate and compare the prevalence of PF4/heparin antibodies in a large sampling of healthy subjects based on literature reports of commercial immunoassays, each used according to its manufacturer’s instructions. Also we sought to characterize, when possible, individual positive responses. We report findings from a total of 11 studies in which a total of 860 healthy subjects were tested for PF4/heparin antibody by immunoassay.

Methods

Identification of literature set

A literature database search was performed in June 2007 using the PubMed database and limited to English language publications and human studies. Search term were “HIT antibodies” or “heparin-platelet factor 4” in combination with “healthy,” “normal,” “volunteers,” “incidence,” “prevalence,” “frequency,” or “laboratory.” The titles and abstracts, when available, of identified publications were screened to determine relevance to our study question. Relevant or possibly relevant articles were retrieved for in-depth review. Articles were eligible if they reported the prevalence in healthy volunteers of PF4/heparin

antibodies, as assessed using a commercial immunoassay (ELISA, gel particle, or particle immunofiltration) according to the manufacturer's instructions. Review articles lacking original data and papers reporting only functional or platelet activation assays were excluded. Additional search strategies included scanning the bibliographies of review articles on HIT or HIT laboratory testing, screening personal files, and querying assay manufacturers. Articles were included in the final literature set upon consensus agreement by the investigators.

Data analysis

From each article in the literature set, pertinent data including the study objective, assay(s) used, description of the healthy volunteers, specimen type, the numbers of tested and test-positive subjects, and details about positive responses were extracted, where available. The proportion (95% confidence interval, CI) of test-positive subjects was calculated by assay type, and comparisons were made using Fisher's exact test. Statistical analyses were conducted using GraphPad Statistical Software (GraphPad Software, Inc., San Diego, CA), and significance was declared at $P < 0.05$.

Results

Literature data set

From the search strategies, 254 articles were identified for consideration. Most ($n = 236$) were excluded because of a lack of original data relevant to our study question. Seven articles were excluded because healthy volunteers were tested using a noncommercial ("in-house") assay for PF4/heparin antibodies. In three of these seven articles, the described assay ultimately served as basis of a commercial assay [12, 13, 19]. The literature set comprised the remaining 11 articles [10, 16, 17, 20-27], each of which reported the prevalence of PF4/heparin antibodies in healthy subjects, as assessed by a commercial immunoassay according to manufacturer's directions (Table 1).

In the literature set (Table 1), the PF4/heparin ELISA was used in nine studies [10, 16, 17, 20-24, 26], the PF4/PVS ELISA in three studies [17, 24, 25], and the PF4/heparin PGIA in three studies [17, 24, 27]. No study used the PIFA. Two studies [17, 24], one of which was conducted in a blinded fashion [17], used the PGIA and both ELISAs. One article [23] described the process and data used by the manufacturer of the PF4/heparin ELISA to establish the normality cut-off. One study [16] presented results for the PF4/heparin ELISA using both the manufacturer's cut-off and a different "in-house" cut-off; the results according to the manufacturer's cut-off are reported herein. The specimen used were sera or plasma in the PF4/heparin ELISA and PGIA, and sera in the PF4/PVS ELISA.

The individual studies evaluated between 20 and 218 healthy subjects (Tables 1 and 2). Across studies, a total of 860 unique, healthy subjects were tested using 1 or more commercial assays, and the majority ($n = 790$) were tested using the PF4/heparin ELISA.

Antibody prevalence and characterization of positive results

The PF4/heparin ELISA was positive, by separate study (nine studies), in 0–30% of healthy subjects, and overall in 17 of 790 (2.2%, 95% CI, 1.1–3.2%) subjects (Table 2). In 14 of 17 test-positive subjects, OD_{492} results were available and ranged from approximately 0.51–1.1, with a median value of 0.63 (Fig. 1). The platelet-activating ability of the antibody was reported in six test-positive subjects: none had a positive serotonin release or platelet aggregation test [17].

The PF4/PVS ELISA was positive by study (three studies) in 0–5% of healthy subjects, and overall in one of 100 (1.0%, 95% CI, 0–3.0%) subjects (Table 2). The single test-positive subject had an OD_{405} of approximately 0.5 (Fig. 1) and had negative serotonin release and platelet aggregation tests [17]. In one of the three studies, none of 50 subjects had a positive PF4/PVS ELISA or serotonin release assay, although in-house assays detected IgG antibody in two subjects and IgM antibody in 33 subjects [25].

The PF4/heparin PGIA was positive by study (three studies) in 0–15% of healthy subjects, and overall in three of 70 (4.3, 95% CI, 0–9.0%) subjects (Table 2). The three test-positive subjects each had an antibody titer (defined as the last positive detection followed by either borderline or negative results for undiluted and serially diluted plasma) of one [27]. The platelet-activating ability of these antibodies was not reported.

No difference in seropositivity among the methods was detected ($P > 0.20$).

Discussion

In this study, we estimated in a large sampling of healthy subjects ($n = 860$, across 11 studies) the prevalence of PF4/heparin antibody by three commercial immunoassays, i.e., the Stago PF4/heparin ELISA, GTI PF4/PVS ELISA, and DiaMed PGIA, and characterized, as possible, the positive responses. Each assay was performed according to the manufacturer's instructions, including the use of the recommended specimen. Test results, at least for the PF4/PVS ELISA, are not appreciably affected by sample preparation or storage [28]. Our literature search identified no study that evaluated healthy subjects using the Akers PIFA. After our analysis was completed, however, a study was published that described "numerous" false-positive reactions with this assay in normal blood donors; the actual data were not reported [29]. Other study limitations include the substantially smaller numbers of subjects tested by the PF4/PVS ELISA ($n = 100$) and the PGIA ($n = 70$) as compared with the PF4/heparin ELISA ($n = 790$), and the inconsistent availability of certain data such as the platelet-activating, functional abilities of detected antibodies, the optical density values by ELISA, imprecision for the testing, and the age, sex, or other factors describing the study subjects.

We found that the seropositivity rate in healthy subjects was 2.2% (95% CI, 1.1–3.2%) by the PF4/heparin ELISA, 1.0% (95% CI, 0–3.0%) by the PF4/PVS ELISA, and 4.3% (95% CI, 0–9.0%) by the PGIA, without significant differences by method ($P > 0.20$). Positive results were typically, but not always, of low OD and close to the cut-off by ELISA or of the lowest titer (titer of 1) by PGIA, that is, they were "weak" positives. However, three of the 14 seropositive subjects by PF4/heparin ELISA with available data had OD_{492} values exceeding 0.8 (including one value > 1.0), higher positive values that are often seen in patients with HIT [13, 16, 17, 20]. An $OD_{405} > 1.0$ in the PF4/PVS ELISA has been associated with thrombotic predisposition [30], although the specific relationship between OD values from the different ELISAs is unclear. To what extent these high false-positive values are from crossreactive antibodies with other antigen specificities [10, 11] or nonspecific binding of IgM isotype is unknown, as these samples were not further characterized for antigen specificity or isotype. Of the seven seropositive subjects who also were tested using a functional assay, each had an ELISA OD value <0.8 and each lacked platelet-activating antibodies.

For the PF4/heparin ELISA, the normality cut-off was set at the 99th percentile of the log transformed OD data of 193 normal volunteers and 95 non-HIT patients [23]. Using this dataset, the manufacturer noted that the two highest OD values occurred in individuals with autoimmune disease (OD_{492} of 0.48) and thrombotic thrombocytopenic purpura (OD_{492} of

0.44), respectively. The data used to set the normality cut-off for the PF4/PVS ELISA are not published. However, according to GTI technical support (Michele Westlake, MT, ASCP, Technical Support Specialist, personal communication), results from 120 healthy individuals were analyzed for normality of the distribution of the OD values, and the cut-off was determined using calculations based on a nonparametric 95% reference interval with a 90% confidence. The modeling used for establishing these cut-offs would suggest that approximately 1% (PF4/heparin ELISA) to 5% (PF4/PVS ELISA) of non-HIT individuals, including healthy subjects, would be predicted to have a positive ELISA result. For the PGIA, the minimum detection limit is not reported. However, preliminary data (Dr. Ronald Orynich, DiaMed-North America, personal communication) suggest that the assay detects antibody when present at a level that would yield an OD_{405} of approximately 0.7 in the PF4/PVS ELISA.

Our analysis considered data from commercial immunoassays for PF4/heparin antibodies that detect IgG, IgM, and IgA classes. The clinical specificity of laboratory testing for HIT is enhanced by measurement of only the IgG class [31], and immunoassays specific for IgG antibodies have recently become commercially available. Overdiagnosis of HIT may occur if laboratory testing is done only using commercial immunoassays, without complementary assays for antibody function [32]. With the immunoassays, the actual OD or antibody titer is often more informative than a simple positive or negative result [33]. Patients, if tested over several days, often seroconvert from borderline negative results to borderline positive results [34].

Higher ELISA OD values are associated with increased thrombotic risk in patients with isolated HIT, with the risk being sixfold greater when the OD_{405} (PF4/PVS ELISA) is ≥1.0 versus 0.4–0.99 [30]. In the test-positive, healthy subjects in our study, antibodies of higher OD or titer were relatively infrequent (one of 15 seropositive subjects with available data had an OD > 1.0), and no functional antibodies were reported. This remains to be further investigated using commercial Ig class-specific assays. Also, the extent to which, if at all, seropositivity in healthy subjects with a normal platelet count is associated with increased thrombotic events or mortality, as has been previously demonstrated in certain patient populations [6, 7], remains to be determined.

One article in our literature set reported that in 50 healthy subjects who were seronegative by both PF4/PVS ELISA and serotonin release assay, in-house, Ig class-specific immunoassays detected IgG PF4/heparin antibodies in two subjects and IgM antibodies in 33 [25]. The authors speculated that this reflected a possible humoral response in the donors to the PF4/heparin complex, prior to exogenous heparin exposure. It is possible that individuals with chronic platelet activation and PF4 release, in the absence of heparin, could become sensitized and manifest naturally occurring PF4/heparin antibodies. Whether PF4/heparin antibodies can develop in such individuals or healthy subjects, as naturally occurring antibodies, remains to be shown. Normal human sera contains naturally occurring antibodies to a variety of self-antigens and are thought to contribute to self-tolerance through functional roles in clearance of antigens from damaged tissues or through neutralizing activities [35]. The relationship of naturally occurring antibodies to autoimmune disease is not clear and how naturally occurring antibodies influence the host response to environmental injury [36] remains to be defined.

Commercial immunoassays detect PF4/heparin antibody in 1.0–4.3% of healthy subjects. Because this “background” prevalence overlaps seropositivity rates with heparin-treated patients in various clinical settings, the normality cut-offs may require further refinement. A prospective study is warranted to better estimate seropositivity in a larger healthy population and help refine normality thresholds.

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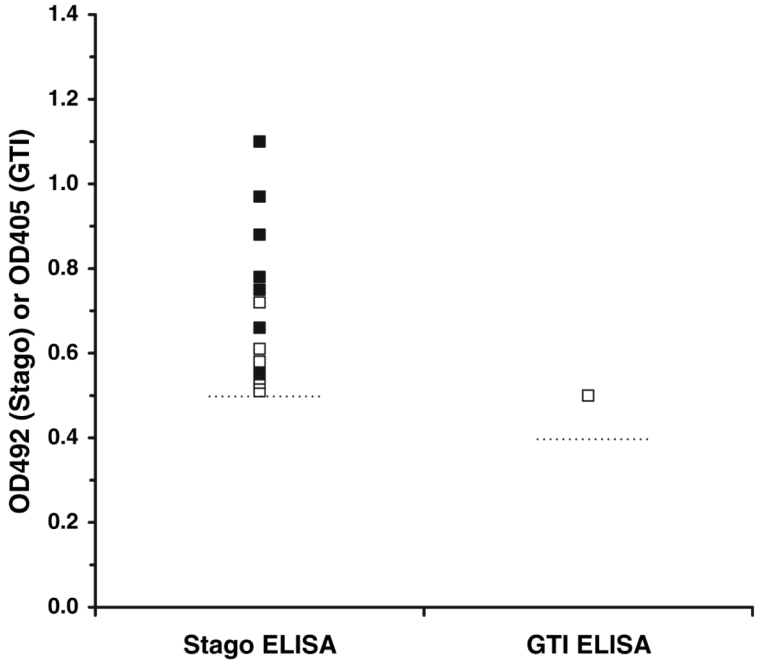

Fig. 1.

ELISA results for 15 seropositive healthy subjects with available data, as determined by the Stago PF4/heparin ELISA ($n = 14$) and GTI PF4/PVS ELISA ($n = 1$). Normality cut-off values for the respective ELISAs ($OD_{492} \geq 0.5$ and $OD_{405} \geq 0.4$) are shown as horizontal, dashed lines. OD values were extracted from published figures [16, 17, 20]. For the seven ELISA-positive subjects further tested for platelet-activating antibody (eachfound to be negative), OD values are shown as open squares

Table 1

Literature set

First author, year	Study objective	Immunoassay(s)	Specimen	Description of healthy subjects
Arepally 1995 [20]	Comparison of PF4/heparin ELISA and serotonin release assay in diagnosis of HIT	PF4/heparin ELISA	Plasma; stored at −70°C before use	Healthy volunteers with no history of heparin exposure or thrombocytopenia
Bachelot-Loza 1998 [21]	Evaluation of FcγRIIa polymorphism in HIT	PF4/heparin ELISA	Plasma, citrated; stored at −80°C before use	Healthy controls with neither history of heparin exposure or thrombocytopenia
Newman 1998 [16]	Evaluation of fluid phase enzyme immunoassay for PF4/heparin antibody	PF4/heparin ELISA	Serum or plasma, citrated or ACD	Healthy volunteers, “normals”
Walenga 1999 [22]	Evaluation of laboratory tests for diagnosis of HIT	PF4/heparin ELISA	Serum	Normals who were not receiving heparin and had not received it during at least the past 6 months; platelet counts were normal
Woodhams 1999 [23]	Establishment of normality cut-off for PF4/heparin ELISA	PF4/heparin ELISA	Not specifically stated (presumably serum or plasma)	Normal volunteers
Eichler 2002 [17]	Comparison of PGIA with functional and antigenic tests for PF4/heparin antibody	PF4/heparin ELISA PF4/PVS ELISA PF4/heparin PGIA	Serum; stored in aliquots at −30°C	Healthy blood donors without any medication; 13 males, seven females; mean ± SD age of 30 ± 10 years
Tazzari 2002 [24]	Comparison of flow cytometry method with commercial assays for PF4/heparin antibody	PF4/heparin ELISA PF4/PVS ELISA PF4/heparin PGIA	Serum	Healthy subjects/donors
Untch 2002 [25]	Determination of isotypes and functionality of PF4/heparin antibody in patients with suspected HIT	PF4/PVS ELISA	Serum; stored at −80°C before use	Normal healthy volunteers
de Larranaga 2002 [10]	Evaluation of PF4/heparin-induced antibodies in patients with autoimmune or alloimmune antiphospholipid antibodies and in healthy controls	PF4/heparin ELISA	Serum	Healthy, normal controls; 25 males, 15 females; mean (range) age of 39.6 (19–57) years
Lee 2003 [26]	Determination of prevalence of PF4/heparin antibody in patients on maintenance dialysis	PF4/heparin ELISA	Serum	Healthy subjects; 20 men, 20 women; mean age 47.9 ± 13.5 years
Alberio 2003 [27]	Evaluation of PGIA in diagnosis of HIT	PF4/heparin PGIA	Plasma, citrated; stored at −70°C before use	Healthy people

Table 2

Test positivity in healthy subjects, by assay

First author, year	Tested, *N*	Positive, *n*	% positive	95% CI	Comment
PF4/heparin ELISA by Stago					
Arepally 1995 [20]	77	1	1.3		Mean OD_{492} 0.19 ± 0.01
Bachelot-Loza 1998 [21]	218	0	0		Each subject: OD_{492}<0.25
Newman 1998 [16]	32	7	21.9		Maximum OD_{490} ~1.1
Walenga 1999 [22]	140	2	1.4		OD_{492} not reported
Woodhams 1999 [23]	193	0	0		OD_{492} 0.01–0.38
Eichler 2002 [17]	20	6	30.0		Maximum OD_{492} ~0.75
Tazzari 2002 [24]	30	0	0		OD_{492} not reported
de Larranaga 2002 [10]	40	1	2.5		OD_{492} not reported
Lee 2003 [26]	40	0	0		OD_{492} not reported
Overall	790	17	2.2	1.1–3.2	
PF4/PVS ELISA by GTI					
Eichler 2002 [17]	20	1	5.0		OD_{405} ~0.5 for single positive
Untch 2002 [25]	50	0	0		Mean ± SEM OD_{410} ~0.28 ± 0.05
Tazzari 2002 [24]	30	0	0		OD_{405} not reported
Overall	100	1	1.0	0–3.0	
PF4/heparin PGIA by DiaMed					
Eichler 2002 [17]	20	0	0		
Tazzari 2002 [24]	30	0	0		
Alberio 2003 [25]	20	3	15.0		Antibody titer of 1 for positives
Overall	70	3	4.3	0–9.0	