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Evaluation of automated immunoassays in the diagnosis of heparin induced thrombocytopenia

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ABSTRACT

Background: Heparin-induced thrombocytopenia (HIT) is caused by platelet-activating antibodies that recognize platelet factor 4/heparin (PF4/hep) complexes. The *in vitro* demonstration of PF4/hep antibodies using functional and immunological methods is essential for optimal management of patients suspected to have HIT. Since functional assays are technically challenging and limited to specialized laboratories, antigen-binding assays are commonly used in routine laboratories.
Study Design: Blood samples from 448 consecutive patients in whom HIT was suspected were investigated using a latex agglutination test HemosIL® HIT-Ab$_{(PF4\text{-}H)}$ (HemosIL-Ab), two chemiluminescence tests HemosIL AcuStar HIT-Ab$_{(PF4\text{-}H)}$ (HemosIL AcuStar-Ab) and AcuStar HIT-IgG$_{(PF4\text{-}H)}$ (HemosIL AcuStar-IgG), an in-house PF4/hep IgG enzyme immunoassay (EIA) and the heparin induced platelet aggregation (HIPA) test.
Results: Antibodies against PF4/hep were detectable in 44 out of 119 samples using HemosIL-Ab among which 20 samples were also reactive in the HIPA; and in 122, 64 and 108 out of 448 sera using HemosIL AcuStar-Ab, HemosIL AcuStar-IgG and in-house PF4/hep IgG-EIA, respectively, among which 52 sera were also reactive in the HIPA. All assays had high sensitivities of >95% for platelet activating antibodies; however, they differed in their specificities. The highest specificity and positive predictive value was observed by HemosIL AcuStar-IgG (96% and 78%, respectively).
Conclusion: Automated immunoassays are useful in the laboratory investigations of HIT and present a potential improvement toward standardization of laboratory investigations of HIT. The high positive predictive capability may justify treating the patient with alternative anticoagulants without waiting for the results of a functional assay.

Introduction

Heparin-induced thrombocytopenia (HIT) is a prothrombotic, immune-mediated adverse reaction that occurs after exposure to unfractionated heparin (UFH), low molecular heparin (LMWH), or other polyanions such as hypersulfated chondroitin sulphate. HIT is usually caused by platelet-activating antibodies that recognize platelet factor 4 (PF4)/heparin-complexes [1,2]. Typical clinical manifestations of HIT are a fall in platelet count by more than 50%, beginning between day 4 to 10 of heparin therapy, often complicated by new thrombotic complications [3–5]. However, the clinical diagnosis of HIT is often difficult, especially in patient populations with a high prevalence of thrombocytopenia such as intensive care patients [6].Thus, the clinical diagnosis must be corroborated by *in vitro* demonstration of heparin dependent antibodies, e.g. platelet activating and/or anti-PF4/heparin IgG antibodies [3,7,8].

Two different classes of assays are available: enzyme immunoassays (EIAs), which detect binding of antibodies to immobilized PF4/heparin complexes and functional assays, which investigate the capability of these antibodies to activate platelets in the presence of heparin. Although functional assays such as the serotonin release assay (SRA) [9] or the heparin-induced platelet activation assay (HIPA) [10], are considered to be the gold standard in the laboratory diagnosis of HIT, they are technically challenging and restricted to specialized laboratories [11]. In contrast, immunoassays are easy to perform and widely available. Because of their excellent negative predictive values (NPV), immunoassays are helpful in excluding HIT. However, these assays usually do not differentiate between pathogenic, i.e. platelet activating, antibodies and clinically irrelevant antibodies and HIT is

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grossly overdiagnosed when only the result of the antigen assay is considered [11]. Immunoassays detecting IgG antibodies only have improved operating characteristics by increased diagnostic specificity for pathogenic antibodies [12–14], however, still only ~50% of anti-PF4/heparin IgG antibodies are platelet activating. An additional approach to increase specificity of PF4/heparin antibody assays is to take the magnitude of the test reactivity (usually given in optical density [OD] values) into account, as it correlates with the likelihood of HIT determined by a scoring system and with the capability of the antibodies to activate platelets [12,14]. Beside their low positive predictive values for HIT, another disadvantage of the currently available immunoassays results from the preferred testing in batches rather than single patient samples for cost considerations and from the lack of standardization.

Three automated PF4/heparin antigen assays, one based on the agglutination of latex particles (HemosIL® HIT-$Ab_{(PF4\text{-}H)}$) and the two others with chemiluminescent detection (HemosIL® AcuStar HIT-$Ab_{(PF4\text{-}H)}$ and HemosIL® AcuStar HIT-$IgG_{(PF4\text{-}H)}$, respectively), have recently been introduced. In the latex agglutination assay, agglutination of PF4/polyvinylsulfonate coated beads by a monoclonal anti-PF4/heparin-antibody is inhibited in the presence of human anti-PF4/heparin antibodies [15]. This assay can only be performed with plasma (the only sample matrix suitable for the ACL TOP® Family System) and it cannot differentiate between IgG, IgM, or IgA antibodies. The chemiluminescence assays are based on binding of anti-PF4/heparin antibodies to PF4/polyvinylsulfonate. They can be performed with serum or plasma and can differentiate between different immunoglobulin classes (IgG only or total antibody). They show a wide range of reactivity and might thus provide additional information compared to the EIAs [16]. These automated assays might allow greater standardization and better comparability of results obtained in different laboratories. Furthermore, they allow single sample testing and provide a rapid turn around time of results of approximately 13 minutes for the latex immunoassay and about 30 minutes on the chemiluminescent system.

In this study we assessed these three assays in comparison with an in-house PF4/heparin EIA and the HIPA test.

Material and Methods

Study design

Blood samples from 491 consecutive surgical and medical patients who received UFH or LMWH and in whom HIT was suspected were evaluated in this study. 94 suspected HIT plasma samples were first referred to the laboratory of the University Hospital of Florence, Italy and 34 suspected HIT plasma samples to the laboratory of the Royal Brompton Hospital, London, United Kingdom. These samples were tested on the day of arrival using the latex agglutination test (HemosIL-Ab). Then the samples were frozen and subsequently sent to the Greifswald laboratory for further investigations in the chemiluminescence tests. In addition the Greifswald laboratory enrolled 363 sera from consecutive patients referred for laboratory testing of suspected HIT. All samples were tested in the Greifswald laboratory using the chemiluminescence assay for IgG, IgM, IgA antibodies (HemosIL AcuStar-Ab) and the chemiluminescence assay for IgG (HemosIL AcuStar-IgG), by an in-house enzyme immunoassay (EIA) for PF4/heparin IgG, IgM, and IgA antibodies separately [17], and by the heparin induced platelet aggregation (HIPA) test. HIPA was considered to be the reference test in this study as clinical information on the patients was sparse.

Latex agglutination assay for PF4/heparin antibodies

HemosIL HIT-$Ab_{(PF4\text{-}H)}$ (HemosIL-Ab) (Instrumentation Laboratory, Bedford, MA, USA) was performed according to the manufacturer's instructions. All assay steps were performed on the ACL TOP Family automated hemostasis analyzer. In brief, plasma samples were incubated with latex beads coated with PF4/polyvinylsulfonate complexes. After binding of the plasma antibodies a monoclonal antibody recognizing PF4/heparin complexes was added. In the presence of human anti-PF4/heparin antibodies, binding of the monoclonal antibody and subsequent agglutination of latex beads is inhibited. Inhibition of agglutination is quantified and reported in arbitrary units (U/mL). The manufacturer suggests that HemosIL-Ab results equal or higher than 1.0 U/mL may indicate the presence of HIT antibodies. In the study, results are classified as negative (0.00-0.99 U/mL), weak positive (1.00-1.99 U/mL), positive (2.00-3.99 U/mL), and strong positive (≥4.0 U/mL).

Chemiluminescent-based assays for HIT

HemosIL AcuStar HIT-$Ab_{(PF4\text{-}H)}$ (HemosIL AcuStar-Ab), and HemosIL Acustar HIT-$IgG_{(PF4\text{-}H)}$ (HemosIL AcuStar-IgG) (Instrumentation Laboratory, Bedford, MA, USA) were performed according to the manufacturer's instructions. In these automated assays, samples are incubated with magnetic particles coated with PF4 complexed to polyvinyl sulfonate, which capture the anti-PF4/heparin antibodies from the sample. After incubation, beads are separated magnetically and washed. Antibody binding is then detected using a mixture of secondary antibodies (anti-human IgG, IgA and IgM) or a specific anti-human IgG antibody in HemosIL AcuStar-Ab and HemosIL AcuStar-IgG, respectively. After a wash step and triggering of the luminescent reaction, the emitted light is measured as relative light units by the ACL AcuStar™ optical system (Instrumentation Laboratory) and arbitrary units (U/mL) are provided within 30 minutes. For both assays, the manufacturer suggests that results equal or higher than 1.00 U/mL may indicate the presence of HIT antibodies. In the study, results are classified as negative (0.00-0.99 U/mL), weak positive (1.00-1.99 U/mL), positive (2.00-3.99 U/mL), and strong positive (≥ 4.0 U/mL).

In-house PF4/heparin EIA

Blood samples were investigated for PF4/heparin antibodies using an in-house EIA that specifically detects IgG, IgA or IgM antibodies as previously described [17]. Reactions with optical density (OD) greater than 0.5 were considered positive. This cut off was defined in different patients populations and in non-heparin treated individuals before [7]. To increase the specificity of antibody detection all IgG positive samples were re-investigated with an inhibition step using a high heparin concentration (100 IU/mL). A reduction of the optical density of greater than 40% was defined as positive inhibition [18].

Heparin-induced platelet activation assay (HIPA)

The HIPA test was performed as described with minor modifications [10]. Briefly, each sample was tested with washed platelets from four different platelet donors in the absence (buffer alone) or presence of heparin (0.2 IU/ml and 100 IU/ml). Reactions were placed in round bottom microtiter wells containing 2 spheres and stirred at approximately 500 rpm. Wells were examined optically at five-minute intervals for loss of turbidity. A serum was interpreted as reactive (positive), if platelets of at least two donors aggregated within 30 minutes in the presence of 0.2 IU/ml, but not with 100 IU/ml heparin. Reading time was 45 minutes. Each test included positive and negative control sera. Plasma samples were recalcified and heat inactivated prior to the investigation using HIPA.

Samples which caused platelet aggregation in the presence of buffer, low and high heparin concentrations were considered as indeterminate, as it remained unclear whether beside the heparin independent activity the sample may contain also heparin dependent antibodies.