Heparin-induced thrombocytopenia (HIT), without or with thrombosis, is an important cause of morbidity and mortality in patients treated with this otherwise extremely useful anticoagulant. Work done in several laboratories has shown that patients with HIT almost invariably have antibodies specific for a platelet derived CXC chemokine, platelet factor 4 (PF4, CXCL4), modified by an external agent, heparin. In the past two decades, much has been learned about the pathogenesis of HIT. However, knowledge of the molecular nature of the immune-response is far from complete and it is unknown why a high percentage of some patient populations treated with heparin form antibodies, yet only a minority experience thrombocytopenia and/or thrombosis.

The immune response in HIT has several unusual features:

1. Thrombocytopenia, usually characterized by a transient fall in platelet count 5-10 days (typical onset) after initiation of treatment or, more rarely, fall in platelet counts beginning soon after heparin is started (rapid onset) in patients previously and recently (generally within the past 2-3 weeks) exposed to heparin;

2. Patients with HIT almost invariably have antibodies specific for complexes consisting of two normal body constituents: heparin and PF4; in addition a significant minority of patients have antibodies that recognize PF4 alone in the absence of heparin;

3. Platelet activation by anti-PF4:heparin immune complexes (IC), engaging with the Fc gamma RIIA receptor on platelets, is integral to the pathogenesis of HIT;

4. Antibodies appear to recognize conformational determinants on PF4 created when the PF4 binds to heparin;

5. The humoral immune response consists of a primary IgM reaction usually followed by secondary formation of IgG and/or IgA antibodies suggesting involvement of helper T cells.

It should be noted that while PF4-heparin complexes are the accepted target for the antibodies associated with HIT, Amiral et al. have speculated that “atypical cases” of HIT can be associated with anti-interleukin 8 (IL8), neutrophil-activating peptide 2 (NAP-2), or anti-protamine-sulfate antibodies (ISTH XXII congress; Abstract n° PP-MO-697; 2009). However, reactions of these antibodies against NAP-2 or IL8 in their normal configurations (not immobilized on plastic) were not described and their relation to antibodies that recognize PF4-heparin complexes is uncertain. In this regard, a clinical relevance of the putative anti-protamine sulfate antibodies in HIT appears unlikely since, on the contrary of PF4 and heparin like molecules, protamine sulfate is not a “normal body constituent”, but is administered as a drug, for a very limited period of time, with the unique purpose of neutralizing injected heparin.

CLINICAL PRESENTATION

As noted, heparin-induced thrombocytopenia is characterized by an unexpected fall in the platelet count occurring five days or more after initiation of treatment with unfractionated heparin. However, in patients with previous heparin exposure, HIT antibodies may be present initially or may increase rapidly in titer, often within 48 hours of heparin administration. This may lead to an acute drop in platelets soon after heparin is started. It has been claimed that, rarely, HIT antibodies may be present in patients never before exposed to heparin and that these patients are also at risk for “acute HIT”. The thrombocytopenia, associated with HIT itself, is rarely severe enough to provoke bleeding. However, a subset of patients develop arterial and/or venous thrombosis and thromboembolism which can be debilitating or fatal.

HIT usually occurs in patients given standard doses of unfractionated heparin, especially of bovine origin, but has been reported in patients treated with low dose, subcutaneous heparin and in patients exposed to heparin “flushes” used to maintain the patency of intravenous lines. Even
minute quantities of heparin released from heparin-bonded catheters appear to be capable of causing the disorder in previously sensitized patients. More recently, several reports of HIT apparently induced by the pentasaccharide fondaparinux (Arixtra) have appeared.

**FUNCTIONAL TESTS**

For many years, it was known that serum from patients with HIT contains immunoglobulins (Ig) that activate platelets in the presence of pharmacologic doses of heparin (0.1-1 units/ml). However, attempts to demonstrate heparin-dependent binding of these Ig to normal platelets were generally unsuccessful. Because the heparin-dependent platelet activation triggered by the antibodies could be blocked by monoclonal antibodies specific for the platelet Fc receptor (FcγRIIA), it was thought for some time that antibodies associated with HIT were specific for heparin and reacted with the anticoagulant to form platelet-activating immune complexes. However, various investigators were unable to demonstrate such complexes. Despite lack of knowledge about the specificity of HIT-associated antibodies, recognition of their platelet-activating properties led to the development of two useful diagnostic procedures – the platelet aggregation test and the serotonin release test (SRA). The latter test is somewhat more sensitive than the former, but it is generally agreed that it is incapable of diagnosing all cases of HIT. Moreover, both of these assays have the disadvantage of requiring fresh platelets, are relatively unstandardized, costly and technically challenging.

In the early 1990s, an important clue to the etiology of HIT was provided by the finding in several laboratories that the antibodies in patients with this condition are specific for complexes containing heparin and PF4 leading to the development of PF4-dependent enzyme immunoassays (PF4-H ELISA). The immunoassays have the advantage of better standardization and do not require normal platelets, but may give positive results in the absence of clinical HIT.

**CLINICAL RELEVANCE OF THE PRESENCE OF PF4-HEPARIN SPECIFIC ANTIBODIES**

The diagnosis of HIT is made clinically with the support from the laboratory. It is important to recognize that a positive PF4-H ELISA test only indicates the presence of antibodies specific for PF4-heparin complexes and is not intended for diagnosis of the clinical entity, HIT. It is important to make a distinction between specificity and sensitivity for “antibody detection” and for “disease diagnosis”. Some reports have claimed that the PF4-H ELISA is “insensitive” and “non-specific” for HIT diagnosis because many patients positive for antibody do not have clinical HIT. However, the assay is intended only for antibody detection and it is very sensitive and specific for that purpose. The high sensitivity of the PF4-H ELISA for antibody detection is not necessarily a drawback because from the clinical standpoint, a missed diagnosis of HIT can be catastrophic for an individual patient. Furthermore, the presence of antibodies specific for PF4-heparin complexes (even if “not clinically significant” at the time a specimen was collected) could prompt a physician to limit exposure to heparin since there is no way to know how many of these patients might develop HIT if heparin treatment is continued. Lastly, it should be noted there are reported cases of patients who were positive in PF4-H ELISA but negative in SRA who had clinical HIT, some with thrombosis (unpublished observations).

A few recent reports have suggested new approaches to obtain an increased correlation of a positive PF4-H ELISA result with both a clinical diagnosis of HIT and a higher incidence of HIT and thrombosis; therefore, having the potential of improving the “clinical specificity” of PF4-H ELISA assays.

Whitlatch et al. provided a fairly persuasive argument for using the “heparin inhibition” step in PF4-H ELISA (high-dose heparin confirmatory procedure) to improve the clinical specificity of the immunoassay. Their findings showed that fewer than 10% of patients judged to have HIT on clinical grounds failed the confirmatory test, whereas approximately one-third of those who did not appear...
to have HIT did so. Furthermore, using statistical
modeling, they derived a nomogram by which the
likelihood of a patient having HIT can be estimated by:

- Using the optical density (OD) value in PF4
ELISA and
- By determining whether or not the reaction is
inhibited by at least 50% when excess heparin is
added.36

Similarly, a close relationship between the optical
density (OD) value in the PF4-H ELISA and the
likelihood that a patient has clinical HIT and is at
risk for thrombosis has been reported by several
laboratories.37,38 To paraphrase Warkentin et al.,
laboratories reporting EIA test results (should)
consider informing clinicians about the actual OD
value of the PF4-H ELISA (rather than just reporting
“positive” or “negative”) and should emphasize the
importance of a strong positive test result.

Because the platelet Fc receptor (FcγRIIA) only
recognizes immunoglobulins G (IgGs), it has been
speculated that detecting only IgG antibodies may
be a measure to improve the clinical specificity of the
PF4-H ELISA. In a very recent report, Greinacher et
al. confirmed the very close correlation of IgG-based
ELISAs with the functional assays, by showing that the
percentage of sera testing positive in the functional
assays strongly correlated with PF4-H-IgG ELISA OD
reactivities.39 Similar observations were also reported
by Warkentin et al.40 On the contrary, it should be
noted that there is not yet a consensus in suggesting
the use of a PF4-H IgG-based ELISA instead of ELISAs
capable of detecting antibodies of any of the three
immunoglobulin classes (IgG, IgA and IgM).41

Moreover, in a retrospective, case-control study
conducted on cardiac surgery patients with
postoperative thrombocytopenia, Kareendi et al. found
serial monitoring of antibody strength, by ELISA
tests, to be useful in making treatment decisions.42 In
this study, patients with borderline or negative initial
assay who had a subsequent second, third, or fourth
assay, 28% ultimately had a positive result on follow-
up tests. Furthermore, 91% of these positive results
occurred on the second or third assay, suggesting the
usefulness of investigating for the presence of PF4-
heparin dependent antibodies at multiple time points
if the clinical suspicion for HIT exists.42 The latter
approach could provide clinicians with a useful tool
to monitor heparin therapy prior to switching to a
different anticoagulant treatment; therefore, possibly
preventing development of HIT had heparin been
continued.

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