

ASSOCIATION OF PROTAMINE SULFATE ANTIBODIES WITH «PSEUDO-HIT» IN HEPARIN TREATED PATIENTS

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OBJECTIVE OF THE STUDY

Heparin Induced Thrombocytopenia (HIT) is usually triggered by heparin dependent antibodies targeted to complexes of Platelet Factor 4 (PF4) and Heparin, mainly of the IgG isotype. Atypical cases associated with Anti-IL-8 antibodies have also been reported.

Clinical and biological presentation of HIT is heterogeneous, which renders the diagnosis of some atypical patients difficult to establish.

We focused on some patients presenting with the clinical suspicion of HIT and which had variable reactivities with immunoassays or platelet activity tests for heparin dependent antibodies.

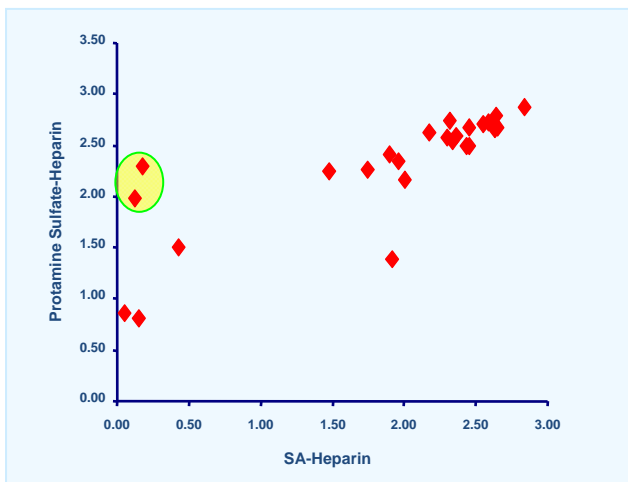
A new "dynamic" immunoassay using heparin immobilized onto a microELISA plate, either through binding to Protamine Sulfate (Heparin being in large excess), or biotinylated and reacted with immobilized Streptavidin, was used for further patient investigations.

Our goal was to investigate which factors could be implicated in the high variability observed with the various immunoassays for heparin dependent antibodies, and to elucidate the reasons for some of the discrepancies.

Our study specifically focused on variability of the heparin dependent antigen, required for HIT antibodies binding.

RESULTS

Comparison of HIT antibody reactivity to Protamine Sulfate-Heparin and to Streptavidin-Heparin (N=25)



Good correlation between the binding of HIT antibodies to Streptavidin (SA)-Biotinylated Heparin and to Protamine Sulfate-Heparin (N=25), excepted for 5 patients presenting a stronger reactivity with Protamine Sulfate-Heparin.

Among these 5 patients, 2 were very strongly positive for the binding to Protamine Sulfate-Heparin, but were totally negative for the binding to Streptavidin-Biotinylated Heparin.

DISCUSSION

- Some patients presenting with clinical and biological symptoms of HIT have antibodies to Protamine Sulfate.
- This reactivity is observed in the presence or absence of Heparin, but is stronger with Heparin.
- Protamine Sulfate used in patients for neutralizing heparin may induce antibodies which behave as heparin dependent antibodies and produce complications close to those observed in HIT (when heparin and Protamine Sulfate are both present in the patient, i.e. during the heparin neutralization step by Protamine Sulfate).
- Protamine Sulfate/Heparin/Antibody complexes may present variable binding in HIT immunoassays, yielding borderline or weak positive results in PF4 Elisass, in the absence of H-PF4 antibodies.
- This observation is in agreement with the early report from Al-Mondhiry et al. (Protamine Sulfate induced thrombocytopenia and leukopenia. *Thromb. Haemost.* 53(1): 60-64; 1985).

MATERIALS AND METHODS:

Assay principle: Heparin is immobilized onto microELISA plates through two different means:

- Large excess of heparin with Protamine Sulfate (PS)
- Biotinylated heparin and Streptavidine (SA)

Tested specimen (plasma or serum 1:100) is introduced into the heparin coated well and mixed with a platelet lysate (source of chemokines).

If antibodies are present, there is a « dynamic » complex formation between antibodies and chemokines onto immobilized heparin. Bound antibodies are revealed with Anti IgG peroxidase conjugate and TMB/H₂O₂ as substrate. A450 is measured.

Control plates: Bovine Serum Albumin (BSA), Protamine Sulfate (PS) or Streptavidine (SA) coated plates were used as controls, in place of heparin coated plates.

Heparin Inhibition studies: They were performed by incubating the tested specimen in the microwell in presence of 2 IU/ml Heparin.

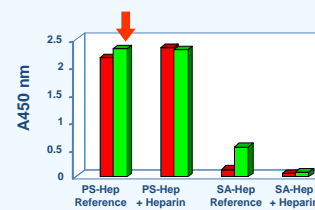
Patients: 25 plasmas from patients with a clinical suspicion of HIT were tested. All plasmas were positive with PAT, positive or borderline with H-PF4 Elisass.

Only the IgG isotype was measured

Antibodies to Protamine Sulfate:

The 2 patients strongly positive to Protamine Sulfate-Heparin and negative to Streptavidin-Biotinylated Heparin were further explored.

Heparin inhibition studies for 2 atypical HIT suspected cases



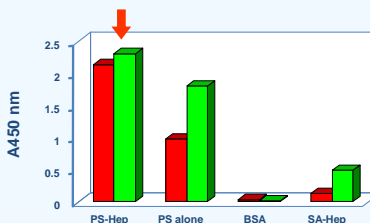
➤ Heparin did not inhibit the binding to Protamine Sulfate-Heparin in these 2 patients.

➔ In 1 patient, there was also a weak reactivity to Hep-SA, which was inhibited by Heparin.

PS: Protamine Sulfate - SA: Streptavidin

Presence of antibodies to Protamine Sulfate is suspected.

Analysis of non specific binding for the 2 atypical patients



➤ These 2 patients developed strong antibodies to Protamine Sulfate, without or with Heparin.

➔ Patient strongly positive for IgG antibodies on day 7 of Heparin therapy (when HIT was suspected) was retrospectively found negative on plasma samples collected from day 1 to day 6.

Both patients present a strong binding to Protamine-Sulfate coated plates, in the presence or the absence of Heparin, but not to BSA or to Streptavidin coated plates.

These patients did not bind to H-PF4, but they presented severe thrombocytopenia and platelet aggregation tests were positive.

Antibodies to Protamine Sulfate, enhanced by heparin, can mimic HIT.

CONCLUSIONS

- IgG antibodies to Protamine Sulfate, non associated with H-PF4 antibodies, can be observed in heparin treated patients when these patients (probably formally sensitized) receive Protamine Sulfate for neutralizing Heparin. They can develop an hyperimmune reaction with generation of IgG antibodies, mimicking HIT.
- A «pseudo HIT symptom» could be induced by antibodies to Protamine Sulfate.
- A special caution should be taken in patients repetitively exposed to heparin, then to Protamine Sulfate.
- Complementary studies are required for characterizing the pathogenic effect of antibodies to Protamine Sulfate in heparin treated patients, or during the heparin neutralization step by Protamine Sulfate, when required (ECC,...).

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Association of Protamine Sulfate antibodies with "Pseudo-HIT" in heparin treated patients.

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Heparin Induced Thrombocytopenia (HIT) is usually triggered by heparin dependent antibodies targeted to complexes of Platelet Factor 4 (PF4) and Heparin, mainly of the IgG isotype. Atypical cases associated with Anti-IL-8 antibodies have also been reported. Studies were performed using a new "dynamic" immunoassay, where heparin is immobilized on a micro-Elisa plate through a complex with Protamine-Sulfate, or biotinylated and bound to coated Streptavidin. Tested specimen (from patients suspected for HIT, plasma or serum) are mixed with normal platelet lysate, as a source of PF4 and of other chemokines. When antibodies are present a dynamic complex forms onto the immobilized heparin, and bound antibodies are then measured with Anti-Immunoglobulins (IgG, or IgA or IgM or IgGAM) coupled to peroxidase, and TMB/H₂O₂ as substrate. Positive samples yield a coloured test above a defined threshold (> 0.5 in standard conditions), which is measured at 450 nm. A group of 25 patients with clinically characterized HIT, and positive in platelet aggregometry assays, was tested with this new approach. Two patients were highly positive only when Heparin-Protamine-Sulfate was used for capture, and negative with Biotinylated Heparin and Streptavidin. These two patients were also positive when Protamine-Sulfate alone was used for coating, but negative with BSA coated plates. In both cases antibodies were of the IgG isotype. These data show that Anti-Protamine-Sulfate antibodies can be present in some patients (formerly exposed to Protamine Sulfate), and in presence of therapeutical doses of heparin a "pseudo-HIT" syndrome can develop. More extensive studies are required in order to document prevalence of this atypical presentation, and association of these antibodies with "pseudo" clinical symptoms of HIT. These data also demonstrate the complexity of HIT presentation, and the variety of "atypical cases", which can produce similar clinical complications to those of "typical HIT", although the mechanisms are slightly different, but always developed in the presence of heparin.