

Heterogeneity of Heparin Induced Antibody Profiles in Patients Suspected of Heparin Induced Thrombocytopenia (HIT)

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Introduction

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 antibodies to IL-8 or Nap-2 or to their complexes with heparin or to Protamine sulfate or to its heparin complexes, have also been reported.

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

Some patients presenting clinical suspicion of HIT have variable reactivities with immunoassays or platelet activity tests for heparin dependent antibodies.

Our goal was to investigate which factors could explain the variability observed with the various immunoassays for HIT, and to elucidate the reasons for some of the discrepancies.

Aim

- ➔ Patients (N=124) with high probability of HIT (clinical diagnosis of HIT, with thrombocytopenia occurring 5-15 days following the onset of heparin therapy) were from different hospitals and were evaluated for the whole of heparin dependent antibodies.
- ➔ These patients had different clinical contexts : Cardiology, Oncology, Surgery.
- ➔ Results were expressed as A450 and positivity was defined by A450 values higher than the Mean value in normals +5 SDs, i.e.:
 - A450 \geq 0.50 for IgG Isotope

Results

Reactivity profile for the different patients tested:

According to antibody binding to the various capture antigens, coated onto the microElisa plate, various reactivity profiles are identified in patients tested for a suspicion of type II HIT, as reported in the here below table:

| Group | N | HIA PS-Hep | | HIA Strep-Hep | | PS | PF4 Alone | H-PF4 | Control BSA |
|--------------|------------|-------------|----------------|---------------|----------------|----|-----------|-------|-------------|
| | | With Lysate | Without lysate | With Lysate | Without lysate | | | | |
| A | 2 | + | - | + | - | - | + | - | - |
| B | 67 | + | - | + | - | - | - | + | - |
| C | 16 | + | - | + | - | - | + | + | - |
| D | 15 | + | + to +/- | + | + to +/- | - | - | - | - |
| E | 12 | + | + | - | - | + | - | - | - |
| F | 12 | + | + | - | - | - | - | - | - |
| Total | 124 | | | | | | | | |

Discussion

Six reactivity profiles were identified in patients with suspicion of HIT.

- ➔ Group B and C correspond to the "typical" type II HIT, where antibodies are targeted to PF4 complexed with Heparin, and a sub group (C) also cross reacts with PF4 alone.
- ➔ Interestingly few patients have antibodies which bind strongly to PF4, and not, or only weakly to its complexes with heparin. These patients usually developed thrombotic complications in addition to thrombocytopenia, during heparin therapy, which lead to suspicion of HIT.
- ➔ As expected, a group of patients with heparin dependent antibodies targeted to non PF4 antigens, was identified (IL8 or NaP2 could be among the other antigens). These profiles are frequently observed in oncology patients.
- ➔ Strikingly, some patients had antibodies reactive with Protamine Sulfate and/or with Protamine Sulfate complexed with heparin. In addition to thrombocytopenia, many of them developed thrombosis under heparin therapy, suggesting the drug as a possible trigger for the complication. In those patients, thrombocytopenia occurs very acutely. Antibodies are observed in patients who had received Protamine Sulfate for neutralizing heparin several times, especially following CPB. These observations suggest a probable deleterious effect of antibodies to Protamine Sulfate or to its complexes with heparin.

Materials and Methods

Various assays are used for testing the binding of antibodies to different heparin dependent antigens

- a) Heparin is immobilized onto microELISA plates through two different means:
 - ➔ Large excess of heparin with Protamine Sulfate (PS-Hep) (Zymutest HIA, IgG Isotype from HYPHEN BioMed)
 - or
 - ➔ Biotinylated heparin coupled to Streptavidin (Strep-Hep)
- b) Other capture antigens are used: PF4-Heparin complexes, PF4 alone, Protamine Sulfate (PS). They are immobilized onto a microELISA plate.

Control plates: Bovine Serum Albumin (BSA) coated plates.

Only the IgG isotype was measured.

Bound antibodies are revealed with Anti IgG peroxidase conjugate and TMB/H₂O₂ as substrate. A450 is measured.

Tested specimen plasma or serum are diluted 1:100 and then:

Mixed with or without a platelet lysate (source of chemokines) into the coated microwell.

Patients:

124 plasmas from patients with a clinical suspicion of HIT were tested.

Methods:

Analysis of antibody binding in plasmas from patients with HIT to: Protamine Sulfate-Heparin (PS-Hep); Streptavidin-Heparin (Strep-Hep); Protamine Sulfate (PS) alone; PF4-Heparin (H-PF4), PF4 alone, Bovine Serum Albumin (BSA).

The different reactivity groups correspond to the following profiles:

| Group | Profile |
|-------|---|
| A | Anti PF4 alone |
| B | Anti PF4-Heparin, but not Anti PF4 |
| C | Anti PF4-Heparin and Anti PF4 |
| D | Non PF4 antigens (Anti-IL8 or Anti-NAP2?) |
| E | Anti Protamine-Sulfate or Anti Protamine-Sulfate Heparin |
| F | Anti Protamine-Sulfate Heparin but not Anti Protamine-Sulfate |

- ➔ Group A, B, and C are reactive only in presence of platelet lysate (source of PF4 or other chemokines). Group D has a variable reactivity without platelet lysate, borderline or negative, but sometimes positive.
- ➔ Group E and F are reactive in the HIA assay, also in the absence of platelet lysate. Heparin dependent antibodies are targeted to Protamine Sulfate and/or to its heparin complexes. Platelet activation assays are usually negative.
- ➔ Most of the patients presented with a strong reactivity (A450 from >1.00 to > 4.00).

Conclusions

- ➔ In addition to the "typical HIT" profile, which concerns the majority of patients developing heparin dependent antibodies, other atypical presentations are possible.
- ➔ In addition to heparin dependent antibodies to PF4 antigen, other antibody profiles can be observed. More especially, isolated strong reactivities to PF4 alone, or to Protamine-Sulfate complexed with heparin, or alone, can be associated with clinical presentations suggesting or mimicking HIT, with frequent thrombosis occurring under heparin therapy.
- ➔ We suggest that in presence of "a clinical probability of HIT", and negative H-PF4 Elisa or platelet activation tests, further investigations need to be performed in order to identify (and to manage) atypical presentations.
- ➔ Further studies are necessary for elucidating the pathological mechanisms triggered by heparin dependent antibodies in these atypical cases.