HEPARIN INDUCED THROMBOCYTOPENIA

New assays for HIT Diagnosis based on the understanding of Heparin antigens function

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INTRODUCTION

- Type II Heparin Induced Thrombocytopenia (HIT), remains the major iatrogenic complication of heparin therapy.

- It is triggered by « Heparin dependent » antibodies targeting heparin-protein (mainly Platelet Factor 4/PF4) complexes.

- It develops more frequently during Unfractionated Heparin (UFH) therapy than during Low Molecular Weight Heparin (LMWH) (ten-fold lower risk).

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CLINICAL INCIDENCE OF HIT

- 0.5 to 2 % (or more?) with UFH therapy.
- 0.1 to 0.2 % with LMWH therapy.
- Expected at 0 % with pentasaccharide.
- Very high incidence of the clinical context (platelet or endothelial activation, inflammation, malignancy) and the therapy duration.
- Low incidence during ECC, despite presence of antibodies (including IgG); protective effect of heparin in excess???
Two types of serological assays are available for laboratory detection of HIT antibodies:

- **Functional (platelet activation) assays** such as the platelet 14C-Serotonin-release assay (SRA) or Platelet aggregation or the Heparin-Induced Platelet Activation (HIPA) test.

- **Enzyme-immunoassays** employing PF4/heparin or PF4/polyvinylsulfonate complexes. These assays can detect antibodies only when PF4 is the heparin dependent protein. We have now developed a new enzyme-immunoassay using a functionally available heparin coated onto a solid surface.
ASSAY PRINCIPLE

Functionally available heparin is coated onto a solid surface. With this approach:

- If chemokines, exhibiting heparin affinity, are present, they bind to heparin, expose neo epitopes, and capture heparin dependent antibodies.

- In addition, if heparin complexes, are present, they can also directly bind to heparin, through the heparin binding protein.
This can be achieved by different means:

- Coating protamine sulfate in the presence of large excess of heparin.
- Coating streptavidin and biotinylated heparin.
- Other: coating heparin covalently bound to a carrier protein (such as albumin) or a polymer.
ASSAY PRINCIPLE FOR THE NEW METHOD

- Sulf Prot.
- UFH
- ELISA Plate
- Streptavidin + Biotinylated Hep
- ELISA Plate
- Sample to be tested
- Platelet or Leucocytes lysates
- IL8 +/− PF4
- ELISA Plate
- IC + TMB
- ELISA Plate

Reading A450 nm

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Assay Protocol

• The plate is coated with heparin functionally available.

• The patient plasma or serum diluted at 1:100 (or more) is incubated in presence or in absence of platelet lysates.

• The second antibody used for the revelation can be an:
  • Anti-IgG,A,M (Screening)
  • Anti-IgG (only for the IgG isotype)
  • Anti-IgM (only for the IgM isotype)
  • Anti-IgA (only for the IgA isotype)

• Substrate: TMB/H$_2$O$_2$, the coloration (5 min.) is stopped with sulfuric Acid.

• The absorbance is measured at 450 nm.
Specificity

- Normal Plasma (N=90)
  - DO: < 0.10
  - SD: 0.04

- Pathological plasma (N=37): HIT or suspicion.
  - A450 ≥ 1
  - Extremes: 1.02 to > 3.00
Correlation with antibodies anti-HPF4 (anti-IgG)

\[ R^2 = 0.890 \]
\[ Y = 1.17x + 0.07 \]
\[ N = 37 \]
Correlation with antibodies anti-PF4 (IgG)

\[ R^2 = 0.06 \]
\[ Y = 0.249X + 0.11 \]
Effect of addition of platelet lysates

n = 27
Curve dose response obtained for 5 pathological Plasmas

A 450 nm

plasma dilutions

P1
P2
P3
P4
P5
Correlation with Asserachrom HPIA

\[ R^2 = 0.83 \]
\[ Y = 0.75X + 0.23 \]

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HEPARINE DEPENDENT ANTIBODY ISOTYPES IN PATIENTS WITH HIT

- 50 patients with HIT (Thrombocytopenia induced by heparin, positive PAT, +/- thrombosis).
  - 31 only IgG
  - 12 IgG and IgA
  - 2 IgG and IgM
  - 4 IgG, IgA and IgM
  - 1 IgM only

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13 patients under ECC have been tested. 54% were positive in IgG,A,M screening.

5 / 7 have IgG isotypes. Despite the presence of IgG, there is a low clinical incidence of HIT. (Possible protective role of large excess of heparin in plasma).
Conclusions

• The new assay for heparin dependent antibodies, responsible for HIT, is easy to carry out as well as economical and soon, it will be automated.

• Good correlation with the functional assay (platelet aggregation) and the current test measuring anti H-PF4 antibodies.

• Potentially sensitive to different antigen targets of the antibodies (studies in progress).

• Possibility of measuring the circulating complexes “Heparin-protein-antibodies”.