NEW APPROACH FOR DETECTION OF HEPARIN DEPENDENT ANTIBODIES AND RISK ASSESSMENT FOR HEPARIN INDUCED THROMBOCYTOPENIA

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Introduction
- This assay uses the potential of immobilized and biologically active heparin to focus and catch antibody-protein (mainly PF4)-heparin complexes. It then mimics the conditions occurring in vivo when heparin dependent antibodies are generated and can induce Heparin Induced Thrombocytopenia (HIT).
- A new assay for measuring heparin dependent antibodies, involved in the development of HIT was developed.
- Various presentations are proposed for the measurement of total antibodies (IgGAM), or for specifically measuring IgG isotypes, or for the total isoloping of IgG, IgA and IgM isotypes.

Assay principle
- Heparin, immobilized onto a solid reactive surface (plate or other), but «functionally available»:
  - Capt chemoekines present into the patient plasma/serum (or supplied exogenously as a platelet lysate), and then forms the reactive anti-antigen, which binds heparin dependent antibodies.
  - Can also bind «heparin-protein-antibody» complexes present in blood circulation.
- «Functionally available» heparin uses one of the following coating procedures:
  - Protamine sulfate complexed with a large excess of heparin.
  - Streptavidin complexed with biotinylated heparin.
  - Heparin chemically coupled to a high molecular weight molecule (natural or synthetic) or polymer.

Assay Protocol
- Plate coated with «functionally available» heparin.
  - 1:100 (or more) diluted plasma or serum ± lysate.
- Second antibody (peroxidase labeled):
  - Anti-IgG (IgG isotype only)
  - Anti-IgGAM (Screening)
  - Anti-IgA
  - Anti-IgM
  - Anti-HPF4 (A450 nm)
- Assay performances

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Conclusions
- New highly sensitive and specific assay for the diagnosis of heparin dependent antibodies involved in HIT, easy to perform and cost effective, offering automation possibilities.
- Good correlation with platelet aggregation tests and measurement of anti-H-PF4 antibodies.
- Potentially sensitive to the various antigenic targets for heparin dependent antibodies (studies in progress).
- Possible measurement of circulating complexes «heparin-protein-antibody» and assay mimicking the heparin dependent antibody binding mechanisms occurring in vivo.
- Very “flexible” assay principle for all laboratory immunological studies on heparin dependent antibodies, which can cause HIT.

Results
Patients: Citrated plasmas from:
- 60 normal individuals
- 37 patients with a clinically diagnosed HIT (platelet course kinetics, positive platelet aggregation tests at low but not at high heparin concentration, recovery of platelet count following heparin withdrawal).

Table 1:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>A450 (N=60)</th>
<th>SD</th>
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<tbody>
<tr>
<td>HIT Plasmas</td>
<td>≥ 1.00</td>
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<td>Range</td>
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<td></td>
<td>1.02 to 2.00</td>
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<td></td>
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Assay performances

References