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 isotyping of IgG, IgA and IgM isotypes.

Assay principle

Heparin, immobilized onto a solid reactive surface (plate or other), but «functionally available»:

- Capt chemokines present into the patient plasma/serum (or supplied exogenously as a platelet lysate), and then forms the reactive auto-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.

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).

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.

Assay Performances

Table 1:

<table>
<thead>
<tr>
<th>HIT Plasmas (N=37)</th>
<th>Anti PF4 (A450 nm)</th>
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<tbody>
<tr>
<td>N=37</td>
<td>≥ 1.00</td>
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<tr>
<td>Range</td>
<td>1.02 to &gt;3.00</td>
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References