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)-Biotynilated Heparin and to Protamine Sulfate-Heparin, 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-Biotynilated 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 Elisas, 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. Throm. Haemost. 53(1): 60-64; 1985).

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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