

Antibodies to Protamine Sulfate or to its complexes with heparin can be the sole ones identified in some patients with suspicion of HIT.

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Introduction

Patients suspected of type II HIT, can present variable assay reactivities and variable clinical profiles, although the major group, with a typical presentation of HIT, has usually homogeneous positive results in immunoassays and platelet functional tests.

However, suspicion of HIT can be associated with atypical clinical presentations and laboratory assays can be difficult to interpret.

In this study we analyze the variability of antibody reactivity to various heparin binding antigens.

Aim

To analyze antibody binding to various heparin dependent antigens in atypical HIT, concerning patients with variable reactivities in laboratory assays, but with clinical presentations suggesting HIT (time of onset of platelet count decrease; thrombocytopenia; thrombosis; no other obvious cause of thrombocytopenia).

Patients:

Medical and cardiology patients with suspicion of HIT during heparin therapy, and presenting variable reactivities in laboratory assays for HIT.

Results

Antibody reactivity

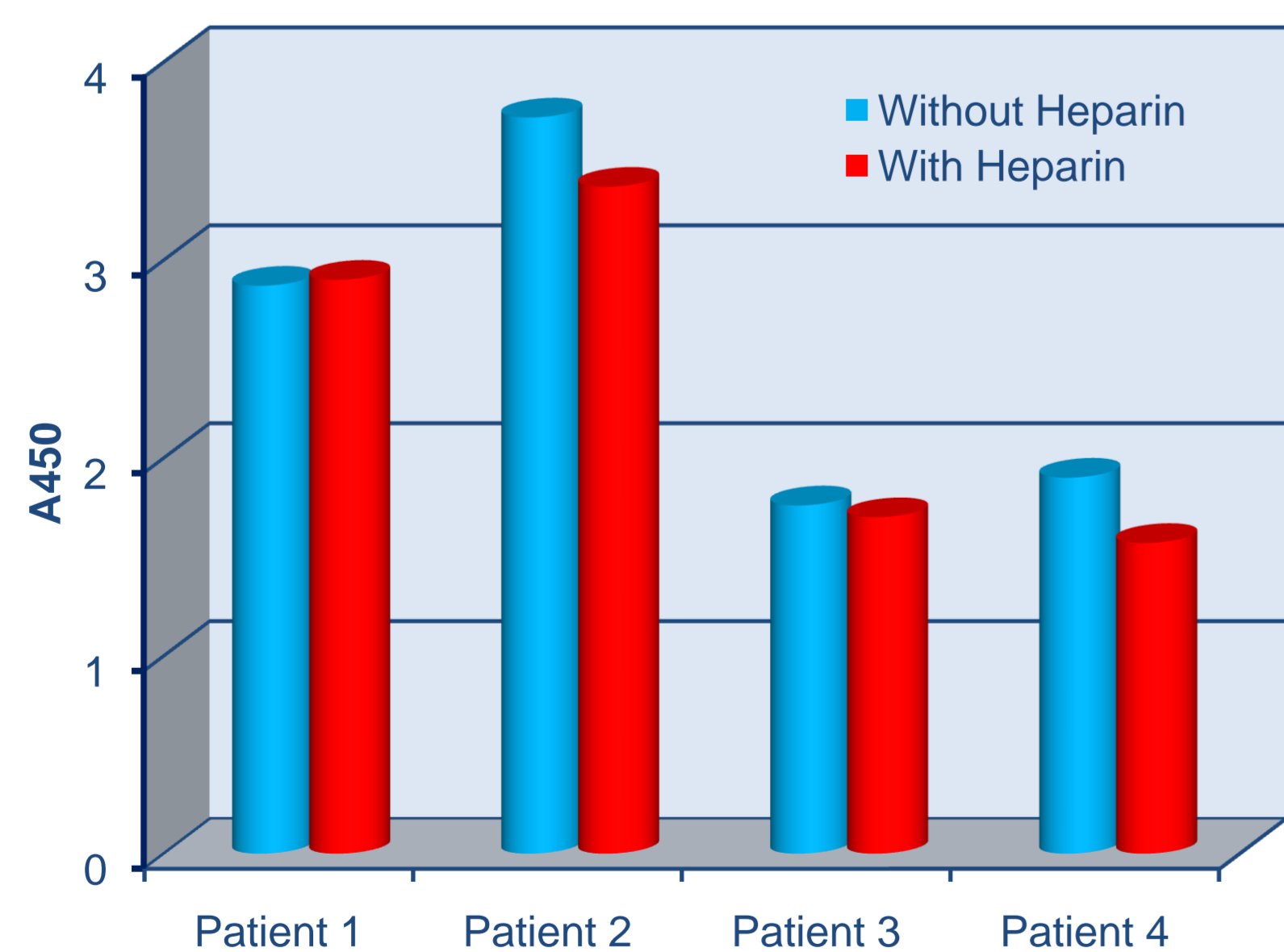
Patients (N=85) are classified according to antibody reactivity to the various capture antigens.

| Antigen | Heparin-protamine sulfate | | Protamine sulfate | BSA | PF4 | Heparin PF4 complexes |
|---------|---------------------------|-------------------------|-------------------|-----|-----|-----------------------|
| | With Platelet lysate | Without Platelet lysate | | | | |
| + | 85 | 16 | 16 | 0 | 47 | 63 |
| - | 0 | 70 | 69 | 85 | 38 | 22 |

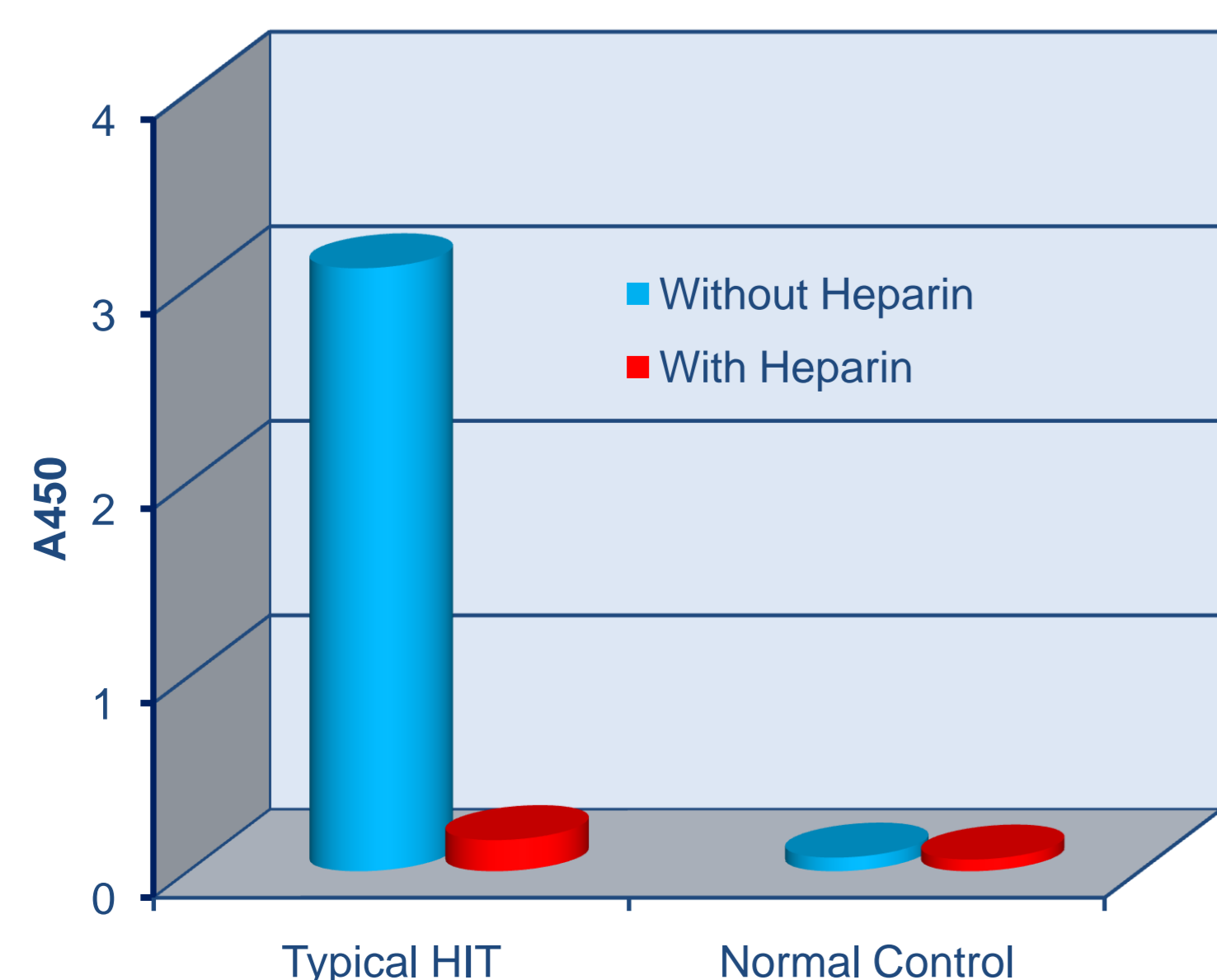
Almost all patients with antibodies to Protamine Sulfate (16) are negative for their binding to Heparin PF4 complexes.

Only 2 out of the 16 patients have antibodies that bind also to H-PF4 (combined antibody reactivity, or capture of Protamine Sulfate-Heparin-antibody complexes by immobilized H-PF4?)

Heparin inhibition studies for the binding to Heparin-Protamine Sulfate complexes (in presence of platelet lysate), performed with the plasma of 4 patients suspected of HIT:



Heparin in excess does not inhibit antibody binding to Protamine Sulfate-Heparin-complexes (or to Protamine Sulfate alone, data not shown), whether Platelet lysate is present or absent.



In typical HIT, excess of heparin totally inhibits antibody binding.

Materials and Methods

85 patients with suspicion of type II HIT and with variable laboratory results (discrepant assays) tested for antibodies binding to:

- ① Heparin-protamine sulfate with or without platelet lysate;
- ② Protamine sulfate (PS);
- ③ Bovine Serum Albumin (BSA);
- ④ Platelet Factor 4 (PF4);
- ⑤ Heparin PF4 complexes (H-PF4)

Plates are saturated with goat serum.

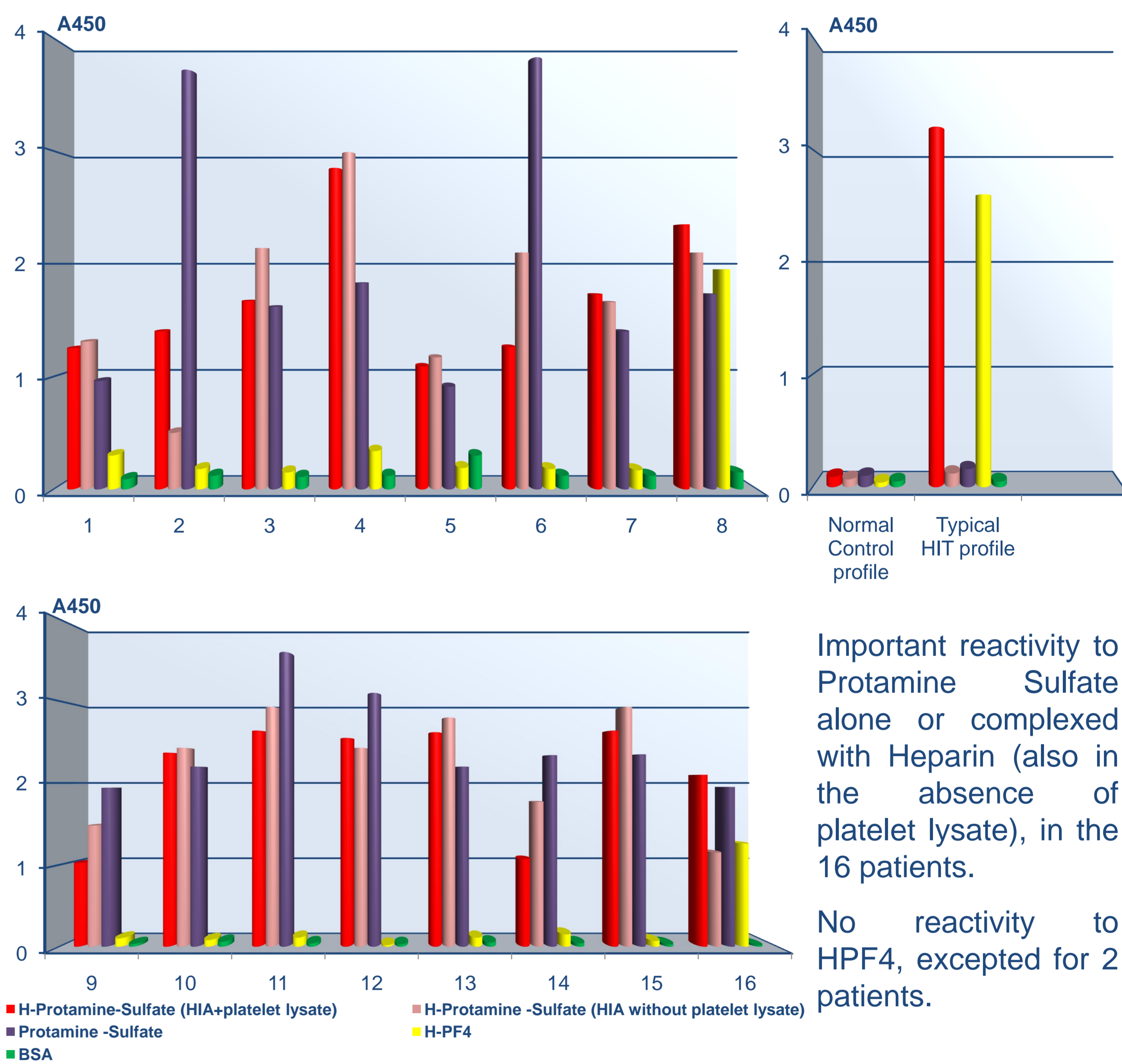
Inhibition tests in presence of an excess of heparin (2 IU/mL), in the 1:100 diluted specimen, were performed.

Only IgG isotypes were evaluated.

All plasma or serum specimen were assayed at 1:100 dilution in the assay diluent (containing 10% goat serum, for blocking non specific interactions).

All patients tested have a clear positive reactivity (A450 from >1.00 to > 3.00) for heparin dependent antibodies (IgG isotypes) tested with the Zymutest HIA, IgG kit (Binding to Heparin-Protamine-Sulfate complexes, in presence of platelet lysate).

Antibody profile in the 16 Patients reactive with Protamine Sulfate



Important reactivity to Protamine Sulfate alone or complexed with Heparin (also in the absence of platelet lysate), in the 16 patients.

No reactivity to HPF4, excepted for 2 patients.

Conclusions

Antibodies to protamine sulfate or to its complexes with heparin are the sole reactivity identified in some patients with suspicion of HIT and presenting its usual clinical associations (thrombocytopenia, kinetics of platelet count decrease, frequently thrombosis). These antibodies are suspected to be pathogenic, but further laboratory studies are required for demonstrating their implication. This adverse immune response requires further investigations.

These antibodies are not inhibited by heparin, as it is the case in typical heparin dependent antibodies in HIT.

Noteworthy, Protamine Sulfate antibodies are observed in a subset of patients presenting all the clinical symptoms of HIT. Most of them are from cardiology and had repeated exposures to Protamine Sulfate.