MEASUREMENT OF HEPARIN DEPENDENT ANTIBODIES WITH IMMUNOASSAYS

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Immunoassays for heparin dependent antibodies have been introduced since the discovery of PF4 as the major target antigen in presence of heparin. Different immunoassay presentations are available, and interestingly heparin can be replaced by polyanions such as Polyvinyl-Sulfonate (PVS), providing evidence that the location of the reactive epitopes of heparin dependent antibodies are on the PF4 molecule. PF4, in presence of heparin (UFH or LMWH) at stoichiometric concentrations, forms ultra-large, multimolecular complexes and its native structure is then altered as shown on **figure 3**.

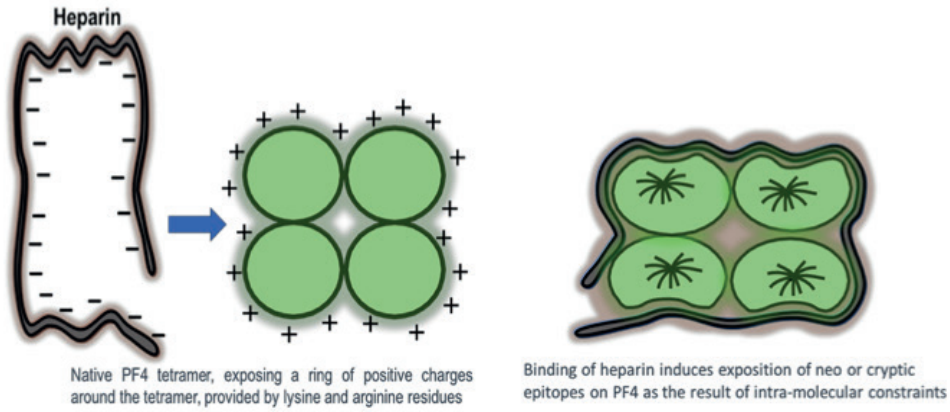
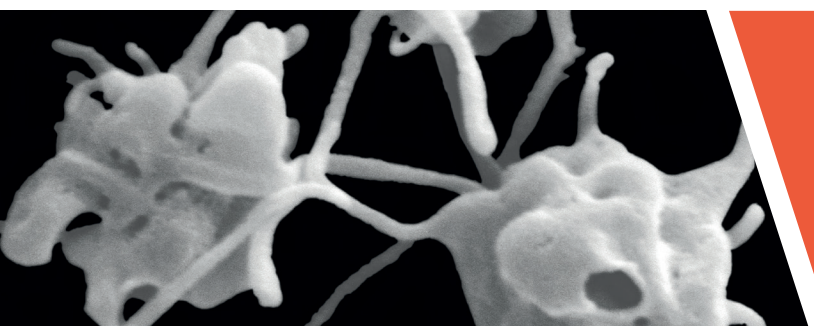


Figure 3:

In presence of heparin, PF4 forms tight complexes, inducing an alteration of the protein structure, which then exposes neo-epitopes; PF4 tetramer exposes a ring of positive charges (due to lysine and arginine residues), which strongly interact with electro-negative sulphate groups on heparin; heparin length has ti be long enough for wrapping around the PF4 tetramer, which requires a structure with at least 12-14 oligosaccharides; optimal formation of macromolecular PF4-Heparin complexes is obtained in presence of 27 IU of heparin (about 150 µg) with 1.0 mg of PF4; however, the electro-negative charge of heparin and the oligosaccharide length prevail on the anti-FXa activity for binding to PF4 and forming ultra-large complexes.

This alteration exposes PF4 epitopes, characteristic of the molecule involvement in heparin complexes, which can bind heparin dependent antibodies. Enzyme Linked Immuno-Sorbent Assays (Elisa) for heparin dependent antibodies, use stoichiometric HPF4 complexes, or complexes of PF4 with PVS for coating the Elisa microwell, which then captures antibodies, when present.

An Anti- IgG linked with peroxidase, or another label, is then introduced for characterizing immobilized IgG antibodies. A more dynamic assay has been introduced in the form of plates coated with a limited amount of protamine sulfate



complexed with an excess of heparin, which is then biologically available (Zymutest HIA). The tested sample is introduced in the heparin containing well, along with a platelet lysate (source of PF4, and other platelet releasable proteins), mimicking what occurs at pathogenic sites where platelets concentrate and are activated, releasing their content. When antibodies are present, platelet PF4 forms complexes with heparin and binds antibodies: the reactivity kinetics for antibodies' binding are directly dependent on their affinity. There is now evidence that heparin dependent / anti-HPF4 antibodies with the IgG isotype are those with the highest significance and association with symptomatic HIT, although in many cases they can remain asymptomatic.

Retrospective analysis in patients diagnosed with HIT, and positive IgG anti-HPF4 antibodies, could help in understanding the risk incidence of asymptomatic IgGs to switch to symptomatic if heparin is continued. If patient plasma or serum samples from the days preceding the diagnosis of HIT are available, testing and isotyping antibodies could help to document this concern. Prospective studies, in patients with prolonged heparin therapy and without HIT, have shown that the various IgG, IgA and IgM isotypes have close kinetics profiles, although IgG reactivity uses to remain low, and rarely increases later. Using an excess of heparin in the Elisa (by diluting the sample in presence of a high heparin concentration) can significantly reduce the antibody reactivity, providing some evidence on the heparin dependence. This is explained by the disruption of HPF4 complexes by a high heparin concentration. However, insome HIT cases, as for example those with a strong reactivity to PF4 alone, there is not, or only a low, inhibition by an excess of heparin. Complementary investigations are then needed for establishing the diagnosis of HIT, especially with the evaluation of the clinical probability. There is nevertheless a clear association between the immunoassay reactivity and the disease risk. For most of the Elisas for heparin dependent antibodies, the clinical cut-off value is defined for an OD of 0.50, but almost all the patients with HIT have an OD > 1.00, or much higher. ■