

INCIDENCE OF THE VARIOUS HEPARIN DEPENDENT ANTIBODY ISOTYPES IN A GROUP OF PATIENTS WITH HEPARIN INDUCED THROMBOCYTOPENIA

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Introduction

- Many controversies are still existing on the incidence of the various heparin dependent antibody isotypes in Heparin Induced Thrombocytopenia (HIT), and on the possible involvement of IgA and/or IgM isotypes.
- Recent reports tend to demonstrate that thrombocytopenia and thrombosis in HIT are mainly associated with the presence of IgG isotypes.
- Our goal was to analyze the incidence of the various isotypes in a group of patients with clinically characterized HIT (kinetics course of platelet counts following onset of heparin therapy, normalization following its withdrawal, positive platelet aggregometry tests) and with positive heparin dependent antibodies, measured with ELISA.

Method

- Patients (N=50) with characterized HIT (clinical diagnosis of HIT, thrombocytopenia associated with heparin therapy and positive platelet aggregation tests) were from three university hospitals and were evaluated for the whole of heparin dependent antibodies (Zymutest HIA or Asserachrom HPIA), then isotyped (Zymutest HIA, IgG, IgA, IgM).
- As controls, 90 normal citrated plasmas were tested.
- Results were expressed as A450 and positivity was defined by A450 values higher than the Mean value in normals +3SDs, i.e.:
 - $A_{450} \geq 0.50$ for the global assay
 - $A_{450} \geq 0.30$ for each separate isotype

Results

- Both global assays correlated well ($r^2=0.92$).
- Normal plasmas yielded $A_{450} < 0.20$ (excepted 1 for IgA: $A_{450}=0.30$ and 2 for IgM: A_{450} of 0.35 and 0.37).
- Among the 50 positive HIT plasmas, 31 were positive only for IgG (Mean $A_{450}= 1.85$), 12 for IgG and IgA, 2 for IgG and IgM, 4 for IgG, IgA and IgM and 1 for only IgM.
- In few cases, A_{450} for IgA were higher than A_{450} for IgG.
- The addition of A_{450} values for IgG, IgA and IgM for each patient matched well with the A_{450} values obtained for the global assay.

Isotype distribution in 50 patients with clinical diagnosis of HIT and heparin dependent antibodies

Patient group	Positive N (%)	Mean A450			A450 range		
		IgG	IgA	IgM	IgG	IgA	IgM
Global	50 (100%)	1.76	0.50	0.36	0.11-2.69	0.20-1.88	>0.20-1.70
IgG only	32 (62%)	1.85	<0.30	<0.30	0.60-2.62	<0.30	<0.30
IgG & IgA	12 (24%)	1.78	1.16	<0.30	0.60-2.69	0.57-1.88	<0.30
IgG & IgM	2 (4%)	1.02	<0.30	0.90	0.90-2.12	<0.30	0.60-1.20
IgG, IgA & IgM	4 (8%)	1.65	0.93	0.96	1.31-1.97	0.60-1.42	0.80-1.30
IgM only	2 (4%)	0.11	0.30	1.50	<0.30	<0.30	1.29-1.70

- In this group, only 2 patients had only IgM.
- Among the 50 patients, 48 had IgG at moderate or high levels, isolated (N=32) or associated with IgA (N=12), or with IgM (N=2) or both (N=4).
- Some patients (N=3) presented with high IgA and low IgGs.

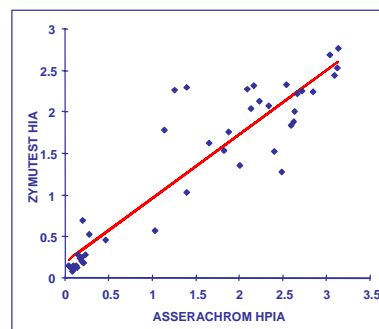
References

- Warkentin TE et al. Impact of the patient population on the risk for heparin-induced thrombocytopenia. Blood 2000; 96(5): 1703-8.
- Lindhoff-Last E et al. Determination of heparin-platelet factor 4-IgG antibodies improves diagnosis of heparin-induced thrombocytopenia. Br J Haematol 2001; 13(4): 886-90.
- Warkentin TE et al. Gender imbalance and risk factor interactions in heparin-induced thrombocytopenia. Blood 2006; 108(9): 2937-41.
- Meyer O et al. Report of a patient with heparin-induced thrombocytopenia type II associated with IgA antibodies only. Clin Appl Thromb Haemost 2006; 12(3): 373-5.
- Greinacher et al. Heparin-induced thrombocytopenia: a prospective study on the incidence, platelet-activating capacity and clinical significance of anti-PF4/heparin antibodies of the IgG, IgM, and IgA classes. J Thromb Haemost May 2007.

Conclusion

- This study shows that in the HIT patient group tested (clinical diagnosis of HIT, positive Platelet Aggregation Tests and presence of heparin dependent antibodies), IgG isotypes are present in almost all of the patients, alone or associated with IgA, IgM or both. Only two patients presented with IgM only.
- In many cases, high levels of IgA isotypes are observed. Their clinical relevance still needs to be determined.
- Type of recruitment (and time of analysis respectively to platelet count drop) can have an important incidence on the isotype distribution.
- Additional investigations are required for better documenting the pathogenicity of the various isotypes, and evaluating if clinical complications of HIT could develop in some patients in the absence of IgG isotypes.

Correlation between both assays for heparin dependent antibodies for total immunoglobulins (HIT & normals)



There is a good correlation between total heparin dependent antibodies measured with ZYMUTEST HIA and global anti-H-PF4 antibodies measured with ASSERACHROM HPIA.

A450 for normals (N=90)

Isotypes	Mean	SD	Range
Total Igs	0.06	0.04	0.04-0.35
IgG	0.05	0.01	0.02-0.09
IgA	0.09	0.04	0.04-0.30
IgM	0.13	0.06	0.05-0.37

A total specificity is demonstrated on the normal group

Discussions

- Isotype distribution in HIT is still discussed. Recent reports suggest that the IgG isotype is far the most significant for association with clinical complications of HIT (thrombocytopenia, skin necrosis and thrombosis).
- In our experience the patient group studied (characterized by the pathologies which are associated with the development of HIT) have a strong incidence on the isotype distribution of heparin dependent antibodies.
- The pathological stage where clinical HIT is suspected or diagnosed can also be an important parameter (early diagnosis with only thrombocytopenia or more advanced complication). This can explain some reports suggesting occurrence of HIT with IgM or IgA or both isotypes only.
- IgA isotype could have some clinical significance. We recently analyzed citrated plasmas from two patients with clinically diagnosed HIT, who had only the IgA isotype at high concentrations (data not shown)