
Immunobiology and Pathophysiology of Heparin-Induced Thrombocytopenia/Thrombosis Syndrome-An Update

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ABSTRACT

Heparin-induced thrombocytopenia (HIT) syndrome is one of the more frequent and dangerous autoimmune complications of heparin therapy in clinical setting. It is now widely accepted that heparins are capable of complexing with endogenous chemokines and modify at molecular level to trigger the HIT-associated antibodies responsible for pathogenesis. Newer evidence suggests a functional heterogeneity in the HIT antibodies. Besides platelet factor IV (PF IV), there are several endogenous factors, which are responsible for the upregulation of HIT antibodies in various prophylactic and therapeutic regimens. While the pathophysiology and the mechanisms of action in HIT are rather complex, the role of IgG subtype antibodies is clearly established in mediating the pathogenesis. Currently available antithrombin drugs seem to be promising therapeutic modalities to combat the severe thrombotic episode and platelet activation associated with HIT. The clinical relevance of the pathologically nonfunctional HIT antibodies and the mechanism(s) of their formation, in terms of both correlative evidence and causal relationships, need further investigation.

Key Words: Autoimmune disorder, Heparin-induced thrombocytopenia (HIT), Antiheparin-PF IV antibodies, Platelet activation, Pathophysiology of HIT, Antithrombin drugs.

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Heparin was originally discovered in 1916 from liver extracts, and currently, it is more commonly prepared from either bovine or porcine mucosal extracts. The mechanism of action of this polyelectrolyte is via binding to antithrombin III, which then inactivates the activated coagulation F IIa, F IXa, and F Xa. Heparins are one of the most commonly administered parenteral therapies in the hospital setting as patients are increasingly being exposed and reexposed to heparins. App-

roximately 1 trillion units of heparins are used per year in the United States, and approximately 12 million patients are exposed to heparin per year^[1]. Despite its remarkable therapeutic spectrum and ubiquity, the use of unfractionated heparin (UFH) has many drawbacks, including nonimmune- and immune-mediated thrombocytopenia^[2,3]. Venous complications are observed more frequently than arterial events in a ratio of approximately 4:1 depending upon the heparin treatment^[4].

Deep venous thrombosis and pulmonary embolism are most commonly treated with UFH; however, UFH is also used in the treatment of other thrombotic disorders as myocardial infarction, unstable angina, and is used with various interventional procedures^[5,6]. The incidence of HIT appears to be greater in patients with underlying hemostatic activation and/or inflammation in various autoimmune and cancer disorders^[7-9].

HIT Classification

Two types of HIT have been classified, namely:

a. Nonimmune-mediated (type I HIT), occurs in approximately 10-30% of patients exposed to UFH^[10]. Typically, mild thrombocytopenia develops within 1-2 days of UFH exposure and normalizes despite continued heparin exposure^[10]. No significant adverse clinical events associated with this syndrome.

b. Immune-mediated (type II HIT), is an adverse drug reaction caused by heparin-dependent IgG antibodies that activate platelets/endothelial cells. It is less common (1-5% incidence); however, its complications are potentially devastating, including a very high association with thrombotic events. Here onwards, the term "HIT" is referred to as immune-mediated HIT syndrome.

Clinical Characteristics

The HIT syndrome is characterized by one or more of the following clinical features:

- i. A platelet count of less than 100,000/ μ L or a 50% decrease from the baseline,
- ii. Normalization of the platelet count following UFH cessation, and
- iii. Clinical identification of associated thrombotic complications.

While hemorrhage is rarely associated with HIT, nearly one-third of the patients will progress to overt thrombosis requiring amputation. The mortality of HIT/thrombosis is approximately 30%^[11].

Immunobiology and Pathophysiology of HIT Syndrome

It is now widely accepted that heparins are capable of complexing with endogenous PF IV and modifying the neoepitope at molecular level to trigger the generation of anti-heparin-PF IV (AHPF IV) antibodies

in the pathogenesis of HIT syndrome^[12,13]. PF IV (~32 kDa) is a basic protein that belongs to the C-X-C family of chemokines, which is found in the α -granules of platelets and has high binding affinity for heparins^[14]. On the other hand, heparin is a highly electro-negatively charged molecule, which wraps around the PF IV tetramer, reacting on a charge basis^[13,15]. It is believed that if appropriate stoichiometric concentrations of PF IV and heparin occur, immunogenic complexes form and antibodies are generated^[10,13,16]. In addition, a variety of other chemokines of the C-X-C family, such as interleukin-8 (IL-8), neutrophil-activating peptide 2 (NAP 2), interferon- γ -inducible protein 10 (gIP 10), platelet basic protein (PBP), and β -thromboglobulin, etc., have also been implicated to form complexes with the circulating heparins and generate respective antibodies, which may or may not cause thrombocytopenia^[13].

The H-PF IV complexes exhibit a neoantigenic behavior and trigger the generation of a heterogeneous group of platelet activating (functional)/nonactivating (nonfunctional) AHPF IV antibodies^[17,18]. Furthermore, PF IV mobilized in certain pathologic conditions is capable of complexing with exposed glycosaminoglycans (GAGs), such as the endothelial and subendothelial heparin sulfate, resulting in the generation of neoepitope to trigger the AHPF IV antibodies. These antibodies exhibit differential interactions with Fc receptors, particularly with Fc γ RIIA (CD32) receptors on platelets/endothelium to lead to thrombocytopenia and/or endothelial/mononuclear (leukocyte) cell dysfunction and secondary pathogenic manifestations^[19,20]. Subsequently, protein kinases (presumably through intracellular Ca⁺² signaling and related second messenger systems) are activated and platelet-derived microparticles are also released. Recently, it has been shown that these platelet microparticles are a significant component of the thrombotic event^[21].

HIT Laboratory Diagnostic Observations

While both UFH and low-molecular-weight heparin (LMWH)s are associated with the generation of AHPF IV antibodies at almost a similar frequency, the prevalence of clinical thrombocytopenia is markedly lower (5.2% vs. 0.8%) in the LMWH-treated patients^[10,18,22]. Also, the results from several clinical tri-

als on heparins suggest that patients' own predisposing factors and pathologic states (such as medical and surgical conditions, sepsis, infection, hypercholesterolemia, and malignancy, etc.) may augment the generation of AHPF IV antibodies and their functionality^[9]. Similarly, the release of endogenous antithrombotic mediators, such as tissue factor pathway inhibitor (TFPI), downregulates the AHPF IV antibodies. Once the antibodies are generated, these antibodies are capable of amplifying further propagation of their production through the generation of various cytokines/growth factors because of the continuous endothelial dysfunction, hypercoagulable state, fibrinolytic deficit, or inflammatory processes, in a given patient during the course of heparin therapy.

In clinically confirmed patients with HIT, the Ig subtyping utilizing ELISA revealed a predominance of IgG subtype antibodies in contrast to the nonsymptomatic high AHPF IV antibodies in patients plasma which showed mostly IgM and/or IgA subtypes in contrast to IgG. In three separate major clinical trials with LMWHs (CORTES, ECHOS, and PLASTERCAST), where clivarine (Reviparin[®],) was used in various prophylactic and therapeutic regimens, the prevalence of AHPF IV antibodies was found to be slightly lower to the one observed with UFH treated patients^[23-25]. Almost all of these antibodies were found to be nonfunctional in terms of platelet activation in HIT laboratory assays, and were predominantly of the IgM and/or IgA subtypes.

UFH was found to generate an increased amount of AHPF IV antibodies in patients who have undergone knee surgery (as compared to the hip surgery), whereas in the same group the LMWHs are devoid of this effect^[24]. Similarly, in cancer patients, UFH produced an increased level of AHPF IV antibodies with a stronger functional index. Additional studies in patients with antiphospholipid syndrome (APS), septicemia, rheumatoid arthritis, pregnancy, and hypertension also revealed a higher prevalence of the AHPF IV antibodies, which were predominantly of the IgM and/or IgA subtypes (nonfunctional). These observations suggest that presumably there are several endogenous factors, which are also responsible for the enhanced upregulation of AHPF IV antibodies in various prophylactic and therapeutic regimens.

Most recently, we determined the subtypes and functionality of AHPF IV antibodies in clinically suspected HIT patients and found that IgG subtype is the principal mediator of platelet activation, with IgA and/or IgM playing less significant role in the immunobiology and pathophysiology of this catastrophic syndrome^[26]. Thus, a test to determine the AHPF IV IgG subtype would be of greater sensitivity in detecting patients with HIT syndrome^[26,27]. However, further experiments on the pathogenicity of IgA and IgM are warranted to better understand the cross-talk among these subtypes/isotypes of antibodies in the HIT pathogenesis. Assuming that if IgA and IgM subtypes do not contribute to the HIT syndrome, or do so on a limited basis, pharmacologic agents that prevent the upregulation of IgG subtype of AHPF IV antibodies may be an appropriate therapeutic option.

Endogenous Factors in HIT Syndrome

To further investigate the pathogenesis of HIT-associated antibodies, several surrogate markers of inflammation [tissue factor (TF), plasminogen activator inhibitor 1 (PAI-1), interleukin-6 (IL-6), IL-2, thrombomodulin (TM), TFPI, tumor necrosis factor- α (TNF α), etc.] were measured in clinically diagnosed HIT patients^[28]. In addition, adhesion molecules (P-, E-, and L-selectins), PF IV, thromboxane B₂, and markers of thrombin generation were evaluated. Varying degrees of upregulation in these markers suggested the poly-pathologic nature of symptomatic HIT-associated antibodies^[28]. In contrast, the LMWHs-treated individuals failed to demonstrate such an increase in many of these cytokines/surrogate markers despite a comparable generation of the ELISA-detectable AHPF IV antibodies^[28].

Synopsis

Based on the current understanding in the literature and our clinical/laboratory experiences, it is clear that the pathophysiology of HIT syndrome is rather complex and involves multiple mechanisms which are dependent not only on the type of heparins used but to a large part determined by the endogenous pathophysiological status of the patient. The fundamental paradox of HIT syndrome is that an anticoagulant turns procoagulant, and thus, an antithrombotic causes thrombosis. The treatment goals based on the known pathophysiology and clinical observations are to interrupt the

immune response by discontinuing heparin, and inhibit thrombin generation. Antithrombin drugs are promising novel agents to treat active thrombosis and prevent new thrombosis. The diagnostic significance of the ELISA-detectable AHPF IV antibodies without the simultaneous considerations of the clinical symptoms and the presence of certain surrogate markers is of crucial value in the management of symptomatic HIT patients. The clinical relevance of the pathologically non-functional AHPF IV antibodies and the mechanism(s) of their formation leading to the HIT syndrome need further investigation.

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REFERENCES

1. Fahey VA. Heparin-induced thrombocytopenia. *J Vasc Nurs* 1995;13:112.
2. Chong BH. Heparin-induced thrombocytopenia. *Aust N Z J Med* 1992;22:145.
3. Hirsh J, Warkentin TE, Shaughnessy SG, et al. Heparin and low-molecular-weight heparin: Mechanisms of action, pharmacokinetics, dosing considerations, monitoring, efficacy, and safety. *Chest* 2001;119:64A.
4. Warkentin TE, Kelton JG. A 14 year study of heparin-induced thrombocytopenia. *Am J Med* 1996; 101:502.
5. Dalen JE, Alpert JS. Natural history of pulmonary embolism. *Prog Cardiovasc Dis* 1975;17:257.
6. Creager MA, Beckman JA. Assessment of arterial and venous thrombosis. In: Loscalzo J, Schafer A, (eds). *Thrombosis and Hemorrhage*. Baltimore: Williams & Wilkins, 1998:491.
7. Ramos RS, Salem BL, Haikal, et al. Critical role of pulmonary angiography in the diagnosis of pulmonary emboli following cardiac surgery. *Cath Cardiovasc Dis* 1995;36:112.
8. Amiral J, Wolf M, Fischer A, et al. Pathogenicity of IgA and/or IgM antibodies to UFH-PF IV complexes with UFH-induced thrombocytopenia. *Br J Haematol* 1996;92:954.
9. Fareed J, Walenga JM, Hoppensteadt DA, et al. Selectins in the HIT syndrome: Pathophysiologic role and therapeutic modulation. *Semin Thromb Hemost* 1999;25(Suppl 1):37.
10. Fabris F, Ahmad S, Cella G, et al. Pathophysiology of heparin-induced thrombocytopenia: Clinical and diagnostic implications-a review. *Arch Pathol Lab Med* 2000;124:1657.
11. Warkentin TE, Chong BH, Greinacher A. Heparin-induced thrombocytopenia: Towards consensus. *Thromb Haemost* 1998;79:1.
12. Amiral J, Bridey F, Dreyfus M, et al. Platelet factor IV complexed to heparin is the target for antibodies generated in heparin induced thrombocytopenia. *Thromb Haemost* 1992;68:95.
13. Amiral J, Marfaing-Koka A, Poncz M, et al. The biological basis of immune heparin-induced thrombocytopenia. *Platelets* 1998;9:77.
14. Brandt E, Flad HD. Structure and function of platelet-derived cytokines of the β -thromboglobulin/interleukin 8 family. *Platelets* 1992;3:295.
15. Nieduszynski I. General physical properties of heparin. In: Lane DA, Lindahl U (eds). *Heparin-Chemical and Biological Properties, Clinical Applications*. Boca Raton: CRC Press Inc, 1989:51.
16. Bock PE, Luscombe M, Marshall SE, et al. The multiple complexes formed by the interaction of platelet factor IV with heparin. *Biochem J* 1980;191:769.
17. Ahmad S, Walenga JM, Jeske WP, et al. Functional heterogeneity of antiheparin-platelet factor IV antibodies: Implications in the pathogenesis of HIT syndrome. *Clin Appl Thromb Hemost* 1999;5(Suppl 1):32A.
18. Ahmad S, Walenga JM, Jeske WP, et al. Lack of thrombocytopenic response after low molecular weight heparins usage is due to the generation of non-functional antiheparin-platelet factor IV antibodies. *J Am Coll Cardiol* 2000;35(Suppl A):316A.
19. Herbert J-M, Savi P, Jeske WP, et al. Effect of SR121566A, a potent GPIIb/IIIa antagonist on the HIT serum-induced platelet mediated activation of human endothelial cells. *Thromb Haemost* 1998; 80:326.
20. Blank M, Shoenfeld Y, Tavor S, et al. Antiplatelet factor IV/heparin antibodies from patients with heparin-induced thrombocytopenia provoke direct activation of microvascular endothelial cells. *Int Immunol* 2002;14:121.
21. Hughes M, Hayward CPM, Warkentin TE, et al. Morphological analysis of microparticle generation in heparin-induced thrombocytopenia. *Blood* 2000; 96:188.
22. Walenga JM, Lewis BE, Hoppensteadt DA, et al. Management of heparin-induced thrombocytopenia and heparin-induced thrombocytopenia with thrombosis syndrome. *Clin Appl Thromb Hemost* 1997;3 (Suppl 1):53A.
23. Breddin HK, Hach-Wunderle V, Nakov, et al. Effects of a low-molecular-weight heparin on thrombus regression and recurrent thromboembolism in patients with deep-vein thrombosis. *N Engl J Med* 2001; 344:626.
24. Ahmad S, Haas S, Hoppensteadt DA, et al. Differential effects of clivarin and heparin in patients undergoing hip and knee surgery for the generation of anti-heparin-platelet factor IV antibodies. *Thromb Res* 2002; in press.
25. Ahmad S, Lietz H, Daud AN, et al. Upregulation of tissue factor pathway inhibitor by a low-molecular-weight heparin, clivarin, is associated with decreased prevalence of antiheparin-PF IV antibodies: Diagnostic and therapeutic implications. *Int Angiol* 2001;20(Suppl 1):42A.
26. Untch B, Ahmad S, Jeske WP, et al. Prevalence, isotype,

- and functionality of anti-heparin-platelet factor IV antibodies in patients treated with heparin and clinically suspected for heparin-induced thrombocytopenia: The pathogenic role of IgG. *Thromb Res* 2002;105:17.
27. Lindhoff-Last E, Gerdson F, Achermann H, et al. Determination of heparin-platelet factor IV-IgG antibodies improves diagnosis of heparin-induced thrombocytopenia. *Br J Haematol* 2001;113:886.
 28. Ahmad S. Endogenous factors contributing to the generation of heparin-PF IV antibodies. Presentation at the 47th Annual Scientific & Standardization Subcommittee (SSC) Session on "Control of Anticoagulation-Part II" of the ISTH, July 7, 2001, Paris, France.

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