Eastern Journal of Medicine 14 (2009) 78-83

Review Article

Diagnostic strategy in heparin induced thrombocytopenia

Meganathan Kannan[a,d*], Sarfraz Ahmad[b], Jawed Fareed[c], Renu Saxena[a]

[a]*Department of Hematology, All India Institute of Medical Sciences, New Delhi, India*
[b]*Florida Hospital Cancer Institute, Orlando, Florida 32804*
[c]*Department of Pharmacology, Loyola University of Medical Center, Chicago, IL 60153*
[d]*Division of Hematology, U.S. Food and Drug Administration, Bethesda, MD 20892*

Abstract. Heparin-induced thrombocytopenia occurs due to the formation of antibodies against the complex formed between heparin and platelet factor 4 (H-PF4) leading to platelet/endothelial cell activation followed by thrombocytopenia. Diagnosis of H-PF4 antibodies are mainly based on two different assays; functional and immunologic assays. While the functional assays are based on the platelet activation, the immunogical assays are based on the binding of IgG antibodies to H-PF4 complexes. Even though ^{14}C-Serotonin Release Assay is high in sensitivity, the most commonly used functional assays are Platelet aggregation test and Heparin-ınduced platelet aggregation The immunologic assays include solid-phase detection, fluid-phase detection, enzyme-linked lmmunosorbent assay, particle gel immunoassay; the commonly used is enzyme-linked ımmunosorbent assay occurrence of HIT may vary not only based on the patients' group but also based on the type of diagnostic technique used. The methodological variations observed in our experience are discussed in this review. In the current review we discuss the diagnostic approach, importance in the diagnosis of FcγRIIa receptor polymorphism, controversies in diagnosis of HIT and preventive measures.

Key words: Trombocytopenia, diagnostic approach, clinical and laboratory diagnosis

1. Introduction

Heparin induced thrombocytopenia (HIT) occurs due to the formation of heparin platelet factor 4 (H-PF4) antibodies that commonly results in platelet count reduction, thrombus formation and thrombocytopenia (1-8). The pathophysiology of HIT is complex due to the functional heterogeneity among the H-PF4 antibodies (9-12). The type I HIT, non-immune mediated, occurs due to the heparin toxicity that occurs immediately after the infusion of heparin whereas the type II HIT, immune mediated occurs after 5-15 days of heparin therapy (13). Diagnosis of HIT antibodies in patients treated with heparin is important because it can be a serious problem, if not diagnosed. The types of diagnostic tests developed, which are mostly based on functional and immunologic assays include Heparin-ınduced platelet aggregation (HIPA), ^{14}C-serotonin release assay (SRA) and Enzyme-Linked Immunosorbent Assay (ELISA) (13-19). The frequency of HIT, in patients treated with heparin, in western countries is between 1% and 5%. Worldwide, however, the prevalence of H-PF4 antibodies vary significantly based on the laboratory diagnosis of HIT (utilizing both immunologic and functional assays), ranging from 10-50%, depending on the type/route of heparin used and also the surgical/medical conditions of the patients (20). Our preliminary study reported the prevalence of HIT among Indians to be about 6% (based only on the HIPA and ELISA test), but the frequency of occurrence rose to 15% when tested the same samples using C-serotonin release assay (SRA) (9). This certainly shows the role of technology used in the diagnosis of HIT antibodies. There are postulations suggesting HIT to have relation with FcγRIIa polymorphism on platelets (21, 22). HIT occurrence may be prone in individuals in

*Correspondence : Dr. Meganathan Kannan, PhD
Division of Hematology,
Center for Biologics Evaluation and Research (CBER),
U.S. Food and Drug Administration (FDA),
Bldg. NIH 29A, Room 2C-10, 8800 Rockville Pike,
Bethesda, MD 20892. USA.
Phone: 301-827-2841 (Off)
Email: kannan.meganathan@fda.hhs.gov

whom homozygous FcγRIIa polymorphism are seen; alternate anticoagulants such as antithrombin agents, fondaparinux or argatroban needs to be chosen in these cases (23-25). Because HIT is life threatening there is a need in timely diagnosis either by clinical or by laboratory methods. Here in this review, we discuss the diagnostic approach, controversies in diagnosis of HIT and preventive measures.

2. Pathophysiology

During the past decade, despite some myths and misconceptions a reasonably good understanding of HIT pathogenesis has been achieved (26-32). Heparin, the negatively charged polysaccharide molecules binds to the positively charged protein tetramer, PF4, to form H-PF4 complexes. As a result, antibodies are formed against H-PF4 complexes that lead to platelet activation followed by thrombocytopenia, activation of endothelial cells and monocytes, and subsequently results in HIT. The binding of heparin to the platelet factor 4 is related to the chain length and degree of sulfation, perhaps explaining why un-fractionated heparin (UFH) is more likely to cause HIT than low molecular weight heparin (LMWH). At the molecular level, HIT is caused by binding of antibodies to the platelet Fcγ receptor that synthesized by the *FcγRIIa* gene, thereby causing platelet activation. It has been postulated that the nucleotide change of Arg-131-His of FcγRIIa receptor may affect the risk of developing HIT, although there are conflicting studies, with some showing an association, whereas others do not. Our previous report suggest the strong association between FcγRIIA polymorphism and development of HIT (33).

3. Diagnostic approach in HIT

3.1.Clinical diagnosis:

The early diagnosis of HIT is largely based on clinical findings, such as platelet count reduction with or without thrombosis in patients exposed to heparin (34). Before the laboratory diagnosis for the presence of H-PF antibodies, HIT diagnosis is made most confidently based on the clinical events that include thrombocytopenia, thrombosis, bleeding and skin lesions. On heparin therapy, the thrombocytopenia itself is, sometimes, enough to make alert hematologists and clinicians in development of HIT. Heparin toxicity results in mild thrombocytopenia but seen within 48 hours of heparin therapy in type I HIT. In the case of Type II HIT, the thrombocytopenia is severe and does not recover unless heparin is discontinued. Sometimes patients may develop risk of bleeding complications which include mucocutaneous bleeding, ranging from petechiae and ecchymoses to gastrointestinal hemorrhage. Though the risk is low, it is estimated to be less than 10%, and is life threatening in the case of open heart surgery. Thrombosis is one of the severe complications of HIT and the thrombotic complications include deep vein thrombosis of legs, stroke, myocardial infarction and pulmonary embolism. Patients developing thrombosis are more prone to high mortality and morbidity. Other clinical syndromes include skin lesion at the site of heparin injection and acute inflammatory reaction such as fever, chills etc. Approximately 10% to 20% of patients, who develop HIT antibodies during subcutaneous heparin injection, develop skin lesion at the site of heparin injection. In our experience, we did not find any HIT patients with thrombosis; rather bleeding complication was observed (35).

3.2. Laboratory diagnosis:

The diagnosis of HIT, based on reduction in platelet count alone, may be fallacious because other diseases such as septicemia, idiopathic immune thrombocytopenia, post-transfusion purpura and drugs like sulfa preparations can also induce thrombocytopenia. Hence, specific laboratory tests are required to confirm diagnosis of HIT. Among the functional and antigen assays, functional assays are considered to be reliable as it diagnose the functional H-PF4 antibodies. The functional tests like heparin induced platelet aggregation (HIPA), serotonin release assay (SRA), and flow cytometric assays take advantage of the ability of diagnosing HPF4 antibodies in the presence of therapeutic amounts of heparin. All these tests require a source of normal donor platelets and are performed with platelet-rich plasma (PRP) of the donor. Even though the advantage of HIPA is a rapid and technically simple, the result strongly varies with donor platelet (pre-determined to be HIT antibody-reactive donors). As the platelet from different healthy donors can respond differently to HIT plasma, it is important to use platelet that are known to respond to HIT plasma and should be included donors from more than one source. The other major factor in HIPA is the use of heparin concentration; because the sensitivity of HIPA test is considered to be dependent on the heparin concentration, care was given to use the therapeutic concentration of heparin in our experiment. From our experience, to get the maximal sensitivity, the heparin concentration

should be performed with 0.25 U/mL. The assay sensitivity can be improved very much if the donor PRP is from 'O' group. The ^{14}C-serotonin release assay (SRA) is based on the principle of heparin induced platelet aggregation and is the most preferred test among all the functional assays due to its high degree of diagnostic sensitivity and specificity (36,37). Furthermore, in this test, using special buffer conditions and performing the assay in microtiter wells permits the simultaneous examination of numerous reaction conditions, thus maximizing the sensitivity (38). It has the major limitation of use of radioactive material as the platelet requires labeling with 0.1µci/ml ^{14}C serotonin. Since SRA is most sensitive and HIPA is a gold standard method for the functional HIT antibody detection, we considered both these techniques for our study of prevalence of H-PF4 antibodies in patients treated with heparin. Our study showed 15% to be positive for HIT and these patients were considered to have classic HIT syndrome, because of the HIPA and SRA positivity along with thrombocytopenia. It has been reported that HIT antibodies induces microparticle formation. HIT induced microparticle formation can be measured by flow cytometry that involves labeling platelets and platelet microparticles FITC-conjugated anti-GPIb. Microparticle release is observed in presence of 0.IU/ml heparin. When compared the microparticle assay with the SRA, the overall agreement between the two assays was 96%. The microparticle assay is as accurate as the SRA and the major advantage of this technique is it does not involve any radioactive material. Moreover, H-PF4-coated polymer beads are now being utilized in some laboratories for a simple and relatively rapid flow cytometry assay, where the beads are exposed to patients' serum – binding of H-PF4 antibodies can be readily detected with fluorescent dye conjugated to human anti-IgG. But its correlation with other standard technique needs to be studies well.

Despite the advantage of the SRA and flow cytometry, the major limitations of these functional assays are their labor-intensive, technically demanding nature and associated high cost. Therefore some of the reference laboratories, particularly in developing countries, are unable to afford these technologies. Additionally, freshly prepared normal donor platelets are pre-requisite for each batch of assays, and all potential donors may not give similar reproducible responses on a day-to-day basis. In fact, only about 40% of all the potential healthy donors are reactive in the functional tests, and that is the reason these assays are poorly standardized between the laboratories. Also, these assays are not well suited for testing large volumes of samples, forcing many reference laboratories to rely on immunoassays. To overcome some of these problems/limitations, newer functional tests for HIT diagnosis at near-bedside are currently under development to provide rapid and more clinically relevant information about the H-PF4 antibody characteristics in a given patient specimen.

Considering the major limitations of the above mentioned functional assays, the rapid, high throughput solid-phase H-PF4 enzyme immunoassays are commonly used for the laboratory diagnosis of HIT. These immunologic tests directly demonstrate antibody binding to the PF4 and polyanion (e.g., heparin) complex, and therefore, merely confirm the presence of the antibody (telling no account of its ability to cause functional responses). The immunologic assays include solid-phase detection, fluid-phase detection, Enzyme-Linked Immunosorbent Assay (ELISA), particle gel immunoassay (PaGIA). The commonly used is ELISA which is a method to measure anti-PF4/heparin antibodies in patients' plasma. Patients' plasma, positive and negative controls are measured simultaneously in this test. Emerging evidence suggests that knowledge of the patient's actual H-PF4 antibody titer (OD value) may be helpful in interpreting the test results (39). While the OD of 0.400 or greater value is considered positive, most often, high OD values (>1.0) are much more likely to be associated with a positive functional test result. There are the studies showing ELISA correlation with the level of circulating thrombin-antithrombin (TAT) complex, indicating an association between higher levels of HPF4 antibodies and thrombin generation (40). This may well correlate with antibody-mediated platelet activation assays leading to higher clinical probability of HIT.

4. Importance of genetic polymorphism (*FcγRIIa)* in HIT

The *FcγRIIa* gene encoding the receptor is highly polymorphic. A nucleotide change of G507A causes an amino acid change Arg to His at position 131 of the FcγRIIa protein. The nucleotide variation of G507A in one allele results in Arg-131-His (heterozygous) and the variation in both the alleles results in Arg-131-Arg (homozygous). In wild type, since there is no nucleotide change, it is Arg-131-Arg (wild type). Ethnic variations in the occurrence of this

FcγRIIa polymorphism are well documented (41). In Western countries, this polymorphism is observed in 74% of the normal population, whereas in the Eastern ones, it is found in 51% of the normal population (11). There are postulations suggesting that prevalence of FcγRIIa polymorphism may affect that of HIT, some studies show this to be correct, whereas others negate the association of FcγRIIa polymorphism with HIT. In our experience, of the 33 patients who underwent heparin therapy for cardiovascular surgery, 10 showed a drastic reduction in platelet count (thrombocytopenia), and all of them carried at least one defective allele on the FcγRIIa platelet receptor (33). One patient with thrombocytopenia was found to be homozygous for FcγRIIA $^{His/\ His}$ polymorphism and rest of the nine patients had at least one defective allele with single amino acid change (Arg-131-His) at FcγRIIa platelet receptor. The occurrence of this polymorphism in Indians was observed to be higher than studies in the East, e.g Japan (51%) but comparable to that in the West (78%) (42). Since all of the thrombocytopenia patients had at least one defective allele, it appears that the occurrence of FcγRIIa polymorphism may predispose to develop HIT in cardiovascular surgery patients on heparin therapy. In other words, the absence of FcγRIIa receptor polymorphism, suggesting low or negligible risk of development of HIT antibodies. Thus it may be true that patients with FcγRIIa polymorphism needs to be given special care before starting heparin therapy and alternate anticoagulants like antithrombin agents, fondaparinux or argatroban needs to be chosen in these patients (43,44). The use of non-heparin anticoagulant in these patients may reduce the risk of poor outcome.

5. Controversy in the diagnosis of HIT

Though several laboratory tests are being developed to diagnose HIT, each technique has its own limitations in diagnosing H-PF4 antibodies. In our experience 9% of HIT that was detected by SRA, could not be detected by HIPA, the gold standard technique for HIT diagnosis. This suggests the existence of methodological variations in the diagnosis of HIT antibodies and it also indicates that there is a need for an additional technique in those cases with negative results. A large number of samples can be processed using ELISA, but may give positive results in patients who do not develop thrombocytopenia and those without history of heparin exposure. There are reports showing 5 to 22% patients with ELISA positive for H-PF4 antibodies, after the heparin therapy, but none developed the HIT syndrome. The negative ELISA results, on the other hand, are also observed in some studies. The negative results may be due to the antibodies against heparin that complexed to cytokines such as IL-8, rather than PF4. In our experience ELISA failed to detect 10% of patients with HIT. Thus it may be necessary to correlate HIT clinical syndrome with the laboratory tests, which are not only rapid but also reliable. Regardless of their demerits or cost-effectiveness, all the antigen (immunologic) tests are considered technically simpler to perform than the functional assays, and moreover the ELISA can be readily automated. Antigen tests can be batched and performed in large test volumes, and being commercially available, and perhaps widely standardized between the laboratories.

6. Preventive measures

Reduction in platelet count, thrombocytopenia, is the first and foremost indicator of the occurrence of possible HIT. While some patients develop thrombocytopenia immediately after the infusion of heparin, some develop after 5 days. Care needs to be given in patients who develop thrombocytopenia after the 48 hours of heparin therapy. There is a chance of developing HIT in patients who have a past history of HIT. Although patients with a past history of HIT do not invariably have recurrent HIT upon heparin re-exposure, it is considered prudent to avoid future exposures to heparin, if at all possible, in these “at risk” patients. In case of a patient with previous history of HIT/heparin exposure, in addition to the clinical observations, they need to be routinely monitored for H-PF4 antibodies using HIT diagnostic assays. Genetic analysis for the FcγRIIa polymorphism needs to be done in these cases to avoid possible HIT. When HIT is suspected, it is highly recommended to discontinue heparin immediately and alternative anticoagulation should be initiated to prevent the occurrence of true HIT. Danaparoid, lepirudin and argatroban can be used as an alternate anticoagulant but recent reports suggest that some of the alternate anticoagulant can cross-react with H-PF4 antibodies, and currently danaparoid is unavailable worldwide. The use of non-heparin anticoagulant in patients with, or at high risk of, HIT may reduce the risk of poor outcome.

Thus, the heparin treated patients need to be monitored routinely both by clinical observation and by laboratory diagnostic assays. It is highly

recommended to immediately discontinue the heparin therapy in HIT suspected patients. The presence of functional H-PF4 antibodies needs to be diagnosed not only by HIPA, the gold standard assay, but also by any of other reliable techniques like SRA. Patients with certain predisposing factors, including immunologic issues, underlying pathogenesis, genetic polymorphism, age and gender are the important factors to be considered in the assessment of the outcome of HIT.

References

1. Warkentin TE. Heparin-induced thrombocytopenia. Hematol Oncol Clin North Am 2007; 21: 589-607.
2. Gitau MM, Arora N. Heparin-induced thrombocytopenia. Mo Med 2009; 106: 50-54.
3. Kelton JG, Warkentin TE. Heparin-induced thrombocytopenia: a historical perspective. Blood 2008; 112: 2607-2616.
4. Unkle DW. Heparin-induced thrombocytopenia. Orthop Nurs 2007; 26: 383-5; quiz 386-387.
5. Amiral J, Marfaing-Koka A, Poncz M, Meyer D. The biological basis of heparin-induced thrombocytopenia. Platelets 1998; 9: 77-91.
6. Swanson JM. Heparin-induced thrombocytopenia: a general review. J Infus Nurs 2007; 30: 232-240.
7. Girolami B, Girolami A. Heparin-induced thrombocytopenia: a review. Semin Thromb Hemost 2006; 32: 803-809.
8. Warkentin TE. Clinical picture of HIT. In: Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenis. 3rd Edition. New York, NY: Marcel Dekker 2004; 53-106.
9. Kannan M, Saxena R, Adiguzel C, Fareed J. An update on the prevalence and characterization of H-PF4 antibodies in Asian-Indian patients. Semin Thromb Hemost 2009; 35: 337-343.
10. Ahmad S, Untch B, Haas S, et al. Differential prevalence of anti-heparin-PF4 immunoglobulin subtypes in patients treated with clivarin and heparin: Implications in the HIT pathogenesis. Mol Cell Biochem 2004; 258: 163-170.
11. Fabris F, Ahmad S, Cella G, Jeske WP, Walenga JM, Fareed J. Pathophysiology of heparin-induced thrombocytopenia: Clinical and diagnostic implications – a review. *Arch Pathol Lab Med* 2000; 124: 1657-1666.
12. Castelli R, Cassinerio E, Cappellini MD, Porro F, Graziadei G, Fabris F. Heparin induced thrombocytopenia: pathogenetic, clinical, diagnostic and therapeutic aspects. Cardiovasc Hematol Disord Drug Targets 2007; 7: 153-162.
13. Kannan M, Ahmed R, Kale S, Ahmad S, Fareed J, Saxena R. Laboratory diagnosis of heparin-induced thrombocytopenia in Asian Indians as investigated with functional and immunologic methods. *Clin Appl Thromb Hemost* 2004; 10: 51-54.
14. Elalamy I, Lecrubier C, Horellou MH, Conard J, Samama MM. Heparin-induced thrombocytopenia: Laboratory diagnosis and management. *Ann Med* 2000; 32: 60-67.
15. Prechel M, Walenga JM. The laboratory diagnosis and clinical management of patients with heparin-induced thrombocytopenia: an update. Semin Thromb Hemost 2008; 34: 86-96
16. Kannan M, Ahmad S, Ahmad F, et al. Functional characterization of antibodies against heparin-PF4 complex in heparin-induced thrombocytopenia patients in Asian-Indians: Relevance to inflammatory markers Blood Coagul Fibrinolysis 2005; 16: 487-490.
17. Walenga JM, Jeske WP, Wood JJ, Ahmad S, Lewis BE, Bakhos M. Laboratory tests for heparin-induced thrombocytopenia: A multicenter study. Semin Hematol 1999; 36: 22-28.
18. Lee DH, Warkentin TE, Denomme GA, Hayward CP, Kelton JG. A diagnostic test for heparin-induced thrombocytopenia: detection of platelet microparticles using flow cytometry. Br J Haematol 1996; 95: 724-731.
19. Risch L, Bertschmann W, Heijnen IA, Huber AR. A differentiated approach to assess the diagnostic usefulness of a rapid particle gel immunoassay for the detection of antibodies against heparin-platelet factor 4 in cardiac surgery patients. *Blood Coagul Fibrinolysis* 2003; 14: 99-106.
20. Warkentin TE, Greinacher A. Heparin-induced thrombocytopenia: Recognition, treatment, and prevention. The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004; 126: 311S-337S.
21. Brandt JT, Isenhart CE, Osborne JM, et al. Anderson, 1995. On the role of platelet Fc gamma RIIa phenotype in heparin-induced thrombocytopenia. Thromb. Haemost 1995; 74: 1564-1572.
22. Lehrnbecher TL, Foster CB, Zhu S, et al. Blauvelt and S.J. Chanock, Variant genotypes of FcgammaRIIIA influence the development of Kaposi's sarcoma in HIV-infected men. Blood 2000; 95: 2386-2390.
23. Harenberg J, Wehling M. Current and future prospects for anticoagulant therapy: inhibitors of factor Xa and factor IIa. Semin Thromb Hemost. 2008; 34: 39-57.
24. Fareed J, Hoppensteadt DA, Fareed D, et al. Survival of heparins, oral anticoagulants, and aspirin after the year 2010. Semin Thromb Hemost 2008; 34: 58-73.
25. Krauel K, Fürll B, Warkentin TE, et al. Heparin-induced thrombocytopenia--therapeutic concentrations of danaparoid, unlike fondaparinux and direct thrombin inhibitors, inhibit formation of platelet factor 4-heparin complexes. J Thromb Haemost 2008; 6: 2160-2167.
26. Selleng K, Selleng S, Greinacher A. Heparin-induced thrombocytopenia in intensive care patients. Semin Thromb Hemost 2008; 34: 425-438.
27. DiGiovanni CW. Current concepts review: heparin-induced thrombocytopenia. Foot Ankle Int. 2008; 29: 1158-1167.
28. Selleng K, Selleng S, Greinacher A. Heparin-induced thrombocytopenia in intensive care patients. Semin Thromb Hemost 2008; 34: 425-438.
29. Hassell K. Heparin-induced thrombocytopenia: diagnosis and management. Thromb Res 2008; 123 11: S16-21.

30. : S16-S21.
31. Kelton JG, Warkentin TE. Heparin-induced thrombocytopenia: a historical perspective. Blood. 2008; 112: 2607-2616.
32. Alberio L. Heparin-induced thrombocytopenia: some working hypotheses on pathogenesis, diagnostic strategies and treatment. Curr Opin Hematol 2008; 15: 456-464.
33. Linkins LA, Warkentin TE. The approach to heparin-induced thrombocytopenia. Semin Respir Crit Care Med 2008; 29: 66-74.
34. Kannan M, Ahmad F, Ahmad S, et al. Heparin-PF4 Antibodies in Heparin Induced Thrombocytopenia: Its Relationship with FcgRIIa Polymorphism. Am J Immunol. 2005; 1: 55-59.
35. Warkentin TE. Clinical picture of HIT. In: Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenis. 3rd Edition. New York, NY: Marcel Dekker 2004; 53-106.
36. Kannan M, Ahmad S, Ahmad F, et al.Functional characterization of antibodies against heparin-PF4 complex in heparin-induced thrombocytopenia patients in Asian-Indians: Relevance to inflammatory markers Blood Coagul Fibrinolysis 2005; 16: 487-490.
37. Sheridan D, Carter C, Kelton JG. A diagnostic test for heparin-induced thrombocytopenia. Blood 1986; 67: 27-30.
38. Walenga JM, Jeske WP, Wood JJ, et al. Laboratory tests for heparin-induced thrombocytopenia: A multicenter study. Semin Hematol 1999; 36: 22-28.
39. Warkentin TE. New approaches to the diagnosis of heparin-induced thrombocytopenia. Chest 2005; 127: 35S-45S.
40. Zwicker JI, Uhl L, Huang WY, et al. Thrombosis and ELISA optical density values in hospitalized patients with heparin-induced thrombocytopenia. J Thromb Haemost 2004; 2: 2133-2137.
41. Chilver-Stainer L, Lammle B, Alberio L. Titre of anti-heparin/PF4 antibodies and extent of in vivo activation of the coagulation and fibrinolytic systems. Thromb Haemost 2004; 91: 276-282.
42. Brieger DB, Mak KH, Kottke-Marchant K. Heparin-induced thrombocytopenia. J. Am. Coll. Cardiol 1998; 31: 1449-1459.
43. Carlsson LE, Santoso S, Baurichter G, et al. Greinacher, Heparin-induced thrombocytopenia: new insights into the impact of the FcgammaRIIa-R-H131 polymorphism. Blood 1998; 92: 1526-1531.
44. Warkentin TE, Kelton JG. A 14 year study of heparin induced thrombocytopenia. Am. J. Med 1996; 101: 502-507.
45. Nand S, Wong W, Yuen B, et al. Heparin Induced thrombocytopenia with thrombosis: Incidence, analysis of risk factors and clinical outcomes in 108 consecutive patients treated as a single institution. Am J Hematol 1997; 56: 12-16.