Incidence of anti-heparin/platelet factor 4 antibodies and heparin-induced thrombocytopenia in medical patients

Medikal hastalarda anti-heparin trombosit faktör 4 antikor ve heparine bağlı trombositopeni sıklığı

Muzaffer Demir[1], Emre Tekgündüz[1], Mustafa Edis[2], Enver Duran[2], Turan Kürüm[3], Ömer Yiğitbaşı[4], Mahmut Yüksel[4]

[1]*Trakya University, Faculty of Medicine, Department of Medicine, Division of Hematology, Edirne, Turkey*

[2]*Trakya University, Faculty of Medicine, Department of Cardiovascular Surgery, Edirne, Turkey*

[3]*Trakya University, Faculty of Medicine, Department of Cardiology, Edirne, Turkey*

[4]*Trakya University, Faculty of Medicine, Department of Nuclear Medicine, Edirne, Turkey*

Abstract

Objective: Heparin-induced thrombocytopenia (HIT) is a life threatening complication of heparin therapy, causing thrombosis. The aim of our study was to find out the frequencies of HIT antibody seroconversion and clinical HIT in Turkish medical patients on different forms of heparins.

Materials and Methods: Our study included 61 patients who were on unfractionated heparin (UFH) (n: 37) and low molecular weight heparin (LMWH) (n: 24) therapies. The frequency of HIT antibody formation was determined by means of antigenic (ELISA), and functional assays (serotonin release assay-SRA).

Results: The seroconversion rates in UFH and LMWH groups were found to be 18.9% and 4.1% (ELISA), and 8.1% and 4.1% (SRA), respectively. One patient (2.1%) on UFH therapy developed deep vein thrombosis. No thromboembolic event was observed in patients taking LMWH.

Conclusion: Seroconversion rates by means of antigenic and functional assays and clinical HIT were more common in patients on UFH than patients on LMWH therapy. *(Turk J Hematol 2009; 26: 171-5)*

Key words: Heparin-induced thrombocytopenia, medical patients, Turkish cohort, unfractionated heparin, low molecular weight heparin.

Received: May 28, 2009 *Accepted: September 29, 2009*

Özet

Amaç: Heparine bağlı trombositopeni (HİT) heparin tedavisinin tromboza neden olan, hayatı tehdit eden bir komplikasyonudur. Çalışmamızın amacı farklı heparin formları kullanan dahili Türk hastalarda HİT antikor serokonversiyonu ve klinik HİT sıklığının saptanmasıydı.

Yöntem ve Gereçler: Çalışmamıza anfraksiyone heparin (AFH) (n: 37) ve düşük molekül ağırlıklı heparin (DMAH) (n: 24) tedavisi alan 61 hasta katıldı. HIT antikor oluşumu antijenik (ELISA) ve fonksiyonel (serotonin salınım testi-SRA) testler ile değerlendirildi.

Address for Correspondence: Prof. Muzaffer Demir, Trakya University, School of Medicine, Division of Hematology, 22030 Edirne, Turkey
Phone: +90 284 235 76 42 – +90 284 235 10 41 E-mail: muzaffer@tnn.net

Bulgular: AFH ve DMAH gruplarında serokonversiyon oranları sırasıyla %18,9-%4,1 (ELISA) ve %8,1-%4,1 (SRA) saptandı. AFH kullanan bir hastada (%2,1) derin ven trombozu gelişti. DMh kullanan hastalarda tromboembolik olay izlenmedi.
Sonuç: aFH kullanan hastalarda dmah kullananlara göre antijenik ve fonksiyonel yöntemlerle serokonversiyon ve klinik HIT oranları daha yüksek saptandı. *(Turk J Hematol 2009; 26: 171-5)*
Anahtar kelimeler: Heparine bağlı trombositopeni, medikal hastalar, Türk hasta grubu, fraksiyone olmayan heparin, düşük molekül ağırlıklı heparin

Geliş tarihi: 28 Mayıs 2009 Kabul tarihi: 29 Eylül 2009

Introduction

Heparin is an important anticoagulant drug which is widely used in almost every discipline of medicine and has saved thousands of lives for over 50 years. The most feared complications of heparin therapy are hemorrhage and heparin-induced thrombocytopenia (HIT). HIT is an acquired, transient, prothrombotic disorder and a life-threatening complication of unfractionated (UFH) and low molecular weight heparin (LMWH) therapy presenting with thrombocytopenia and/or complicating venous or arterial thromboembolism that is associated with increased in vivo thrombin generation [1]. HIT is a clinicopathologic condition and adverse drug reaction caused by platelet-activating antibodies of mostly pathogenic IgG class which are directed against a molecular complex formed by heparin and platelet α-granule protein, platelet factor 4 (PF4) [2]. The frequency of HIT is dependent on four factors: duration of heparin use (>1 week versus<1 day), type of heparin (UFH>LMWH>fondaparinux which is synthetic heparin pentasaccharides), type of patient population (surgery> medical>pregnancy), and patient gender (female>male) [3]. HIT occurs in 3% to 5% and 0.5% of patients receiving UFH and LMWH, respectively [4]. In the absence of alternative anticoagulation, the risk of thrombosis is ~ 5% to 10% per day in the first few days after cessation of heparin [5] and mortality from HIT ranges from 18-50% [6]. In medical conditions, the frequency of both antibody formation and thrombocytopenia or/and thrombosis are much less than in surgical settings [7,8]. The confirmation of HIT by laboratory methods is complex and difficult but is a sine qua non of HIT diagnosis. To date, there are two types of assays used to measure the antibody formation (immunologic and functional assays) [9-11]. The frequency of AHPF4 formation is much greater than the risk of HIT and only minority of antibodies detected with EIA are able to activate platelets in vivo. The interrelationship between antibody formation and clinical HIT has been demonstrated with an iceberg model [11]. We recently reported the frequency of AHPF4 antibody formation and clinical HIT in Turkish patients undergoing cardiac surgery [12]. Our study was designed to establish the laboratory methods of HIT and to determine the frequency of antibody generation and clinical HIT events in medical patients in Turkey.

Materials and Methods

Patients Selection: This prospective study enrolled a total of 61 consecutive medical patients who were treated at a single institution (Trakya University Hospital) during 2004-2005 and no history of recent (< 100 days) heparin/LMWH exposure. 37 patients with acute coronary syndrome (ACS) and venous thromboembolism (VTE) (deep vein thrombosis (DVT) and/or pulmonary embolism (PE)) who were treated with UFH for at least 5±2 days, and 24 DVT patients who were on therapeutic dose LMWH therapy for at least 6±2 days were included. Patients of LMWH therapy arm were treated with nadroparin (n=5), dalteparin (n=5), and enoxaparin (n=14). Written informed consent was obtained from all patients. The study was conducted according to the Declaration of Helsinki.

Sample collections: Blood samples were taken on 10±2. days of heparin therapy. Daily and alternate day platelet counts were performed on patients belonging to UFH and LMWH therapy arm, respectively. Thromboembolic attacks were evaluated on clinical grounds. In suspected cases radiological (Doppler ultrasonography and/or spiral computerized tomography), and scintigraphic (ventilation-perfusion scan) confirmations were made. The first day of heparin exposure was considered day 0. Ten ml of blood without anticoagulant was taken and allowed to incubate at room temperature for 1 hour to assure blood clot formation. The samples were centrifuged at 3000/min for 15 minutes. All serum samples were inactivated by heat inactivation at 56 ºC for 45 minutes and stored at -85 ºC until tested.

Laboratory assays: For determining the antibodies, both antigenic and activation assays were carried out. The determination of anti-heparin/platelet factor 4 antibodies (HPF4-A) was performed as previously described by using a commercially available ELISA kit (Asserachrome HPIA; Diagnostica Stago, France) [11]. The positive results were defined as an absorbance value of equal or greater than 0.5 optical density units at A_{492} nm. This ELISA kit detected two other anti-human immunoglobulin (Ig) classes (Ig A and Ig M) besides Ig G.

Heparin-PF4 dependent platelet reactive antibodies were detected with two functional assays, the platelet aggregation test (PAT) and the serotonin release assay (SRA). The patient samples were tested in duplicate with different platelet donors. A pool of ten normal-healthy-previously tested donors' platelets were used in these activation assays. PAT was performed as previously reported [9,13]. In this method, platelet rich citrated plasma was used and the samples were considered positive if ≥20% maximum aggregation was met at 0.1 to 1.0 U/ml heparin concentrations. Aggregation response was monitored for 20 min. SRA was performed as previously described [9,14]. For the SRA, the samples were considered positive if all the following criteria were met: 1) ≥20% serotonin release at 0.1U/ml heparin and 2) inhibition of platelet activation at high concentration of heparin (100 U/ml) 3) appropriate activation profiles observed with positive and negative control samples. If

a patient sample was negative by PAT or SRA, it was repeated in duplicate with a different platelet donor.

Definition of HIT: HIT was defined according to the following criteria. 1) unexplained absolute or relative thrombocytopenia with a decrease of 50% from baseline platelet counts after day 5 of heparin therapy for no other reason except heparin treatment. 2) new thromboembolic complications such as venous or arterial thrombosis associated with heparin exposure and not due to surgery, thrombophilia, DIC, etc. 3) laboratory evidence of anti-heparin-platelet factor 4 antibodies.

Statistical analysis: All statistical calculations were performed by using Microsoft Excel software. Results were expressed as mean ± standard deviation. The positive results were given as percent value of total patient number. Chi-square test was used to compare seroconversion rates among groups. The p value if equal or lower than 0.05 was considered as significant.

Results

The study cohort included 37 patients (11 female, 26 male) with acute coronary syndrome (n:29) and VTE (n:8) (DVT and/or PE), and 24 DVT patients (12 female, 12 male). Mean age of patients on UFH and LMWH therapy were 65±9.5 years and 61±7.2 years, respectively. The frequency of HIT antibody generation measured by ELISA in patients taking UFH and LMWH were found to be 18.9% and 4.1%, respectively. SRA detected HIT antibodies in 8.1% of patients on UFH and 4.1% of patients on LMWH therapy. PAT was positive in 8 patients (21.6%) on UFH and 1 (4.1%) patient on LMWH therapy. One patient (2.1%) with ACS who was on UFH therapy developed DVT. No thromboembolic attack was observed in patients taking LMWH (Figure 1 and 2). Seroconversion rates by means of ELISA (p: 0.01), PAT (p: 0.02) and SRA (p: 0.04) and clinical HIT were more common in patients on UFH than patients on LMWH therapy (Figure 3).

Discussion

HIT is a very strong risk factor for arterial and venous thromboembolism, both in relative (odds ratio for thrombosis = 20-40) and absolute (thrombosis risk 30-45%) terms, depending on the patient population affected [15]. As HIT is a clinicopathologic syndrome its diagnosis depends on any clinical event related to heparin exposure like thrombocytopenia, thrombosis, anaphlaxis and demonstration of HIT antibody serocoversion by serologic and functional assays. Only a minority of patients with antibody seroconversion develops HIT. In one third to one half of the cases isolated HIT-defined as HIT presented with thrombocytopenia but without thrombosis- is subsequently complicated by thrombosis [16]. Therefore simply discontinuing heparin is not enough to prevent thromboembolic complications and an alternative nonheparin anticoagulant should be commenced immediately in case of highly probable or confirmed HIT. In order to prevent HIT overdiagnosis a clinical scoring system (4Ts) is very useful for predicting pretest probability of HIT [17].

In general both seroconversion, thrombocytopenia and thrombosis rates are lower among medical patients compared to surgical patients. Because we did not perform baseline search for HIT antibodies, we cannot exclude that some patients were already seropositive before study entry. In three different reports on ACS patients who were treated with UFH

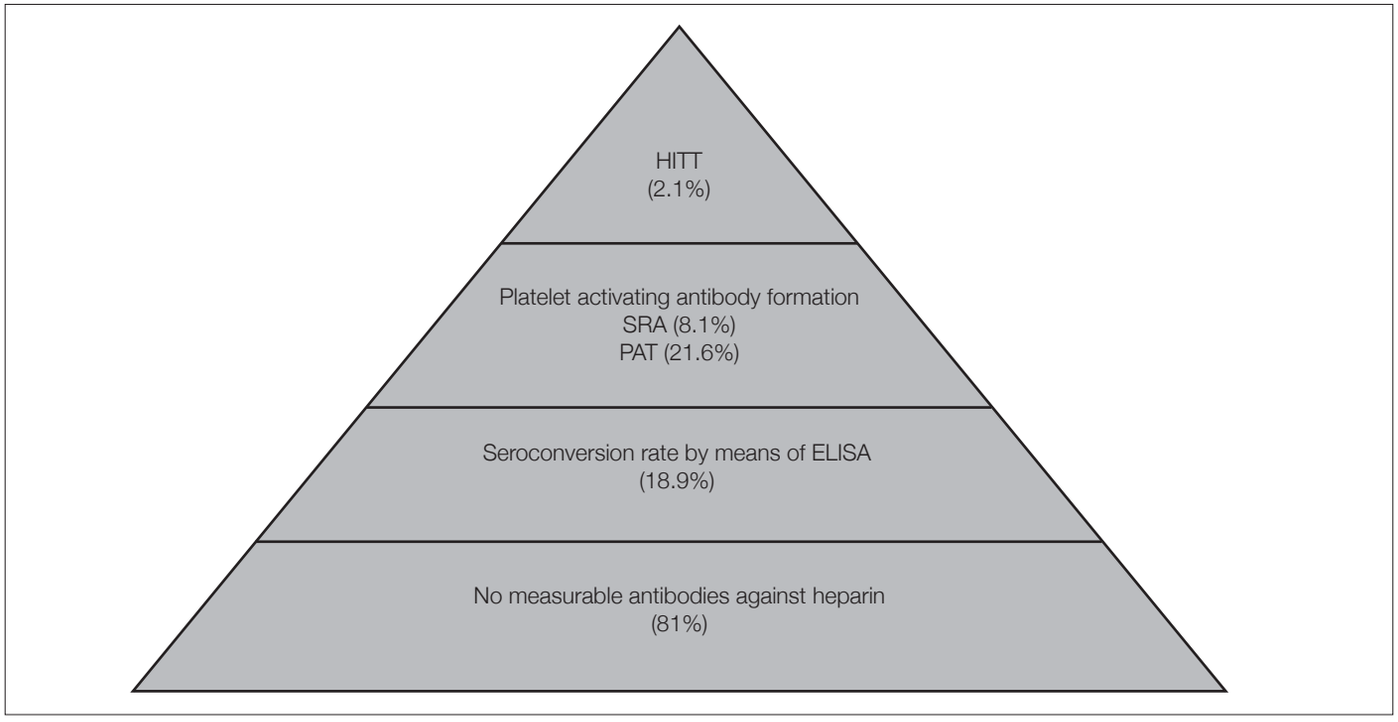

Figure 1. Data on the frequency of antibody generation and clinical HIT in medical patients on UFH therapy are presented as an iceberg model. SRA: Serotonin release assay; PAT: Platelet aggregation assay; HITT: Heparin induced thrombocytopenia thrombosis

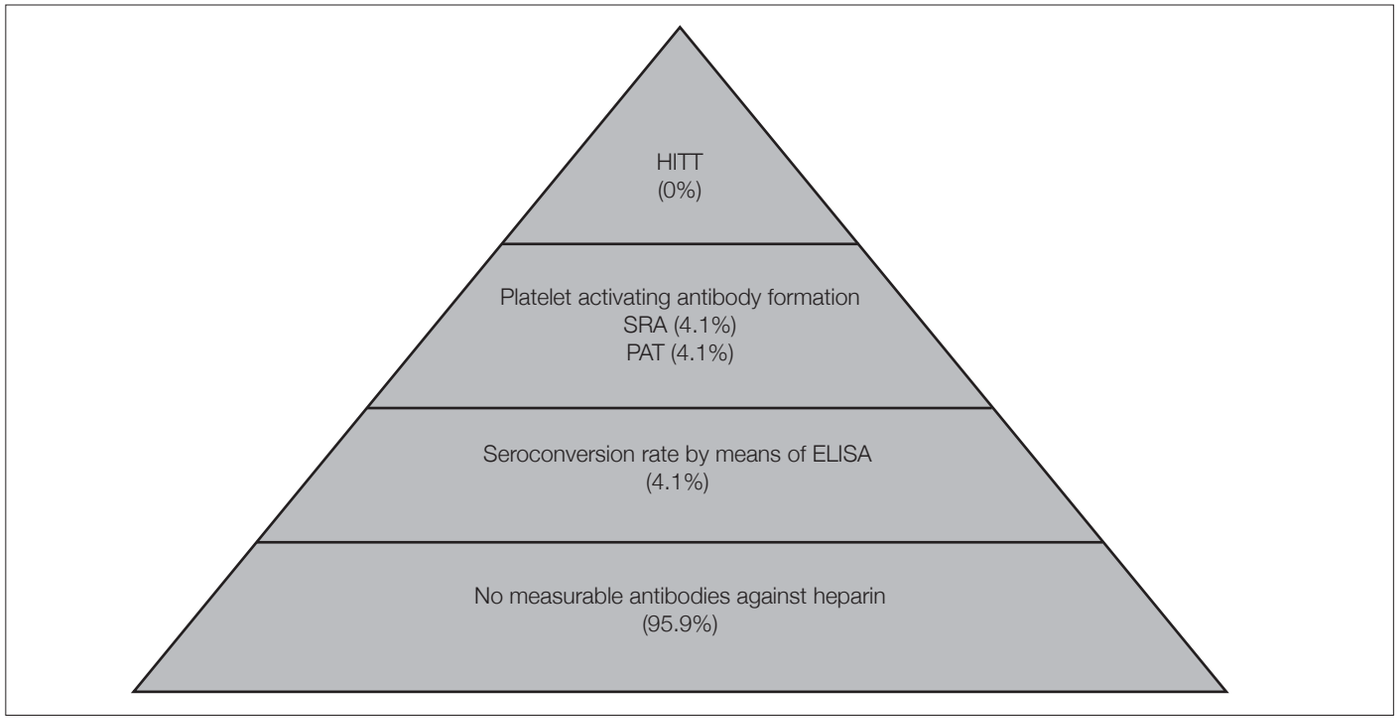

Figure 2. Data on the frequency of antibody generation and clinical HIT in medical patients on LMWH therapy are presented as an iceberg model. SRA: Serotonin release assay; PAT: Platelet aggregation assay; HITT: Heparin induced thrombocytopenia thrombosis

Table 1. The clinical and laboratory results of SRA positive samples

Age /Sex	Heparin type	ELISA (OD)	SRA (% release)	Platelet count at study entry (mm^3)	Minimum platelet count during study period (mm^3)	Thromboembolic event
77/F	UFH	0,870	74	222000	192000	-
75/M	UFH	2,870	85	267000	212000	DVT
66/M	UFH	0,807	65	288000	292000	-
62/F	LMWH	0,555	59	198000	188000	-

F: Female; M: Male; OD: Optical density; SRA: Serotonin release assay; DVT: Deep vein thrombosis

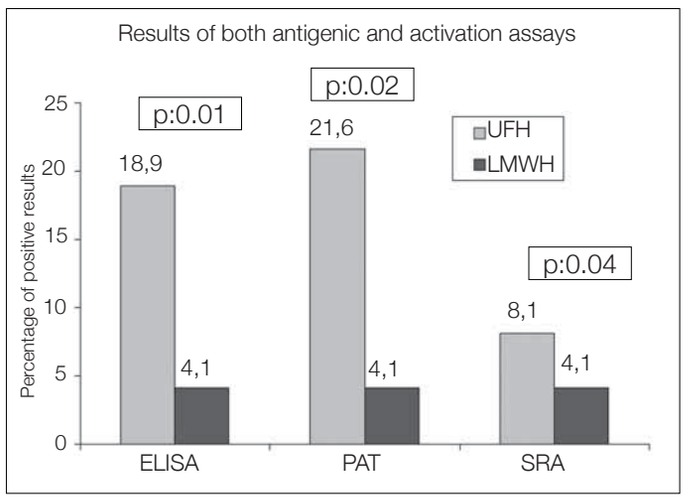

Figure 3. Results of serologic and functional assays on heparin and LMWH

seroconversion rates were found to be 8.7%, 10.6% and 30%, respectively [11,18,19]. There is a trend for increased seropositivity with time both in surgical and medical patients on heparin and LMWH therapy [12,20]. In their multicenter study Lindhoff-Last E et al. [20] evaluated a total of 1137 patients with DVT who were randomly assigned to UFH or reviparin for 5-7 days. On day 5-7 the incidence of AHPF4 were found to be 9.1% and 3.7%, who were on UFH and LMWH therapy, respectively. These values were 20.7% (UFH) and 7.5% (LMWH) on day 21. In our study seroconversion rates were 18.9% and 4.1% for patients on UFH and LMWH, respectively. As we did not perform serologic assays on different time periods we were not able to demonstrate this phenomenon. Ig A and IgM antibodies cannot activate platelets by way of Fcγ (IgG) receptors and therefore not pathogenic. As our ELISA kit detected both IgA and IgM antibodies besides IgG, our results could be an overestimation of true seroconversion rates. The incidence of HIT associated thrombosis among medical patients varies between 0.8%-1.6% and 0.53%-0.8% in

patients on UFH and LMWH treatment, respectively [7,8,18,20]. Seroconversion rates by means of antigenic and functional assays and clinical thrombosis on our patient cohort are compatible with the vast majority of reports on HIT in the literature.

In conclusion, seroconversion rates and clinical HIT were more common in patients on UFH than patients on LMWH therapy. The small patient number, the most important shortcoming of our study, make it difficult to draw any firm conclusions. Nevertheless, as far as we know this is the first effort evaluating the frequency of HIT among Turkish medical patients on different heparin preparations.

No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

References

1. Warkentin TE. Think of HIT. Hematology (Am Soc Hematol Educ Prog) 2006;408-14.
2. Warkentin TE. Heparin-induced thrombocytopenia: a clinicopathologic syndrome. Thromb Haemost.1999; 82: 439-47.
3. Warkentin TE. Heparin-induced thrombocytopenia. Hematol Oncol Clin N Am 2007;21:589-607.
4. Rice L. Heparin-induced thrombocytopenia. Myths and misconceptions (that will cause trouble for you and your patient). Arch Intern Med. 2004;164:1961-4.
5. Greinacher A, Eichler P, Lubenow N, Kwasyn H, Luz M. Heparin-induced thrombocytopenia with thromboembolic complications: metaanalysis of 2 prospective trials to assess the value of parenteral treatment with lepirudin and its therapeutic aPTT range. Blood 2000;96:846-51.
6. Kuo KHM, Kovacs MJ. Fondaparinux: A potential new therapy for HIT. Hematology 2005;10:271-75.
7. Girolami B, Prandoni P, Stefani PM, Tandou C, Sabbion P, Eichler P, Ramon R, Baggio G, Fabris F, Girolami A. The incidence of heparin-induced thrombocytopenia in hospitalized medical patients treated with subcutaneous unfractionated heparin: a prospective cohort study. Blood 2003;101:2955-9.
8. Prandoni P, Siragusa S, Girolami B, Fabris F. BELZONI Investigators Group. The incidence of heparin-induced thrombocytopenia in medical patients treated with low-molecular-weight heparin: a prospective cohort study. Blood 2005;106:3049-54.
9. Walenga JM, Jeske WP, Fasanella AR, Wood JJ, Bakhos M. Laboratory tests for the diagnosis of heparin-induced thrombocytopenia. Semin Thromb Hemost 1999; 25 (Suppl 1): S43-9.
10. Demir M, Ahmad S, Walenga JM, Fareed J. Laboratory diagnosis of heparin-induced thrombocytopenia. Turk J Haematol 2000;17(3 Suppl 3):181-90.
11. Warkentin T and Sheppard JAI. Testing for HIT antibodies. Transfusion Medicine Reviews 2006; 20: 259-72.
12. Demir M, Duran E, Yigitbasi O, Vural O, Kurum T, Yuksel M, Turgut B, Walenga JM, Fareed J. Incidence of antiheparin-platelet factor 4 antibodies and heparin-induced thrombocytopenia in Turkish patients undergoing cardiac surgery. Clin Appl Thromb Hemost 2007;13:279-84.
13. Chong BH, Burgess J, Ismail F. The clinical usefulness of the platelet agregation test for the diagnosis of heparin-induced thrombocytopenia. Thromb Haemost. 1993;69:344-50.
14. Sheridan D, Carter C, Kelton JG. A diagnostic test for heparin-induced thrombocytopenia. Blood 1986;67:27-30.
15. Warkentin TE, Greinacher A, Koster A and Lincoff AM. Treatment and prevention of heparin-induced thrombocytopenia. The 8th ACCP conference on antithrombotic and thrombolytic therapy. Chest 2008; 133:340S-380S.
16. Warkentin TE, Greinacher A. Heparin-induced thrombocytopenia: recognition, treatment and prevention. The 7th ACCP conference on antithrombotic and thrombolytic therapy. Chest 2004;126: 311S-337S.
17. Keeling D, Davidson S, Watson H. Management of heparin-induced thrombocytopenia. Br J Haematol. 2006; 133: 259-69.
18. Matsuo T, Tomaru T, Kario K, Hirokawa T and on behalf of HIT Research Group of Japan. Incidence of heparin-PF4 complex antibody formation and HIT in ACS. Thrombosis Research 2005; 115: 475-81.
19. Mascelli MA, Macselli MA, Deliargyris EN, Damaraju LV, Barnathran ES, Sane DC. Role of anti-PF4/heparin antibodies in recurrent thrombotic events after ACS. Seminars in Thrombosis and Hemostasis 2004; 30: 347-50.
20. Lindhoff-Last E, Nakov R, Misselwitz F, Breddin HK, Bauersachs R. Incidence and clinical relevance of heparin-induced antibodies in patients with deep vein thrombosis treated with unfractionated heparin or low-molecular-weight heparin. Br J Haematol 2002; 118: 1137-42.