Elevated levels of D-dimer and fragment 1+2 upon central venous catheter insertion and factor V Leiden predict subclavian vein thrombosis

Flip H. Jansen
Hanneke M. van der Straaten
Mark Roest
Fred Haas
Philip G. de Groot
Rob Fijnheer

Background and Objectives. Subclavian vein thrombosis is a well-recognized complication following central venous catheter insertion. We studied whether the determination of D-dimer levels, fragment 1+2 levels and factor V Leiden can identify patients at high risk of developing subclavian vein thrombosis.

Design and Methods. The presence of central venous catheter associated thrombosis was analyzed in 235 patients undergoing allogeneic bone marrow transplantation, of whom 30 (13%) developed thrombosis. A case-control study was performed with 30 patients matched for age, gender, and type of transplantation who did not develop thrombosis. Blood was sampled 3-5 days after catheter insertion. D-dimer levels were determined using a latex microparticle assay and an enzyme linked immunosorbent assay (ELISA). An ELISA was used to determine fragment 1+2 levels. The factor V genotype was determined by polymerase chain reaction.

Results. The levels of D-dimer and fragment 1+2 were significantly elevated in the patients who developed thrombosis. Five patients tested positive for factor V Leiden and all 5 developed subclavian vein thrombosis. Patients with high D-dimer levels (> 1300 μg/L measured by latex agglutination and >350 μg/L measured by ELISA) had a 7.0 and 6.0 times higher risk of developing subclavian vein thrombosis, respectively. A 5.5-fold increased risk of thrombosis was observed in patients with a fragment 1+2 level higher than 1.300 nmol/L. This resulted in positive predictive values of 0.78, 0.80 and 0.83 for the fragment 1+2, D-dimer and D-dimer latex agglutination assays, respectively. The accompanying negative predictive values were 0.39, 0.40 and 0.42, respectively.

Interpretations and Conclusions. We conclude that the measurement of D-dimer and fragment 1+2 levels after central venous catheter insertion, as well as factor V Leiden determination, can be used to identify patients at high risk of developing symptomatic subclavian vein thrombosis.

Key words: central venous catheter, factor V Leiden, subclavian vein thrombosis, bone marrow transplantation, D-dimer, fragment 1+2.

Haematologica 2005; 90:499-504

From the Department of Hematology, (FHJ, HMvdS, PGdG, RF); Research Laboratory of the Department of Clinical Chemistry (MR); University Medical Center Utrecht (UMCU), Utrecht, Netherlands Department of Clinical Chemistry, St Antonius Hospital, Nieuwegein, the Netherlands (FH).

Correspondence:
Dr. Rob Fijnheer, Department of Haematology, University Medical Center Utrecht (UMCU), Heidelberglaan 100, 3584 CX Utrecht, the Netherlands.
E-mail: r.fijnheer@azu.nl

Patients undergoing allogeneic bone marrow transplantation require long-term venous access for the administration of medication, blood products, parenteral hyperalimentation and blood sampling. Therefore central venous catheters (CVC) with a double or triple lumen, mostly Hickman catheters, are inserted. Numerous complications have been reported after insertion of central venous catheters, the most significant ones being infection and thrombotic complications. Thrombotic complications are found in 4-42% of all cases.[1-9] The risk of developing thrombosis or bacteremia seems to be higher with the use of triple-lumen CVC than with double or single lumen catheters.[3-5] Predictive testing to identify patients who are at risk of developing thrombosis could be helpful to develop antithrombotic strategies.

A *hypercoagulable* or *prethrombotic* state,[10] which is characterized by an imbalance between coagulation and anticoagulation, may be a preliminary stage leading to the formation of a thrombus. Coagulation is triggered by tissue factor and after several steps activated factor X is formed. Prothrombin fragment 1+2 is released when activated factor X cleaves prothrombin to produce thrombin. Free thrombin converts fibrinogen into fibrin, which is cross-linked by thrombin-activated factor XIII. Cross-linked fibrin is lysed by plasmin producing the proteolytic derivative, D-dimer. Thus levels of fragment 1+2 are increased on activation of coagulation, while D-dimer can be used as a marker of fibrinolytic activity. The congenital factor V Leiden mutation leads to hypercoagulability and manifests as resistance to activated protein C. It is the most common

defect underlying venous thromboembolism, with a prevalence of 3-5% in The Netherlands.[11] We have previously shown that patients with factor V Leiden have a 7.7 (95% CI 3.3-17.9)-fold increased risk of developing CVC-associated thrombosis.[12]

We analyzed the presence of CVC-associated thrombosis in 235 consecutive patients undergoing bone marrow transplantation. A case-control study was performed to determine whether levels of D-dimer, fragment 1+2 and factor V Leiden can identify patients at risk of developing subclavian vein thrombosis.

Design and Methods

Patients

A cohort of 255 consecutive patients who received an allogeneic bone marrow transplantation was studied from July 1996 until April 2002 at the Hematology Department of the University Medical Center Utrecht (UMCU). All 255 patients received a double or triple lumen Hickman catheter. Twenty patients were excluded from the analysis because plasma samples were lacking. Of the remaining 235 patients, 172 patients had an HLA-identical donor and 63 patients a matched unrelated donor. Thirty out of 235 patients developed thrombosis after the insertion of the central venous catheter. These 30 patients were matched with another 30 patients, also from this group of 235 patients, who had not developed thrombosis. They were matched by age, gender and type of bone marrow transplantation. The patients' characteristics are listed in Table 1. The physicians were not aware of the factor V Leiden status, D-dimer or fragment 1+2 levels and those performing the D-dimer, fragment 1+2 and factor V Leiden testing were blinded to clinical outcome. All patients gave informed consent.

Table 1. Underlying diseases and characteristics of 60 patients (30 patients with thrombosis and 30 patients without thrombosis).

	Thrombosis	*No thrombosis*
Disease (%)		
Acute myeloid leukemia (AML)	9 (30)	4 (13)
Acute lymphoid leukemia (ALL)	1 (3)	8 (27)
Chronic myeloid leukemia (CML)	9 (30)	5 (17)
Myelodysplastic syndrome (MDS)	2 (7)	2 (7)
Severe anaplastic anemia (SAA)	4 (13)	1 (3)
Non-Hodgkin's lymphoma (NHL)	2 (7)	4 (13)
Multiple myeloma (MM)	3 (10)	6 (20)
Type of transplantation (%)		
HLA-identical	21 (70)	21 (70)
Matched unrelated donor (MUD)	9 (30)	9 (30)
Mean age in years (range)	41 (23-55)	40 (21-62)
Male gender (%)	15 (50)	15 (50)

Treatment regimen

All patients had a tunnelled Hickman catheter (Bard Benelux NV, Nieuwegein, The Netherlands), inserted under sterile conditions in the operating room. Patients receiving an HLA-identical bone marrow transplant had a double-lumen catheter inserted, while a triple-lumen catheter was used in patients receiving a matched unrelated bone marrow transplant. A standard insertion technique was used, consisting of direct puncture of the infraclavicular vein, introduction of a guide wire, radiological confirmation of the correct position of the guide wire, dilatation of the entry route, creation of a subcutaneous tunnel, and insertion of the catheter. All catheters were correctly positioned with the tip in the superior vena cava. Each lumen was tested for adequate aspiration and infusion characteristics and flushed with heparin-saline (100 IU/mL). A postoperative chest X-ray was performed routinely to detect inadvertent pneumothorax and to confirm correct placement of the catheter. All patients received thrombosis prophylaxis: a daily dose of 5700 IE/day of nadroparin s.c. was started before insertion of the central venous catheter and continued for a period of 10 days. All patients received 2 g of cephalotin intravenously one hour before insertion of the catheter. Catheters were flushed with 9 mL of 0.9% saline solution followed by 7 mL of heparin (100 IU/mL). Flushing was performed once a day. The catheter was also flushed after blood withdrawal and after administration of medicines or blood products. Conditioning therapy consisted of cyclophosphamide and total body irradiation. All patients with a matched unrelated donor received anti-thymocyte globulin and low dose heparin (100 IU/kg) for a period of 4 weeks, starting 5 days before the transplantation, in order to prevent veno-occlusive disease of the liver. All patients were hospitalized during the period of transplantation. Catheters were removed before leaving the hospital. All patients had a standardized outpatient follow-up for at least 3 months and were seen twice a week.

Diagnosis and clinical signs of thrombosis

In all cases of clinical signs of thrombosis, i.e. swelling and/or redness of the limb or venous engorgement, a color flow Doppler imaging examination was performed.[13-15] If the clinical signs of thrombosis persisted but the initial color flow Doppler imaging was negative, the Doppler examination was repeated. Thereafter contrast venogra-

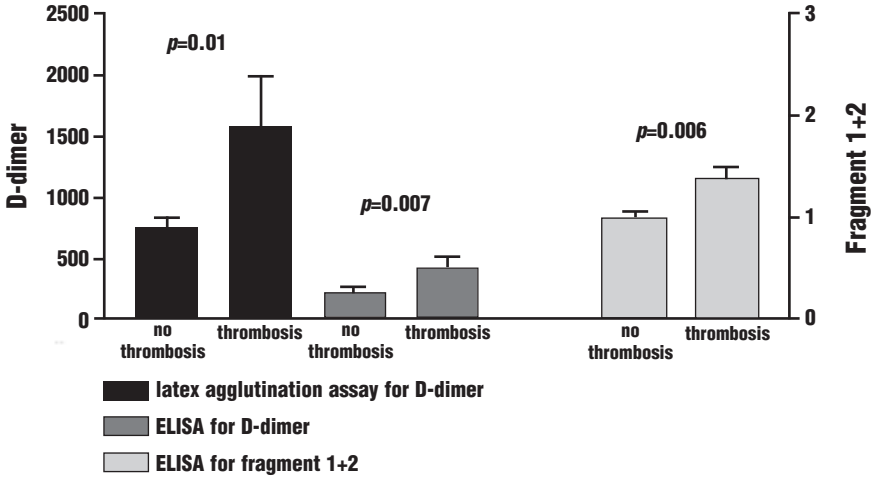

Figure 1. Mean d-Dimer and fragment 1+2 levels in plasma samples taken 3-5 days after catheter insertion comparing patients who developed central venous catheter associated thrombosis to patients without central venous catheter associated thrombosis.

phy was performed (n=2). In patients with a documented thrombus the catheter was removed and heparin was given i.v. for 5 days, followed by anticoagulant therapy for 3 months.

Blood samples and hemostatic parameters

Blood samples were collected 3-5 days after insertion of the venous catheter, immediately prior to the conditioning regimen of the bone marrow transplantation. Blood was taken from the catheter. Samples were stored at -80°C.

The D-dimer levels were determined using an automated microparticle latex agglutination D-dimer assay, Tina-quant® (Roche Diagnostics, Germany) and an ELISA, TintElize® (Biopool International, Sweden). The Tina-quant D-dimer assay has a normal value of 500 μg/L and the normal value of the TintElize D-dimer assay is 39 ng/mL.

Fragment 1+2 levels were assayed using Enzygnost® Fragment 1+2 micro (Dade Behring, Germany). The factor V Arg506/Gln506 genotype was determined in pre-bone marrow transplantation DNA by polymerase chain reaction analysis and hybridization with allele-specific oligonucleotides.[16]

Statistical analysis

Contingency table analyses were performed with a standard χ^2 test. The relation between the continuous variables was investigated by linear regression analysis. The risk of thrombosis for levels of D-dimer or fragment 1+2 and factor V Leiden was investigated by multivariate logistic regression analysis. Cut-off points for the D-dimer and fragment 1+2 assays were based upon optimal sensitivity and specificity, using receiver operating characteristics (ROC) curves. Statistical evaluation was performed using the SPSS package, version 10.0 for Windows (SPSS INC, Chicago, USA).

Results

Thirty of 235 patients (13%) developed a CVC-associated thrombosis. The mean time to thrombosis after insertion of the central venous catheter was 36 days (range 8-95 days), the median time was 26 days. Based on optimal sensitivity and specificity we established a cut-off point for the D-dimer latex agglutination assay of 1300 μg/L, a cut-off point of 350 μg/L for the D-dimer ELISA and a cut-off point of 1.300 nmol/L for the fragment 1+2 assay.

D-dimer (latex agglutination assay)

Three to five days after catheter insertion, the mean level of D-dimer in the patients who developed CVC-associated thrombosis was 1573 μg/L (range=186-13067 μg/L), whereas the mean value in the group of patients without thrombosis was 742 μg/L (range 0-2298 μg/L) (p=0.01) (Figure 1). Ten out of 12 patients (83%) with D-dimer levels higher than 1.300 μg/L developed catheter-associated thrombosis while 20 out of 48 patients (42%) with D-dimer levels lower than 1300 μg/L developed thrombosis giving a positive and negative predictive value of 0.83 and 0.42, respectively. The odds-ratio was 7.0 (95% CI=1.4-35.5). The sensitivity and specificity were 0.33 and 0.93, respectively (Table 2A). The mean time to thrombosis was 35.9 days for patients with D-dimer levels higher than 1300 μg/L and 35.4 days for patients with D-dimer levels lower than 1300 μg/L (p=0.96).

D-dimer (ELISA)

The mean level of D-dimer in the patients who developed CVC-associated thrombosis was 438 μg/L (range 66-2727 μg/L), whereas the mean value in the group of patients without thrombosis was 233 μg/L

Table 2A. Results of the D-dimer, fragment 1+2 and factor V Leiden assays.

	Cut-off point	PPV	NPV	Sens.	Spec.	OR	95% CI
D-dimer (latex agglutination)	1300 µg/L	0.83	0.42	0.33	0.93	7.0	1.4-35.5
D-dimer (ELISA)	350 µg/L	0.80	0.40	0.40	0.90	6.0	1.5-24.3
Fragment 1+2 (ELISA)	1.300 nmol/L	0.78	0.39	0.47	0.86	5.5	1.5-19.6
Factor V Leiden	–	1.0	0.45	0.16	1.0	–	–

Table 2B. Results for the D-dimer and fragment 1+2 assays, excluding factor V Leiden patients.

	Cut-off point	PPV	NPV	Sens.	Spec.	OR	95% CI
D-dimer (latex agglutination)	1300 µg/L	0.82	0.36	0.36	0,90	7.9	1.5-41.0
D-dimer (ELISA)	350 µg/L	0.75	0.37	0.36	0.93	5.1	1.2-21.5
Fragment 1+2 (ELISA)	1.300 nmol/L	0.75	0.34	0.48	0.86	5.8	1.5-21.5

PPV: positive predictive value; NPV: negative predictive value; Sens.: sensitivity; Spec. : specificity; OR: odds-ratio; CI: confidential interval.

(range 39-965 µg/L) (p=0.007) (Figure 1). Twelve out of 15 patients (80%) with D-dimer levels higher than 350 µg/L developed catheter-associated thrombosis while 18 out of 45 patients (40%) with D-dimer levels lower than 350 µg/L developed thrombosis giving a positive and negative predictive value of 0.80 and 0.40, respectively. The odds-ratio was 6.0 (95% CI=1.5-24.3). The sensitivity and specificity were 0.40 and 0.90, respectively (Table 2A).

The mean time to thrombosis was 38.4 days for patients with D-dimer levels higher than 350 µg/L and 31.8 days for patients with D-dimer levels lower than 350 µg/L (p=0.47).

Fragment 1+2 (ELISA)

The mean level of fragment 1+2 in the patients who developed CVC-associated thrombosis was 1.434 nmol/L (range 0.390-3.690 nmol/L), whereas the mean value in the group of patients without thrombosis was 1.004 nmol/L (range 0.400-1.960 nmol/L) (p=0.006) (Figure 1). Fourteen out of 18 patients (78%) with fragment 1+2 levels above 1.300 nmol/L developed a catheter-associated thrombosis compared to 16 out of 41 patients (39%) with fragment 1+2 levels lower than 1.300 nmol/L giving a positive and negative predictive value of 0.78 and 0.39, respectively. The odds-ratio was 5.5 (95% CI=1.5-19.6). The sensitivity and specificity were 0.47 and 0.86, respectively (Table 2A).

The mean time to thrombosis was 45.3 days for patients with fragment 1+2 levels above 1.300 nmol/L and 24.8 days for patients with fragment 1+2 levels lower than 1.300 nmol/L (p=0.017).

Factor V Leiden

Five patients in the thrombosis group tested positive for factor V Leiden (17%), while all the patients in the group without thrombosis were negative for the mutation (p<0.02), giving a sensitivity and specificity of 0.16 and 1.0 and a positive and negative predictive value of 1.0 and 0.45, respectively.

We found no significant correlation between factor V Leiden and the levels of D-dimer and fragment 1+2. The associations between levels of D-dimer and fragment 1+2 and CVC-associated thrombosis still existed after the exclusion of all patients with the factor V Leiden mutation (Table 2B).

Discussion

This study was designed to demonstrate whether a hypercoagulable state upon central venous catheter insertion could be used to predict an increased risk of developing symptomatic thrombosis.

To confirm thrombosis in symptomatic patients we used color flow Doppler imaging. This technique is known to have a sensitivity and specificity of 56%-100% and 82%-100%, respectively.[13-15] This relatively low sensitivity of ultrasonography could lead to a high number of false-negatives, thus underestimating the incidence of upper-extremity deep venous thrombosis. Therefore, if clinical signs persisted despite the initial color flow Doppler imaging being negative, the ultrasound examination was repeated and, finally, in two patients venography was performed. In order to prevent a possible disturbance or influence of the catheter insertion and/or chemotherapeutic intervention we analyzed plasma samples taken 3-5 days after catheter insertion and immediately prior to administration of the conditioning regimen of the bone marrow transplantation. Our study shows that measuring levels of D-dimer and fragment 1+2 as well as factor V Leiden determination can predict a risk of subclavian vein thrombosis. Patients with high D-dimer levels (>1300 µg/L, measured by latex agglutination and >350 µg/L measured by ELISA) had 7.0 and 6.0 times higher risk of developing subclavian vein thrombosis,

respectively. We found a 5.5-fold increased risk of thrombosis in patients with fragment 1+2 levels higher than 1.300/nmol/L. The five patients who were found to be positive for factor V Leiden all developed subclavian vein thrombosis after catheter insertion. The mean time between thrombosis and the insertion of the central venous catheter was 36 days (range 8-95 days).

D-dimer measurements are now widely used in clinical practice. This study shows that D-dimer assays can be used to predict an increased risk of thrombosis, in contrast to D-dimer analysis in deep venous thrombosis and pulmonary embolism in which the absence of elevated levels of D-dimer is used to exclude the presence of thrombosis.[17,18] However, it is difficult to make general statements on D-dimer levels because of a lack of standardization between the various assays used in clinical practice. The difference we noted between the D-dimer cut-off values of the latex agglutination assay and the ELISA may be caused by differences in antibody specificity and a preference for cross-linked fibrin derivatives over non-cross-linked fibrinogen- or fibrin degradation products.[19,20] At present, ELISA is considered the gold standard for measuring D-dimer levels, but this technique is labor intensive, expensive, and time-consuming.[21] A latex agglutination assay for D-dimer is less expensive, not as time-consuming (<45 min) and easy to perform. Therefore, because we found similar results for the latex agglutination assay and the ELISA for D-dimer, we recommend the latex agglutination assay for measuring D-dimer levels in further clinical studies. As mentioned before, five of the patients in our group of patients who developed subclavian vein thrombosis had the factor V Leiden mutation.

The role of factor V Leiden in CVC-associated thrombosis has been previously reported.[12] Contrary to what could be expected, we did not find that levels of D-dimer and fragment 1+2 were higher in factor V Leiden positive patients than in patients without factor V Leiden who developed subclavian vein thrombosis. Even after exclusion of the patients positive for factor V Leiden the association between high levels of D-dimer and fragment 1+2 and CVC-associated thrombosis did not alter.

There could be two explanations for the increased levels of D-dimer and fragment 1+2 that were found after catheter insertion and the development of subclavian vein thrombosis. First, a systemic prothrombotic state could already pre-exist in the patient, which, triggered by catheter insertion, finally leads to the development of thrombosis. A second explanation could be that there is no pre-existent prothrombotic state, but a hypercoagulable state is induced by catheter insertion, and clinically overt thrombosis develops in a few weeks.

In conclusion, all three assays employed in this study showed that increased activation of coagulation soon after central venous catheter insertion predicts subclavian vein thrombosis. Based on this study we conclude that the measurement of D-dimer and fragment 1+2 levels after central venous catheter insertion, as well as factor V Leiden determination, can be used to identify patients at high risk of developing symptomatic subclavian vein thrombosis. Further studies are warranted in order to develop anti-thrombotic strategies, for example low doses of low molecular weight heparin, for this high-risk category of patients.

FHJ: conception and design, analysis and interpretation of data, drafting of the article: HMvdS: analysis and interpretation of data, critical revision of the article for important intellectual content, administrative and technical support; MR: analysis and interpretation of data, statistical expertise; FH: provision of study materials, administrative, technical and logistic support; PGdG: analysis and interpretation of data, critical revision of the article for important intellectual content: RF: conception and design, analysis and interpretation of data, critical revision of the article for important intellectual content, final approval of the article, provision of study materials an patients.

Manuscript received October 21, 2004. Accepted March 10, 2005.

References

1. Cassidy FP Jr, Zajko AB, Bron KM, Reilly JJ Jr, Peitzman AB, Steed DL. Noninfectious complications of long-term central venous catheters: radiologic evaluation and management. Am J Roentgenol 1987;149:671-5.
2. Kaye CG, Smith DR. Complications of central venous cannulation. Br Med J 1988;297:572-3.
3. Conlan MG, Haire WD, Lieberman RP, Lund G, Kessinger A, Armitage JO. Catheter-related thrombosis in patients with refractory lymphoma undergoing autologous stem cell transplantation. Bone Marrow Transplant 1991;7:235-40.
4. Eastridge BJ, Lefor AT. Complications of indwelling venous access devices in cancer patients. J Clin Oncol 1995; 13:233-8.
5. Lagro SW, Verdonck LF, Borel Rinkes IH, Dekker AW. No effect of nadroparin prophylaxis in the prevention of central venous catheter (CVC)-associated thrombosis in bone marrow transplant recipients. Bone Marrow Transplant 2000;26:1103-6.
6. Tesselaar ME, Ouwerkerk J, Nooy MA, Roosendaal FR, Osanto S. Risk factors for catheter related thrombosis in cancer patients. Eur J Canc 2004;40:2253-9.
7. Pessa ME, Howard RJ. Complications of Hickman-Broviac catheters. Surg Gynecol Obstet 1985;161:257-60.
8. Boraks P, Seale J, Price J, Bass G, Bass G, Ethell M, Keeling D, et al. Prevention of central venous catheter associated thrombosis using mini-dose warfarin in patients with haematological malignancies. Br J Haematol 1998;101:483-6.
9. Thomas JH, MacArthur RI, Pierce GE, Hermreck AS. Hickman-Broviac catheters. Indications and results. Am J Surg 1980;140:791-6.
10. Bauer KA, Rosenberg RD. The pathophysiology of the prethrombotic state in humans: insights gained from studies using markers of hemostatic system activation. Blood 1987; 70: 343-50.
11. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994;330:517-22.

12. Fijnheer R, Paijmans B, Verdonck LF, Nieuwenhuis HK, Roest M, Dekker AW. Factor V Leiden in central venous catheter-associated thrombosis. Br J Haematol 2002;118:267-70.
13. Prandoni P, Polistena P, Bernardi E, Cogo A, Casara D, Verlato F, et al. Upper-extremity deep vein thrombosis. Risk factors, diagnosis, and complications. Arch Intern Med 1997;157:57-62.
14 . Baarslag HJ, van Beek EJR, Koopman MMW, Reekers JA. Prospective study of color Duplex ultrasonography compared with contrast venography in patients suspected of having deep venous thrombosis of the upper extremities. Ann Intern Med 2002;136:865-72.
15. Mustafa BO, Rathbun SW, Whitsett TL, Raskob GE. Sensitivity and specificity of ultrasonography in the diagnosis of upper extremity deep vein thrombosis. Arch Intern Med 2002;162:401-4.
16. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64-7.
17. Hirsh J, Lee AY. How we diagnose and treat deep vein thrombosis. Blood 2002;99:3102-10.
18. Bounameaux H, de Moerloose P, Perrier A, Miron MJ. D-dimer testing in suspected venous thromboembolism: an update. QJM 1997; 90: 437-42.
19. Ellis DR, Eaton AS, Plank MC, Butman BT, Ebert RF. A comparative evaluation of ELISAs for D-dimer and related fibrin(ogen) degradation products. Blood Coagul Fibrinol 1993;4:537-49.
20. Dempfle CE, Zips S, Ergul H, Heene DL. The fibrin assay comparison trial (FACT): evaluation of 23 quantitative D-dimer assays as basis for the development of D-dimer calibrators. FACT study group. Thromb Haemost 2001;85:671-8.
21. Lee AY, Ginsberg JS. Laboratory diagnosis of venous thromboembolism. Baillieres Clin Haematol 1998;11:587-604.