

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/25654>

Please be advised that this information was generated on 2019-03-22 and may be subject to change.

D-Dimer Determination to Assess Regression of Deep Venous Thrombosis

M. C. H. Janssen¹, H. Verbruggen², H. Wollersheim¹, B. Hoogkamer², H. van Langen³, I. R. O. Nováková⁴

From the Department of Medicine, Divisions of ¹General Internal Medicine and ⁴Haematology, ²Central Haematological Laboratory, ³Clinical Vascular Laboratory, University Hospital Nijmegen, The Netherlands

Summary

A number of studies evaluating deep venous thrombosis (DVT) have demonstrated that plasma levels of thrombotic and fibrinolytic parameters change during treatment, but the relationship between thrombus regression and evolution of these markers remains unknown. The objective of the present study was to correlate levels of D-Dimer (DD) with thrombus regression as assessed by duplex scanning.

From 44 patients treated for acute DVT, DD were determined at diagnosis and at the end of initial heparin therapy of at least 5 days. Thrombus regression was measured by repeated duplex scanning at diagnosis and after 1 and 3 months.

DD significantly decreased during heparin treatment as compared with values at presentation. DD levels were significantly higher in the group of patients without normalization of the DVT after 3 months ($p = 0.003$). A ninefold excess tendency was seen for DD levels >1200 ng/ml at the end of initial treatment to be associated with poor resolution of the DVT [odds ratio 9.0, 0.95 confidence interval (CI) 2.3-35.4]. When the patients with an established malignancy were excluded, the differences were even more significant ($p = 0.0004$ for DD levels after initial treatment and an odds ratio of 17.5, 0.95 CI 3.3-92.5).

These results suggest that increased DD levels after the initial phase of treatment are related to poor resolution of DVT after 3 months. These findings contribute to further insight into the process of thrombus regression. Furthermore high DD levels might help to identify the patients with a poor prognosis and could be useful to judge the efficacy of anticoagulant treatment.

Introduction

Deep venous thrombosis (DVT) is a common disease with considerable morbidity. Patients with acute DVT are usually treated with an initial course of heparin (5 to 10 days) followed by at least 3 months of oral anticoagulant therapy (1, 2). The extent of thrombus regression at the time of withdrawing anticoagulant therapy is usually not known.

The past few years duplex scanning (DS) has become a reliable technique to diagnose DVT. Because of its non-invasive nature, the technique is also useful for follow-up of DVT. A few studies have prospectively assessed the outcome of acute DVT by means of DS. It

was demonstrated that more than 50% of vein thrombi undergo complete resolution 3 to 6 months after diagnosis (3-5).

Regression of DVT depends on a balance between haemostatic and fibrinolytic factors. Measurement of D-Dimer (DD), a specific fibrin degradation product, has proven to be useful for the diagnostic management of DVT (6-8). A number of studies have demonstrated that plasma levels of DD and other markers decrease during heparin therapy, but the relationship between thrombus regression and evolution of the markers remains unknown (9-11).

A plasma marker that has predictive value for the outcome of a DVT is valuable for the long-term management of DVT. The objective of this study was to establish the relation between DD levels in the acute phase of DVT and the resolution of DVT after 3 months as assessed by serial DS.

Methods

Patients

Forty-four patients (24 men and 20 women) with a mean age of 53 years (range 28-82) with a first acute DVT confirmed by DS were enrolled in a prospective study after informed consent was obtained. All patients received continuous intravenous heparin infusion according to a standard protocol for at least five days. Heparin level was monitored by the assessment of heparin level with a chromogenic assay (target level 0.4-0.8 IU/ml) (12). Oral coumarin derivatives were started within 24 h and continued for a period of at least 3 months at doses adjusted to target an international normalized ratio (INR) between 2.0 and 4.0. All patients had been instructed to wear graduated compression stockings for the whole period.

Vascular Testing

The patients underwent repeated DS at the time of diagnosis and after 1 and 3 months. Patients were placed in the reverse Trendelenburg position to examine the iliac, common femoral and superficial femoral vein. Popliteal, posterior tibial and remaining distal veins were studied with the leg in a dependent position. Images of all veins were obtained with transverse and longitudinal views. The technique of DS and the criteria for interpretation have been previously described (13).

To measure thrombus mass the technique proposed by Prandoni et al. was used (4); maximum compressibility of the vein was assessed, in the transverse section, by pressing on the vein with the transducer probe. A freeze frame image was obtained and the residual vein diameter was measured on line, expressed in millimeters. This finding was interpreted as an indicator of thrombus mass. Regarding the DVT outcome, worsening was reported if the clot extended to vein segments previously unaffected or if increased thrombus mass was detected. No change indicated the presence of an unaltered clot. Improvement was reported if a decreased thrombus mass was measured. Veins were considered to be normalized if they were fully compressible. According to the results of DS obtained at 3 months patients were divided in 2 groups: (1) normalized, which indicates complete resolution and (2) not normalized, which means incomplete resolution of the DVT.

Correspondence to: Dr. M. C. H. Janssen, Department of Medicine, Division of General Internal Medicine, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands - Tel: +31 24 3614782; Fax: +31 24 3541734

Table 1 Patients' characteristics

	Normalization n=23	No normalization n=21
age	46 (28-75)	60 (31-82)
male / female	12 / 11	12 / 9
distal / proximal DVT	5 / 18	0 / 21
mean days heparin therapy	6.7 ± 1.7	6.7 ± 1.7
hours until optimal heparin level	13.1 ± 9	17.7 ± 13.8
malignancy (%)	5 (22%)	3 (14%)
average heparin level (IU/ml)	0.59 ± 0.12	0.59 ± 0.10
average dose of heparin (U)	284237 ± 101787	278583 ± 160979
DD before treatment (ng/ml)	3763 ± 4314	4767 ± 4186
DD after heparin (ng/ml)	1382 ± 1818	1990 ± 1439

Laboratory Testing

At the time of diagnosis and at the end of initial therapy from all patients 4.5 ml of blood was drawn into a vacutainer tube containing 0.5 ml CTAD solution (Becton Dickinson). Platelet poor plasma was prepared within one hour of sampling by centrifugation at 4000 × g for 10 min at 5° C. The supernatant plasma was snap-frozen in aliquots and stored at -70° C until assayed. DD-concentrations were measured with an ELISA method (Fibrinostica FbDP, Organon Teknika). The laboratory technician and the vascular technician were blind for each other's results. All assays were performed with one single batch of the ELISA test kit.

Analysis

A Receiver Operating Curve (ROC) for DD levels at the end of heparin therapy was constructed by plotting the sensitivity (true positive fraction) towards 1-specificity (false positive fraction). For within subject comparison of the blood tests at the end of heparin therapy compared to baseline a paired t-test was used. The Wilcoxon-Mann-Whitney test was used to compare the blood tests depending on the resolution of DVT. A p-value of 0.05 or less was considered significant. The posterior probability of poor resolution in relation to different DD levels is presented as the odds ratio with 95% confidence intervals (0.95 CI).

Results

After 3 months 23 out of 44 patients (52%) showed complete resolution on DS. Table 1 shows the characteristics of the 2 groups of

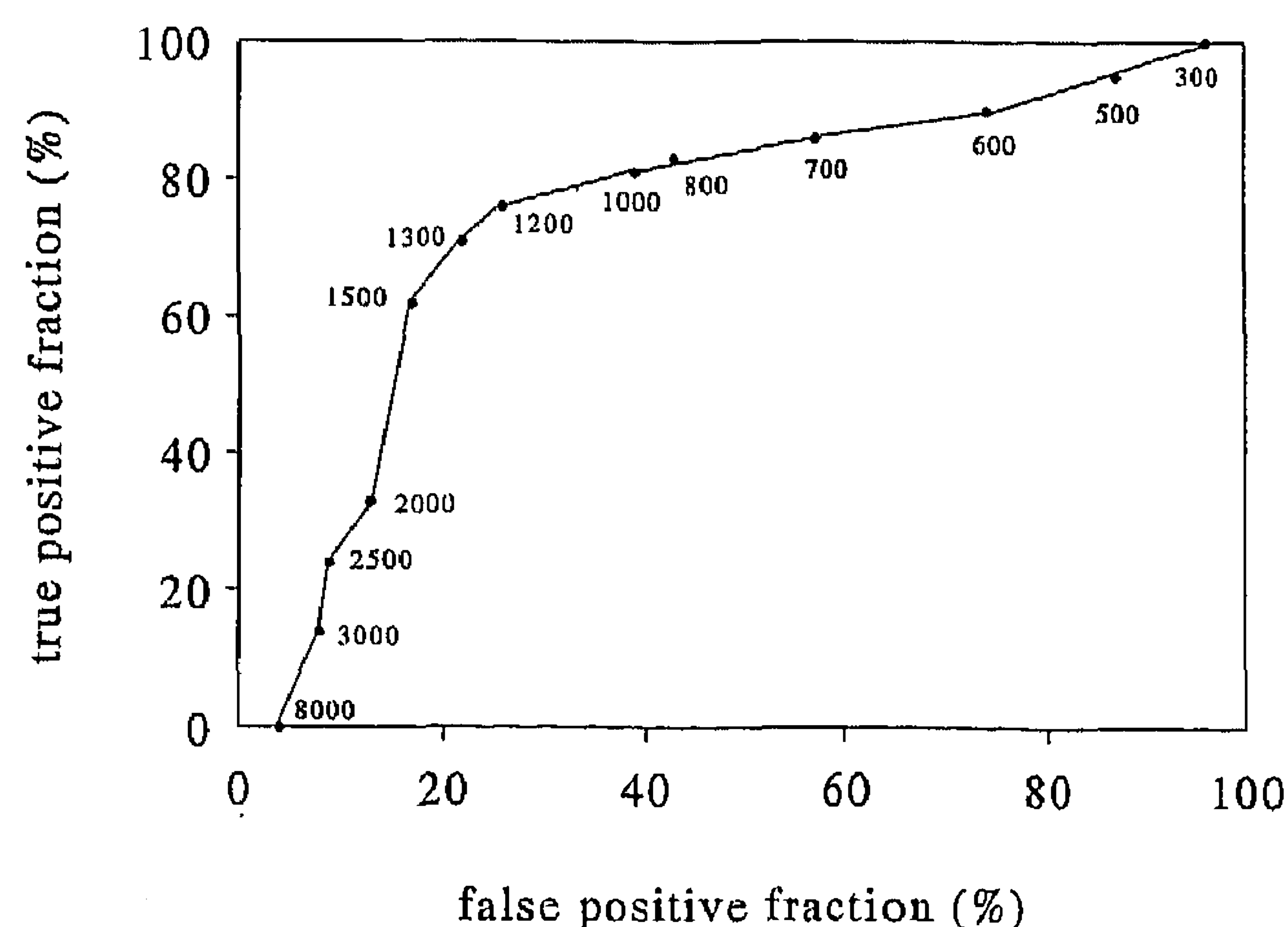


Fig. 1 ROC curve analysis of D-Dimer levels (ng/ml) at the end of heparin therapy associated with poor resolution of DVT

patients: the number of days of heparin treatment, the mean heparin level and the mean dose of heparin used were not different. There was a small difference in time until an adequate heparin level was achieved and the mean age of the patients was higher in the group that did not normalize. All patients with distal DVT normalized within 3 months.

For the whole group of patients DD decreased significantly during heparin treatment ($p = 0.0001$). There was no significant relation between the location of initial thrombus and DD levels.

The ROC curve for DD levels at the end of heparin therapy is shown in Fig. 1. A cut-off value of 1200 ng/ml was chosen based on optimal sensitivity and minimal false positive fraction.

Figure 2 shows the comparison of DD levels before and after heparin treatment between the group of patients that normalized after three months and the group that did not normalize. DD levels at the end of initial therapy were significantly higher in the group of patients that did not normalize ($p = 0.003$). However, there was a large overlap of individual values between the groups. Mean values of the markers at the end of initial therapy remained elevated. While the patients with distal DVT ($n = 5$) were excluded DD levels at the end of heparin treatment were still significant ($p = 0.006$).

Figure 3 shows the DD levels of the same group of patients when the patients with an established malignancy ($n = 8$) are excluded. It shows that the differences in DD levels between the group that normalized and the group that did not normalize were even more significant ($p = 0.0004$).

Table 2 shows the odds ratios of different DD levels to be associated with poor resolution of DVT for the whole group of patients and for the patients without malignancy. A ninefold excess tendency was seen for DD levels > 1200 ng/ml after initial therapy to be associated with poor resolution of the DVT (odds ratio 9.0, 0.95 CI 2.3-35.4). The odds ratio for levels > 1200 ng/ml after initial therapy for the group of patients without malignancy was 17.5, 0.95 CI 3.3-92.5.

Discussion

The objective of this study was to assess the time course of DD, a specific fibrinolytic parameter, in patients presenting an acute DVT treated with standard anticoagulant treatment. The second objective was to investigate the predictive value of this parameter for regression of DVT, because incomplete regression might lead to long-term complications like recurrent DVT or the post-thrombotic syndrome.

The recent development of assays for activation markers of the haemostatic and fibrinolytic system allows rapid and specific determination of these parameters in plasma. We used DD as a specific fibrin degradation product being increasingly used as a screening test for diagnosis of DVT because DD is elevated in the presence of active thrombosis. A high sensitivity and negative predictive value of DD assays has been reported in large series of patients (6-8).

In agreement with Mirshahi et al. (11) and Arcelus et al. (14) we found a trend but no significant correlation of DD levels with the location of the DVT. An explanation for this might be the influence of the age of the thrombus and the fact that the relation between fibrin mass and fibrin-split products also depends on the activity of the fibrinolytic system, which varies among patients. Also extravascular fibrin deposition may contribute to elevated DD. Because of these confounders DD levels are probably of no value for the prediction of thrombus size in individual patients.

Previous reports indicate that, at initiation of heparin therapy, a sharp decrease in DD levels occurs within 1-3 days, followed by a continuous and slow decline towards the normal range (9-11). There

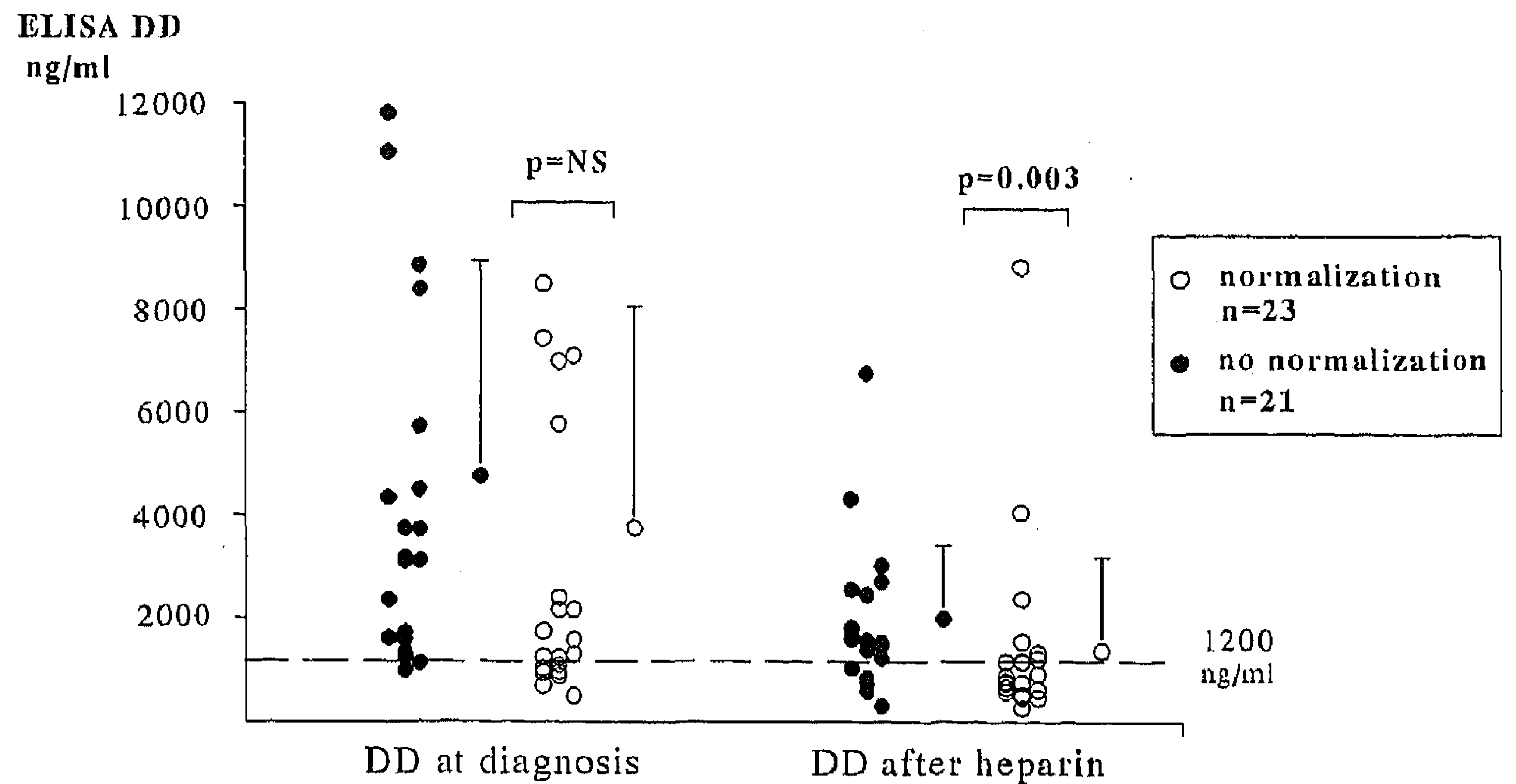


Fig. 2 Evolution of plasma DD levels during initial heparin therapy in patients with and without normalization after 3 months. Mean values \pm SD are also indicated

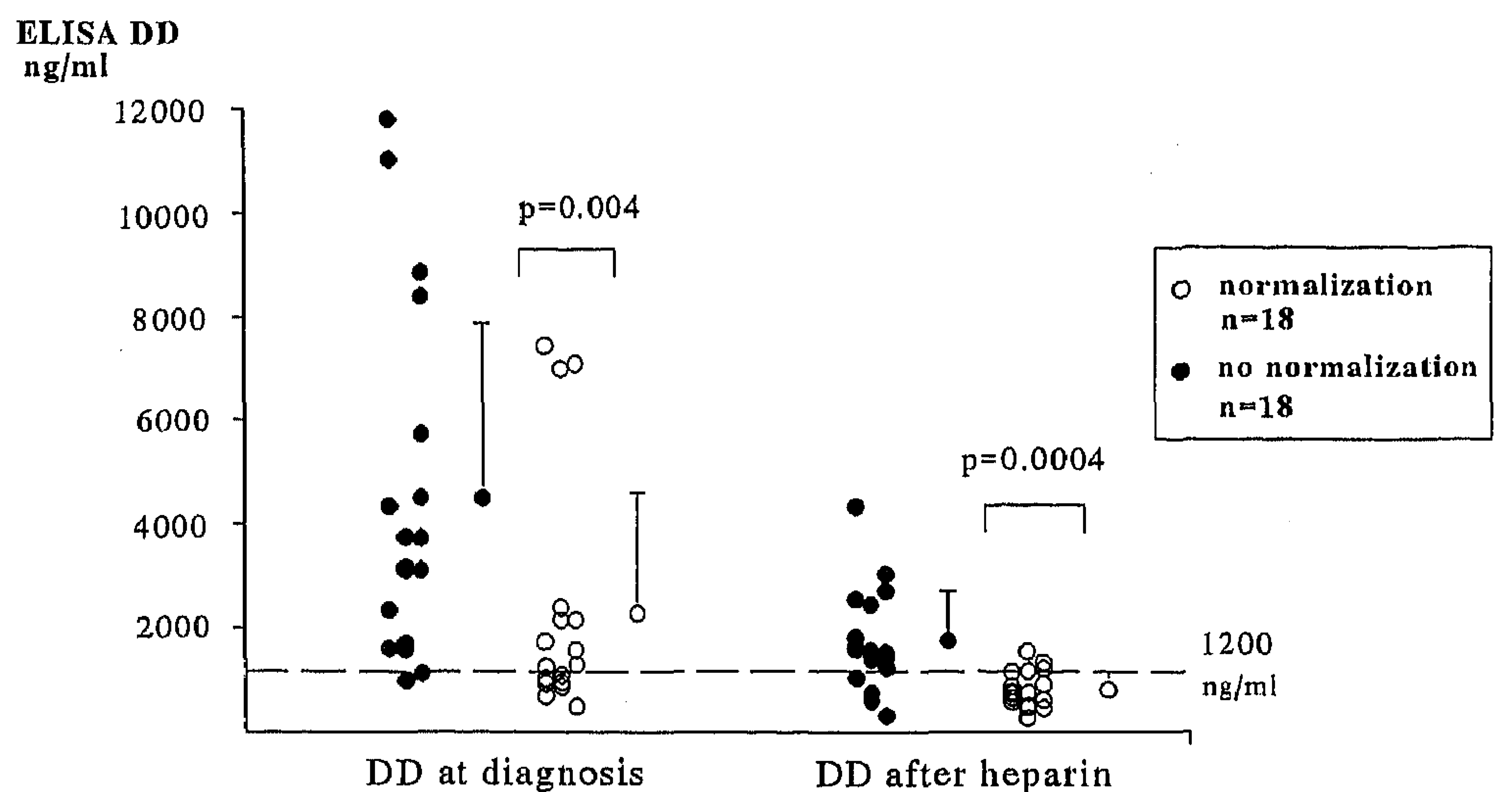


Fig. 3 Evolution of plasma DD levels in the group of patients without malignancy during initial heparin therapy in patients with and without normalization after three months. Mean values \pm SD are also indicated

are anecdotal reports on patients with thromboembolic recurrence and secondary increase of DD levels (9). In the current study DD also decreased significantly during the initial phase of treatment. Remarkably in only 4 patients DD was completely normalized (<500 ng/ml) at the end of initial heparin therapy. An explanation could be that fibrinolysis is a long lasting phenomenon which occurs independently of thrombin generation and of heparin therapy. Another possibility could be linked to the relatively long DD half-life (8 h).

We decided to evaluate outcome of DVT after 3 months because that is currently the recommended time to withdraw anticoagulant treatment after a first period of DVT (15). There are two other studies comparing levels of plasma markers to clinical outcome. The first study compares DD levels with venography after 10 days (10). During this period a relation was found between decrease of DD and regression of DVT. Arcelus et al. evaluated plasminogen activator inhibitor (PAI) and DD levels with trombus regression during 6 months (14). They found a significant relation between PAI levels and a trend between DD levels and outcome of DVT. Our results demonstrate a significant relation between DD levels after initial therapy and regression of DVT, even though patients with distal DVT were excluded. Also a ninefold excess tendency was seen for DD levels >1200 ng/ml to be associated with poor resolution of the DVT (odds ratio 9.0, 0.95 CI 2.3-35.4).

Figure 3 shows that the differences between the group that normalized and the group that did not normalize were even more significant when patients with an established malignancy ($n = 8$) were excluded.

Table 2 Odds ratios of DD for poor resolution of DVT at different cut-off levels

cut-off level (ng/ml)	Odds ratio (all patients)	Odds ratio (exclusion of patients with malignancy)
500	3.0	3.4
700	4.6	5.0
900	5.5	10.0
1000	6.6	13.0
1200	9.0	17.5
1500	7.7	26.7
2000	3.3	6.9
3000	1.8	2.0
4000	1.1	1.1

For this group of patients the odds ratio for DD levels >1200 ng/ml associated with poor resolution was 17.5 (0.95 CI 3.3-92.5). This is not surprising concerning the fact that different types of malignancy also cause elevated DD levels (16-18); it was demonstrated that the accuracy of DD in diagnosis of DVT is also less in patients with a malignancy (19).

In conclusion, this study provides evidence that increased DD levels during the initial heparin treatment, might be related to outcome of DVT after 3 months, especially in patients without malignancy. To estimate the predictive value of an elevated DD validation in a separate group of patients is needed. These findings may contribute to further insight into the process of thrombus regression. Also it could help to identify the patients with a poor prognosis and may be useful to judge the efficacy of anticoagulant treatment. Whether or not serial measurement of DD is useful for monitoring and predicting regression of DVT, at what time interval this should be performed and what should be the best cut-off value are questions for future studies in larger patient populations.

Acknowledgments

The authors are indebted to Mrs M. Willemsen for performing the DD assays.

References

1. ten Cate JW, Koopman MMW, Prins MH, Büller HR. Treatment of venous thromboembolism. *Thromb Haemost* 1995; 74: 197-203.
2. Janssen MCH, Nováková IRO, Verbruggen H, Wollersheim H, Thien T. New developments in the treatment of deep venous thrombosis. *Neth J Med* 1997; 50: 36-45.
3. Caprini JA, Arcelus JJ, Hoffman KN, Size G, Laubach M, Traverso CI, Coats R, Finke N, Reyna JJ. Venous duplex imaging follow-up of acute symptomatic deep vein thrombosis of the leg. *J Vasc Surg* 1995; 21: 472-6.
4. Prandoni P, Cogo A, Bernardi E, Villalta S, Polistena P, Simioni P, Noventa F, Benedetti L, Girolami A. A simple ultrasound approach for detection of recurrent proximal-vein thrombosis. *Circulation* 1993; 88: 1730-5.
5. Manton M. Deep venous thrombosis: Longitudinal study with Duplex US. *Radiology* 1991; 179: 271-3.
6. Bounameaux H, de Moerloose P, Perrier A, Reber G. Plasma measurement of D-dimer as diagnostic aid in suspected venous thromboembolism: an overview. *Thromb Haemost* 1994; 71: 1-6.
7. Elias A, Aptel I, Huc B, Chale JJ, Nguyen F, Cambus JP, Boccalon H, Boneu B. D-Dimer test and diagnosis of deep vein thrombosis: A comparative study of 7 assays. *Thromb Haemost* 1996; 76: 518-22.
8. Janssen MCH, Heebels AE, Metz de M, Verbruggen H, Wollersheim H, Janssen S, Schuurmans MMJ, Nováková IRO. Reliability of five rapid D-Dimer assays compared to ELISA in the exclusion of deep venous thrombosis. *Thromb Haemost* 1997; 77: 262-6.
9. Speiser W, Mallek R, Koppensteiner R, Stumpflen A, Kapiotis S, Minar E, Ehringer H, Lechner K. D-dimer and TAT measurement in patients with deep venous thrombosis: utility in diagnosis and judgement of anticoagulant treatment effectiveness. *Thromb Haemost* 1990; 64: 196-201.
10. Alexandre P, Amiral J, Bienvenu J, Borg JY, Combe S. Markers of hemostatic system activation in acute deep venous thrombosis-evolution during the first days of heparin treatment. *Thromb Haemost* 1993; 70: 909-14.
11. Mirshahi M, Soria C, Soria J, Faivre R, Kieffer Y, Bassand JP, Toulemonde F, Caen J. Changes in plasma fibrin degradation products as a marker of thrombus evolution in patients with deep vein thrombosis. *Thromb Res* 1988; 51: 295-302.
12. Fischer J, Verbruggen B, Wessels H, Haanen C. Interference of coumarin therapy with the "Heptest" owing to declining prothrombin concentrations. *Clin Chem* 1989; 35: 483-6.
13. Lensing AW, Prandoni P, Brandjes D, Huisman PM, Vigo M, Tomasella G, Krekt J, Wouter ten Cate J, Huisman MV, Büller HR. Detection of deep-vein thrombosis by real-time B-mode ultrasonography. *N Engl J Med* 1989; 320: 342-5.
14. Arcelus JJ, Caprini JA, Hoffman KN, Fink N, Size GP, Fareed J, Hoppensteadt D. Laboratory assays and duplex scanning outcomes after symptomatic deep vein thrombosis: preliminary results. *J Vasc Surg* 1996; 23: 616-21.
15. Sarasin FP, Bounameaux H. Duration of oral anticoagulant therapy after proximal deep vein thrombosis: a decision analysis. *Thromb Haemost* 1994; 71: 286-91.
16. Zurborn KH, Duscha H, Gram J, Bruhn HD. Investigations of coagulation system and fibrinolysis in patients with disseminated adenocarcinomas and non-Hodgkin's lymphomas. *Oncology* 1990; 47: 376-80.
17. Edwards CM, Warren J, Armstrong L, Donnelly PK. D-dimer: a useful marker of disease stage in surgery for colorectal cancer. *Br J Surg* 1993; 80: 1404-5.
18. Mirshahi SS, Pujade Lauraine E, Soria C, Mirshahi M, Fretault J, Bernadou A, Soria J. D-dimer and CA125 levels in patients with ovarian cancer during antineoplastic therapy. Prognostic significance for the success of anti-cancer treatment. *Cancer* 1992; 69: 2289-92.
19. Raimondi P, Bongard O, de Moerloose P, Reber G, Waldvogel F, Bounameaux H. D-dimer plasma concentration in various clinical conditions: implication for the use of this test in the diagnostic approach of venous thromboembolism. *Thromb Res* 1993; 69:125-30.

Received February 10, 1997 Accepted after resubmission April 8, 1997