Venous Thrombosis and D-dimers

A new approach in diagnostic management

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Venous Thrombosis and D-dimers

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Veneuze trombose en D-dimeren
Een nieuwe diagnostische benadering
(met een samenvatting in het Nederlands)

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- There is no pleasure in having nothing to do;
the fun is in having lots to do and not doing it -

Mary Wilson Little

CONTENTS

CHAPTER 1

Introduction

INTRODUCTION

The golden standard for diagnosing deep venous thrombosis (DVT) has been venography for many years. Venography is an invasive, expensive and time-consuming procedure which is not available in every hospital and not suitable for emergency practice. Compression ultrasonography (CUS) has mainly replaced the venography in many hospitals. The sensitivity and negative predictive value of CUS are respectively 95% and 98% for proximal DVT (1), but are considerably lower (36-93%) for distal DVT (2-4). The incidence of pulmonary embolism (PE) in distal DVT is 20-30%, but almost all emboli are asymptomatic and clinically unimportant (5). A distal DVT, however, can expand to the proximal system which has a PE incidence of 50% (5). This has resulted in the general recommendation to perform a second CUS within one week after the initial CUS to detect a possible progression from a distal DVT to the proximal system. Only about 25% of all patients suspected for DVT actually has thrombosis (6;7), which makes the diagnostic procedure of repeated CUS inefficient and expensive. Newer non-invasive strategies to reduce the need for CUS in the diagnostic management of venous thromboembolism are warranted. Major developments in the management of screening for DVT are the renewed interest for the clinical score and the introduction of measurement of the D-dimer concentration.

The pre-test clinical probability score

The clinical appearance of DVT is heterogeneous and for a long time, the clinical parameters have been considered to be useless. The introduction of a new clinical assessment score (Table) has made it possible to classify patients with a low, moderate or high pre-test clinical probability (PCP) score, with respectively a 3%, 17% and 75% chance of having DVT (8). The PCP score alone however, is not sensitive enough for replacing CUS. It is also subjected to the investigators' clinical view and thus inter-observer variances. The PCP score may, however, have an important role in a diagnostic strategy where other non-invasive tools are combined.

D-dimers

Plasma D-dimers are degradation products of cross-linked fibrin and thus markers of fibrinolysis (Figure). As thrombus formation simultaneously activates fibrinolysis, the D-dimer concentration can be used as an indirect measurement of thrombus formation. The D-dimer has a high sensitivity in the exclusion of thrombosis (95-100%), according to the type of D-dimer assay used (9-13). It has been suggested that a normal D-dimer concentration can be used to exclude thromboembolic processes (14), where abnormal D-dimer concentrations necessitate further investigations such as ultrasonography or venography.

The major disadvantage of the test is the frequent finding of a positive test: increased concentrations of D-dimer levels are observed in many conditions, such as infection and malignancy. This implicates that the specificity of the test is low.

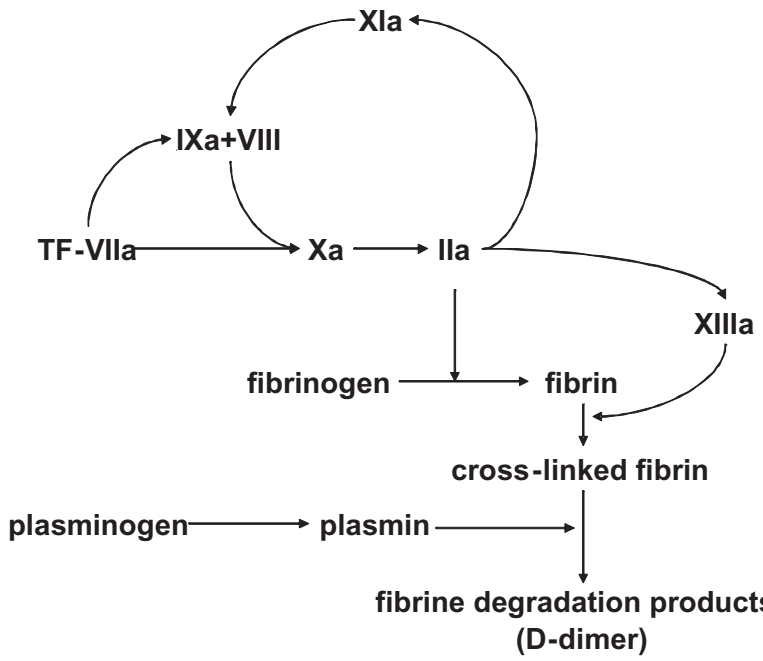
So far, no consensus exists about the exact place of the PCP score and the D-dimer in reducing the need for CUS in the management of patients suspected for having DVT.

Table. Pre-test clinical probability score for deep vein thrombosis (8)

Features	Score
Active cancer	1
Paralysis, paresis or recent plaster immobilisation	1
Bedridden >3 days or major surgery within 4 weeks	1
Localised tenderness along distribution of the deep venous system	1
Entire leg swollen	1
Calf swelling >3cm compared with asymptomatic leg	1
Pitting oedema (greater in symptomatic leg)	1
Non-varicose collateral superficial veins	1
Alternative diagnosis	-2

A score ≤ 0 is considered to be a low, 1-2 a moderate and ≥ 3 a high probability for having deep venous thrombosis of the leg.

Figure



When tissue damage occurs, Tissue Factor (TF) is released, that will directly bind with activated factor VII (VIIa) to form the TF-VIIa complex. This complex activates factor X, that will transform prothrombin into thrombin (IIa). The activation of factor X by the TF-VIIa complex not only takes place by direct activation, but also by activation of factor IX. Thrombin stimulates its own production by activation of factor XI and IX. The subsequent generated thrombin will split fibrinogen into fibrin monomers. Under the influence of activated factor XIII, stable cross-linked fibrin will be formed. Fibrinolysis of this cross-linked fibrin is induced by plasmin, and results in the formation of fibrin degradation products. One of these products is the D-dimer. (This model is strongly simplified)

OUTLINE OF THE THESIS

The subject of this thesis is to refine the role of the D-dimer assay in the diagnostic management of DVT.

CHAPTER 2 discusses laboratory aspects of the D-dimer test. Can the D-dimer concentration be measured using heparin plasma on routine chemical analyzers and do variances in the (pre)analytic process influence the D-dimer determination?

The question about the time of increase and decrease of D-dimer concentration after formation of a clot is addressed in **CHAPTER 3**. The early course of D-dimer concentration is described after initiation of an artificial clot in patients with hereditary hemorrhagic teleangiectasia who underwent embolisation of pulmonary arteriovenous malformations.

In **CHAPTER 4**, the clinical performance of a latex D-dimer assay in the diagnosis of DVT is studied. It discusses whether certain clinical or laboratory conditions result in a lower sensitivity or specificity of the test with the possibility to restrict the use of the D-dimer test in selected patients.

The key-question is whether the D-dimer test can replace or reduce the need for CUS in outpatients suspected for having DVT. **CHAPTER 5** describes a multicenter management study on diagnosing DVT using the combination of the PCP score, the D-dimer test and CUS. Results of the safety of exclusion of DVT by a non-high PCP score and a normal D-dimer concentration are given.

As several D-dimer assay are available, it is of interest to be informed about the performances of different tests in the same study population. In **CHAPTER 6**, five different D-dimer assays are compared using primary clinical outcomes as sensitivity, negative predictive value and specificity for DVT.

For the early treatment of VTE, low molecular weight heparin (LMWH) has proven to be equally effective as unfractionated heparin (UFH) from a clinical point of view (15-30). **CHAPTER 7** describes whether this is also true for changes in coagulation markers during the first days of treatment of PE. The effects of LMWH versus UFH on different coagulation and fibrinolytic

markers as fragment 1+2, thrombin-antithrombin complexes, fibrin monomers, D-dimers and clot lysis times are described, as well as early changes in perfusion abnormalities.

In 60-80% of all DVT, there is an identifiable underlying cause that is responsible for the thrombophilic state. The association of thrombosis with cancer is well known. Screening for malignancy in every patient with DVT is laborious and expensive. In **Chapter 8**, the question is addressed whether the height of the initial D-dimer concentration or the early course of the D-dimer concentration during treatment for DVT can be used as a predictor for underlying malignancy in patients with DVT.

An overview of recent published articles on the diagnostic management of deep venous thrombosis is given in **Chapter 9.**

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CHAPTER 2

No influence of heparin plasma and other (pre)analytic variables on D-dimer determination

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SUMMARY

Background Sample material for D-dimer, as for most other coagulation tests, is citrate plasma, which is inconvenient when the test is performed on a clinical chemistry analyzer. Other (pre)analytic variables might influence the D-dimer determination, but are not studied extensively. We investigated the effect of heparin plasma and different (pre)analytic variables on the D-dimer concentration.

Methods Heparin plasma for D-dimer concentration, using the Tina-quant® latex assay, was compared with citrate plasma in 190 patients. The possible influence of pneumatic mail was investigated in heparin plasma from 25 patients: one tube was transported by pneumatic mail and the other was carried by hand. For sample stability analysis, D-dimer concentration in random citrate plasma samples was measured immediately, after 16 and 24 hours and after having been frozen once or stored for two years at –70°C. For analyzer analysis, we compared D-dimer results from a Hitachi 917 and an Integra 700 analyzer in citrate and heparin samples from 190 patients; in 145 other heparin samples, we compared the data obtained from a Hitachi 717 and an Integra 400 analyzer.

Results D-dimer concentration in citrate and heparin plasma showed a perfect correlation (r=1.00). The D-dimer concentrations in heparin were 19% higher than in citrate plasma, which can be attributed to the dilution by the citrate solution. No influences of pneumatic dispatch, time of measurement, freezing or type of analyzer on D-dimer concentrations were found.

Conclusions Our data show the validity of the determination of D-dimer concentrations using heparin plasma. The D-dimer assay remains valid under different (pre)analytic conditions.

INTRODUCTION

D-dimer, a classical coagulation parameter, is increasingly used in the exclusion of venous thromboembolism and the evaluation of coagulopathies. As with all other coagulation tests, the sample material is citrate plasma. In particular when the D-dimer test is performed on a clinical chemistry analyzer, the use of citrate plasma is a burden. Depending on whether other coagulation tests are requested, either an extra tube for the D-dimer determination or sample splitting is needed. Furthermore, as citrate plasma used for coagulation tests is centrifuged differently from heparin plasma and serum, it disturbs the clinical chemistry workflow. Because D-dimer will be used in an emergence setting, where a short turn-around time (TAT) is mandatory, it would be advantageous if D-dimers could also be determined in heparin plasma.

Although many investigators have studied different D-dimer assays mainly in search of clinical outcomes as sensitivity and negative predictive value (1-3), it is possible that differences in the (pre)analytic processes can lead to different results of the D-dimer assay. The influence of (pre)analytic variables on the D-dimer concentration has not been studied extensively. There are reports on the effect of freezing on D-dimer concentration (4-7), but the effects of transport and type of analyzer have been less studied (8;9).

The first objective of this study is the validation of D-dimer determinations using heparin plasma. The second objective of this study is to investigate whether the results of a D-dimer assay are influenced by different (pre)analytic processes, such as transportation, time of measurement, freezing and the type of analyzer used.

METHODS

D-dimer measurement

We used the Tina-quant® (Roche, Germany) quantitative latex assay for determination of D-dimer concentrations. Samples were collected into S-Monovette® 9NC tubes (0.106 mol/L sodium citrate; Sarstedt, Rommelsdorf, Germany) and S-Monovette® lithium heparin tubes (15 IU/mL lithium-heparin). Citrate samples were centrifuged according to the standard protocol for coagulation samples (3000g for 15 minutes without brake) and lithium-heparin samples were centrifuged according to the protocol for clinical chemistry samples (2200g for 10 minutes with brake). Measurements were performed on a Roche Hitachi 917 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) and a Cobas Integra 700 analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Pre-analytic conditions

Comparison of citrate versus heparin plasma:

Citrate and heparin plasma samples were randomly obtained from 190 patients (139 in Munich and 51 in Nieuwegein). The samples in Munich were analyzed on a Hitachi 917 and the samples in Nieuwegein were analyzed on an Integra 700 analyzer.

Method for transport conditions:

From 25 patients, 2 tubes of heparin plasma were collected. One of the heparin-plasma samples was sent to the laboratory by pneumatic mail (a distance of approximately 100m); the other sample was carried by hand. The samples were centrifuged as clinical chemistry samples. D-dimer concentration was measured on an Integra 700 analyzer. In another experiment, transport was simulated by placing sample tubes on an oscillating roller. One tube citrate plasma and two tubes of lithium-heparin plasma from three healthy donors were obtained. The citrate sample and one of the lithium-heparin samples were centrifuged immediately. The citrate sample was left standing at room temperature. The centrifuged lithium-heparin plasma was split into two tubes; one was left standing at room temperature, the other tube was placed on a roller. The second lithium-heparin tube was not centrifuged but placed directly on the roller (anticoagulated whole

blood) and centrifuged afterwards. D-dimer concentration in all samples was determined using a Hitachi 917 analyzer.

Method for sample stability:
Random clinical citrate (n=15) and heparin (n=17) plasma samples were collected from patients. The D-dimer concentration was measured directly and measured again after the samples had stood at room temperature for 16 and 24 hours and after having been frozen once (snap-frozen in liquid nitrogen). In addition, five citrate plasma samples from a two year old clinical study, which had been stored at –70°C, were measured. All measurements except for the long term stability study were performed on a Hitachi 917. For the 2 year stability study, the D-dimer measurement was done on an Integra 700.

Analytic conditions

Comparison of analyzers:
The first experiment was to compare a Hitachi 917 and an Integra 700 analyzer by which D-dimer concentration was measured using citrate and heparin plasma of 190 patients.
In a second experiment, from 145 patients fresh heparin samples were taken. D-dimer concentrations were measured on an Integra 400 and a Hitachi 717 analyzer. The standard centrifugation protocol for clinical chemistry samples was used, as described previously.

Statistics

For comparison of D-dimer results, the Pearson and Kendall correlation coefficient and the Passing-Bablok regression were used. The Student t-test was used to determine the influence of simulated transport on the D-dimer concentration and to determine the sample stability of D-dimer concentration.

RESULTS

Pre-analytic conditions

Comparison of citrate versus heparin plasma:

In Fig. 1, D-dimer concentrations in citrate plasma are compared with heparin plasma. We found that the results in heparin were higher than in citrate plasma: mean 2.51 vs 2.06 mg/L. There was a high correlation between the citrate- and heparin plasma samples and between the two analyzers (r=1.0, slope=1.196, Kendall tau=0.959, md(95)=0.18).

Influence of transport:

The effect of transporting heparin samples by pneumatic mail is shown in Fig. 2. We found no difference between the two sample groups (mean 1.57 vs 1.59 mg/L; r=0.999, slope=0.993, Kendall tau=0.967, md(95)=0.09, p=0.4). The results of simulated transport by placing sample tubes on an oscillating roller are given in Table 1; there was no difference in D-dimer concentrations among the samples.

Sample stability:

Results of sample stability are given in Fig. 3 for citrate plasma and Fig. 4 for heparin plasma. No differences were found between the different times of measurement (p=0.4 for 16 h and 0.557 for 24 h in citrate plasma; p=0.3 for 16 h and 0.3 for 24 h in heparin plasma). There was no effect of freezing (p=0.3 for citrate-plasma samples frozen once; p=0.1 for citrate-plasma samples stored frozen for 2 years; p=0.3 for heparin-plasma samples frozen once).

Figure 1. D-dimer concentration in citrate versus heparin plasma on Integra 700 (□) and Hitachi 917(△) analyzers (n=190)

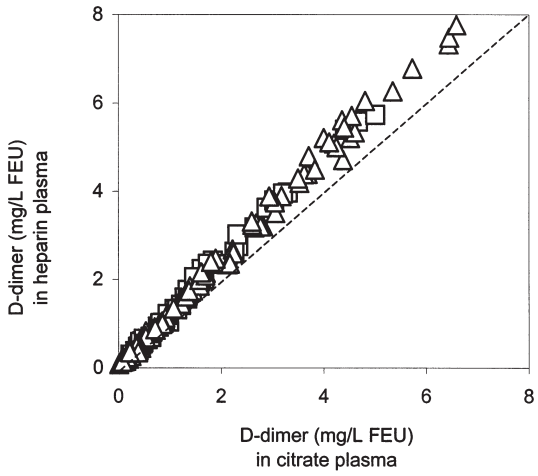


Figure 2. D-dimer concentration in lithium-heparin plasma with and without transport by pneumatic mail (n=25)

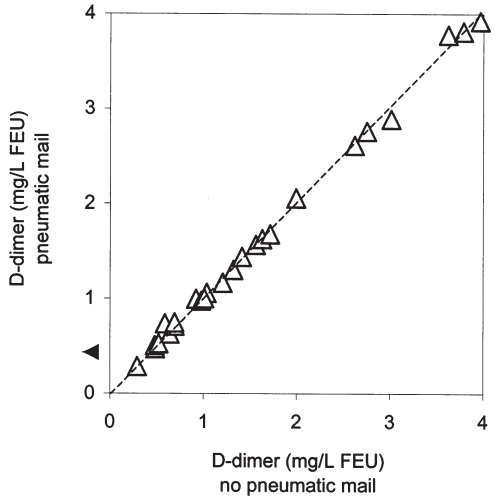


Figure 3. Sample stability in citrate plasma (○ after 16 hours (n=11), □ after 24 hours (n=13), △ frozen 1 time (n=9), ✳ frozen 2 years (n=5))

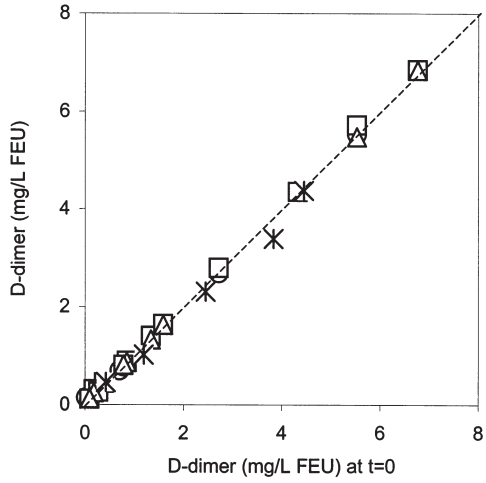


Figure 4. Sample stability in heparin plasma (○ after 16 hours (n=13), □ after 24 hours (n=16), △ frozen 1 time (n=10))

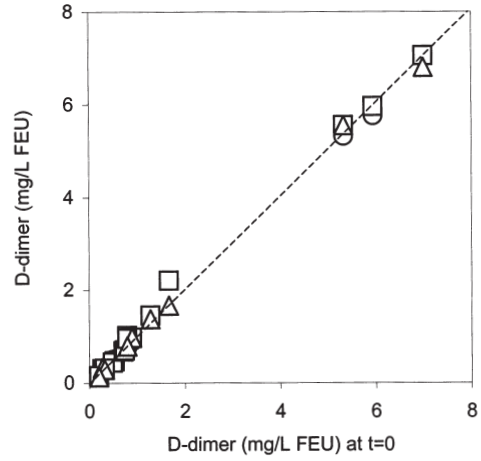


Table 1. Sample stability in simulated transport by an oscillating roller (average of 3 donors). Values in lithium-heparin plasma have been corrected for citrate references. Values are reported as mean ± SD. P-values indicate differences compared to citrate plasma.

Material and condition	D-dimer (µg FEU/mL)	P-value
Citrate plasma[a]	0.207 ± 0.081	
Lithium-heparin plasma[b]	0.253 ± 0.045	0.5
Lithium-heparin plasma roller[c]	0.217 ± 0.081	0.2
Lithium-heparin plasma whole blood roller[d]	0.223 ± 0.076	0.1

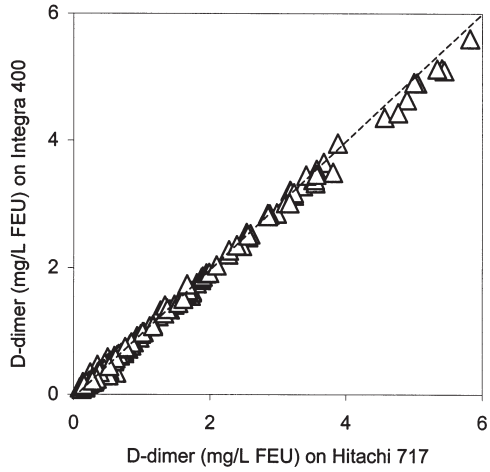
[a] centrifuged immediately and left standing at room temperature
[b] centrifuged immediately and left standing at room temperature
[c] centrifuged immediately and placed on an oscillating roller
[d] immediately placed on a roller (anti-coagulated whole blood) and centrifuged afterwards.

Analytic conditions

Comparison of analyzers:

Fig. 1 shows the comparison of D-dimer measurements on a Hitachi 917 and an Integra 700 analyzer. A good correlation was found (r=1.000, slope=1.196, Kendall tau=0.959). Fig. 5 shows the comparison of D-dimer measurements in heparin plasma on a Hitachi 717 and Integra 400 analyzer. No differences were found (r=0.999, slope=1.0165, Kendall tau=0.950).

Figure 5. D-dimer determination in lithium-heparin plasma on Integra 400 and Hitachi 717 analyzers (n=145)



DISCUSSION

Our data confirm and extend the previous report of Vukovich et al (10) on the validity of performing the Tina-quant D-dimer test using heparin plasma rather than the standard measurements in citrate plasma. This finding substantially decreases the TAT of the D-dimer test by reducing pre-analytic processing. We estimate that, in our hospital, this TAT has been reduced from 60 to 30 minutes. Our observation has an important consequence for the use of D-dimer measurements in emergency settings, where it is a prerequisite to perform a reliable test with a short TAT. It also implicates that a D-dimer test performed on a chemical analyzer can compete with the newer point-of-care D-dimer tests: the latter D-dimer tests have less extensively been validated and their advantage from a time-consuming point of view is now being diminished.

We found the D-dimer concentration in heparin plasma to be higher than in citrate plasma, with an average of 19%. This can be explained by the fact that there is no dilution in the heparin plasma, whereas this is the case in the citrate solution. This percentage was identical in both centers that participated in this study. As all reports in literature are based on the standard citrate solution, we therefore multiply the results in the heparin samples by a factor of 0.84 (=1/1.19) to avoid the need for a change in reference and cut-off values. This correction factor is in accordance with the correction factor that has been found by Vukovich et al (10) and corresponds with what one would theoretically expect when 1 part citrate solution and 9 parts blood with a hematocrit of 42% are taken together (11).

The D-dimer concentration in lithium-heparin plasma in our study is unaffected by transport by pneumatic mail or by rough handling, simulated by placing the sample on an oscillating roller. This opens the possibility of pneumatic dispatch of the sample tubes and thus a gain in time at the emergency department. It also indicates that samples obtained by general practitioners or local hospitals with limited capacity can be transported to a central laboratory for D-dimer measurements without decreasing their quality. That means that patients do not necessarily have to go to the emergency room for D-dimer determinations.

We found that the D-dimer concentrations in citrate and heparin plasma were stable regardless of the time of measurement, freezing or the type of analyzer used. This indicates that it is valid to perform studies on frozen

material and that test results of the Tina-quant® assay, obtained under different (pre)analytic conditions, can be compared without loss of reliability.
In conclusion, measurement of D-dimer concentrations with the Tina-quant® D-dimer test using heparin plasma is valid and provides a reduction in TAT. The D-dimer assay remains valid under different (pre)analytic conditions.

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CHAPTER 3

The early course of D-dimer concentration following pulmonary artery embolisation

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SUMMARY

Background There is little information about the course of D-dimer concentration in patients during the first hours of a thrombotic event. We measured D-dimer concentrations following pulmonary artery embolisation in patients with pulmonary arteriovenous malformations.

Methods We studied D-dimer concentration before and during an 8-hour time period after embolisation in 13 patients; the control group were 14 patients with a diagnostic heart catheterisation without embolisation. We investigated the extent of D-dimer concentration in relation to the total volume of the parts of the embolised arteries, the improvement in pO2 and the reduction in shunt fraction.

Results The patients had significantly increased D-dimer levels at t=2, 4 and 8 hours after the procedure as compared to baseline ($p=0.001$) and to the controls ($p=0.047$). We found a correlation between D-dimer concentration and the calculated volume of the embolised arteries ($R^2=0.74$).

Conclusions There is no relevant lag time between the increase in D-dimer concentration and pulmonary embolisation. The relation between D-dimer concentration and thrombus size deserves confirmational studies.

INTRODUCTION

The onset of venous thromboembolic processes is accompanied by an increase in the concentration of D-dimers, which are products of the plasmin-mediated proteolysis of cross-linked fibrin. Measurement of D-dimer concentration is considered to be a promising tool in the reduction of additional tests to rule out venous thromboembolism (VTE). Sensitivity and negative predictive value of D-dimer tests have been investigated in several management studies (1-4). Although some authors suggest that anticoagulant therapy can be withheld in patients with normal D-dimer levels (5;6), studies about the accuracy of different D-dimer tests show large variations in sensitivity and negative predictive value (7-9). A source of variation in D-dimer concentration may be the time of D-dimer determination in relation with the duration of symptoms or the onset of the thromboembolic process (10;11). Data about the course of D-dimer concentration in patients with VTE are limited to a study during the first days of heparin treatment in patients with deep venous thrombosis (12). It is of clinical importance to be informed about the time of increase and decrease in D-dimer concentration to establish the optimal time of D-dimer testing and to define the time-period before and after which a D-dimer test may have reduced accuracy. In addition, it is of interest to be informed about the correlation between the size of the thrombus and the extent of D-dimer concentration, as large thrombi may lead to a more aggressive therapeutic approach and to a more careful monitoring.

We studied the course of D-dimer concentrations in patients undergoing embolisation of pulmonary arteries because of pulmonary arteriovenous malformations (PAVM) that caused hypoxemia and right-to-left shunts. We also calculated the correlation between D-dimer concentrations and the estimated size of the thrombus.

PATIENTS AND METHODS

Patients

We studied 13 patients with hereditary hemorrhagic teleangiectasia (HHT) or Rendu-Osler-Weber disease who underwent embolisation because of PAVM causing hypoxemia and increased right-to-left shunts. Embolisation in patients with PAVM induces thrombus formation due to local haemostasis in the presence of thrombogenic fibres attached to coils. The patient group consisted of 13 individuals (3 men and 10 women) with a mean age of 39.9 ± 12.2 years (range: 23-66 years). The control group consisted of 14 patients (8 men and 6 women) with a mean age of 56.3 ± 17.5 years (range: 23-82 years) who underwent a diagnostic right and/or left heart catheterisation procedure with pulmonary or coronary angiography without any intervention. The individuals in the control group were suspected of aortic or mitral insufficiency (n=4), aortic stenosis (n=1), coronary insufficiency (n=2), HHT (n=3), pulmonary hypertension (n=2), Scimitar syndrome (n=1) or screened for lung transplantation (n=1). Patients and controls did not use oral anticoagulants.

Methods

The catheterisation in both groups was carried out by right heart catheterisation through a femoral approach. The quantity of heparin to flush the catheter in the embolised group was at least 3000 IU with a maximum of 5000 IU depending on the length of the procedure. The control group received a standard dose of 5000 IU heparin. There was no significant difference in the median dose of heparin used in patients and controls. The duration of the procedure was 1-3 hours in both groups. For the embolisation, we used coils made of platinum with and without synthetic fibres (Cook, Denmark and Target Therapeutics, Ireland). Blood was collected two hours before, at the start of and two, four and eight hours after the procedure. We used the Asserachrom® ELISA (Diagnostica Stago, France) for the determination of D-dimer concentrations. All patients gave informed consent and the medical ethical committee of the St. Antonius Hospital approved the study protocol.

The size of the thrombus induced by the coils was estimated in three different ways. We calculated the total volume of the parts of the pulmonary arteries that were embolised ($\sum \pi r^2 x$ length).

We calculated the reduction of the right to left shunt measured with 100% oxygen, as described previously (13-15). Finally, we measured differences in pO2 (in kPascal) before and after the embolisation.

Statistics

Between groups repeated measurements ANOVA was used (α-levels were 0.05). Data are given as mean ± standard error of the mean. For calculation of correlation Pearson's correlation and p-values were used.

RESULTS

The course of D-dimer concentration determined by the Asserachrom® ELISA assay in the patient and control group is shown in Fig. 1. The results at t=-2 and t=0 hours were similar and combined at t=0 hours. At the start of the procedure, no differences in D-dimer concentration were found between the two groups. At t=2 hours, D-dimer concentration in the patient group was significantly higher as compared to D-dimer concentration at baseline (p=0.001). The D-dimer values obtained at t=4 hours and t=8 hours remained significantly higher when compared to baseline (p=0.02 and p=0.001 respectively). The control group showed no significant increase in D-dimer concentration as compared to baseline. The increase in D-dimer concentration in the patient group differed significantly from the control group (p=0.047).

We found a correlation between D-dimer concentration and the calculated volume of the parts of the pulmonary arteries that were embolised (R^2=0.74, p<0.001) (Fig. 2), but not between D-dimer concentration and the decrease of the right to left shunt or the increase in pO2 (R^2=0.26, p=0.1 and R^2= 0.06, p=0.5 respectively).

Figure 1. Course of D-dimer concentration following pulmonary embolisation in patients with pulmonary arteriovenous malformations. Controls under went diagnostic heart catheterisation without intervention.

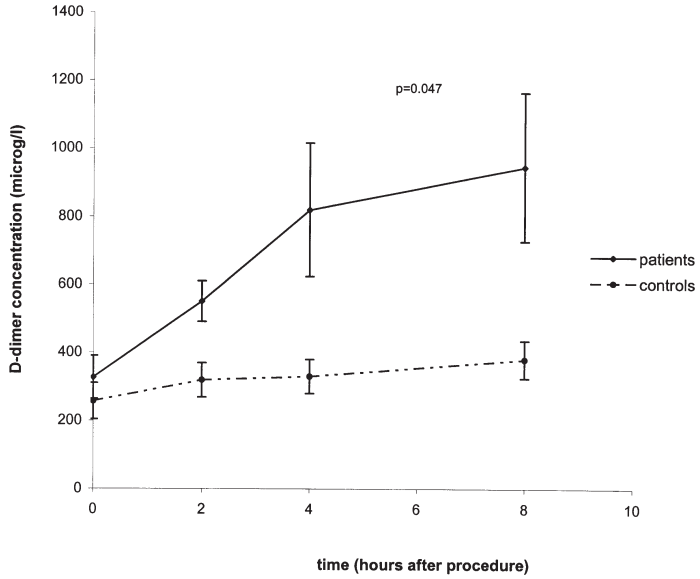
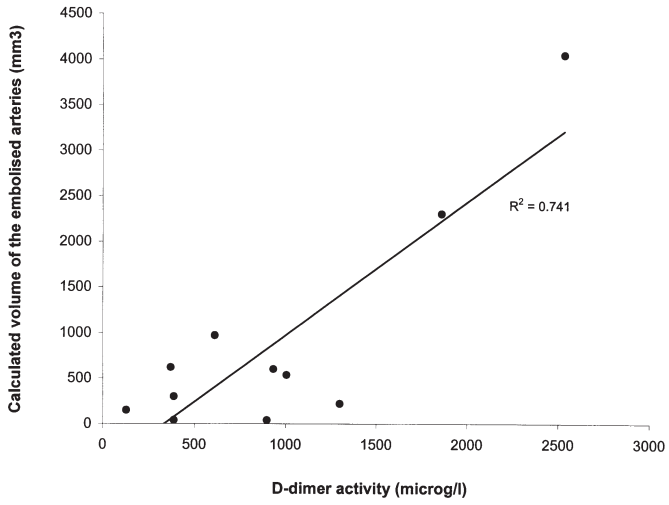


Figure 2. Relationship between D-dimer concentration and calculated surfaces of the parts of the embolised arteries following pulmonary embolisation.



DISCUSSION

Determination of D-dimer concentration is increasingly used as diagnostic tool for exclusion of VTE. The moment of D-dimer determination in pulmonary embolism (PE) varies from an immediate diagnostic procedure in patients with sudden onset of dyspnoea and chest-pain to a delayed procedure in patients with unexplained thoracic complaints for a longer period. The exact time after which D-dimer concentrations begin to rise and fall after the formation of a clot in humans is unknown. Because PE is often preceded by the formation of a thrombus in the legs, our model of PAVM-embolisation may not be identical to the events in patients with PE. Our study mimics, however, some of its aspects and provides data about the D-dimer concentration in the first hours after a thromboembolic event.

In this study in patients with PAVM undergoing embolisation, we observed a significant increase in D-dimer concentration at 2 hours after the start of embolisation, which remained significantly elevated during 8 hours. This implicates that D-dimer concentrations show an almost immediate increase after the start of embolisation and makes the existence of a lag time unlikely. A significant time by group interaction was present, which means that the differences in D-dimer concentration between patients and controls increased during the observation period of 8 hours and that the increase of D-dimer was not due to the catheterisation alone. The clinical implication of this finding is that D-dimer concentrations remain elevated for at least the first 8 hours after the onset of thrombosis.

In a previous study, a trend but not a significant correlation was found between D-dimer levels and the location of VTE (16). In the present study, we found a correlation between D-dimer concentrations and the calculated volume of the parts of the pulmonary arteries that were embolised. This may indicate higher D-dimer levels to be present in larger thrombus formations. The fact that we did not find a correlation between D-dimer levels and differences in shunt fraction and pO2 after embolisation may be due to the fact that the latter two are indirect estimates of thrombus formation, while measurement of the volume of the arteries is a more direct approach. Furthermore, there may be inter-individual differences in fibrinolytic activity after a thromboembolic event. However, as functional impairment is more relevant than the size of the thrombus, it is -in our opinion- not correct to delay further tests (such as pulmonary angiography) in patients suspected

for having PE with only small increases in D-dimer concentration and non-diagnostic ventilation-perfusion scans.
In conclusion, we found no relevant lag time between the increase in D-dimer concentration and pulmonary embolisation in patients with PAVM. D-dimer concentrations remain elevated during at least the first 8 hours of a thrombotic event. The D-dimer concentration may be helpful to predict the extent of thrombus formation.

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CHAPTER 4

Usefulness of a semiquantitative D-dimer test for the exclusion of deep venous thrombosis in outpatients

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SUMMARY

Background The D-dimer test is used commonly in diagnostic strategies to reduce the need for ultrasonography in patients suspected of having deep venous thrombosis. We studied several clinical and laboratory variables that might limit the accuracy of a semiquantitative D-dimer test.

Methods In this retrospective cohort study, 704 outpatients suspected of having deep venous thrombosis underwent a semiquantitative D-dimer test and ultrasonography. The performance of the D-dimer test was calculated in patients using anticoagulants (n=61), patients with previous thrombosis (n=127), and patients with malignancy (n=47), including 39 patients with more than one of these characteristics. The 508 remaining patients were considered to be the reference group.

Results A total of 254 patients (36%) had evidence of deep venous thrombosis. The D-dimer test had a sensitivity of 99% (174/176; 95% confidence interval [CI]: 96% to 100%) and a negative predictive value of 98% (98/100; 95% CI: 93% to 100%) in the reference group. The sensitivity of the D-dimer test in patients using oral anticoagulants was 75% (6/8; 95% CI: 35% to 97%; p=0.01 compared with the reference group). Test sensitivity was 96% (51/53; 95% CI: 87% to 100%) in patients with previous thrombosis, and 100% (29/29; 95% CI: 88% to 100%) in patients with cancer. However, 553 (79%) of all patients, including 43 of the cancer patients (91%), had an abnormal D-dimer test.

Conclusions The semiquantitative D-dimer test in this study has a high sensitivity and negative predictive value in the exclusion of deep venous thrombosis, except perhaps among patients using oral anticoagulants. D-dimer tests in patients with cancer and in patients over 70 years old may not be worthwhile, as the tests are usually positive.

INTRODUCTION

Diagnosing deep venous thrombosis remains a challenge. The current diagnostic strategy usually consists of performing an ultrasonography of the femoral and popliteal veins; this test is repeated within 1 week if the initial examination was negative. It is safe not to anticoagulate patients with repeated normal ultrasonography (1;2). However, only 15% to 28% of patients suspected of having deep venous thrombosis actually have a thrombosis (1-4), and only 1% of patients with an initially normal ultrasonography develop deep venous thrombosis within 1 week (1;2). This has led to a search for a more efficient diagnostic strategy.

Plasma D-dimer levels, which measure the degradation products of cross-linked fibrin, are a valuable diagnostic test for the exclusion of deep venous thrombosis, and it is safe to withhold anticoagulant treatment in patients with a single negative ultrasonography and a negative D-dimer test (2). In addition, the combination of clinical assessment and a normal D-dimer test might also reduce the need for ultrasonography (5-7). There is a wide range, however, in the diagnostic accuracy of D-dimer tests. The first generation of rapid latex agglutination assays, which can be used in an emergency and bedside setting, has considerably lower sensitivity and negative predictive values than do the classic enzyme linked immunosorbent assays (ELISA) (8-10). Newer, fully-automatic, quantitative rapid latex and ELISA tests may be more accurate (8-10), but are more expensive and are not widely available.

A major disadvantage of measuring D-dimer levels is the frequent finding of a positive test; thus ultrasonography is still needed in the majority of patients suspected of having deep venous thrombosis. It might save both time and health care costs to avoid measuring D-dimer levels in patients with a high a priori likelihood of having a positive test. Therefore, we sought to determine the usefulness of a latex D-dimer test in excluding deep venous thrombosis, as well as to define clinical variables (such as use of oral anticoagulants, previous deep venous thrombosis, malignancy, age) or laboratory variables (renal function, complete blood count) that may affect its usefulness in a cohort of 704 outpatients suspected of having deep venous thrombosis.

PATIENTS AND METHODS

Patients and methods

We retrospectively identified 711 consecutive outpatients who had been suspected of having deep venous thrombosis from November 1994 to December 2000. We excluded 3 women who were pregnant and 4 patients who had a history of a previous deep venous thrombosis without documented recanalization. At initial presentation, all 704 remaining patients underwent ultrasonography of the symptomatic leg and a D-dimer test. The ultrasonography was repeated within 1 week if the first examination was negative for deep venous thrombosis. If the ultrasonography was positive for deep venous thrombosis, the patient was treated with anticoagulants. Data from a 6-month follow-up were obtained by visit or telephone contact with the patient or the general practitioner. We recorded patients' sex, age, use of oral anticoagulants, previous venous thromboembolism, and active malignancy (defined as receiving any treatment for cancer or documented recurrent or metastatic disease). Patients without use of oral anticoagulants, previous venous thromboembolism, or malignancy were assigned as the reference group. We also recorded serum creatinine levels and complete blood counts at the time of presentation. The medical ethics committee of our hospital approved the study protocol.

Ultrasonography, using real-time B-mode with compression, was performed with a 7.5-MHz or a 5.0-MHz transducer. Two areas of the leg were examined: the common femoral vein at the inguinal ligament and the popliteal vein at the knee-joint line traced down to the point of the trifurcation of the calf veins. Veins were scanned in the transverse plane only. Lack of compressibility was the sole criterion for an abnormal result; a vein was considered fully compressible if no residual lumen was seen.

The D-dimer test used was the Minutex® (Biopool, Umeå , Sweden), used as a semiquantitative test by dilutions. We made a range of samples: the undiluted sample and the sample diluted with buffer 1 + 1, 1 + 3, and 1 + 7. The results are reported as negative, 1+, 2+, 3+, or 4+; 1+ or more was considered to be a positive test result. The tests were performed under standard conditions, using a magnifying glass with a lamp, a black background, and positive and negative control plasma samples.

Laboratory measurements of creatinine were performed by routine methods using an Integra 700 analyzer (Roche, Mannheim , Germany).

Complete blood count measurements were performed using Gen S (Beckman Coulter, Fullerton, California).

Statistics

We calculated the sensitivity, specificity, negative predictive value, and positive predictive value, including 95% confidence intervals, for the D-dimer test. The Fisher exact test (two-tailed) was used to compare proportions. The Cochran-Armitage test for trend was used to compare multiple proportions. For the analysis of the association between age and D-dimer levels, only patients without proven deep venous thrombosis (n=450) were included to eliminate bias from the higher prevalence of thrombosis in elderly patients. General linear regression models were used to compare continuous laboratory variables with categorical D-dimer results. SAS software (Cary, North Carolina) and Confidence Interval Analysis (London, United Kingdom) were used for all calculations.

RESULTS

The sample consisted of 704 patients with a mean (± SD) age of 59 ± 17 years. Of the 464 patients with an initial normal ultrasonography, 187 (40%) underwent a second ultrasonography. Deep venous thrombosis was diagnosed in 254 patients (36%), 240 of whom were diagnosed at presentation and 14 during 6-month follow-up (10 by the second ultrasonography within 1 week, 2 at 8 weeks, 1 at 3 months, and 1 at 5 months). All 14 of these patients had a positive D-dimer test at presentation. In the 464 patients with an initial normal ultrasonography, 6-month follow-up data were obtained by visit in 361 patients (78%) and by telephone contact in 103 patients (22%).

Performance of the D-dimer test

The sensitivity and negative predictive value of the D-dimer test were high (≥ 97%), particularly among patients who did not use anticoagulants or have a history of deep venous thrombosis or cancer (the reference group; Table). A deep venous thrombosis was detected by ultrasonography in 4 patients with a negative D-dimer test, of whom 2 used oral anticoagulants. Sensitivity was lower among the 61 patients using oral anticoagulants; of the 8 patients with deep venous thrombosis in this group, 2 had a negative D-dimer test. Anticoagulated patients were more likely to have a negative D-dimer test than was the reference group (46% [28/61] vs. 20% [100/508], $p<0.001$). Sensitivity and negative predictive value did not differ statistically among patients with a previous deep venous thrombosis (n = 127) when compared with the reference group (Table). Both of the 53 patients with thrombosis in this group who had a negative D-dimer test were using oral anticoagulants.

All 29 of the 47 patients with cancer who had a deep venous thrombosis had a positive D-dimer test; however, so did all but 4 of those without venous thrombosis, a specificity of only 22% (Table). Excluding the 7 patients with cancer who used oral anticoagulants did not significantly improve the specificity.

The positive predictive value of the D-dimer test increased with higher D-dimer concentrations (Figure 1). The prevalence of a positive D-dimer test increased with age ($p<0.001$; Figure 2). This association remained after adjustment for the use of oral anticoagulants, previous venous thromboembolism, cancer, and sex. Of the 156 patients aged ≥70 years, 79% (n =

123) had a positive D-dimer test, compared with 61% (180/294) of younger patients (p=0.0001).
There was a significant inverse correlation between D-dimer level and the hemoglobin concentration (R=-0.17, p=0.01). Of the 704 patients, data on hemoglobin concentration were available in 513 patients. The mean hemoglobin concentration in the 114 patients with a negative D-dimer test was 13.7 ± 1.4 g/dL, compared with 13.0 ± 1.9 g/dL in the 109 patients with a D-dimer level of 4+ (p=0.01). No correlation was found between D-dimer level and the leukocyte count (R=0.06, p=0.7), platelet count (R=0.05, p=0.9), or serum creatinine concentration (R=0.14, p=0.06).

Table. Performance of the D-dimer Test in Patients Suspected of Deep Venous Thrombosis in Different Subgroups.

Characteristic	Total (n = 704)	Reference Group* (n = 508)	Current Users of Oral Anticoagulants (n = 61)	P Value†	Previous Venous Thromboembolism (n = 127)	P Value†	Active Malignancy (n = 47)	P Value†
	Percentage (Number) or Mean ± SD				Percentage (Number) or Mean ± SD		Percentage (Number) or Mean ± SD	
Female sex	62 (437)	65 (330)	48 (29)	0.01	66 (84)	0.8	26 (12)	<0.0001
Age (years)	59 ± 17	58 ± 17	66 ± 15	<0.001	58 ± 18	0.8	70 ± 12	<0.0001
Venous thrombosis	36 (254)	35 (176)	13 (8)	<0.001	42 (53)	0.1	62 (29)	<0.001
Sensitivity	98 (250/254)	99 (174/176)	75 (6/8)	0.01	96 (51/53)	0.2	100 (29/29)	1.0
Specificity	33 (147/450)	30 (98/332)	49 (26/53)	<0.01	45 (33/74)	0.01	22 (4/18)	0.6
Negative predictive value	97 (147/151)	98 (98/100)	93 (26/28)	0.2	94 (33/35)	0.3	100 (4/4)	1.0
Positive predictive value	45 (250/553)	43 (174/408)	18 (6/33)	<0.01	55 (51/92)	0.03	67 (29/43)	<0.01
Negative D-dimer test	21 (151)	20 (100)	46 (28)	<0.001	28 (35)	0.05	9 (4)	0.1

* The reference group consists of patients without use of anticoagulants, previous thrombosis, or malignancy. Thirty-nine patients had more than one characteristic: 7 patients used oral anticoagulants and had a malignancy, 6 patients had previous venous thromboembolism and malignancy, and 26 patients used oral anticoagulants because of previous venous thromboembolism.

† Compared with reference group.

Figure 1. The positive predictive value of the D-dimer test according to D-dimer concentrations among outpatients suspected of having deep venous thrombosis.

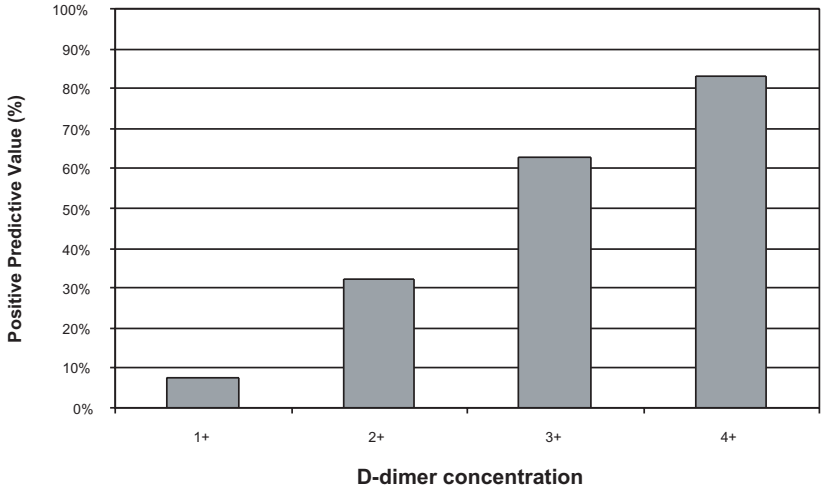
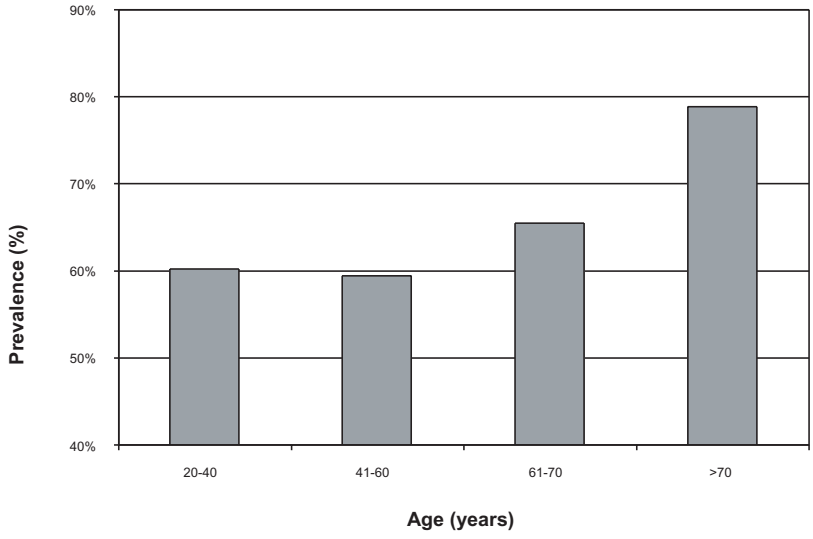


Figure 2. The prevalence of a positive D-dimer test according to age among outpatients suspected of having deep venous thrombosis, who did not have thrombosis after objective testing.



DISCUSSION

To develop practical guidelines for the use of the D-dimer test in the management of patients suspected of having a deep venous thrombosis, we studied several clinical and laboratory variables that might affect the test's sensitivity or specificity. The rapid latex D-dimer assay that we used had excellent sensitivity and negative predictive value in outpatients, with the possible exception of those who used oral anticoagulants. The previously reported lower sensitivity and negative predictive value of this latex D-dimer assay may be the result of differences in the preanalytic and analytic process (8-10). For example, Janssen et al. performed this test on frozen and stored samples (8). A more common problem with the rapid latex D-dimer test is that its interpretation is influenced by inter- and intraobserver variability. In our laboratory, we used standard conditions and skilled technicians.

Patients using oral anticoagulants had a significantly lower sensitivity when compared with the reference group, although there were only 8 patients with a deep venous thrombosis in this group. This finding may be explained by a reduced capacity for thrombin generation and plasma D-dimer formation in patients taking oral anticoagulants (11;12). This is consistent with the significant increase in D-dimer levels after cessation of anticoagulant therapy (13) and the normalization of elevated D-dimer levels after beginning their use (14). Although larger studies are warranted to confirm our findings, we have decided not to use the D-dimer test to exclude deep venous thrombosis in patients using oral anticoagulants.

We also found that the D-dimer test can be used to exclude renewed thrombotic activity in patients with previous venous thrombosis, since the sensitivity and negative predictive value of the test were not different from the reference group. As an initial screening in patients with a history of deep venous thrombosis, the D-dimer test is probably more reliable than an ultrasonography when renewed thrombotic activity is suspected. Ultrasonography is less sensitive in the case of distal thrombosis (15) and has a high rate of false-positive results due to persisting venous abnormalities (16).

Only 4 of the 47 patients with malignancy had a negative D-dimer test. Elevated D-dimer levels in patients with malignancy might be the result of production of procoagulant proteases by tumor cells, leading to subclinical intravascular coagulation (17). However, local rather than systemic fibrin

formation may be a more likely cause of elevated D-dimer levels (18). In our opinion, the high prevalence of a positive D-dimer test result, along with its low specificity, suggest that the assay may not be useful as a screening test in patients with cancer. We did not, however, confirm the results of a study that reported the low negative predictive value of a D-dimer test in patients with cancer (19).

As has been reported (20-23), D-dimer tests were more likely to be positive with aging, perhaps due to a greater prevalence of comorbid conditions. Of the 156 patients older than 70 years who did not have deep venous thrombosis, almost 80% (n = 123) had a positive D-dimer test, compared with 61% (180/294) of patients younger than 70 years. Therefore, it is questionable whether the D-dimer test is useful in older patients. The cost-effectiveness of the D-dimer test in elderly patients should be determined. In addition, hemoglobin levels were indirectly proportional to D-dimer levels, perhaps because anemia often occurs in chronic inflammatory disorders that also may lead to increased D-dimer levels.

In conclusion, the sensitivity and negative predictive value of this rapid latex D-dimer assay for the exclusion of deep venous thrombosis are high. The D-dimer test has a good performance in patients with previous venous thromboembolism, but we do not recommend its use in patients using oral anticoagulants because of the reduced sensitivity of the test. D-dimer tests in patients with cancer and in patients over 70 years old may not be worthwhile, as the tests are usually positive.

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CHAPTER 5

The combination of a normal D-dimer concentration and a non-high pretest clinical probability score is a safe strategy to exclude deep venous thrombosis

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SUMMARY

Background Serial ultrasonography is reliable for the diagnosis of deep venous thrombosis in symptomatic patients, but the low prevalence of thrombosis in this group renders this approach costly and inconvenient to patients. We studied the clinical validity of the combination of a pretest clinical probability score and a D-dimer test in the initial evaluation of patients suspected of deep venous thrombosis.

Methods Patients with a normal D-dimer concentration (< 500 FEU µg/L) and a non-high probability score (< 3) had no further testing. Patients with a normal D-dimer concentration and a high probability score (≥ 3) underwent one ultrasonography. Serial ultrasonography was performed in patients with an abnormal D-dimer concentration. Patients were followed for 3 months.

Results A total of 812 patients were evaluable for efficacy. Only 1 of 176 patients (0.6%; 95% CI: 0.02-3.1%) with a normal D-dimer concentration and a non-high probability score developed thrombosis during follow-up. A normal D-dimer concentration and a high probability score were found in 39 patients; 3 of them (7.7%; 95% CI: 1.6-20.9%) had thrombosis at presentation, and one (2.8%; 95% CI: 0.07-14.5%) developed pulmonary embolism during follow-up. In 306 of 597 patients (51.3%) with an abnormal D-dimer concentration, thrombosis was detected by serial ultrasonography. Six patients (2.1%; 95% CI: 0.8-4.4%) developed thrombosis during follow-up. No deaths due to thromboembolism occurred during follow-up. The total need for ultrasonography was reduced by 29%.

Conclusions The combination of a non-high pretest clinical probability score and a normal D-dimer concentration is a safe strategy to rule out deep venous thrombosis and to withhold anticoagulation.

INTRODUCTION

Deep venous thrombosis can be diagnosed or rejected accurately by serial compression ultrasonography. In two studies with a follow-up period of, respectively, 3 and 6 months, venous thromboembolic complications were found in only 0.6% and 0.7% of patients with suspected deep venous thrombosis after normal serial ultrasonography (1;2). Serial ultrasonography, however, is inefficient because only 17 to 24% of patients suspected for deep venous thrombosis actually has it (1-6), and only 0.9% develop it after the initial normal ultrasonogram (2). Other non-invasive diagnostic tests, such as the pretest clinical probability score (5-7) and the D-dimer measurement (4;7-14), are subject of studies to reduce the need for ultrasonography. It has been proven safe to withhold anticoagulant treatment in patients with a low pretest clinical probability score and normal initial ultrasonogram (6) and in patients with a normal D-dimer and normal ultrasonography at first presentation (3;11;14). The next step, which is the subject of the present study, is to investigate the safety of the combination of a non-high pretest clinical probability score and a normal D-dimer to replace of ultrasonography as the initial test in the diagnostic management of patients suspected of having deep venous thrombosis.

PATIENTS AND METHODS

Patients

This investigation was a prospective cohort study in 4 large, nonacademic, teaching hospitals in the Netherlands. All outpatients with clinical symptoms of deep venous thrombosis of the leg, who were referred by their general practitioners, were potentially eligible for the study. Exclusion criteria were pregnancy, previous deep venous thrombosis in the ipsilateral leg without documentation of recanalization, a concomitant clinical suspicion of pulmonary embolism, the use of unfractionated heparin, low-molecular-weight heparin or any form of oral anticoagulant in the past month, geographic impossibility for follow-up and life expectancy less than 3 months. Patients entering the study were asked for informed consent. The medical ethical committees of the participating centers approved the study.

Methods

Eligible patients who gave informed consent were assessed by one of the study physicians or local residents and a single pretest clinical probability score according to Wells et al was performed (5). Patients were considered to have a low or moderate ("non-high") suspicion for having deep venous thrombosis when the score was < 3 and at high suspicion when the score was ≥ 3. We performed the Tina-quant® quantitative latex D-dimer assay (Roche, Germany) in all patients. Previous reports have validated this test using venography (12;15). An abnormal test was defined as a D-dimer-value > 499 Fibrin Equivalent Units (FEU) µg/L. The pretest clinical probability score was performed by physicians who were not previously aware of the D-dimer concentration.

Ultrasonography, using real-time B-mode with compression, was done with a 7.5 MHz and/or a 5.0 MHz transducer. Two areas of the leg were examined: the common femoral vein at the inguinal ligament and the popliteal vein at the knee-joint line traced down to the point of the trifurcation of the calf veins. Veins were scanned in the transverse plane only. Lack of compressibility was the sole criterion for an abnormal result; a vein was considered fully compressible if no residual lumen was seen.

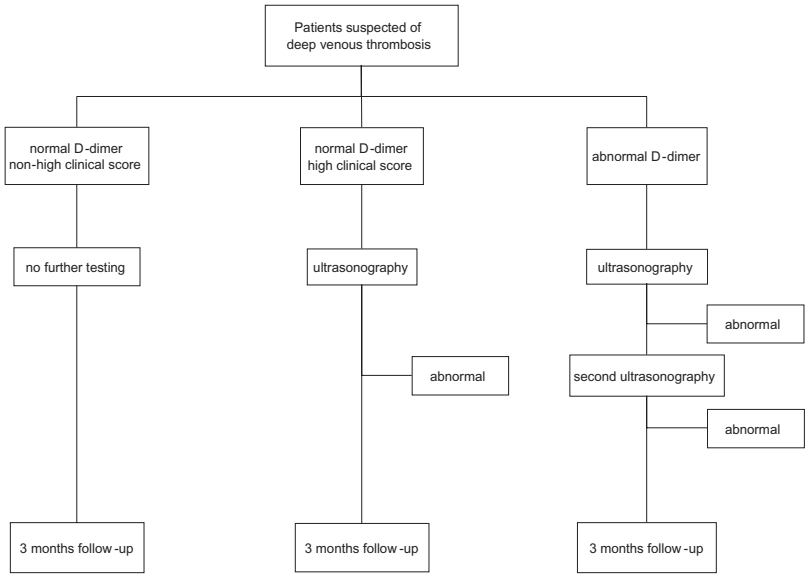
Patients with a non-high pretest clinical probability score and a normal D-dimer concentration had no further investigations (Figure). The patients with a high pretest clinical probability score and a normal D-dimer concen-

tration underwent a single ultrasonogram at presentation. Patients with an abnormal D-dimer concentration (irrespective their pretest clinical probability score) had repeated ultrasonography (with a second ultrasonography after 1 week). Patients who had no further investigations and patients with normal ultrasonography results did not receive anticoagulation. These patients were followed for 3 months and data from this 3-month follow-up were obtained by visit or telephone contact with the patient or the general practitioner. Patients were instructed to contact their physician when symptoms of their leg worsened or when symptoms of pulmonary embolism occurred. Worsening of complaints of the leg, new complaints of the leg or respiratory tract, or an objective clinical change in the patient were followed by objective testing using ultrasonography, ventilation-perfusion scintigraphy or pulmonary angiography. The primary outcome of this study was the development of thromboembolic processes (deep venous thrombosis or pulmonary embolism) during the 3-months follow-up period.

Statistics

For calculation of 95% confidence intervals (CI), the exact binomial method was used.

Figure. Diagnostic flowchart for patients with suspected deep venous thrombosis.



RESULTS

During the study period, 902 consecutive outpatients with suspected deep venous thrombosis were referred. Of these patients, 827 (92%) were eligible and gave informed consent. There were 75 ineligible patients (Table 1). Of the 827 patients included in the study, 812 patients were fully evaluable (mean age 59 ±17 years; 518 (64%) women). None of the patients used tranexamic acid. The 15 patients that were not fully evaluable were excluded from the final analysis of the study (i.e. the 3-month thromboembolic risk): one patient (with normal serial ultrasonography) was lost to follow up, 6 patients received oral anticoagulation for reasons other than thromboembolism during follow up, 4 patients had protocol violation, and 4 patients died during follow-up. The 4 patients that died all had a positive D-dimer and normal serial ultrasonography, and the cause of death was not related to thrombosis. None of these 15 patients had venous thromboembolism at time of exclusion from the study.

The main results of this study are given in Table 2. Deep venous thrombosis or pulmonary embolism was diagnosed in 317 (39%) patients during the entire study period. Deep venous thrombosis was diagnosed at initial screening in 309 patients by single or serial ultrasonography; 8 patients of the remaining 503 patients (1.6%; 95% CI: 0.7-3.1%) developed deep venous thrombosis or pulmonary embolism during the 3-months follow-up period. No deaths due to venous thromboembolism were observed during the follow-up period.

In the group of patients with a non-high pretest clinical probability score and normal D-dimer concentration, deep venous thrombosis was found during follow-up in one patient (0.6; 95% CI: 0.02-3.1%). This patient returned to the hospital because of worsening of complaints 2 days after first presentation; at that time the D-dimer was abnormal and a deep calf vein thrombosis was detected by a single ultrasonogram. This patient seemed to be heterogeneous for the factor V Leiden mutation. The diagnostic strategy performed in this group had a negative predictive value of 99.4% (95% CI: 96.9-100%). There were no deaths in this group. The prevalence of a low or moderate clinical probability score in this group was 49% (87 of 176 patients) and 51% (89 of 176 patients), respectively.

Three of the 39 patients (7.7%; 95% CI: 1.6-20.9%) with a normal D-dimer concentration and a high pretest clinical probability score had deep venous thrombosis on ultrasonography at presentation; one out of the

remaining 36 patients (2.8%; 95% CI: 0.07-14.5%) developed pulmonary embolism at 71 days after initial screening. At that time, the D-dimer concentration was abnormal and repeated ultrasonograms remained normal. This patient seemed to have an abdominal localized non-Hodgkin lymphoma that impaired lymph drainage from the legs. The negative predictive value of this regimen was 97.2% (95% CI: 85.5-99.9%). No deaths were reported in this group.

In patients with an abnormal D-dimer concentration (n=597), 291 had deep venous thrombosis detected by the first ultrasonogram, and 15 patients had it detected by the second ultrasonogram. Six of the 291 patients (2.1%; 95% CI: 0.8-4.4%) with normal serial ultrasonography developed venous thromboembolism during the 3-month follow-up period (3 deep venous thromboses and 3 pulmonary embolisms). Of these 6 patients, one was heterogeneous for the factor V Leiden mutation, one had a non-Hodgkin lymphoma and one had a previous history of venous thromboembolism and was using oral anticonceptives. The diagnostic strategy in the group with serial ultrasonography had a negative predictive value of 97.9% (95% CI: 95.6-99.2%). The prevalence of a low, moderate, or high clinical score in the group with abnormal D-dimer concentrations was 19% (114 of 597 patients), 41% (243 of 597 patients), and 40% (240 of 597 patients), respectively. The prevalence of DVT in these three clinical probability groups was 22%, 51%, and 68%, respectively.

If exclusion of deep venous thrombosis would have been based on a normal D-dimer concentration alone (ie, not using the pretest clinical probability score), we would have missed deep venous thrombosis in 5 of 215 patients (2.3%; 95% CI: 0.8-5.3%) with a normal D-dimer concentration; this strategy would have a negative predictive value of 97.7% (95% CI: 94.7-99.2%). The sensitivity of the D-dimer was 98.4% (95% CI: 96.4-99.5%; 312 of 317 patients), and its specificity 42.4% (95% CI: 38.1-46.8%; 210 of 495 patients).

Analysis of the clinical probability score in the 812 patients showed that 24.8% (201 of 812 patients) had a low probability score, 40.9% (332 of 812 patients) a moderate and 34.3% (279 of 812 patients) a high probability score. The prevalence of deep venous thrombosis in the three probability categories was 12.9%, 37.7%, and 59.5%, respectively. The sensitivity of the D-dimer in patients with a low probability score was 96.2%, the negative predictive value was 98.9%, and specificity was 51.4%. In patients with a moderate score, the values were 100%, 100% and 39.6%, respec-

tively. In patients with a high probability score, the D-dimer had values of 97.6%, 90.5% and 33.6%, respectively.

The total number of ultrasonograms performed in this study was 949; if a strategy of serial ultrasonography was performed in all patients in this study, we would have performed 1335 ultrasonograms. Our diagnostic strategy led to a reduction of 29% in the need for ultrasonograms.

Table 1. Reasons for exclusion in 75 patients.

Reason for exclusion	N
Pregnancy	1
Previous deep venous thrombosis in the ipsilateral leg without documentation of recanalization	6
Concomitant clinical suspicion of pulmonary embolism	3
The use of unfractionated heparin, low-molecular-weight heparin or any form of oral anticoagulant in the past month	50
Geographic impossibility for follow-up	3
Life expectancy < 3 months	1
No informed consent obtained (8 refused and 3 were mentally incapable of signing).	11

Table 2. Prevalence of venous thromboembolism according to D-dimer result and pretest clinical probability score. Values are reported as number (percentage;95% confidence intervals).

Group	n (%)	VTE during follow up, n (%; 95% CI)	Overall VTE, n (%; 95% CI)
Normal D-dimer and non-high score	176 (22%)	1 (0.6%;0.02-3.1)	1 (0.6%;0.02-3.1)
Normal D-dimer and high score	39 (5%)	1 (2.8%;0.07-14.5)	4 (10.3%;2.9-24.2)
Normal D-dimer	215 (26%)	2 (0.9%;0.1-3.4%)	5 (2.3%;0.8-5.3%)
Abnormal D-dimer	597 (74%)	6 (2.1%;0.8-4.4)	312 (52.3%;48.3-56.3)
Total	812 (100%)	8 (1.6%;0.7-3.1)	317 (39.0%;35.7-42.4)

VTE indicates venous thromboembolism and includes deep venous thrombosis and pulmonary embolism

DISCUSSION

This study indicates that it is relatively safe to withhold anticoagulation in outpatients suspected of having deep venous thrombosis who have a normal D-dimer test and a non-high pretest clinical probability score. This approach will reduce the need for compression ultrasonography. The role of the pretest clinical probability score and/or the D-dimer concentration in the diagnostic management of deep venous thrombosis has been the objective of many different studies (4-14;16;17). All suggested diagnostic regimens may not exceed the failure rate (percentage of missed deep venous thrombosis) of 0.6% and 0.7% that have been reported in 2 landmark studies on the safety of serial ultrasonography (1;2). The failure rate of 0.6% (CI 0.02-3.1%) in our study for patients with a non-high pretest clinical probability score and a normal D-dimer concentration is comparable with the studies in which serial ultrasonography has been used. Therefore, we conclude that this regimen can replace serial ultrasonography in this subgroup of patients with suspected deep venous thrombosis.

A part of the follow-up in our patients was provided by their general practitioner. In The Netherlands, patients see their general practitioner on a regular basis and the general practitioner provides follow-up of their patients in many diseases. In case of hospitalization, the general practitioner will always be notified. At 3 months, patients were seen by a physician in one of the participating institutes or by their general practitioner or they were contacted by telephone by a physician in one of the participating institutes. If a telephone contact revealed continues complaints of the leg, patients were seen by their general practitioner or a physician. A likewise method of follow-up has also been used in other management studies on the diagnosis of venous thromboembolism (5;18).

The prevalence of deep venous thrombosis in our study population was relatively high (39%), especially compared with other recent studies (1;2;5-7;14;16;17). In our institutes, this prevalence has been that high for several years. Since the participating centers in this study are the only institutes for diagnosing thrombosis in the region and because we have used consecutive patients, we do not believe that this high prevalence is due to some kind of bias. A possible explanation for this prevalence might be the referral pattern of the general practitioner. Despite this high prevalence, exclusion of deep venous thrombosis without performing an ultrasonography was possible in 22% of the patients. The need for ultrasonography in the whole study pop-

ulation was reduced by approximately 30%, which means an economical advantage and less inconvenience for patients and radiology staffs. Our results also open the possibility for general practitioners to rule out deep venous thrombosis in a substantial number of patients without presenting those patients to the emergence wards.

Where previous reports excluded deep venous thrombosis using the combination of a normal D-dimer concentration and only a low pretest clinical probability score (16;17), we show that deep venous thrombosis can also be excluded in patients with a normal D-dimer concentration and a low and moderate pretest clinical probability score. However, a D-dimer assay with a high sensitivity is an important prerequisite in our strategy. Because the sensitivity of the D-dimer assay in previous reports (using the SimpliRed D-dimer assay) was only 85%, one needs to rely more extensively on the pretest clinical probability score for a safe exclusion of deep venous thrombosis. However, due to the probability of an alternative diagnosis, this score is highly subjective and shows large interobserver variability (19). The D-dimer in our study had a sensitivity of 98.4% and a negative predictive value of 97.7%. For exclusion of deep venous thrombosis in patients with a non-high pretest clinical probability score and a normal D-dimer concentration, we recommend the use of a D-dimer assay with at least a sensitivity and negative predictive value as high as the one used in the present study.

Leaving out the pretest clinical probability score from our strategy, would have led to an increase in the percentage of missed venous thromboembolism from 0.6% (95% CI: 0.02-3.1%) to 2.3% (95% CI: 0.8-5.3%) in patients with a normal D-dimer concentration. Because of the small number of missed thromboembolic processes, however, the confidence intervals overlap. However, this possible additional value of a simple pretest clinical probability score was achieved despite the fact that it was performed by more than 20 junior residents in 4 different hospitals. Therefore, we recommend using the combination of the pretest clinical probability score and a D-dimer test in stead of using the D-dimer alone to exclude deep venous thrombosis, as suggested by some authors (11). The use of the pretest clinical probability score had only a limited effect on the total number of patients in whom serial ultrasonography can be avoided: 39 of the 215 patients (18.1%) with a normal D-dimer concentration underwent a single ultrasonogram because of a high pretest clinical probability.

The overall failure rate of the diagnostic strategy in our study was 1.6% (95% CI: 0.7-3.1%). In the patients in our study in whom serial ultrasonog-

raphy was performed, the failure rate was 2.1% (95% CI: 0.8-4.4%). This higher percentage can be explained by the fact that only patients with a positive D-dimer assay and, therefore, a higher a priori chance of having deep venous thrombosis underwent serial ultrasonography.

In conclusion, the combination of a non-high pretest clinical probability score and a normal D-dimer concentration is a safe strategy to rule out deep venous thrombosis without performing ultrasonography in symptomatic outpatients. The need for ultrasonography can be reduced by approximately 30% using this strategy.

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CHAPTER 6

The usefulness of five D-dimer assays in the exclusion of deep venous thrombosis

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SUMMARY

Background D-dimer measurement is a promising tool in the exclusion of venous thrombosis. New D-dimer assays have been introduced, but need clinical validation.

Our objective was to evaluate the clinical usefulness of four relatively new D-dimer assays and a classical ELISA in outpatients suspected for deep venous thrombosis.

Methods In 537 patients, participants in a large prospective management study using a clinical probability score and a D-dimer measurement (Tina-quant®), additional samples were taken for D-dimer measurement using the Asserachrom ELISA®, the VIDAS New®, the STA-LIA® and the Miniquant® assay. Performances of each test were calculated using clinical data during a 3-months follow-up.

Results Thrombosis was detected in 224 patients (42%). The area under the ROC curve was significantly higher for the Tina-quant® as compared to the other assays. Using standard cut-off values, sensitivity, negative predictive value (NPV) and specificity of the Asserachrom® were 97, 94 and 33% respectively. For the VIDAS New®, values were 100, 96 and 8% respectively. The Tina-quant® showed values of 99, 98 and 41% respectively and the STA-LIA® 98, 95 and 32%. Values for the Miniquant® were 95, 94 and 52%.

Conclusions The D-dimer assays in our study all show a high sensitivity and negative predictive value, but none of the assays reached a NPV of > 98% at standard cut-off values. D-dimer assays with a low specificity still necessitate additional diagnostic tests in the majority of the patients.

INTRODUCTION

Measurement of the concentration of D-dimers is a promising tool in the non-invasive diagnostic management of patients suspected for having deep venous thrombosis (DVT). Retrospective analyses show a high sensitivity and negative predictive value of D-dimer for the exclusion of venous thromboembolism (VTE) (1-13). Two management studies have proven the safety of withholding anticoagulants in patients with a normal D-dimer and a normal initial ultrasonography (14;15). Various combinations of pretest clinical probability scores and a normal D-dimer test or even a normal D-dimer alone have been suggested to be accurate enough for the exclusion of VTE (8;16-19). However, the choice for a diagnostic management strategy depends largely upon the reliability of the D-dimer test.

Many D-dimer assays are available. The classical enzyme linked immunosorbent assays (ELISA) are considered to be the golden standard in determining D-dimer (1;2;4-7;9;13;20;21), but they are time-consuming and not suitable for emergency use. The early latex assays have too little sensitivity to be used in clinical practice (1;22) and are replaced by newer latex assays with improved sensitivity and negative predictive value (5;6;10;12;13;16;17;23-25).

The negative predictive value and the sensitivity of the D-dimer assay represent its safety in the exclusion of thrombosis in case of a normal D-dimer. The specificity reflects its clinical usefulness from an economical point of view. A low specificity, which is the result of a lot of false-positive test results, still implies additional diagnostic procedures in the majority of the patients.

The purpose of this study is to evaluate the accuracy in terms of sensitivity and negative predictive value and the clinical usefulness in terms of specificity of four relatively new different D-dimer assays (VIDAS New®, Tinaquant®, STA-LIA® and Miniquant®) and a classical ELISA in symptomatic outpatients suspected for DVT.

PATIENTS AND METHODS

Patients

In total, 537 eligible patients entered the current study. Exclusion criteria were pregnancy, previous deep venous thrombosis in the ipsilateral leg without documentation of recanalisation, a concomitant clinical suspicion of pulmonary embolism (PE), the use of unfractionated heparin, low molecular weight heparin or any form of oral anticoagulant in the past month, geographic impossibility for follow-up and life expectancy less than 3 months. The patients participated in a prospective multi-centre cohort study in outpatients with suspected DVT(26) and belonged to two of the four participating centres. Patients were categorized according to their D-dimer concentration (using the Tina-quant® D-dimer assay) and their clinical probability score, according to Wells et al (27). In patients with a normal D-dimer concentration and a non-high clinical score, no further testing was done. Patients with a normal D-dimer concentration and a high clinical score underwent a single compression ultrasonography (CUS). In case of an abnormal D-dimer concentration, serial CUS was performed. Primary outcome was the development of venous thromboembolism (DVT or PE) during a 3-months follow-up period.

Methods

D-dimer concentration was measured immediately using the Tina-quant® D-dimer assay (Roche, Mannheim, Germany), which is a fully automated quantitative immunoturbidimetric latex assay adapted for the Integra 70°C (Roche, Mannheim, Germany). Additional samples were taken within 2 hours of presentation and frozen at -70ºC until further study analysis. The mean storage duration was 724 days (range 75-1296). The other D-dimer determinations were done using the Asserachrom® ELISA (Diagnostica Stago, Asnière, France), the VIDAS D-dimer New® ELISA (bioMerieux, Marcy l'Etoile, France), the STA-LIA® fully automated quantitative immunoturbidimetric latex assay measured with the STA-R® (Diagnostica Stago, Asnière, France) and the Miniquant® fully automated quantitative immunoturbidimetric latex assay measured with the Miniquant™-1 analyser (Biopool, Umeå, Sweden). All D-dimer assays were performed according to the manufacturer's instructions by technicians who were unaware of the clinical outcomes of the patients. Test results for all D-dimer

assays but the Miniquant® are reported as fibrin equivalent units (FEU) mg/L; results of the Miniquant® are reported as D-dimer equivalent units in mg/L. The cut-off value as recommended by the manufacturers was 0.5 FEU mg/L for the Asserachrom®, the VIDAS New®, the Tina-quant® and the STA-LIA® D-dimer assays, and 0.25 mg/L for the Miniquant® assay.
For detection of DVT, ultrasonography using real-time B-mode with compression was done with a 7.5 MHz and/or a 5.0 MHz transducer. Two areas of the leg were examined: the common femoral vein at the inguinal ligament and the popliteal vein at the knee-joint line traced down to the point of the trifurcation of the calf veins. Veins were scanned in the transverse plane only. Lack of compressibility was the sole criterion for an abnormal result; a vein was considered fully compressible if no residual lumen was seen.
If a patient was suspected for PE after entering the prospective management study, a ventilation-perfusion scintigraphy was performed using routine methods. A normal scintigraphy ruled out PE, where a high probability scan confirmed the diagnosis. An intermediate probability scintigraphy was followed by pulmonary angiography.

Statistics

Sensitivity, negative predictive value (NPV) and specificity of the five D-dimer assays were calculated in relation to the results of the clinical outcomes, i.e. having venous thromboembolism at presentation or during follow-up. For calculation of 95% confidence intervals (CI), the exact binomial method was used. The Fisher's exact test was used for comparison of the sensitivity, NPV and specificity between the D-dimer assays. Receiver Operator Characteristics (ROC) curves were constructed by plotting sensitivity (true positive fraction) versus 1-specificity (false positive fraction) using Analyse-it® Software for Microsoft Excel (Leeds, United Kingdom). The area under the curve (AUC) was then calculated and compared using the Hanley and McNeil method (28). Agreement between two methods to classify a patient having a negative or positive test result was estimated by calculation of the kappa coefficient: a value of > 0.81 represents an excellent concordance, 0.80-0.61 a good concordance, 0.60-0.41 a moderate concordance, 0.40-0.21 a mediocre concordance, 0.20-0 a poor concordance and < 0 very poor concordance (29).

RESULTS

The study population consisted of 537 patients. VTE was diagnosed in 224 patients (prevalence 42%): 210 patients had DVT at presentation, 11 patients had DVT detected by the second CUS, and three developed VTE during follow-up (one DVT and two PE). Both cases of PE were detected by ventilation-perfusion scintigraphy.

Figure 1 shows the ROC curves displaying sensitivity and specificity for the different D-dimer assays at different cut-off levels. Using the Asserachrom® ELISA as the reference test, the AUC of the VIDAS New®, STA-LIA® and Miniquant® assays were not different (p=0.6, p=0.8 and p=0.1 respectively) (Table 1). The AUC of the Tina-quant® was significantly higher compared with the other assays; p=0.003 for comparison with the Asserachrom®, p=0.004 with the VIDAS New®, p<0.0001 with the STA-LIA® and p=0.01 with the Miniquant®.

The performance of the different D-dimer assays is given in Table 2, showing sensitivity, NPV and specificity with 95% confidence intervals according to different cut-off values for each assay. Given the cut-off values as recommended by the manufacturers, all D-dimer assays had a sensitivity of 95% or higher and a NPV of 94% or higher. The highest NPV (98%) was seen with the Tina-quant®; this was not significantly different as compared to the Asserachrom® (p=0.08), the VIDAS New® (p=0.4) and the STA-LIA® (p=0.2), but higher as compared to the Miniquant® (p=0.047). The highest sensitivity (100%) was seen with the VIDAS New® assay: this was not statistically different as compared to the Asserachrom® (p=0.07), the Tina-quant® (p=1.0) and the STA-LIA® (p=0.2), but higher as compared to the Miniquant® (p=0.006). Sensitivity of the Miniquant® (95%) was lower as compared to the Tina-quant® (p=0.02). By changing the cut-off values, only in the Tina-quant® a NPV of 100% could be reached. Slight (not statistically significant) improvements of the NPV were seen in the Asserachrom® and STA-LIA® assays by lowering the cut-off values, but they led to significant reductions in specificity. A low specificity was seen for the VIDAS New® D-dimer assay: its specificity of 8% was lower than the other D-dimer assays that showed values of 32-52% (p<0.0001 when compared to the Asserachrom®). The number of patients with a negative VIDAS New® D-dimer test result was 27/537 as compared to 110/537 negative Asserachrom® tests (p<0.0001), 131/537 negative Tina-quant®

tests (p<0.0001), 106/537 negative STA-LIA® tests (p<0.0001) and 174/537 negative Miniquant® tests (p<0.0001).
Table 3 shows the kappa coefficients between the 5 D-dimer assays. The concordance of the Asserachrom® with the Tina-quant® and the STA-LIA® is good, with the Miniquant® moderate and with the VIDAS New® mediocre. The concordance of the VIDAS New® with the other D-dimer assays was mediocre or worse. Concordance between the Tina-quant®, STA-LIA® and Miniquant® was good.

Figure 1. ROC curve analysis of the accuracy of measurement of plasma D-dimer concentrations using different D-dimer assays.

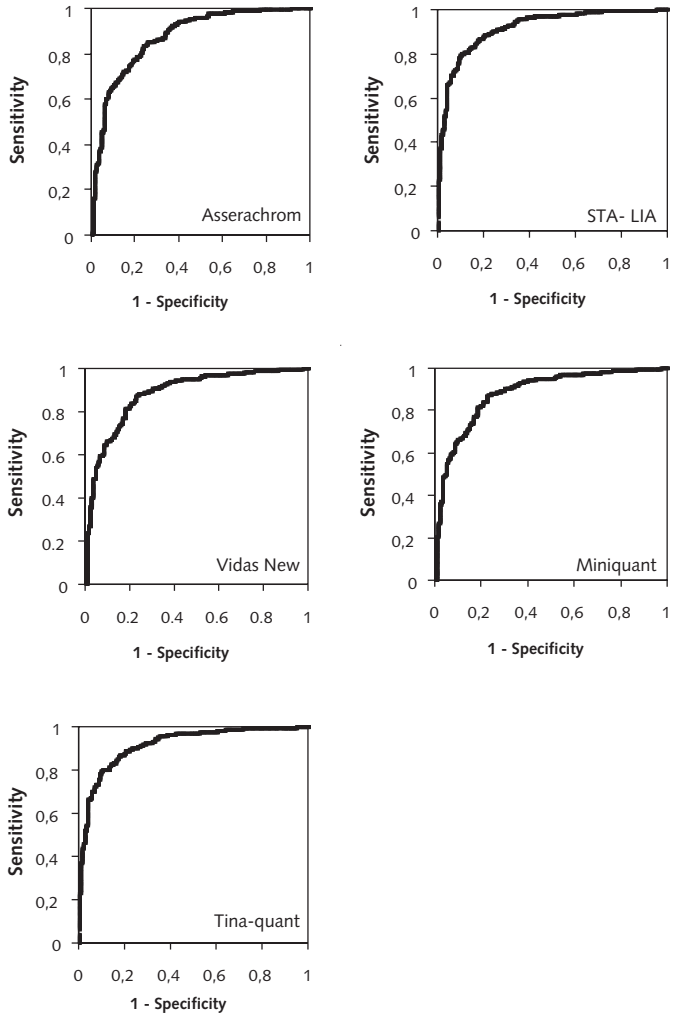

Table 1. Comparison of the area under ROC curves (AUC) for the 5 D-dimer assays, reported as p-values.

	AUC (95%CI)	VIDAS New®	Tina-quant®	STA-LIA®	Miniquant®
Asserachrom®	0.879 (0.850-0.908)	0.6	0.003	0.8	0.1
VIDAS New®	0.887 (0.859-0.916)		0.004	0.7	0.2
Tina-quant®	0.922 (0.900-0.945)		<0.0001	0.01	
STA-LIA®	0.883 (0.854-0.911)			0.07	
Miniquant®	0.902 (0.874-0.930)				

Table 3. Kappa coefficients between the 5 D-dimer assays.,

	VIDAS New®	Tina-quant®	STA-LIA®	Miniquant®
Asserachrom®	0.33	0.63	0.70	0.59
VIDAS New®		0.24	0.31	0.18
Tina-quant®			0.70	0.72
STA-LIA®				0.62

For the calculation of the accuracy indices, quantitative D-dimer results lower than the cut-off value were considered to be negative. Cut-off values for the Asserachrom®, VIDAS New®, Tina-quant® and STA-LIA® were 0.5 FEU mg/L. The cut-off value for the Miniquant® was 0.25 D-dimer equivalent units mg/L.

Table 2. Clinical performance of the different D-dimer assays. Values are reported as percentage and their 95% confidence intervals. Cut-off values as recommended by the manufacturers have been given in bold.

D-dimer assay	Cut-off value	Sensitivity	Negative predictive value	Specificity
Asserachrom®	1.0 FEU mg/L	88 (84-93)	89 (85-93)	68 (63-73)
	0.5 FEU mg/L	**97 (94-99)**	**94 (87-97)**	**33 (28-38)**
	0.4 FEU mg/L	99 (96-100)	96 (89-99)	24 (20-29)
	0.3 FEU mg/L	99 (97-100)	96 (85-99)	13 (10-17)
	0.2 FEU mg/L	100 (98-100)	95 (75-100)	6 (4-9)
VIDAS New®	1.0 FEU mg/L	98 (96-100)	96 (90-99)	29 (24-34)
	0.5 FEU mg/L	**100 (98-100)**	**96 (81-100)**	**8 (6-12)**
	0.4 FEU mg/L	100 (98-100)	93 (66-100)	4 (2-7)
	0.3 FEU mg/L	100 (98-100)	88 (47-100)	2 (1-5)
	0.2 FEU mg/L	100 (98-100)	0 (0-100) 0	(0-12)
Tina-quant®	1.0 FEU mg/L	93 (89-96)	94 (90-96)	70 (65-75)
	0.5 FEU mg/L	**99 (97-100)**	**98 (95-100)**	**41 (36-47)**
	0.4 FEU mg/L	100 (98-100)	99 (94-100)	31 (26-36)
	0.3 FEU mg/L	100 (98-100)	100 (94-100)	18 (14-22)
	0.2 FEU mg/L	100 (98-100)	100 (74-100)	4 (2-7)
STA-LIA®	1.0 FEU mg/L	94 (90-97)	93 (89-96)	60 (55-66)
	0.5 FEU mg/L	**98 (95-99)**	**95 (89-99)**	**32 (27-37)**
	0.4 FEU mg/L	99 (97-100)	98 (91-100)	25 (20-30)
	0.3 FEU mg/L	100 (98-100)	98 (89-100)	15 (11-19)
	0.2 FEU mg/L	100 (98-100)	-	0 (0-1)
Miniquant®	0.5 mg/L	90 (86-94)	91 (86-94)	72 (67-77)
	0.25 mg/L	**95 (91-98)**	**94 (89-97)**	**52 (47-58)**
	0.2 mg/L	96 (93-98)	94 (88-97)	43 (37-48)
	0.15 mg/L	95 (91-97)	93 (87-97)	35 (29-40)
	0.1 mg/L	98 (96-100)	93 (83-98)	17 (13-21)

DISCUSSION

The diagnostic strategy in patients suspected for DVT or PE is being challenged by the development of highly sensitive D-dimers and the revival of the pretest clinical probability score. Recent studies have demonstrated the safety of withholding anticoagulant treatment in patients with a normal D-dimer and a normal single ultrasound (14;15). Even the combination of a low clinical score and a normal D-dimer concentration can be considered a safe strategy to exclude thrombosis and to withhold anticoagulant therapy in patients suspected for venous thromboembolism (8;15-17;19;26;30-32). When trying to replace accepted strategies like venography or serial ultrasound, their failure rates of, respectively, 1.3% (33) and 0.6-0.7% (34;35) may not be exceeded by the new diagnostic strategy. Therefore, a highly sensitive D-dimer test is mandatory for the exclusion of thrombosis in every new diagnostic strategy. Differences between D-dimer assays are thought to be caused by antibody specificity, especially concerning the preference for high- or low molecular weight fibrin derivates and for crosslinked and non-crosslinked fibrin derivates (36). Other causes of discrepancies between D-dimer assays are time-dependence of neo-epitope expression in the course of fibrin formation and dissolution, assay format, purity or heterogeneity of the calibrator, matrix effects of plasma on epitope presentation and interference by irrelevant analytes (37).

In our study, ROC curve analysis showed a significantly higher AUC for the Tina-quant® D-dimer assay as compared to the other four assays. At standard cut-off values, the Tina-quant® also had the highest NPV with 98% (although not statistically different), with a high sensitivity of 99%. The performance of the Tina-quant® in our study was comparable with previous reports (1;5;13). Using standard cut-off values, the Miniquant® reached a sensitivity of 95% with a NPV of 94%, which is comparable with previous reports (38-40). It had significant lower values of sensitivity and NPV as compared to the Tina-quant® and a significant lower sensitivity as compared to the VIDAS New®. Results of the STA-LIA® assay showed a high sensitivity of 98% and NPV of 95%. This is higher than a previous report that found a sensitivity of 89% and NPV of 86% (41) but comparable with other reports (42-44). The VIDAS New® had an excellent sensitivity of 100% and a NPV of 96%, which was better than a previous report (45) but comparable with a study from de Moerloose et al (46).

It is well known that the D-dimer tests have positive results in case of various co-morbid conditions, such as infections and malignancy. Due to these frequent false-positive test results, the specificity of the D-dimer is low. It is the specificity that determines the usefulness of the D-dimer as an exclusion criterion for thrombosis. A low specificity of a D-dimer implies that CUS will still be necessary in the majority of the patients. Although the sensitivity and NPV of the VIDAS New® were high, a remarkably low specificity of 8% was found. This low specificity is in disagreement with two previous reports on the VIDAS New® (45;46) that found a specificity of 42% and 33% respectively at the same cut-off level. Furthermore, the kappa coefficients of the VIDAS New® compared to the other four assays were mediocre to poor. This is in disagreement with the kappa coefficients for the original VIDAS compared to the STA-LIA®, Asserachrom® and Tina-quant® as found by van der Graaf et al (13). As the VIDAS New® assay is fully automated and not dependent on technician skills, it is not likely that variables in the analytic process have lead to the large number of false-positive results in our study. A previous report has seen no effect of transportation or storage at room temperature on the results of the VIDAS® D-dimer assay (47). We showed recently that freezing of samples or type of chemical analyser had no influence on D-dimer concentration (48). A considerable improvement of the performance of the VIDAS New® was achieved after raising the cut-off level to 1.0 FEU mg/L, resulting in a sensitivity of 98%, a NPV of 96%, and a specificity of 29%.

The D-dimer assays in our study all showed generally high sensitivity and NPV. In general, the sensitivity could be improved by lowering the cut-off values, but the subsequent decrease in specificity makes the assays less useful in clinical practice. Despite changes in cut-off values none of the tests reached a NPV of > 98%, except for the Tina-quant®. Using standard cut-off values, none of the tests reached a NPV of > 98%. When the use of D-dimer is restricted to patients with a low clinical probability score only, it has been shown for the SimpliRed® and Tina-quant® assay that the NPV of the D-dimer assay will further increase (18;26;30). It is therefore to be expected that the combination of a clinical probability score and the other D-dimer tests from our study will also increase the NPV and be a safe strategy to exclude DVT in outpatients.

In conclusion, the D-dimer assays in our study all show a high sensitivity and negative predictive value. However, using standard cut-off values, none of the assays reached a NPV of > 98%. For exclusion of DVT, we

therefore recommend combining the D-dimer assay with other non-invasive tests in order to reach failure rates less than 1.0%. The use of D-dimer assays with a very low specificity might not be worthwhile from an economical point of view, as a positive test will necessitate additional testing in the majority of the patients.

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CHAPTER 7

Low molecular weight heparin is equally effective as unfractionated heparin in reducing coagulation activity and perfusion abnormalities during the early treatment of pulmonary embolism

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SUMMARY

Background: Little is known about differences between UFH and LMWH on coagulation activity during treatment for pulmonary embolism. The objective of this study was to compare UFH and LMWH in the early treatment of pulmonary embolism in terms of control of coagulation markers and perfusion abnormalities.

Methods: 37 patients with acute pulmonary embolism were randomised to receive intravenous UFH or subcutaneous dalteparin, both together with acenocoumarol. Daily samples were obtained for measurement of thrombin generation (fragments 1+2, TAT-complexes and fibrin monomers) and fibrinolysis (D-dimer concentrations and clot lysis times). Ventilation-perfusion scintigraphies were performed from which the percentage-of-vascular-obstruction scores (PVOs) were calculated at day 0 (PVOsD0) and day 5 (PVOsD5).

Results: The INR was therapeutic in both groups at day 3. At day 3, anti-Xa levels were 0.21 IU/ml in the UFH group (n=19) and 0.29 IU/ml in the LMWH group (n=18) (p=0.1). F1+2 and TAT-complexes rapidly normalised without differences between the groups (p=0.5 and p=0.4 resp.). Fibrin monomer levels did not decrease, and showed an increase in the UFH group from day 3 ($p<0.05$ for differences between the groups). D-dimer levels decreased over time, without differences between the groups (p=0.6). Clot lysis times were shorter in the UFH group ($p<0.05$). The PVOsD0 and PVOsD5 were not different (p=0.5 and p=0.8 resp), but the decrease of the PVOs over time was higher in the LMWH group (p=0.04).

Conclusion: LMWH is at least equally effective as UFH in reducing coagulation activity and perfusion abnormalities in the early treatment of pulmonary embolism.

INTRODUCTION

Intravenous administration of unfractionated heparin (UFH) has proven its efficacy in the early treatment of venous thromboembolism (1-3). The purpose of using heparin in the treatment of thrombosis is to inhibit thrombin generation. The use of UFH, however, has several inconveniences. Intravenous administration of UFH requires hospitalisation and frequent monitoring of the activated partial thromboplastin time (aPTT) for optimal dose-adjustment. Low molecular weight heparin (LMWH) has the advantage of a subcutaneous administration route, more stable plasma heparin concentrations, the absence of the need for monitoring and a lower incidence of heparin-induced thrombocytopenia (4). Treatment of deep venous thrombosis of the leg with LMWH is considered to be equally safe as UFH when looking at clinical outcomes (5-20). LMWH also appears to be safe in the treatment of pulmonary embolism from a clinical point of view (10;16;21-24). Less agreement exists about the effect of LMWH versus UFH in reducing coagulation activity in venous thrombosis. Some studies report a more rapid decrease in coagulation activity by UFH as compared to LMWH (25;26), whereas others find the opposite (27;28) or no differences (29-31). These studies mainly concern patients with deep venous thrombosis or healthy donors. The effect of LMWH versus UFH on early changes in haemostatic markers in patients with pulmonary embolism has not yet been studied. As many clinicians still use UFH in the treatment of pulmonary embolism, the finding of equal or more effectiveness of LMWH on coagulation markers and perfusion abnormalities might further support the use of LMWH in the initial treatment of pulmonary embolism.

The purpose of this study is to compare UFH and LMWH in their capacity to inhibit thrombin generation in the early treatment of pulmonary embolism by measuring prothrombin fragments 1+2 (F1+2), thrombin-antithrombin complexes (TAT) and fibrin monomers. Secondly, we compare UFH and LMWH in their effects on fibrinolysis by measuring D-dimer concentrations and clot lysis times. Finally, we compare UFH and LMWH in terms of changes in perfusion abnormalities during the early days of treatment for pulmonary embolism.

PATIENTS AND METHODS

Study design

This was a randomised, non-blinded study where continuous infusion of dose adjusted unfractionated heparin was compared to a once daily subcutaneous injection of dalteparin in terms of changes in coagulation markers and changes in perfusion abnormalities. Informed consent was obtained from all participating patients and the medical ethical committee of our institute approved the study protocol. The primary endpoint of this study was the time course of different coagulation and fibrinolytic markers in the two heparin regimens. The secondary endpoint was the evaluation of changes in perfusion abnormalities during the early treatment with the two heparins.

Patients and study protocol

Patients with proven symptomatic pulmonary embolism (as described later) were eligible for this study. Exclusion criteria were active malignancy (defined as currently receiving any treatment for cancer or documented recurrent or metastatic disease), recent surgery or large trauma in the past month, the use of any form of anticoagulant in the past month, pregnancy, a history of heparin-induced thrombocytopenia and the presence of a contraindication for anticoagulation. Patients were randomised to receive UFH or LMWH (dalteparin). UFH was given within 1 hour after diagnosis as an intravenous bolus of 5000 IU, followed by continuous infusion, starting with 25000 IU/d. Dosage adjustment was made by monitoring the ratio of the activated partial thromboplastin time (aPTT). The first aPTT ratio was measured 6 hours after the start of UFH and was followed by monitoring twice a day with a target range of 2.0-3.5. In patients who were randomised for LMWH, dalteparin was given through a subcutaneous injection within 1 hour after diagnosis and after that once a day at 17h00. Dosages of dalteparin were body weight-adjusted: < 55 kg: 10.000 IU; 55-65 kg: 12.500 IU; 65-85 kg: 15.000 IU; > 85 kg: 18.000 IU. Within 24 hours of the start of the study, oral coumarin derivates were started (acenocoumarol). Treatment with UFH or dalteparin was stopped when the International Normalized Ratio (INR) was > 2.0 on two consecutive days with a minimum duration of five days.

Laboratory assessments

Before the start of any anticoagulant therapy, and following daily between 08h00 and 09h00 the next consecutive 5 days, blood was drawn. The day of presentation and before treatment was started, was considered to be day 0. The INR was measured daily using the Hepato Quick® (Diagnostica Stago, Asnière, France) and the aPTT using the Cephotest™ (Axis-Shield PoC AS, Oslo, Norway). Extra samples were stored and frozen at –70°C until further assessment. Anti-Xa levels were measured at day 3 using the Staclot® Heparin (Diagnostica Stago, Asnière, France).

Thrombin generation was estimated by measurement of prothrombin fragments 1+2 (F1+2) using the ELISA Enzygnost® F1+2 (Dade Behring, Schwalbach, Germany) with normal values 0.4-1.1 nmol/l, thrombin-antithrombin complexes (TAT) using the ELISA Enzygnost® TAT micro (Dade Behring, Schwalbach, Germany) with normal values 1.0-4.1 µg/l and fibrin monomers using Berichrom® FM (Dade Behring, Schwalbach, Germany) with normal values <3.4-14.5 mg/l.

Effects on fibrinolysis were estimated by measuring D-dimer concentration using the ELISA Asserachrom® (Diagnostica Stago, Asnière, France) with normal values < 500 µg/ml Fibrin Equivalent Units (FEU) and plasma clot lysis times using previously described methods (32;33), where lysis of a tissue-factor induced clot by exogenous t-PA was studied by monitoring changes in turbidity during clot formation and subsequent lysis. The contribution of thrombin activatable fibrinolysis inhibitor (TAFI) to the clot lysis time was assessed after addition of 25 µg/ml carboxypeptase inhibitor (CPI, Calbiochem, La Jolla, CA) to the plasma, which is a specific inhibitor of activated TAFI (34).

Laboratory assessments were done by skilled personnel who were unaware of the treatment regimens.

Diagnosis of pulmonary embolism

In all patients, a ventilation-perfusion scintigraphy was performed at presentation (day 0). Lung scans were performed in 4 standard views: anterior, posterior and right and left posterior oblique. Additional right and left lateral views were performed at indication. For interpretation of the scans, Hull's criteria were used (35), as well as a lung segment reference chart (36). A normal scintigraphy ruled out pulmonary embolism, where a high probability scan confirmed the diagnosis. An intermediate probability scintigraphy was followed by pulmonary angiography. During this angiography, a stan-

dard intravenous dose of 5.000 IU UFH was given. The ventilation-perfusion scintigraphy was repeated at day 5. These results were compared with the results obtained at day 0 by two nuclear specialists who were unaware of the treatment regimens. The percentage-of-vascular-obstruction score (PVOs), as described previously (37;38), was calculated at day 0 (PVOsD0) and at day 5 (PVOsD5).

Statistics

For comparison of baseline characteristics between the two groups, the Fisher's exact test and the Mann-Whitney U test were used with two-tailed p-values. We used the repeated measurement MANOVA for comparison of groups over time with the SAS software (SAS version 8.2, Cary, NC, USA). The Mann-Whitney U test with two-tailed p-values was used to compare daily values between the groups using SPSS software (SPSS version 10.0.5, Chicago, USA). Bivariate correlation between laboratory values and PVOs was calculated using SPSS software.

RESULTS

Forty patients with proven pulmonary embolism were included in this study. In three patients, more than one sample was missing; these 3 patients were excluded from further analysis. Of the remaining 37 patients, 19 patients received UFH and 18 patients received LMWH. For baseline characteristics, see Table 1. The two groups were comparable considering gender, the PVOs and the number of angiographies performed. Patients receiving dalteparin were of older age. Except for F1+2 levels, which were higher in the LMWH group, the coagulation and fibrinolytic markers were comparable.
The INR reached therapeutic values in both groups at day 3: the median in the LMWH group was 2.3 and in the UFH group 2.1 (p=0.4), the means were 2.4 ± 0.8 and 2.1 ± 0.8. The mean aPTT ratio in the UFH group after 24 hours was 2.0 ± 0.7, where 8/19 (42%) had values > 2.0. At day 3, 15/19 (79%) had values > 2.0, at day 4 17/19 (89%) and at day 5 all patients had an aPTT ratio > 2.0. Anti-Xa levels at day 3 were 0.29 ± 0.15 IU/ml in the LMWH group and 0.21 ± 0.1 IU/ml in the UFH group (p=0.1). No bleeding complications were observed in the total study population.

Table 1. Baseline characteristics in patients with pulmonary embolism initially treated with intravenous unfractionated heparin or subcutaneous dalteparin. Values are reported as median, unless indicated. (F1+2 = fragments 1+2, TAT = thrombin-antithrombin, FEU= Fibrin Equivalent Units, PVOs = percentage-of-vascular-obstruction score)

	Unfractionated heparin N = 19	Dalteparin N = 18	P-value
Women/men (n)	12/7	7/11	0.2
Age (median;range)	46 (28-74)	60 (30-82)	0.02
Angiography (n)	3	3	1.0
F1+2 (nmol/l)	1.4	2.2	0.03
TAT-complexes (µg/l)	6.6	6.7	0.9
Fibrin monomer (mg/l)	22.6	21.6	0.6
D-dimer (µg/ml FEU)	1950	2611	0.7
Clot lysis time (min)	52	45	0.9
PVOs (%)	28.6	22.9	0.5

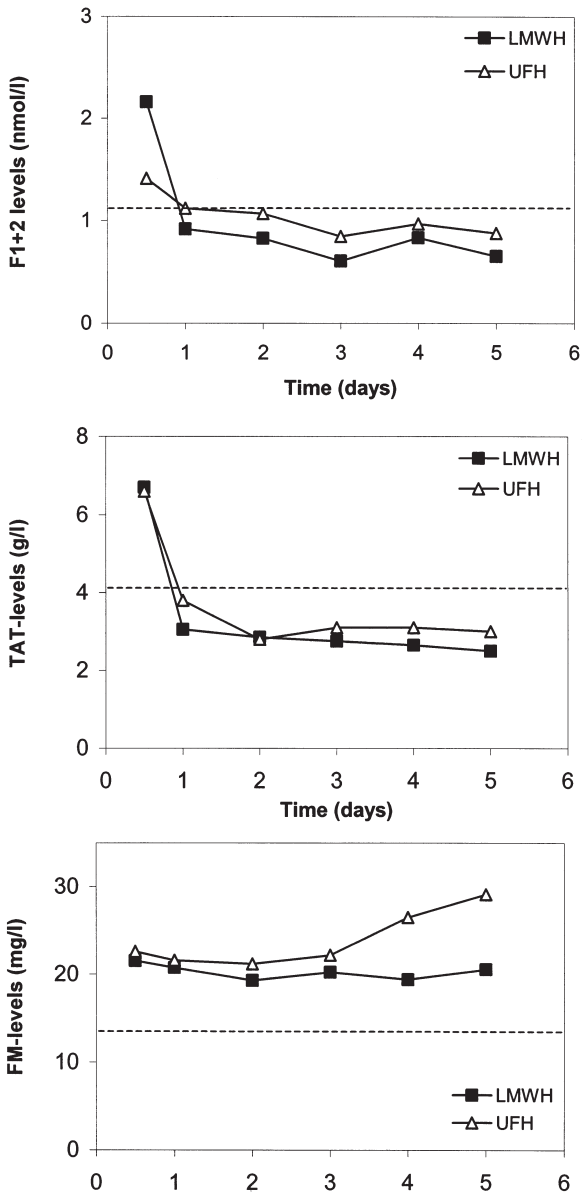
Evaluation of thrombin generation

The levels of F1+2 decreased over time in the total group ($p<0.0001$) (Figure 1a). Baseline values were higher in the LMWH group as to compared to the UFH group ($p=0.03$). After treatment, F1+2 levels normalised rapidly in both groups. No differences were seen at day 1 ($p=0.3$), day 2 ($p=0.052$), day 3 ($p=0.2$), day 4 ($p=0.4$) and day 5 ($p=0.2$).

A rapid normalisation of TAT complexes was seen in both groups compared to baseline values ($p=0.02$) (Figure 1b). After day 1, the levels of TAT complexes remained normal. Between the groups, there was no difference over time ($p=0.5$). Comparison of daily values between the groups showed no significant differences ($p=0.9$ at day 0, $p=0.3$ at day 1, $p=1.0$ at day 2, $p=0.4$ at day 3, $p=0.4$ at day 4 and $p=0.3$ at day 5).

Fibrin monomer levels did not decrease to normal values during treatment in both heparin regimens. They remained stable in the LMWH group, but increased again in the UFH group. Differences were significant at day 3 ($p=0.04$), day 4 ($p=0.04$) and day 5 ($p=0.003$) (Figure 1c).

Figure 1. The effect of low molecular weight heparin (LMWH) and unfractionated heparin (UFH) on thrombin generation in patients with pulmonary embolism. Dotted lines represent upper normal values.
a) Fragment 1+2 levels (F1+2), b) Thrombin-antithrombin (TAT)-complexes, c) Fibrin monomer (FM) levels



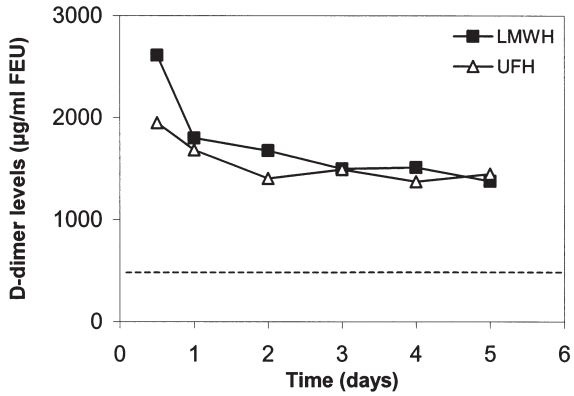
Evaluation of fibrinolysis

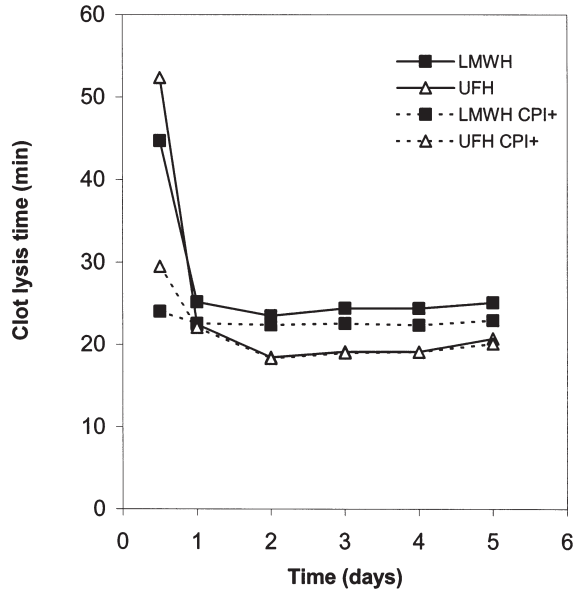
The course of the D-dimer levels showed a significant decrease in both groups over time ($p<0.0001$), but there were no differences between the two groups over time ($p=0.6$) (Figure 2a). Comparing daily values, there were no differences between the groups ($p=0.7$ at day 0, $p=0.7$ at day 1, $p=0.9$ at day 2, $p=1.0$ at day 3, $p=0.8$ at day4 and $p=0.6$ at day 5).

Plasma clot lysis times are shown in Figure 2b. There was a significant difference between the groups over time, with longer clot lysis times in the LMWH group ($p<0.0001$). Lysis times did not differ at day 0 and 1 ($p=0.9$ and $p=0.1$ respectively), but from day 2 they were longer in the LMWH group ($p=0.003$ at day 2, $p<0.001$ at day 3, $p=0.001$ at day 4 and $p=0.02$ at day 5).

After addition of CPI, there was a similar decrease in clot lysis times at day 0 in both groups ($p=0.28$). Clot lysis times between the groups were not different at day 0 and day 1 ($p=0.052$ and $p=0.06$ respectively), but they were higher from day 2 in the LMWH group ($p=0.007$ at day 2, $p=0.001$ at day 3, $p=0.006$ at day 4 and $p=0.048$ at day 5). There was no additional effect of CPI on clot lysis times after the start of UFH. In the LMWH group, CPI resulted in shorter clot lysis times at day 0 and day 1 ($p=0.001$ and $p=0.04$ respectively), but not at day 2, 3, 4 and 5 ($p=0.2$, $p=0.2$, $p=0.1$ and $p=0.1$ respectively).

Figure 2. The effect of low molecular weight heparin (LMWH) and unfractionated heparin(UFH) on fibrinolysis in patients with pulmonary embolism.
Dotted line in figure 2a represents upper normal value.
CPI = carboxypeptase inhibitor
a) D-dimer levels, b) Clot lysis time



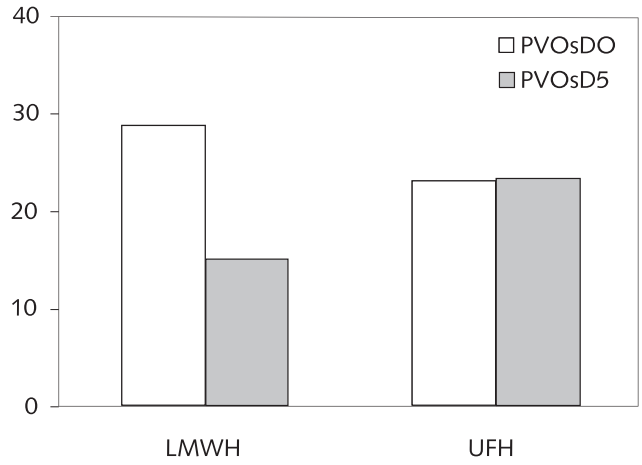


Perfusion abnormalities

Six patients did not have a second ventilation-perfusion scintigraphy at day 5 (3 in the UFH and 3 in the LMWH group). They were excluded from PVOs analysis. In the remaining 31 patients, the median of the PVOsD0 was 28.6% in the LMWH group and 22.9% in the UFH group (p=0.5) (Figure 3). At day 5, the values were 15.0% and 23.2% respectively (p=0.8). In the LMWH group, there was a reduction in PVOs although not statistically significant (p=0.09). In the UFH group, no reduction was seen (p=0.6). Although values at day 0 and 5 did not differ statistically between the groups, there was a significant difference in the decrease of the PVOs over time in favour of the LMWH group (p=0.04).

There was a correlation between the PVOsD0 and the initial F1+2 levels at presentation (p=0.009) and between the PVOsD0 and the initial D-dimer levels at presentation (p=0.04). No correlation was found between the PVOsD0 and other laboratory variables. There was no correlation between the PVOsD5 and any laboratory marker at day 5.

Figure 3. Percentage-of-vascular-obstruction score (PVOs) at day 0 (PVOsD0) and day 5 (PVOsD5) in patients with pulmonary embolism treated with low molecular weight heparin (LMWH) or unfractionated heparin (UFH)



DISCUSSION

Inhibiting thrombin generation is the main purpose of treatment of venous thrombosis with heparins. The safety of UFH and LMWH in the treatment for venous thrombosis has been demonstrated in a number of trials (5-24). Few data exist on the effectiveness of both heparin regimens in controlling coagulation markers in deep venous thrombosis. A recent large report showed the efficacy of LMWH in reducing thrombin generation at 1 and 3 weeks (39), but for pulmonary embolism no such studies have been conducted.

In this study, we demonstrate that in patients with pulmonary embolism, both UFH and LMWH immediately decrease levels of F1+2 fragments and TAT-complexes to normal values. The decrease by UFH is in agreement with previous reports (40,41) and the similar decrease in both heparin regimens has been found by others (30,31). The DVTENOX trial, however, showed more rapid decreases of TAT-complexes and F1+2 in patients treated with enoxaparin compared to UFH (27). On the other hand, in a smaller study, Stricker et al found the opposite (25). Some authors seek an explanation for the differences between the studies in the degree of anticoagulation and the moment of reaching therapeutic ranges (25;27).

Interestingly, fibrin monomer levels did not decrease in both heparin regimens. In patients treated with UFH, they even showed an increase after 3 days of treatment. This might implicate that UFH inhibits fibrin formation in a lesser extent over time than LMWH. As the increase in fibrin monomers in UFH is seen from day 3, this cannot be explained by non-therapeutic ranges of UFH, as therapeutic ranges were reached after 3 days. However, anti-Xa levels in the LMWH group were higher at day 3; although this difference was not statistically significant, it might be clinically relevant. One study reported higher anti-Xa levels and lower fibrin monomer levels during infrainguinal bypass surgery in patients treated with LMWH compared to UFH (42). Another explanation may be found in fibrin-bound thrombin. After generation of fibrin, activated thrombin can bind to fibrin. This fibrin-bound thrombin retains its procoagulant activity, but is protected from antithrombin-induced inhibition through the formation of ternary thrombin-fibrin-heparin complexes (43-46). Furthermore, fibrin is a potent modulator of heparin activity in vivo by inhibiting heparin-catalyzed thrombin-antithrombin complex formation through formation of these ternary complexes (47). One could postulate that the formation of this ternary

complex might differ between UFH and LMWH and therefore might influence thrombin inactivation and subsequent fibrin formation. Indeed, Hogg et al found that heparins with lower molecular weight are less effective in promoting thrombin binding to fibrin polymer (48). Furthermore, during LMWH therapy, higher antithrombin levels are maintained compared to UFH (39,49,50). This implicates that in patients treated with LMWH, more thrombin will be inhibited by antithrombin and less fibrin will be generated. Although this could explain the differences in fibrin monomer levels between UFH and LMWH in our study, the increase of fibrin monomer levels in the UFH group is still not clear.

The role of heparin in the enhancement of fibrinolysis is thought to be through inhibition of thrombin-dependent generation of activated TAFI. As a result, stabilisation of a clot by TAFI will be diminished, leading to shortening of clot lysis times. In our study, we found that both heparins resulted in shorter clot lysis times after treatment. In vitro inactivation of TAFI by CPI eliminated any further effect of heparin on clot lysis times, confirming the findings of Lisman et al (51), that the effect of heparin on the clot lysis time is mainly through TAFI. However, the clot lysis times were longer in the LMWH group when compared to the UFH group ($p<0.0001$); this difference remained significant after inhibition of TAFI by CPI. The fact that the lysis times in our study were shorter after treatment with UFH, independent of TAFI activity, raises the question whether there are other factors involved through which UFH has more influence on clot lysis times than LMWH. Urano et al found that addition of factor Xa together with calcium shortens clot lysis times (52). The lower anti-Xa levels in our UFH group (although not statistically significant) might therefore be the explanation for our findings. Another possibility could be the tissue-plasminogen activator (t-PA) levels. It has been shown that heparin shortens clot lysis times through an increase in t-PA (53). If UFH increases t-PA more than LMWH, this will result in shorter clot lysis times. Unfortunately, we were not able to perform these tests retrospectively.

Reports on the course of D-dimer levels during treatment with heparin and the differences between UFH and LMWH are heterogeneous (25,27,31,39,40). In our study, we found a decrease of D-dimer levels over time in both groups ($p<0.0001$), without differences between the groups. This implicates that LMWH has a similar effect on fibrinolysis in vivo as UFH.

As a clinical endpoint of our study, we calculated the PVOs before and at the end of treatment. There was no significant difference between the groups at day 0 and day 5. However, there was a decrease in the LMWH group, while no decrease was seen in the UFH group (p=0.04). These findings might argue in favour of LMWH as initial treatment for pulmonary embolism. From a clinical point of view, it is of interest to be informed about possible correlations between laboratory variables and perfusion abnormalities. We found a correlation between F1+2 and D-dimer levels and perfusion abnormalities, where higher levels of F1+2 or D-dimer correlated with higher vascular obstruction scores. Therefore, the initial height of F1+2 or D-dimer might give an indication of the severity of the pulmonary embolism. This is in agreement with a previous report (54). At day 5, we found no correlation between any laboratory value and perfusion abnormalities. This implicates that the coagulation and fibrinolytic markers in our study are not suitable for clinical follow-up evaluation.

In conclusion, LMWH (dalteparin) is at least equally effective as UFH in reducing coagulation activity and in prohibiting further increases in perfusion abnormalities in the early treatment of pulmonary embolism. Our findings support the use of LMWH as initial treatment of pulmonary embolism.

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CHAPTER 8

High D-dimer concentrations can predict malignancy in patients presenting with deep venous thrombosis

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SUMMARY

Background Venous thromboembolism can be related to malignancy, but routine screening for cancer in patients with deep venous thrombosis (DVT) is a matter of debate.

Methods In consecutive outpatients with proven DVT, D-dimer measurement was done at presentation. Patients were followed for a median of 31 months and the occurrence of malignancy was documented. In a proportion of the patients, daily measurements of D-dimers were performed for 4 consecutive days.

Results Forty-nine (22%) of 218 patients with DVT had or developed cancer: 28 patients were known with cancer, in 8 patients cancer was discovered at time of presentation with DVT and during folow-up 13 patients developed cancer. The prevalence of cancer in patients with initial D-dimer levels < 4000 µg/l was 16% as compared to 35% in patients with D-dimer levels > 4000 µg/l (p=0.005). After 4 days of treatment, the prevalence of cancer was 14% when D-dimer levels were < 4000 µg/l, compared to 46% when they were still > 4000 µg/l (p=0.02). Of the patients with thrombosis and cancer, 78% (38/49) was older than 60 years. D-dimer levels at age > 60 did not disciminate in the presence or absence of cancer. In younger patients, initial D-dimer levels of < 4000 µg/l were associated with a cancer prevalence of 3% compared to 23% at levels > 4000 µg/l (p=0.001). After 4 days of treatment, cancer prevalences were 0% and 100% in younger patients with D-dimer levels < and > 4000 µg/l respectively (p=0.003).

Conclusions High D-dimer concentrations at presentation and persistently high D-dimer levels during the first days of treatment are indicators of an increased change for overt or occult forms of cancer, especially in patients younger than 60 years.

INTRODUCTION

The development of venous thromboembolism is associated with transient risk factors for thrombosis like surgery or trauma, and permanent risk factors (such as factor V Leiden, the prothrombin mutation, protein C and S deficiency, antithrombin deficiency, lupus anticoagulant and hyperhomocysteinemia). Venous thromboembolism can also be related to malignancy (1-5). The incidence of malignancy in patients with thrombosis is 7-26% (6-10). Patients with cancer are at higher risk of developing venous thromboembolism, which has been observed especially in postoperative patients with cancer and in patients receiving chemotherapy (11).

Although several studies have tried to define clinical conditions in which cancer is more frequent in venous thromboembolism (3,6,10,12,13), there is still debate about routine screening for occult malignancy. It would be of interest if certain patients with thrombosis could be identified as having an increased risk of having malignancy. The mechanism of the hypercoagulable state in cancer is complex and many markers of blood clotting activation are disturbed (2,14,15). Increased concentrations of D-dimers, specific markers of fibrinolysis, can be found in patients with cancer (18-19). As in many patients suspected for venous thromboembolism a D-dimer measurement is now being performed according to more recently developed diagnostic strategies (20-24), it is of interest to know whether the D-dimer concentration can be used as a predictor for cancer as cause for venous thromboembolism in these patients.

The aim of this study is to investigate whether the initial height of the D-dimer level in patients with deep venous thrombosis or early changes in D-dimer levels during treatment for deep venous thrombosis can differentiate between patients with and without cancer.

PATIENTS AND METHODS

Consecutive outpatients with proven deep venous thrombosis of the leg were included in this study from July 1998 to October 2001. Ineligibility criteria were the use of anticoagulants, pregnancy and not documented recanalisation after a previous history of deep venous thrombosis in the ipsilateral leg. Thrombosis was diagnosed using real-time B-mode compression ultrasonography, where lack of compressibility was the criterion for an abnormal result; a vein was considered fully compressible if no residual lumen was seen. In all patients, a D-dimer measurement was done using the Tina-quant® quantitative latex assay (Roche, Mannheim, Germany) at presentation. D-dimer values above 8000 µg/l FEU (Fibrinogen Equivalent Units) were not diluted further and were reported as > 8000 µg/l FEU. Treatment for thrombosis was started immediately and was given as a once daily subcutaneous injection with a low molecular weight heparin (dalteparin; dose adjusted to weight). Oral acenocoumarol was started simultaneously. Patients were treated with dalteparin for at least 5 subsequent days until the INR was > 2.0. We documented the presence of previously known malignancy and the type of malignancy that was detected at the moment of thrombosis or developed during follow-up. Data on the follow up period were obtained by checking the medical records in the participating hospital from July 1998 to February 2002, by sending a questioner to the patient's general practitioner and by checking the national database network and registry of histo- and cytopathology.

We had the intention to measure D-dimer levels daily for a period of at least four days after the start of anticoagulation. The day of presentation was considered to be day 0. This study was, however, restricted to the first 57 patients, because in 1999, we lost the opportunity for this clinical follow-up and daily D-dimer measurements due to the decision to treat patients with deep venous thrombosis at home.

Statistics

As D-dimer values > 8000 µg/l FEU were not diluted, no linear comparison between the groups could be made. We have made clusters for the D-dimer levels, with ranges 0-2000, 2001-4000, 4001-6000 and > 6000 µg/l FEU. We used the Fisher's exact method with two-tailed p-values for comparison between the clusters.

RESULTS

In total, 218 (102 men and 116 women) consecutive outpatients with proven deep venous thrombosis of the leg were included. Of the total of 218 patients, 49 (22%) patients had or developed cancer; 28 were already known with cancer at the moment of thrombosis, 8 had cancer discovered at presentation with thrombosis and 13 developed malignancy during follow up (Table 1). The median follow up was 31 months (range 4-54 months). In the 8 patients who had cancer discovered at presentation, all but one had metastatic disease. In the 13 patients who developed cancer during follow-up, 3 had metastatic disease. The median time to the development of cancer in the latter 13 patients was 15 months (range 3-36 months). The total prevalence of cancer in patients with deep venous thrombosis that were not previously known with cancer was 11% (21/190). The prevalence of cancer during follow-up was 7% (13/182).
Of the 218 patients, 102 (47%) was older than 60 years. In these 102 patients, the prevalence of cancer was 37% (38/102). This was significantly higher than the prevalence of cancer in patients younger than 60 years (10% (12/116); p=0.0003). The median age in patients with cancer was higher than in patients without cancer: 67.9 years (range 30-88) versus 55.0 years (range 18-90) (p<0.001). Of the patients with thrombosis and cancer, 78% (38/49) was older than 60 years. Of the 13 patients that developed cancer in the follow up, 85% (11/13) was older than 60 years.
At initial D-dimer levels of 0-2000 µg/l FEU, the prevalence of cancer in the total group was 12% (7/59), at levels 2001-4000 µg/l FEU 19% (13/69), at levels 4001-6000 µg/l FEU 33% (9/27) and at levels > 6000 µg/l FEU 32% (20/63). Comparing the lowest with the highest cluster, this difference was statistical significant (p=0.009). The prevalence of cancer in patients with a D-dimer < 4000 µg/l FEU was 16% (20/128) as compared to 32% (29/90) (p=0.005) in patients with D-dimer levels > 4000 µg/l FEU (Table 2). In patients older than 60 years, there was no difference in cancer prevalence between patients with D-dimer levels above or under 4000 µg/l FEU (p=0.7). In patients younger than 60 years, the prevalence of cancer was 3% when D-dimer levels were < 4000 µg/l FEU, and 23% when D-dimer levels were > 4000 µg/l FEU (p=0.001).

D-dimer levels were measured at 4 consecutive days after the start of anticoagulation in 57 patients with proven deep venous thrombosis. All

patients had D-dimer levels > 500 µg/l FEU at presentation. There was a significant decrease in D-dimer levels over time. At presentation (day 0), 25 (44%) of the 57 patients had a D-dimer concentration in the highest cluster (> 6000 µg/l FEU); at day 4 only 8 (14%) patients had a D-dimer concentration > 6000 µg/l FEU (p<0.001). Similarly, at presentation 10 (18%) of the 57 patients had D-dimer levels in the lowest cluster (0-2000 µg/l FEU), where at day 4 these values were 23/57 (40%) (p=0.01). However, at day 4 only 2 (4%) of the 57 patients returned to normal D-dimer levels (< 500 µg/l FEU).

Of these 57 patients, there were 12 (21%) patients with cancer; 6 were already known with cancer, 2 had cancer discovered at presentation and in 4 patients cancer developed during follow up. In 4 of the 6 patients who were not previously known with cancer, the D-dimer levels remained > 4000 µg/l FEU at day 4. Comparing the D-dimer levels in patients with and without cancer, there was a significant difference at day 4. At day 4, 6 (50%) of the 12 patients with cancer had D-dimer levels > 4000 µg/l FEU, where in the 45 patients without cancer only 7 (16%) had D-dimer levels > 4000 µg/l FEU (p=0.02). The prevalence of cancer in patients with D-dimer levels < 4000 µg/l FEU at day 4 was 14% (6/44), as compared to 46% (6/13) in patients with D-dimer levels > 4000 µg/l FEU (p=0.02) (Table 2).

Of these 57 patients, 29 (51%) were over 60 years old. In these patients, 10 (34%) had cancer. A D-dimer concentration > 4000 µg/l FEU at day 4 in these patients was associated with a cancer prevalence of 36% compared to 33% at lower D-dimer concentrations (p=1.0). In patients younger than 60 years (n=28), 2 had cancer. These two patients both had D-dimer concentrations > 4000 µg/l FEU at day 4. The remaining 26 patients had no cancer and they all had a D-dimer concentration < 4000 µg/l FEU at day 4. These differences were statistical significant (p=0.003) (Table 2).

Table 1. Types of malignancy in outpatients with deep venous thrombosis. Of the 49 patients with cancer, 28 were previously known with cancer, 8 had cancer discovered at presentation with deep venous thrombosis and 13 developed cancer during follow-up.

	Previously known (n)	At presentation (n)	During follow-up (n)	Total (n)
Prostate	7	1	3	11
Non-Hodgkin lymphoma	8	-	1	9
Colorectal	3	1	4	8
Other haema-tologic	4	-	-	4
Lung	3	1	1	5
Stomach	1	-	1	2
Pancreas	-	2	-	2
Breast	1	1	-	2
Ovarium	-	-	2	2
Other	1	2	1	4
Total	28	8	13	49

Table 2. Prevalence of cancer in patients with deep venous thrombosis according to D-dimer concentrations at presentation and after 4 days of treatment and according to age. Values are reported as percentage (n).

	D-dimer concentration (µg/l FEU)					
	At presentation			At day 4		
	< 4000	> 4000	P value	< 4000	> 4000	P value
Total group	16 (20/128)	32 (29/90)	0.005	14 (6/44)	46 (6/13)	0.02
≥ 60 years	35 (18/52)	40 (20/50)	0.7	33 (6/18)	36 (4/11)	1.0
< 60 years	3 (2/76)	23 (9/40)	0.001	0 (0/26)	100 (2/2)	0.003

DISCUSSION

This study shows that in patients with deep venous thrombosis, high D-dimer concentrations at presentation and during the first days of treatment are indicators of an increased change for overt or occult forms of cancer. The prevalence of cancer in patients with thrombosis reported in literature varies from 7 to 26% (6-10) and corresponds well with the cancer prevalence of 22% in our study. Reports on the incidence of occult cancer in idiopathic deep venous thrombosis vary from 8% to 26% (9,25,26). So far, routine screening for cancer in every patient with deep venous thrombosis has not been recommended (12). There are some reports that identify patients with thrombosis with an increased risk of having cancer. A recent report identified the presence of bilateral deep venous thrombosis as an independent predictive factor of cancer discovery with a hazard ratio of 6.28 compared with unilateral one (27). The risk of developing cancer in the first year after diagnosis of thrombosis is 4 to 7 times higher in patients with idiopathic thrombosis compared to patients with secondary thrombosis (25,28).

We found that if the initial D-dimer concentration was > 4000 µg/l FEU, the prevalence of cancer was significantly higher than at D-dimer levels < 4000 µg/l FEU (32% versus 16%). Also, if the D-dimer levels after 4 days of treatment were still > 4000 µg/l FEU, the prevalence of cancer was significantly higher than if D-dimer levels were < 4000 µg/l FEU: 46% versus 14%. This implicates that the D-dimer concentration at presentation and after 4 days of treatment with oral anticoagulants can select patients that are at a higher risk of having cancer. Cushman et al found no association of baseline D-dimer with the occurrence of cancer-associated venous thrombosis (29). However, that study was conducted in unsymptomatic patients with a high prevalence of a normal D-dimer concentration. They used quintiles where the lowest quintile (D-dimer 2-69 µg/l) was compared with the highest quintile (278-7429 µg/l). In our study, performed in symptomatic patients with proven deep venous thrombosis, the D-dimer levels were all > 500 µg/l. Another difference is that the comparison of D-dimers in our study was performed at the time of the thrombosis, and not in the periods before.

We confirm the findings of Ranft et al, who found that 71% of patients with deep venous thrombosis and occult cancer were over 60 years old (8). In our study, 78% of the patients with thrombosis and cancer were older

than 60 years and of the patients that developed cancer in the follow up, 85% was older than 60 years. Similarly, in a recent study in patients with thrombosis, 21% of the patients over 60 years old developed cancer, compared to 5% in patients younger than 60 years old (10). This shows that the chance of having cancer in patients with deep venous thrombosis increases with aging and this might be a stimulus to screen for occult malignancy in patients older than 60 years. In our study, determination of a D-dimer has no value in defining a subgroup of elderly with an increased prevalence of cancer. However, in younger patients, we found a significant difference in the prevalence of cancer when comparing D-dimer levels above and under 4000 µg/l: 23% versus 3% for D-dimers at presentation and 100% versus 0% for D-dimers at day 4 of treatment. Therefore, especially in younger patients with deep venous thrombosis, D-dimers might be useful as indicators for underlying malignancy. A possible explanation for this age-dependency may be the fact that D-dimer concentrations will be frequently elevated in the elderly due to co-morbidity and general vessel damage (16;30-34).

In conclusion, we found that patients with deep venous thrombosis and a D-dimer concentration > 4000 µg/l at presentation and after 4 days of treatment are at higher risk for developing cancer, especially when they are younger than 60 years. Cancer-related deep venous thrombosis most frequently occurs in patients older than 60 years. Our results argue for a larger study towards cost-effectiveness of screening for a malignancy in patients older than 60 years and in patients younger than 60 years with initially high and persistently elevated D-dimer levels.

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CHAPTER 9

Simplification of the diagnosis of deep venous thrombosis by using the clinical score and D-dimer concentration

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SUMMARY

Serial ultrasonography of the leg is a safe but inefficient method in diagnosing patients suspected for deep venous thrombosis (DVT). With the introduction of the clinical score and the D-dimer, the diagnosis of DVT is ready for a change. It is safe to withhold anticoagulation in patients with a single normal ultrasonography in combination with a low clinical score or a normal D-dimer concentration. Recent studies show the safety of excluding DVT based on the combination of a normal D-dimer and a low clinical score alone. This article presents an overview of recent studies about the diagnostic management of patients with deep venous thrombosis.

INTRODUCTIE

Vereenvoudiging van diagnostiek van diepveneuze trombose door gebruik van klinische score en D-dimeer concentratie

In de diagnostiek van diepveneuze trombose (DVT) is de seriële compressie ultrasonografie (CUS) een veilige, maar inefficiënte methode gebleken. Met de introductie van de klinische score en de D-dimeer is de diagnostiek toe aan een verandering. Het blijkt veilig om antistolling te onthouden aan patiënten met een enkele normale CUS in combinatie met een lage klinische score of een normale D-dimeer concentratie. Recente studies tonen aan dat het veilig is om DVT uit te sluiten op basis van de combinatie van een lage klinische score en een normale D-dimeer concentratie alleen. Dit artikel geeft een overzicht van recente studies betreffende de diagnostiek van patiënten met DVT. Diepveneuze trombose (DVT) is een relatief vaak voorkomende aandoening met een incidentie van 1.8/1000 in de normale populatie (1). De flebografie is lang als gouden standaard voor de diagnostiek van DVT beschouwd. Flebografie is echter invasief, duur en met een kleine kans op contrastallergie en iatrogene trombose. Onderzoek heeft aangetoond dat flebografie vervangen kan worden door seriële echografie van de beenvaten (2). Recent is dit bevestigd in twee studies, waaruit blijkt dat de kans op het ontwikkelen van een DVT na twee normale echografieën slechts 0.6-0.7% is (3;4). Echter, DVT komt in deze studies slechts voor bij 17-24% van de patiënten en de tweede echografie levert slechts bij 0.4-0.7% van de patiënten een DVT op (3;4). De seriële echografie is dus een betrouwbare, maar inefficiënte methode in de diagnose van DVT. In Nederland zullen jaarlijks ongeveer 144.000 patiënten worden verwezen in verband met de verdenking op DVT. Dit verklaart de aandacht voor snelle en efficiënte diagnostiek.
Nieuwe niet-invasieve strategieën zijn ontwikkeld met als doel de noodzaak tot echografie te reduceren. Hierbij wordt gebruik gemaakt van de klinische score en het meten van de D-dimeer concentratie in plasma.

KLINISCHE SCORE

Wells et al ontwikkelden een score waarmee meer inzicht werd verkregen in de a priori kans op het bestaan van DVT (Tabel 1). Zij hebben aangetoond dat 55-57% van de voor DVT verdachte patiënten een lage score heeft; hiervan heeft slechts 3-5% een DVT (5;6). Een gemiddelde score wordt gevonden bij 27-33%, met een kans van 17-33% op een DVT. Bij een hoge score (12-16%) is de kans op DVT 75-85%. Het voordeel van deze klinische score is dat deze snel kan worden aangeleerd en eenvoudig kan worden toegepast. Het nadeel is de subjectiviteit en variabiliteit: overeenstemming tussen verschillende clinici wordt slechts bereikt in 57% (7).

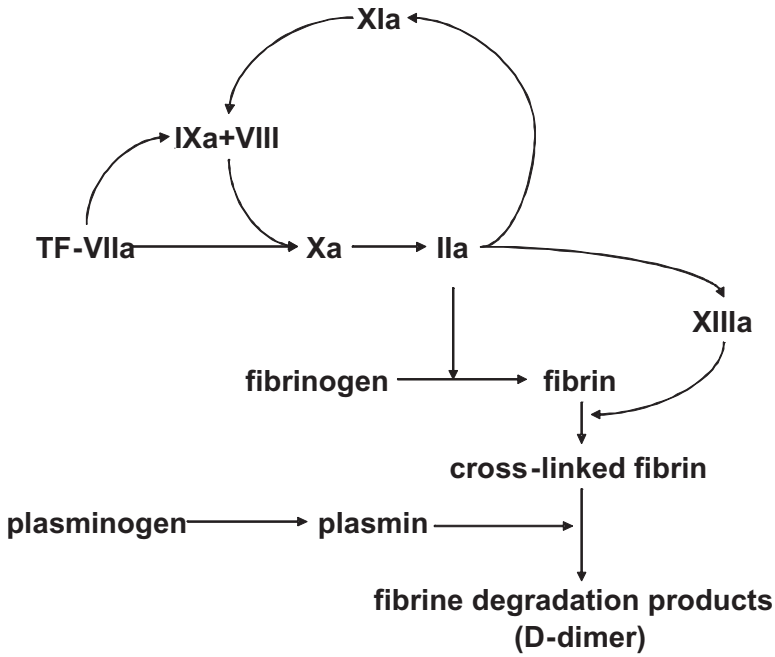
Tabel 1. Klinische score bij verdenking op diepveneuze trombose volgens Wells et al (5).

gegevens uit anamnese en lichamelijk onderzoek	**score**
maligniteit	1
paralyse, parese of recent gips aan been	1
recente bedrust (>3 dagen) of grote operatie (<4 weken)	1
lokale drukpijn over diep veneus vaatbed	1
hele been gezwollen	1
kuit opgezet >3 cm tov andere kuit	1
pitting oedeem beperkt tot symptomatisch been	1
uitgezette oppervlakkige venen (niet variceus)	1
alternatieve diagnose waarschijnlijker dan DVT	-2
hoog risico	≥ 3
matig risico	1-2
laag risico	≤ 0

D-DIMEREN

Als afbraakproduct van cross-linked fibrine neemt de D-dimeer concentratie toe bij het optreden van fibrinolyse (Figuur 1). De toename van de concentratie D-dimeren is dus een indirecte maat voor stolselvorming. De sensitiviteit en negatief voorspellende waarde (NPV) van D-dimeren voor trombose zijn hoog, maar afhankelijk van de test die gebruikt wordt. De klassieke ELISA testen hebben een hoge sensitiviteit (97%) en NPV (93%) (8-17). Zij zijn echter bewerkelijk en daardoor niet geschikt voor gebruik op de spoedeisende hulp. De oudere latex-aggluttinatie testen zijn sneller, maar hebben een aanzienlijk lagere sensitiviteit (60-80%) (8;10). Snelle ELISA testen (zoals VIDAS®) zijn nu beschikbaar met een hoge sensitiviteit (98-99%) en NPV (98%) (10-13;17-20). De nieuwere latex testen (zoals Tina-quant® en STA-LIA®) zijn snel hebben nu ook een hogere sensitiviteit (98-99%) en NPV (98%) (12;13;17;21;22). De kwalitatieve agglutinatie test (SimpliRed®) heeft een sensitiviteit van 83% en een NPV van 93% (8;11;12;23-29).
Een belangrijk aspect is het feit dat D-dimeren vaak verhoogd zijn bij oudere patiënten en bij aandoeningen zoals infecties en maligniteiten (30-35). Dit resulteert in een lage specificiteit voor trombose. De D-dimeer is dan ook vooral van waarde als een exclusie test. Een normale D-dimeer sluit trombose uit, een verhoogde D-dimeer concentratie vereist aanvullend beeldvormend onderzoek. De specificiteit bepaalt de klinische toepasbaarheid: een D-dimeer test met een lage specificiteit noodzaakt aanvullend onderzoek (echografie) en is dus minder kosteneffectief.

Figuur 1. Schematische weergave van de stollingscascade.



Door weefselbeschadiging komt Tissue Factor (TF) vrij, dat bindt met geactiveerd factor VII (VIIa) en het TF-VIIa complex vormt. Dit complex activeert factor X, welke vervolgens protrombine omzet in trombine (IIa). Trombine zal zijn eigen productie bevorderen via activatie van factor XI en IX. De activatie van factor X door het TF-VIIa complex gebeurt niet alleen door directe activatie maar ook door activatie van factor IX. Het gevormde trombine zet fibrinogeen om in fibrine. Dit fibrine wordt met behulp van factor XIII omgezet in het stabielere cross-linked fibrine. De afbraak hiervan onder invloed van plasmine (de fibrinolyse) resulteert in het ontstaan van fibrine afbraak producten, waarvan de D-dimeren een onderdeel zijn (sterk vereenvoudigd model).

NIEUWE DIAGNOSTISCHE COMBINATIES

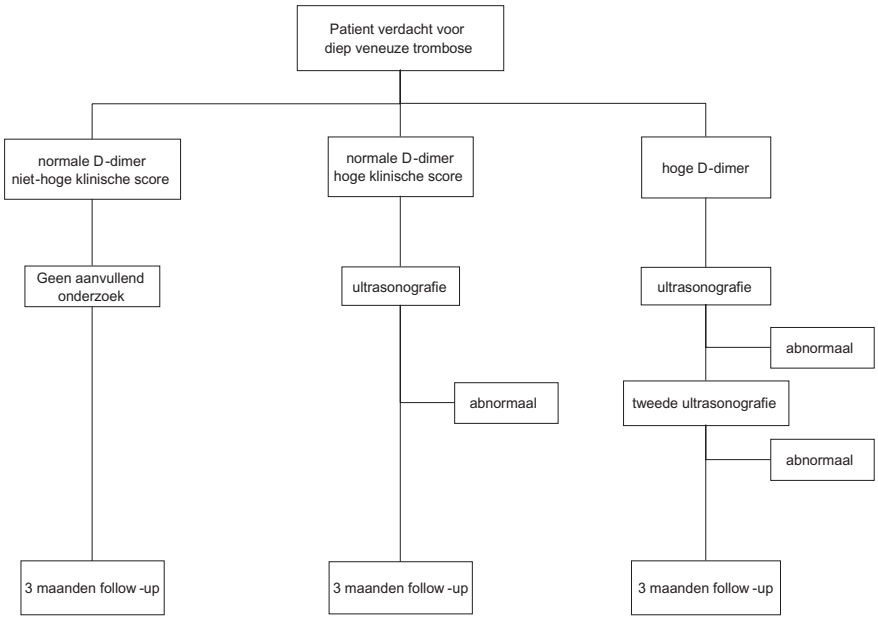
A. Echografie en de klinische score:
In een studie van Wells et al werd bij 593 patiënten echografie gecombineerd met de klinische score (6). Bij een lage klinische score (n=329) werd één echografie verricht: DVT werd gevonden bij 9 patiënten. Gedurende de follow-up van 3 maanden werd bij slechts 1 (0.3%) van de 320 een DVT geconstateerd. Patiënten met een gemiddelde score (n=193) ondergingen twee echografieën; de eerste toonde 27 maal een DVT aan, de tweede 3. In de follow-up ontwikkelden 2 (1.2%) van de 163 patiënten een DVT. Patiënten met een hoge score (n=71) ondergingen één echografie; 49 hadden een DVT. Een normale echografie werd gevolgd door een flebografie die bij 4 patiënten een DVT aantoonde. In de follow-up kreeg niemand van de overigen in deze groep een DVT. Deze studie toont aan dat het veilig is om antistolling te onthouden aan patiënten met een lage klinische score en één normale echografie.
Tick et al (36) publiceerden in een serie van 811 patiënten onder andere de resultaten van de follow-up van 280 (35%) patiënten met een lage klinische score. Eénmalig echografisch onderzoek liet een DVT zien bij 30 van de 280 patiënten. Bij 5 (2.0%) van de 250 personen met een normale echografie werd gedurende de follow-up van 3 maanden een DVT geconstateerd. Dit percentage ligt hoger dan bij de studie van Wells et al (6). Wellicht wordt dit verklaard door de hogere prevalentie van DVT in deze studie: 41% versus 16%. Een mogelijke verklaring hiervoor is dat er een selectie van patiënten heeft kunnen plaatsvinden in de studie van Tick et al door tussenkomst van de huisarts.

B. Echografie en D-dimeren:
In een studie van Bernardi et al (37) ondergingen 946 patiënten een echografie; 260 patiënten hadden een DVT. Van de overige 686 patiënten hadden 598 een normale D-dimeer concentratie; bij hen werd afgezien van een tweede echografie. De incidentie van DVT na een normale D-dimeer en normale echografie was 0.2%.
In een vergelijkbare studie van Kraaijenhagen et al (38) werden 1739 patiënten onderzocht. Middels een echografie werd bij 391 patiënten een DVT aangetoond. Van de overige 1348 patiënten hadden 828 een normale D-dimeer concentratie. Hiervan ontwikkelde slechts 0.6 % een trombose in de follow-up.

Deze twee studies laten zien dat het veilig is om DVT uit te sluiten bij een normale D-dimeer concentratie en een enkele normale echografie.

C. Combinatie van de klinische score en D-dimeren:
De vraag of een DVT kan worden uitgesloten op basis van een niet-hoge klinische score en een normale D-dimeer concentratie werd onderzocht in een recente Nederlandse studie (21). Bij 812 patiënten met de verdenking op een DVT werden een klinische score volgens Wells en een D-dimeer concentratie bepaald. De klinische score werd onderverdeeld in niet-hoog (< 3) en hoog (≥ 3). Het diagnostische traject is weergegeven in Figuur 2. De totale prevalentie van trombose in deze studie was 39% (309 DVT bij presentatie en 8 veneuze tromboembolie tijdens follow-up). De resultaten zijn weergegeven in Tabel 2. Van de 176 patiënten met een normale D-dimeer en een niet-hoge score kreeg 1 (0.6%) patiënt een kuitvenetrombose na 1 week (NPV van 99.4%). Bij 3 van de 39 patiënten met een normale D-dimeer en een hoge score was sprake van een DVT bij presentatie en één patiënt ontwikkelde een longembolie na 71 dagen (NPV 97.2% in deze groep). De overige 597 patiënten ondergingen herhaald echografisch onderzoek: de eerste echografie was afwijkend bij 291 patiënten en de tweede bij 15 patiënten. Bij 6 (2.1%) van de 291 met twee normale echografieën werd trombose tijdens de follow-up gevonden (NPV 97.9%). In deze studie werd het aantal echografieën gereduceerd met 29%. Deze studie laat zien dat het veilig is om DVT uit te sluiten op basis van een normale D-dimeer concentratie en een niet-hoge klinische score. Een soortgelijke conclusie werd getrokken in 3 vergelijkbare studies (Tabel 3). In de studie van Janes et al (24) werd een gemodificeerde Well's score gebruikt, en in de studies van Kearon et al (25) en Anderson et al (39) de originele Well's score. Voor de exclusie van DVT werd door Schutgens et al een lage of gemiddelde score gebruikt, terwijl in de overige studies alleen een lage score werd gebruikt. Het verschil tussen de eerstgenoemde studie en de laatste 3 studies is dat in de eerste studie een D-dimeer test is gebruikt met een beduidend hogere sensitiviteit en NPV (Tina-quant® versus SimpliRed®). Het gebruik van een hoog sensitieve D-dimeer test draagt er toe bij dat ook bij patiënten met een gemiddelde klinische score en een normale D-dimeer een DVT kan worden uitgesloten. Bij gebruik van een minder sensitieve test wordt meer nadruk gelegd op de klinische score: alleen in geval van een lage score en een normale D-dimeer kan een DVT worden uitgesloten.

Figuur 2. Diagnostisch traject voor patiënten verdacht voor DVT, zoals gebruikt in de studie van Schutgens et al (21).



Tabel 2. Prevalentie van veneuze tromboembolie volgens D-dimeer concentratie en klinische score (21).

Groep	N	VTE in follow-up	Totaal VTE
Normale D-dimeer en niet-hoge score	176 (22%)	1 (0.6%;0.02-3.1)	1 (0.6%;0.02-3.1)
Normale D-dimeer en hoge score	39 (5%)	1 (2.8%;0.07-14.5)	4 (10.3%;2.9-24.2)
Normale D-dimeer	215 (26%)	2 (0.9%;0.1-3.4%)	5 (2.3%;0.8-5.3%)
Abnormale D-dimeer	597 (74%)	6 (2.1%;0.8-4.4)	312 (52.3%;48.3-56.3)
Totaal	812 (100%)	8 (1.6%;0.7-3.1)	317 (39.0%;35.7-42.4)

Waarden zijn gerapporteerd in aantallen (percentage;95% betrouwbaarheids intervallen). VTE betekent veneuze tromboembolie (diepveneuze trombose en longembolie).

Tabel 3. Overzicht van management studies naar de diagnostiek van diepveneuze trombose met behulp van de klinische score en D-dimeren.

Studie	N	Prevalentie VTE	Exclusie van DVT op basis van D-dimeer en klinische score	NPV
Schutgens et al (21)	812	39%	176 (22%)	99.4%
Janes et al (24)	404	24%	98 (24%)	99.0%
Kearon et al (25)	445	14%	177 (40%)	99.4%
Anderson et al (39)	1075	18%	316 (29%)	98.4%

VTE = veneuze tromboembolie, DVT = diepveneuze trombose, NPV = negatief voorspellende waarde van de combinatie van de klinische score en D-dimeren.

D. D-dimeren:

Perrier et al suggereerden veneuze trombo-embolieën uit te sluiten op basis van alleen een normale D-dimeer concentratie (20). Bij 23% van de 918 patiënten bij wie de diagnose DVT of longembolie (LE) werd vermoed werd een trombose gevonden. In dit onderzoek hadden 288 (31%) patiënten een normale D-dimeer en werd in principe geen aanvullend onderzoek verricht. Als veiligheidsmaatregel werd bij deze patiënten echter alsnog een echografie verricht. Bij 2 (0.7%) van de 288 patiënten werd hierdoor een DVT ontdekt. In de follow-up was er niemand die een DVT ontwikkelde. In deze studie was bij 474 patiënten alleen verdenking op een DVT; 127 van deze 474 patiënten hadden een normale D-dimeer. Geen van deze patiënten ontwikkelde een DVT in de follow-up. Deze studie suggereert dat het veilig is om trombose uit te sluiten op basis van een normale D-dimeer concentratie alleen, hoewel dit niet in andere, prospectieve studies is bevestigd.

Conclusies en aanbevelingen

In de huidige consensusstrategie voor DVT wordt uitgegaan van herhaalde echografieën. Deze strategie zal echter moeten worden aangepast op basis van de resultaten van bovengenoemde studies.

Het is veilig om de tweede echografie achterwege te laten in geval van een normale eerste echografie en een lage klinische score of normale D-dimeer bij presentatie. Recente studies tonen aan dat het ook veilig is om DVT uit sluiten op basis van een lage klinische score in combinatie met een normale D-dimeer zonder aanvullende beeldvormende diagnostiek en, waarschijnlijk, ook op basis van een gemiddelde klinische score met een normale, hoog sensitieve, D-dimeer test. Lokale factoren, zoals type D-dimeer test, ervaring met de klinische score en prevalentie van DVT zullen de keuze voor een diagnostische strategie bepalen.

Routinematig gebruik van de D-dimeer test bij alle patiënten wordt niet aangeraden. De sensitiviteit van de D-dimeer test daalt aanzienlijk (van 99% naar 75%) bij patiënten die orale antistolling gebruiken (34). Bij deze patiënten dient het gebruik van de D-dimeer te worden vermeden. In patiënten met kanker lijkt de NPV gelijk aan patiënten zonder kanker (34;40), hoewel een andere studie het tegendeel bewijst (41). De specificiteit in deze patiënten lijkt verlaagd (34;41), hoewel dit niet in elke studie wordt gevonden (40). Nader onderzoek naar de waarde van D-dimeren in deze patiënten is vereist. Daarnaast zijn alle bovenstaande management studies gedaan bij ambulante patiënten. Bij patiënten die zijn opgenomen in een ziekenhuis zal de D-dimeer concentratie vaak verhoogd zijn door comorbiditeit. Dat leidt tot een sterke afname van de specificiteit van de D-dimeer test. Routinematig gebruik van deze test is niet aan te raden bij deze patiëntencategorie. Een lage specificiteit is ook gevonden bij patiënten op hoge leeftijd (30-35). Ook bij deze populaties levert een D-dimeer bepaling weinig winst in het diagnostisch traject op. Overigens is de sensitiviteit in deze groep patiënten niet verminderd (34).

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SUMMARY

Measurement of D-dimer concentrations is supposed to be a promising tool in the diagnostic workup of venous thromboembolism (VTE). The subject of this thesis is to refine the role of the D-dimer in the exclusion of venous thrombosis.

The sample material for measurements of D-dimer concentration is citrate plasma. This is inconvenient for the clinical chemistry workflow, as an extra sample for D-dimer measurements is needed and citrate samples are centrifuged separately from heparin plasma. Furthermore, little is known about the effect of (pre)analytic processes on the outcome of D-dimer measurements. In **chapter 2**, we compared D-dimer measurements in citrate and heparin plasma from 190 patients. We found a high correlation between the two samples (r=1.0, slope=1.196, Kendall tau=0.95). The D-dimer concentrations in heparin were 19% higher than in citrate plasma, which was totally explained by the extra dilutional effect of citrate plasma. We found no effect of transport in 25 samples by dispatching them by pneumatic mail and by placing them on an oscillating roller. Sample stability was measured in samples that were left standing at room temperature for 16 and 24 hours, in samples that were snap-frozen once and in samples that were frozen at –70°C. No differences in D-dimer concentrations were found between these samples. Finally, we performed D-dimer measurements on 4 different analyzers: a high correlation between the samples was found.
This study shows that it is valid to measure D-dimer concentrations using heparin plasma on routine chemical analyzers. A correction factor of 0.84 has to be applied to avoid the need for a change in reference and cut-off values, as all reports in literature are based on the standard citrate solution. This finding implicates a significant improvement in reporting the D-dimer results by a reduction of the turn-around time. Furthermore, we found D-dimer concentrations to remain stable under various (pre)analytic conditions, making comparison of different studies more feasible.

It is of clinical importance to be informed about the time of increase and decrease in D-dimer concentration in venous thromboembolism. This allows to establish the optimal time of D-dimer testing and to define the time-period before and after which a D-dimer test may have reduced accuracy. In

addition, it is of interest to be informed about the correlation between the size of the thrombus and the extent of D-dimer concentration, as large thrombi may lead to a more aggressive therapeutic approach and to a more careful monitoring. These questions have been addressed in **chapter 3**, where we studied the course of D-dimer concentrations in patients undergoing embolisation of pulmonary arteries because of pulmonary arteriovenous malformations (PAVM) and calculated the correlation between D-dimer concentrations and the estimated size of the thrombus. In 13 patients with hereditary hemorrhagic teleangiectasia (HHT) or Rendu-Osler-Weber disease who underwent embolisation because of PAVM causing hypoxemia and increased right-to-left shunts, we measured D-dimer concentrations at 2, 4 and 8 hours after the embolisation. The control group consisted of 14 patients who underwent diagnostic heart catheterisation without any intervention. At t=2 hours, D-dimer concentrations in the patient group were significantly higher as compared to baseline (p=0.001), and remained significantly higher at t=4 and t=8 hours. The control group showed no significant increase in D-dimer concentration as compared to baseline. The increase in D-dimer concentration in the patient group differed significantly from the control group (p=0.047).
We found a correlation between D-dimer concentration and the calculated volume of the parts of the pulmonary arteries that were embolised, but not between D-dimer concentration and the decrease of the right to left shunt or the increase in pO2.
This study shows that D-dimer concentrations increase almost immediately after formation of a clot and that they remain elevated for at least 8 hours after onset. Furthermore, the height of the D-dimer concentration might help to predict the extent of the thrombus.

Although high sensitivity and negative predictive value of D-dimers in venous thrombosis have been reported in outpatients, its clinical usefulness will also be determined by the specificity of the test. In **chapter 4**, we tried to define clinical and laboratory variables that might restrict the use of the D-dimer in terms of reduced sensitivity, negative predictive value or specificity. In 704 outpatients suspected of having DVT, the performance of a latex agglutination D-dimer test (Minutex®) was calculated in patients using anticoagulants (n=61), patients with previous thrombosis (n=127), and patients with malignancy (n=47), including 39 patients with more than one of these characteristics. The 508 remaining patients were considered to

be the reference group. A total of 254 patients (36%) had evidence of deep venous thrombosis. The D-dimer test had a sensitivity of 99% and a negative predictive value of 98% in the reference group. The sensitivity of the D-dimer test in patients using oral anticoagulants was 75% (p=0.01 compared with the reference group) and the negative predictive value was 93%. Anticoagulated patients were more likely to have a negative D-dimer test as compared to the reference group (46% vs. 20%, p<0.001). Test sensitivity and negative predictive value were respectively 96% and 94% in patients with previous thrombosis, and 100% in patients with cancer. In cancer patients, 91% had an abnormal D-dimer test. The prevalence of a positive D-dimer test increased with age. In patients aged ≥ 70 years, 79% had a positive D-dimer test, compared with 61% of younger patients (p=0.0001).
This study shows that the overall sensitivity and negative predictive value of this rapid latex D-dimer assay for the exclusion of deep venous thrombosis are high. The D-dimer test has a good performance in patients with previous venous thromboembolism, but we do not recommend its use in patients using oral anticoagulants because of the reduced sensitivity of the test. The D-dimer tests in patients with cancer and in patients over 70 years old are usually positive and may not be worthwhile in terms of low specificity.

Serial ultrasonography is reliable for the diagnosis of deep venous thrombosis in symptomatic patients, but the low prevalence of thrombosis in this group renders this approach costly and inconvenient to patients.
In **chapter 5**, we studied the clinical validity of the combination of a pretest clinical probability score and a D-dimer test in the initial evaluation of patients suspected of deep venous thrombosis. In 812 consecutive outpatients, a clinical score and a D-dimer test (Tina-quant®) were performed. A total of 176 patients had a normal D-dimer concentration (< 500 FEU µg/L) and a non-high probability score (< 3) and had no further testing. Only 1 (0.6%) of these patients developed thrombosis in the 3 months follow-up period. A normal D-dimer concentration and a high probability score were found in 39 patients; in these patients, a single ultrasonography was performed. Three of these patients (7.7%) had thrombosis at presentation, and one (2.8%) developed pulmonary embolism during follow-up. Serial ultrasonography was performed in patients with an abnormal D-dimer concentration. In 306 of 597 patients (51.3%) with an abnormal D-dimer con-

centration, thrombosis was detected by serial ultrasonography. Six patients (2.1%) developed thrombosis during follow-up. No deaths due to thromboembolism occurred during follow-up. Deep venous thrombosis or pulmonary embolism was diagnosed in 317 (39%) patients during the entire study period.
This study shows the safety of withholding anticoagulation in patients suspected of having deep venous thrombosis with the combination of a normal D-dimer concentration and a non-high clinical score. This strategy reduces the need for ultrasonography by approximately 30%.

For measurement of D-dimer concentrations, several assays are available. Where earlier D-dimer assays were time consuming or showed less sensitivity for thrombosis, new D-dimer assays have now been introduced. They claim to be fast and highly sensitive, but need clinical validation.
In **chapter 6**, we evaluated the clinical usefulness of four relatively new D-dimer assays and a classical ELISA in 537 outpatients suspected for deep venous thrombosis. Patients were participants in a large prospective management study using a clinical probability score and a D-dimer measurement (Tina-quant®). Additional samples were taken for D-dimer measurement using the Asserachrom ELISA®, the VIDAS New®, the STA-LIA® and the Miniquant® assay. Performances of each test were calculated using clinical data during a 3-months follow-up. Thrombosis was detected in 224 patients (42%). The area under the ROC curve was significantly higher for the Tina-quant® as compared to the other assays. Using standard cut-off values, sensitivity, negative predictive value and specificity of the Asserachrom® were 97, 94 and 33% respectively. For the VIDAS New®, values were 100, 96 and 8% respectively. The Tina-quant® showed values of 99, 98 and 41% respectively and the STA-LIA® 98, 95 and 32%. Values for the Miniquant® were 95, 94 and 52%. Lowering the cut-off values resulted in an improvement of sensitivity and negative predictive value, but in a decrease of specificity, rendering the tests less useful in clinical practice.
This study shows that the D-dimer assays, used in this study, all show a high sensitivity and negative predictive value, but none of the assays reached an NPV of > 98% at standard cut-off values. For exclusion of DVT, we therefore recommend combining the D-dimer assay with other non-invasive tests in order to reach failure rates less than 1.0%. The use of D-dimer assays with a very low specificity might not be worthwhile from an

economical point of view, as a positive test will necessitate additional testing in the majority of the patients.

Intravenous administration of unfractionated heparin (UFH) has proven its efficacy in the early treatment of venous thromboembolism. The use of UFH, however, has several inconveniences, such as the need for hospitalisation and frequent monitoring of activated partial thromboplastin times. Treatment of deep venous thrombosis and pulmonary embolism with low-molecular-weight heparin (LMWH) is considered to be equally safe as UFH when looking at clinical outcome, but little is known about differences between UFH and LMWH on coagulation activity during treatment for pulmonary embolism. In **chapter 7**, we compare UFH and LMWH in the early treatment of pulmonary embolism in terms of control of coagulation markers and perfusion abnormalities in a randomised, non-blinded study. Therefore, 37 patients with acute pulmonary embolism were randomised to receive intravenous UFH or subcutaneous dalteparin; both groups also receive acenocoumarol. Blood samples were obtained before treatment and daily for 4 consecutive days. Thrombin generation was assessed by measuring fragments 1+2 (F1+2), thrombin-antithrombin (TAT)-complexes and fibrin monomers. F1+2 and TAT-complexes rapidly normalised without differences between the groups. Fibrin monomer levels did not decrease, and showed an increase in the UFH group from day 3. Fibrinolysis was analysed by measuring D-dimer concentrations and clot lysis times. D-dimer levels decreased over time, without differences between the groups. Clot lysis times were shorter in the UFH group. In vitro inactivation of thrombin activatable fibrinolysis inhibitor (TAFI) by carboxypeptidase-inhibitor eliminated any further effect of heparin on clot lysis times, confirming that the effect of heparin on the clot lysis time is mainly through TAFI. Ventilation-perfusion scintigraphies were performed from which the percentage-of-vascular-obstruction scores (PVOs) were calculated at day 0 (PVOsD0) and day 5 (PVOsD5). The PVOsD0 and PVOsD5 were not different, but the decrease of the PVOs over time was higher in the LMWH group.
This study shows that LMWH (dalteparin) is at least equally effective as UFH in reducing coagulation activity and in prohibiting further increases in perfusion abnormalities in the early treatment of pulmonary embolism. Our findings support the use of LMWH as initial treatment of pulmonary embolism.

Venous thromboembolism can be related to malignancy, but routine screening for cancer in every patient with deep venous thrombosis is a matter of debate. In **chapter 8**, we investigated if the initial height of the D-dimer concentration or the D-dimer concentrations during treatment for DVT can be used as a predictor for (occult) malignancy. We studied 218 consecutive outpatients with proven DVT, in whom a D-dimer test was performed. Patients were followed for a median of 31 months and the occurrence of malignancy was documented. In total, 49 (22%) patients had or developed cancer; 28 were already known with cancer at the moment of thrombosis, 8 had cancer discovered at presentation with thrombosis and 13 developed malignancy during follow up. The prevalence of cancer in patients with an initial D-dimer < 4000 µg/l FEU was 16% (20/128) as compared to 32% (29/90) (p=0.02) in patients with D-dimer levels > 4000 µg/l FEU. The prevalence of cancer in patients with D-dimer levels < 4000 µg/l FEU at day 4 was 14% (6/44), as compared to 46% (6/13) in patients with D-dimer levels > 4000 µg/l FEU (p=0.02). Furthermore, the prevalence of cancer in patients older than 60 years was higher compared to younger patients (37% vs. 10%; p=0.0003). Of the patients with thrombosis and cancer, 78% (38/49) was older than 60 years. Of the 13 patients that developed cancer in the follow up, 85% (11/13) was older than 60 years. In patients older than 60 years, there was no difference in cancer prevalence between patients with D-dimer levels above or under 4000 µg/l FEU (p=0.7). In patients younger than 60 years, the prevalence of cancer was 3% when initial D-dimer levels were < 4000 µg/l FEU, and 23% when D-dimer levels were > 4000 µg/l FEU (p=0.001). After 4 days of treatment, cancer prevalences were 0% and 100% in younger patients with D-dimer levels < and > 4000 µg/l respectively (p=0.003).
This study shows that high D-dimer concentrations at presentation and during the first days of treatment are indicators of an increased change for overt or occult forms of cancer, especially in patients younger than 60 years. Cancer-related deep venous thrombosis is more frequent in patients older than 60 years. The D-dimer concentration in patients older than 60 years does not contribute to discriminate between patients with and without cancer.

An overview of recent published articles on the diagnostic management of deep venous thrombosis is given in **Chapter 9**.

FUTURE PERSPECTIVES

The diagnosis of deep venous thrombosis (DVT) is subject to important changes with the introduction of D-dimers and the clinical score. It has now been established that it is safe to withhold anticoagulant treatment in patients suspected for having DVT with a low/moderate clinical score and a normal D-dimer concentration. This thesis has given more insight in the use of D-dimers in the diagnosis and management of venous thrombosis. However, certain questions yet remain unanswered.

Do we have to use D-dimers on a routine base in the diagnostic management of every patient suspected for having DVT?
In this thesis, we showed the decrease in sensitivity of the D-dimer test in patients with oral anticoagulation (chapter 4). Decreases in sensitivity and specificity of the D-dimer test have also been reported in hospitalized patients (1;2). Larger prospective studies are needed in hospitalized patients and in patients on anticoagulants before firm conclusions can be made. Reports about the sensitivity and negative predictive value (NPV) of the D-dimer tests in patients with cancer vary. Some authors suggest that the NPV of the D-dimer test in patients with cancer is comparable with that of patients without cancer (3;4), whereas others report the contrary (5). The specificity of the D-dimer test in the patients with a malignancy is found to be lower in two studies (3;5), which was, however, not confirmed in another study (4). More studies about the reliability of the D-dimer test to exclude DVT in cancer patients are warranted.

What is the optimal diagnostic strategy in DVT?
Several diagnostic strategies have been suggested in literature, varying from the use of D-dimers alone to several combinations of venography, ultrasonography, pretest clinical score and D-dimer tests. In this thesis, we presented the safety of diagnosing DVT using combinations of the clinical score, the D-dimer and ultrasonography (chapter 5). In our model, however, the second ultrasound showed DVT in only 15 (4.9%) out of 306 patients. We suggest a new diagnostic strategy for patients suspected for DVT, based on our studies and some recently published studies from other groups (Figure 1). The challenge for further research will be the reduction of the need for the second ultrasound in this strategy.

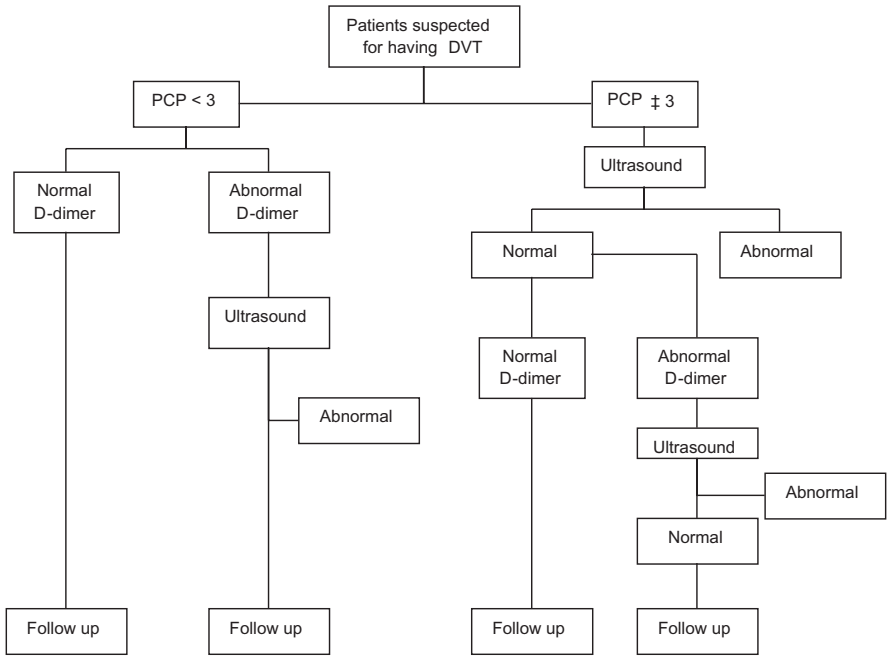
How to improve the clinical use of the D-dimer in the elderly?
Several reports (this thesis included) have documented the increase in D-dimer concentrations in the elderly (6-11). Age-adjusted cut-off values may improve the specificity of the D-dimer test. Another possibility is to restrict the use of the D-dimer test in the elderly to patients without co-morbidity in an attempt to improve the specificity of the D-dimer test.

Are D-dimers the only laboratory markers for excluding thrombosis?
Although most studies have focused on the D-dimer tests in the exclusion of thrombosis, it is of interest to know if there are other coagulation or fibrinolytic markers that can be used for this purpose. Previous reports on fibrin monomers in the exclusion of DVT were discouraging (12) to promising (13;14). A recent report discussed the potential role of fibrin monomer-fibrinogen complexes in the diagnosis of thrombosis (15). Whether fibrin monomers could be used in combination with D-dimers to further reduce the need for ultrasound remains to be investigated. Other markers for thrombin generation, like thrombin-antithrombin complexes and fragments 1+2, were inferior to the D-dimer concentration (16;17). Larger prospective studies are needed to determine the possible role of these coagulation markers as tools in the exclusion of thrombosis.

Can D-dimers be used to predict recurrent thrombosis or occult diseases?
Recent reports have showed that persistently elevated D-dimer concentrations can predict recurrence of thrombosis (18;19). We suggest that high D-dimer levels are associated with an increased the risk for cancer. Repeated measurement of D-dimers concentrations during the treatment of patients with DVT might be used to select a subgroup of patients in whom an extended search for occult cancer can be justified.

The D-dimer test forms an intriguing contribution to the diagnostic management of patients suspected for having DVT. We support the incorporation of the D-dimer test in the diagnostic standards of care for these patients. The place of the D-dimer test in several subgroups of patients needs further attention.

Figure 1. Diagnostic flowchart for patients suspected for having deep venous thrombosis (DVT).



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SAMENVATTING IN HET NEDERLANDS

Diepveneuze trombose (DVT) is een aandoening waarbij er een stolsel (trombose) ontstaat in één van de bloedvaten van het been of in het kleine bekken (of in zeldzame gevallen op andere plaatsen). Wanneer er een deel van dit stolsel loslaat, kan dit via de bloedstroom meegevoerd worden, waarna het stolsel blijft steken in de kleine bloedvaten van de long. Dit verschijnsel heet een longembolie.
Het stellen van de diagnose van DVT wordt veelal gedaan door middel van een echografisch onderzoek. Echter, dit onderzoek kan kleine stolsels in de kuit missen. Daarom wordt een tweede echografie verricht na een week, om een eventueel gemiste trombose alsnog te detecteren. Hoewel dit een veilige methode is gebleken, is het erg inefficiënt: slechts een kwart van de patiënten heeft daadwerkelijk een trombose en het rendement van de tweede echografie is erg laag.
Het meten van D-dimeer concentraties is een veelbelovend hulpmiddel in de diagnostiek van DVT. D-dimeren zijn afbraakproducten van fibrine (een stolsel bestaat voornamelijk uit fibrine), en aldus een indirecte maat voor het ontstaan van trombose. Bij iedereen is er een bepaalde hoeveelheid D-dimeer aanwezig, maar in geval van een stolsel zal de concentratie stijgen. Het onderwerp van dit proefschrift is de rol van D-dimeren bij de diagnostiek van DVT te verfijnen.

Het materiaal waarin D-dimeren in het laboratorium worden gemeten is citraat plasma, in tegenstelling tot heparine plasma waarin de routine bepalingen worden gemeten. Dit is onhandig voor de klinisch chemische analyse, omdat hiervoor een extra buis dient te worden afgenomen en omdat citraat plasma een andere bewerking dan heparine plasma ondergaat hetgeen vooral veroorzaakt wordt door een andere (langere) wijze van centrifu-geren. Verder is weinig bekend over de effecten van de wijze waarop het materiaal behandeld wordt op de D-dimeer concentratie. In **hoofdstuk 2** vergeleken wij D-dimeer bepalingen in citraat en heparine plasma van 190 patiënten. We vonden een hoge correlatie tussen de twee bepalingen. De D-dimeer concentraties waren 19% hoger in heparine plasma dan in citraat plasma, wat volledig verklaard kan worden door de extra verdunning die plaatsvindt in citraat plasma. Er was geen effect van transport in 25 buisjes die door middel van buizenpost werden verstuurd of op een bewegende roller werden geplaatst. De stabiliteit van de bepaling werd

gemeten in buisjes die op kamertemperatuur werden bewaard gedurende 16 en 24 uur, in buisjes die éénmalig kortdurend werden ingevroren en in buisjes die 2 jaar lang bewaard werden bij een temperatuur van -70ºC. Er werd tussen deze bepalingen geen verschil gevonden in D-dimeer concentratie. Tenslotte werden vier verschillende technieken gebruikt voor de bepaling; er werd geen verschil gevonden in de gebruikte apparatuur.
Deze studie toont dat D-dimeren bepaald kunnen worden in heparine plasma op routine chemische machines. Een correctiefactor van 0.84 dient te worden gebruikt om een verandering in referentiewaarden en grenswaardes te vermijden, aangezien alle literatuur gebaseerd is op standaard citraat plasma. Dit gegeven betekent een duidelijke verkorting van de reportagetijd van de D-dimeer uitslagen hetgeen leidt tot een kortere wachttijd op de eerste hulp. Tevens toonden wij aan dat de D-dimeer concentratie stabiel blijft onder verschillende omstandigheden. Aangezien ingevroren materiaal representatieve gegevens blijft geven, is het verantwoord om met dit materiaal verder onderzoek te verrichten.

Het is van belang om geïnformeerd te zijn over het tijdstip van ontstaan en verdwijnen van de D-dimeren in geval van veneuze trombose. Dit helpt het optimale tijdstip voor een D-dimeer bepaling in te schatten en de periode te bepalen waarvoor en waarna een D-dimeer test minder accuraat kan zijn. Verder is het van belang te weten of er een samenhang is tussen de hoogte van de D-dimeren en de grootte van het stolsel, aangezien grotere stolsels mogelijk aanleiding kunnen zijn voor een intensievere behandeling. Deze vragen stellen wij in **hoofdstuk 3**, waar wij het beloop van de D-dimeer concentraties hebben bestudeerd bij patiënten die een behandeling ondergingen van afwijkende bloedvaten in de long (pulmonale arterioveneuze malformaties [PAVM]) ten gevolge van de ziekte van Rendu-Osler-Weber. De behandeling bestaat uit het afsluiten van de afwijkende bloedvaten door het creëren van een stolsel in het bloedvat (=embolisatie). Tevens hebben wij de relatie tussen de D-dimeer concentraties en de geschatte grootte van het stolsel berekend. Bij 13 patiënten met de ziekte van Rendu-Osler-Weber die een embolisatie ondergingen van PAVM, werden D-dimeer concentraties gemeten vóór, 2, 4 en 8 uur na de embolisatie. De controle groep bestond uit 14 patiënten die een hartkatheterisatie ondergingen zonder verdere ingreep. Twee uur na aanvang van de ingreep (t=2 uur), waren de D-dimeer concentraties in de patiënten groep reeds hoger dan de uitgangswaarden (p=0.001) en deze waarden bleven ver-

hoogd op t=4 en 8 uur. De controle groep toonde geen toename van de D-dimeer concentratie. De stijging van de D-dimeer concentraties in de patiënten groep was hoger dan in de controle groep. Wij vonden eveneens een relatie tussen de hoogte van de D-dimeer concentratie en het berekende volume van de gedeeltes van de arteriën die werden geëmboliseerd, maar geen relatie tussen de D-dimeer concentratie en de afname in de rechts-links shunt of de stijging van het zuurstofgehalte.
Deze studie toont aan dat de D-dimeer concentraties vrijwel direct stijgen na het ontstaan van een (kunstmatig) stolsel en dat ze minstens 8 uur na ontstaan verhoogd blijven. De hoogte van de D-dimeer concentratie zou een inschatting kunnen geven van de grootte van het stolsel.

In de literatuur is beschreven dat de gevoeligheid (sensitiviteit) en de negatief voorspellende waarde van de D-dimeertest voor veneuze trombose hoog is, maar de klinische toepasbaarheid wordt mede bepaald door de specificiteit. In **hoofdstuk 4** hebben wij geprobeerd om klinische variabelen te definiëren welke de toepasbaarheid van de D-dimeer zouden kunnen doen verminderen en zouden leiden tot een verminderde sensitiviteit of specificiteit. Bij 704 patiënten, bij wie een DVT werd vermoed, werd de waarde van een D-dimeertest (Minutex®) berekend. Deze groep patiënten werd onderverdeeld in een groep met bloedverdunnende middelen (n=61), een groep met een eerder doorgemaakte trombose (n=127) en een groep patiënten met kanker (n=47). De referentiegroep bestond uit patiënten zonder bloedverdunners, eerdere trombose of kanker. In totaal werd bij 254 patiënten (36%) een trombose aangetoond. De D-dimeertest had een sensitiviteit en negatief voorspellende waarde van respectievelijk 99% en 98% in de referentiegroep. De sensitiviteit bij patiënten met bloedverdunners was met 75% aanzienlijk lager dan de referentiegroep. Tevens hadden de patiënten die bloedverdunners gebruikten vaker een normale D-dimeertest in vergelijking met de referentiegroep (46% versus 20%). Bij patiënten met eerdere trombose was de sensitiviteit 96% en de negatief voorspellende waarde 94%; bij patiënten met kanker waren deze beide waarden 100%. Bij patiënten met een vorm van kanker was in 91% sprake van een verhoogde D-dimeer concentratie. De kans op een verhoogde D-dimeeruitslag stijgt met de leeftijd: 79% van de patiënten ouder dan 70 jaar had een verhoogde D-dimeer waarde in vergelijking met 61% in de jongere patiënten.
Deze studie toont aan dat de gebruikte D-dimeertest een hoge sensitiviteit en negatief voorspellende waarde voor trombose heeft. De D-dimeertest is

van waarde bij patiënten met eerdere trombose, maar is waarschijnlijk minder betrouwbaar bij patiënten met bloedverdunners. De D-dimeertest is vaak verhoogd bij patiënten met kanker en bij oudere patiënten.

Het herhaald verrichten van een echografie van de beenvaten is een veilige, maar inefficiënte methode gebleken bij de diagnostiek van DVT.
In **hoofdstuk 5** bestuderen wij de waarde van de combinatie van een klinische score en de D-dimeer bij de beoordeling van patiënten met een verdenking op DVT. Bij 812 patiënten (uit 4 verschillende ziekenhuizen) werd een klinische score en een D-dimeer (Tina-quant®) meting verricht. Er waren 176 patiënten met een niet-hoge score en een normale D-dimeer concentratie; bij deze patiënten werd geen aanvullend onderzoek verricht. Slechts 1 (0.6%) van deze patiënten kreeg een trombose in de daarop volgende follow-up periode van 3 maanden. Negenendertig patiënten hadden een normale D-dimeertest en een hoge klinische verdenking op DVT; bij deze groep werd eenmalig een echografie van de beenvaten verricht. Drie (7.7%) van deze patiënten had een DVT bij presentatie, en 1 (2.8%) kreeg een longembolie in de follow-up periode. Er werd tweemaal een echografie verricht bij patiënten met een verhoogde D-dimeer concentratie. Bij 306 van deze 597 patiënten werd hierbij een DVT geconstateerd. Zes (2.1%) patiënten ontwikkelden een trombose gedurende de follow-up van 3 maanden. Gedurende de gehele studieperiode werd bij 317 patiënten (39%) een trombose gevonden.
Deze studie toont aan dat het veilig is een DVT uit te sluiten op basis van de combinatie van een niet-hoge klinische score en een normale D-dimeer concentratie. Deze methode resulteert in een afname van bijna 30% in het verrichten van een echografie.

Er zijn verschillende testen beschikbaar om de D-dimeer concentratie te meten. Waar oudere testen arbeidsintensief of te weinig sensitief waren, zijn er nu nieuwe testen ontwikkeld die sneller en betrouwbaarder zouden zijn. Nieuwe testen moeten echter klinisch gevalideerd worden. In **hoofdstuk 6** onderzochten wij vier relatief nieuwe D-dimeer testen en vergeleken deze met een klassieke D-dimeer test in opgeslagen bloed van 537 patiënten bij wie een DVT vermoed werd. Deze patiënten maakten deel uit van de studie die in hoofdstuk 5 beschreven is, en bij wie de D-dimeer concentratie in eerste instantie gemeten werd met behulp van de Tina-quant® D-dimeer test. Extra bloed werd verzameld om de D-dimeer concentraties op

een later tijdstip te meten met behulp van de Asserachrom ELISA®, VIDAS New®, STA-LIA® en Miniquant® D-dimeer testen. Trombose werd ontdekt bij 224 (42%) patiënten. ROC curves (sensitiviteit versus 1-specificiteit) werden berekend voor elke test. De oppervlakte onder de curve is een maat voor de bruikbaarheid van de test en was het hoogst voor de Tina-quant®. Bij standaard (door de fabrikant aangegeven) grenswaarden waren sensitiviteit, negatief voorspellende waarde en specificiteit van de Asserachrom® respectievelijk 97, 94 en 33%. Deze waarden waren res-pectievelijk 100, 96 en 8% voor de VIDAS New®; de Tina-quant® had waarden van respectievelijk 99, 98 en 41%, de STA-LIA® respectievelijk 98, 95 and 32% en de Miniquant® respectievelijk 95, 94 en 52%. Aanpassing van de grenswaarden waaronder de test normaal wordt geacht, resulteerde in een verbetering van sensitiviteit en negatief voorspellende waarde, maar dat ging (uiteraard) ten koste van de specificiteit.
Deze studie toont aan dat de D-dimeer testen die hier gebruikt zijn allen een hoge sensitiviteit en negatief voorspellende waarde hebben, maar geen van allen bereikt een voorspellende waarde van > 98% bij standaard grenswaarden. Voor het uitsluiten van trombose bevelen wij daarom aan de D-dimeer test altijd te blijven gebruiken in combinatie met andere methoden (zoals de klinische score) om het missen van de diagnose DVT te beperken tot minder dan 1.0%. Het gebruik van een D-dimeer test met een lage specificiteit is klinisch minder waardevol, omdat een positieve test altijd gevolgd dient te worden door aanvullend onderzoek.

Voor de behandeling van veneuze trombose is de effectiviteit van ongefractioneerde heparine (UFH) via een continu infuus bewezen. Het nadeel is echter dat de patiënt hiervoor in een ziekenhuis dient te worden opgenomen, en dat UFH een frequente meting van stollingparameters vraagt voor een optimale dosisaanpassing. De behandeling met een éénmaal daagse, onderhuidse injectie van een laag-moleculair-gewicht heparine (LMWH) heeft als voordeel dat dit thuis kan gebeuren en dat monitoring van stollingsparameters niet noodzakelijk is. De behandeling van longembolieën met LMWH is veilig gebleken, maar weinig is bekend over verschillen tussen UFH en LMWH in veranderingen in de stolling gedurende de eerste dagen na aanvang van de therapie. In **hoofdstuk 7** vergelijken wij de effecten van UFH en LMWH op de stolling en de afwijkingen op de longscan gedurende de eerste dagen van de behandeling van longembolie. Zevenendertig patiënten met een acute longembolie wer-

den behandeld met UFH (n=19) of met dalteparine, een LMWH (n=18). Dagelijks werd op een vast tijdstip bloed afgenomen gedurende een periode van 5 dagen. De vorming van het stolsel (trombine productie) werd gemeten door middel van fragmenten 1+2 (F1+2), trombine-antitrombine (TAT)-complexen en fibrine monomeren. F1+2 en TAT-complexen normaliseerden snel na start van de behandeling zonder verschil tussen de twee groepen. De gemiddelde concentratie van fibrine monomeren daalde in beide groepen niet, maar toonde zelfs een toename in de UFH groep vanaf dag 3. De afbraak van het stolsel (fibrinolyse) werd gemeten door bepaling van de D-dimeren concentratie en de clot-lysis tijd. De gemiddelde D-dimeer concentratie daalde in de loop van de tijd, zonder verschil tussen de twee groepen. De clot lysis tijden waren korter in de UFH groep. Na inactivatie van thrombin activatable fibrinolysis inhibitor (TAFI) door carboxypeptidase-inhibitor bleek heparine geen additioneel effect meer te hebben op de clot lysis tijd. Dit bevestigt de gedachte dat het effect van heparine op de clot lysis tijd voornamelijk door inactivatie van TAFI te verklaren is. Ventilatie-perfusie scans werden verricht waaruit de "percentage-of-vascular-obstruction" scores (PVOs; een maat voor de grootte van de longembolie) berekend werden op dag 0 (PVOsD0) en dag 5 (PVOsD5). De daling van de PVOs op dag 5 ten opzichte van dag 0 was groter in de LMWH groep.
Deze studie toont aan dat LMWH (dalteparine) minstens even effectief is als UFH in het verminderen van de stollingsactivatie en de preventie van toename van longafwijkingen. Dit ondersteunt het gebruik van LMWH in de vroege behandeling van longembolie.

Veneuze trombose kan gepaard gaan met kanker, maar het routinematig screenen op kanker bij iedere patiënt met een DVT is een punt van discussie. In **hoofdstuk 8** onderzoeken wij of de initiële hoogte van de D-dimeer concentratie of het beloop van de D-dimeer concentratie tijdens behandeling van een DVT een voorspellende waarde heeft voor het ontstaan of aanwezig zijn van kanker. Wij bestudeerden 218 patiënten met een bewezen DVT, bij wie een D-dimeer test werd verricht. Patiënten werden gevolgd gedurende gemiddeld 31 maanden en het ontstaan van kanker werd gedocumenteerd. In totaal hadden of kregen 49 (22%) patiënten kanker; 28 waren al bekend met kanker op het moment van de DVT, bij 8 patiënten werd kanker ontdekt op het moment van de DVT en 13 patiënten ontwikkelden een kwaadaardige aandoening gedurende de

follow-up periode. Bij een initiële D-dimeer concentratie kleiner dan 4000 µg/l kwam kanker bij 16% (20/128) van de patiënten voor, vergeleken met 32% (29/90) in patiënten met D-dimeer concentraties groter dan 4000 µg/l. Als de D-dimeer concentratie na 4 dagen behandeling kleiner dan 4000 µg/l was, kwam kanker voor bij 14% (6/44) van de patiënten, vergeleken met 46% (6/13) van de patiënten met D-dimeer concentraties groter dan 4000 µg/l. Een kwaadaardige aandoening werd vaker geconstateerd bij patiënten ouder dan 60 jaar (37%) in vergelijking met jongere patiënten (10%). Van alle patiënten met trombose en kanker bleek 78% (38/49) ouder dan 60 jaar te zijn. Van de 13 patiënten die kanker kregen gedurende de follow-up, was 85% (11/13) ouder dan 60 jaar. Bij patiënten ouder dan 60 was er geen verschil in voorkomen van kanker tussen patiënten met D-dimeer concentraties boven of onder 4000 µg/l. Bij jongere patiënten daarentegen kwam kanker voor in 3% bij initiële D-dimeer concentraties kleiner dan 4000 µg/l en in 23% bij D-dimeer concentraties groter dan 4000 µg/l. Indien de D-dimeer concentratie gemeten werd op dag 4 van de behandeling, bleek bij jongere patiënten met een waarde kleiner dan 4000 µg/l een kwaadaardige aandoening in het geheel niet (0%) voor te komen, terwijl alle (100%) jongere patiënten met D-dimeer waarden groter dan 4000 µg/l kanker bleken te hebben. De onderzoeksgroep was niet groot, maar indien onze bevindingen in een grotere studie bevestigd kunnen worden dan zou screening op een kwaadaardige aandoening vooral verricht moeten worden bij oudere patiënten en bij jongeren patiënten bij wie in het begin of op dag 4 van de behandeling de D-dimeer concentratie hoger is dan 4000 µg/l.

Een overzicht en interpretatie van de recente literatuur betreffende de diagnostiek van DVT en met name de rol van de D-dimeertest en de klinische score wordt gegeven in **hoofdstuk 9**.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 15 augustus 1970 te Leiden. In 1989 behaalde hij het Gymnasium B diploma aan het Bernardinus college te Heerlen, waarna hij de studie geneeskunde startte aan de Katholieke Universiteit Leuven te België. Het artsendiploma werd in juni 1996 met onderscheiding behaald. Aansluitend volgde een arts-assistentschap inwendige geneeskunde in het Groene Hart Ziekenhuis te Gouda. De perifere opleiding tot internist ving aan op 1 januari 1998 in het Sint Antonius Ziekenhuis te Nieuwegein (opleiders: dr. H.C.M. Haanen en dr. P.Th.J. Slee). Tijdens deze perifere opleidingsperiode werden de ideeën voor dit proefschrift verzameld en de daarbij behorende studies uitgevoerd. In 2002 werd de opleiding voortgezet in het Universitair Medisch Centrum Utrecht (opleider: prof. dr. D.W. Erkelens), alwaar de opleiding in april 2003 werd voltooid en hij thans werkzaam is als fellow haematologie (opleider: prof.dr. A.H. Hagenbeek).

ABBREVIATIONS

CUS =	compression ultrasonography
DD =	D-dimer
DVT =	deep venous thrombosis
ELISA =	enzyme linked immunosorbent assay
FEU =	fibrinogen equivalent unit
FM =	fibrin monomer
HHT =	hereditary hemorrhagic teleangiectasia
LMWH =	low molecular weight heparin
NPV =	negative predictive value
PAVM =	pulmonary arteriovenous malformations
PCP =	pre-test clinical probability score
PE =	pulmonary embolism
PPV =	positive predictive value
PVOs =	percentage of vascular obstruction score
Sens =	sensitivity
Spec =	specificity
TAFI =	thrombin activated fibrinolysis inhibitor
TAT =	thrombin-antithrombin
UFH=	unfractionated heparin
US =	ultrasound
VTE =	venous thromboembolism