

# Performance of a New Fibrin Monomer Assay to Exclude Deep Vein Thrombosis in Symptomatic Outpatients

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## Summary

In this study we prospectively assessed the reliability of a new fibrin monomer assay in 106 outpatients with clinically suspected deep venous thrombosis of the lower limb. According to the results of the objective tests and using different cut-off points we calculated the sensitivity, specificity and negative predictive value of the fibrin monomer assay. The prevalence of deep vein thrombosis was 44.3% (31.1% proximal, 13.2% distal). Using a cut-off level of plasma fibrin monomer of 3.5 µg/ml, a sensitivity, specificity and negative predictive value of 100% (95% CI: 94-100%), 35.6% (95% CI: 23-48%) and 100% (95% CI: 86-100%), respectively, were obtained. The exclusion rate was 19.8% (95% CI: 12-27%) of all referred patients. These accuracy indices compared favourably with the respective results of a routine D-dimer ELISA used for comparison. Conclusion: This new fibrin monomer assay appears to be a reliable method for the exclusion of deep vein thrombosis in symptomatic outpatients.

## Introduction

Of all patients with suspected deep vein thrombosis (DVT) of the lower extremities only one third will prove to have the disease when objective diagnostic methods such as contrast venography or sonography are applied. Therefore it is of considerable interest to have a reliable screening test to accurately identify patients without the disease to obviate unnecessary diagnostic work-up (1, 2). Assays that measure cross-linked fibrin derivatives such as D-dimer or soluble fibrin have shown potential clinical utility. It has been advocated that normal plasma levels of D-dimer or soluble fibrin can be used for the exclusion of venous thromboembolism (3-7).

Increased plasma levels of circulating soluble fibrin are considered as molecular markers of an impending thrombotic event resulting from increased thrombin formation (8-11). Therefore, it is reasonable to hypothesize that patients with DVT have elevated plasma levels of soluble fibrin. In contrast to D-dimer, production of soluble fibrin is neither dependent upon factor XIIIa-mediated cross-linking of fibrin nor upon a normal fibrinolytic system (7).

Fibrin monomer (FM), a component of soluble fibrin, is produced when thrombin sequentially cleaves fibrinopeptides A and B from the amino-termini of the A $\alpha$ - and B $\beta$ -chains of fibrinogen, respectively. Several assays that measure soluble fibrin or FM are available, including functional tests based on the enhancement of t-PA-induced

plasminogen activation and immunologic tests detecting fibrin-specific neoantigens (7, 8, 12-14). Potential neo-antigens for immunologic detection of FM are the aminoterminal of the  $\alpha$ -chain which is temporarily uncovered by thrombin-mediated cleavage of fibrinopeptide A, the t-PA binding site A $\alpha$ -(148-160) which is uncovered by conformational changes induced by fibrinopeptide A release and the sequence  $\gamma$  (312-324) of the gamma chain which becomes accessible after cleavage of the fibrinopeptides (15-17). The advantage of measuring FM in comparison with fibrinopeptide A is the much longer half life of the former (5-6h) (8).

Enzymun-Test FM<sup>®</sup> is a new ELISA-based fibrin monomer assay using a specific monoclonal IgG antibody directed against the temporarily uncovered aminoterminals of the  $\alpha$ -chains of fibrinogen following cleavage of fibrinopeptide A (18).

In the present study we evaluated the performance of this new FM assay for the exclusion of DVT in a series of consecutive symptomatic outpatients who were referred by primary health care physicians.

## Patients and Methods

### Patients

Consecutive outpatients referred for diagnostic work-up for clinically suspected deep vein thrombosis of the lower limb between December 1996 and January 1998 were included. All patients had their diagnosis confirmed or refuted within 1 day of referral by the general practitioner. Patients were excluded if they were receiving therapeutic or prophylactic anticoagulation (oral anticoagulation or heparin/low molecular weight heparin (LMWH)) for more than 24 h prior to study enrollment. Pregnant women and patients having been hospitalized in the preceding 3 days were also excluded. All patients gave informed consent. From all patients history was obtained and all underwent physical examination. The study protocol was approved by the local ethical committee.

### Diagnosis of Deep Vein Thrombosis

DVT was diagnosed either by colour-coded duplex sonography, performed by an experienced sonographer, or by contrast venography. Patients were considered not to have DVT when the initial test was normal and 3-month follow up was uneventful. DVT was classified as "proximal" when it was localized in the popliteal and/or iliofemoral veins and as "distal" when localized exclusively in the calf veins.

### Laboratory Analysis

All blood samples were collected into 10 ml plastic tubes (Monovette<sup>®</sup>, Sarstedt Nuembrecht, Germany) containing 1 ml 0.106 M trisodium citrate. Blood samples were centrifuged twice at 1500  $\times$  g for 10 min at room temperature and frozen within one hour after blood sampling. Plasma was stored in aliquots using polypropylene tubes at -70 °C.

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Fibrin monomer antigen was measured using Enzymun-Test FM® (Boehringer Mannheim AG, Mannheim, Germany) as previously described (11, 18). Briefly, fibrin monomer is measured by a specific monoclonal IgG<sub>1</sub> antibody (2B5) raised against the neo-epitope, which is temporarily uncovered by the thrombin-mediated cleavage of fibrinopeptide A from fibrinogen. It is a two-step sandwich assay in streptavidin-coated polystyrene tubes using monoclonal antibody 2B5 as biotin-conjugated capture antibody and peroxidase-labelled tagging antibody. A sample pretreatment step with thiocyanate leads to the dissociation of soluble fibrin complexes. The test was performed on an automated ELISA instrument system (ES 300, Boehringer Mannheim GmbH, Mannheim, Germany).

Asserachrom D-Dimer® ELISA (STAGO, Asnières, France) was used for comparison (cut-off level: 500 ng/ml). It was performed according to the instructions of the manufacturer. D-dimer values are expressed in ng/ml of fibrinogen equivalent units.

**Statistical Analysis**

The accuracy indices [sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)] of different fibrin monomer cut-off levels were calculated. The 95% confidence intervals (CI) were calculated according to the normal approximation of the binomial distribution. The Receiver Operating Characteristic (ROC) curve was constructed by plotting the sensitivity (true positive fraction) versus 100-specificity (false positive fraction).

**Results**

During the study period a total of 123 consecutive outpatients with clinically suspected deep vein thrombosis were referred. Seventeen patients had to be excluded because of therapeutic or prophylactic anti-coagulation for more than 24 h prior to study enrollment. Finally, a total of 106 patients were included into the study. The median age was 56.3 years (range 16 to 88 years), 51% were female. The mean delay from first symptoms to study enrollment was 8 days. The overall prevalence of deep vein thrombosis using objective tests was 44.3% (47/106 patients). DVT was proximal in 33 (31.1%) and distal in 14 (13.2%) patients. Clinical signs of pulmonary embolism were present in 4 (8.5%) patients with confirmed DVT. Fourteen patients (13.2%) had received one or two doses of heparin or low molecular weight heparin prior to blood sampling, 10 (21.3%) in the DVT group and 4 (6.8%) in the group without DVT.

The distribution of the fibrin monomer results of all the 106 patients is shown in Fig. 1. The indices of accuracy of the fibrin monomer assay

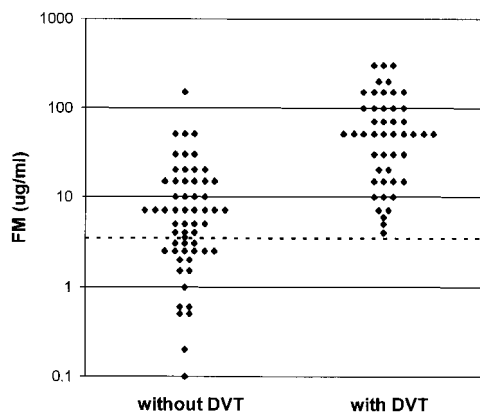


Fig. 1 Distribution of fibrin monomer (FM) levels in patients (n = 106) with and without deep vein thrombosis (DVT). The horizontal dashed line indicates the cut-off at 3.5 µg/ml

Table 1 Accuracy indices of several cut-off levels of fibrin monomer test (Enzymun-Test FM®) and of Asserachrom D-Dimer® ELISA used for comparison

	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
<b>Fibrin monomer</b>				
<b>Cut-off</b>				
3.5 ug/ml	100 (94-100)	35.6 (23.4-47.8)	55.3 (44.7-65.9)	100 (86-100)
5.0 ug/ml	97.9 (93.7-100)	42.4 (29.8-55.0)	57.5 (46.7-68.3)	96.2 (88.6-100)
7.5 ug/ml	91.5 (83.5-99.5)	61.0 (48.6-73.5)	65.2 (53.7-76.7)	90.0 (80.7-99.3)
10.0 ug/ml	87.2 (77.7-96.8)	64.4 (52.2-76.6)	66.1 (54.4-77.9)	86.4 (76.2-96.5)
<b>Asserachrom D-Dimer ELISA</b>				
<b>Cut-off</b>				
500 ng/ml	95.7 (90.0-100)	61.0 (48.6-73.5)	66.2 (54.9-77.4)	94.7 (87.6-100)
370 ng/ml	100 (94-100)	39.0 (26.5-51.4)	56.6 (46.0-67.3)	100 (87-100)

PPV: positive predictive value  
NPV: negative predictive value

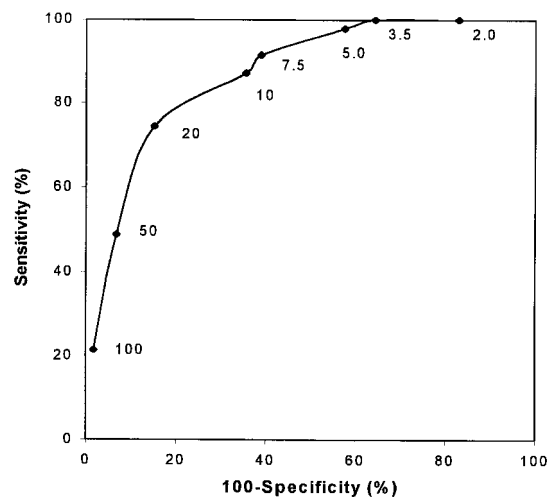


Fig. 2 Receiver Operating Characteristic (ROC) curve of accuracy indices of fibrin monomer plasma concentrations for the presence of deep vein thrombosis. The given numbers indicate different plasma fibrin monomer cut-off levels in µg/ml

are given in Table 1 and in the ROC analysis in Fig. 2. Using a cut-off level of 3.5 µg/ml, the fibrin monomer assay had a sensitivity of 100% (95% CI: 94-100%) and a NPV of 100% (95% CI: 86-100%) for the diagnosis of deep vein thrombosis. Taking this cut-off level the Enzymun-Test FM® was able to adequately exclude the presence of deep vein thrombosis in 21 of 106 symptomatic outpatients, giving an exclusion rate of 19.8% (95% CI: 12-27%).

A separate analysis performed after the exclusion of the patients in whom therapy with heparin or LMWH had been started within 24 h prior to study enrollment revealed a similar performance of the FM assay at a cut-off level of 3.5 µg/ml: sensitivity 100% (95% CI: 92-100%), specificity 36.7% (95% CI: 24-49%), NPV 100% (95% CI: 86-100%) and exclusion rate 21.7% (95% CI: 13-30%) (n = 92).

A further analysis at a cut-off level of 3.5 µg/ml restricted to patients with proximal DVT yielded again a similar performance of the Enzymun-Test FM® as compared to the overall performance: sensitivity 100% (95% CI: 91-100%), NPV 100% (95% CI: 86-100%), specificity 35.6% (95% CI: 23-48%) and exclusion rate 22.8% (95% CI: 14-31%), respectively (n = 92).

The sensitivity, NPV and exclusion rate of the Asserachrom D-Dimer® ELISA were 95.7% (95% CI: 90-100%), 94.7% (95% CI: 88-100%) and 34% (95% CI: 25-43%), respectively, using a cut-off

level of 500 ng/ml. Taking a lower cut-off level of 370 ng/ml, in order to achieve a sensitivity and NPV of 100%, the specificity and the exclusion rate of the D-dimer ELISA were 39.0% (95% CI: 27-51%) and 21.7% (95% CI: 14-30%), respectively (Table 1).

## Discussion

The aim of our study was to evaluate a new fibrin monomer test for its usefulness to safely exclude deep vein thrombosis in symptomatic outpatients. The present study indicates that the Enzymun-Test FM<sup>®</sup> is an accurate method for this purpose. Using a cut-off level for fibrin monomer of 3.5 µg/ml a sensitivity of 100% (95% CI: 94-100%) and a negative predictive value of 100% (95% CI: 86-100%) were obtained. Thus, none of the patients with proven DVT had a normal fibrin monomer test result. Although the optimal cut-off point of the Enzymun-Test FM<sup>®</sup> has still to be definitely established, these results show that patients who presented with suspected DVT and showed a fibrin monomer result below the cut-off level of 3.5 µg/ml were unlikely to have DVT.

Over the past years several investigations have been performed to evaluate the potential role of cross-linked fibrin derivatives or soluble fibrin in the diagnostic work-up of patients with suspected DVT. ELISA-based D-dimer assays have shown potential clinical utility. In our study, we compared the accuracy indices of the Enzymun-Test FM<sup>®</sup> with the Asserachrom D-Dimer<sup>®</sup> ELISA. The sensitivity (100%) and the negative predictive value (100%) of the fibrin monomer assay were equal to the respective results of the D-dimer ELISA (95.7% and 94.7%, respectively; cut-off level 500 ng/ml). Two patients with proven distal DVT had a D-dimer value below the cut-off level. On the other hand, the fibrin monomer assay showed a lower specificity (35.6%) and exclusion rate (19.8%) than the D-dimer assay (61% and 34%, respectively). However, fixing a lower cut-off level for D-dimer (370 ng/ml) in order to achieve an equivalent sensitivity and NPV of 100% for the D-dimer assay, resulted in a decrease of the specificity and the exclusion rate of the Asserachrom D-Dimer<sup>®</sup> ELISA to 39.0% (95% CI: 27-51%) and 21.7% (95% CI: 14-30%), respectively. Thus, with the precondition of an optimal sensitivity and NPV the performances of both assays were equivalent. The low specificity and exclusion rate of the fibrin monomer assay probably resulted from the fact that in several clinical conditions, such as infection, trauma or malignancy, which might have coexisted in our patients, circulating fibrin monomers are generated in similar amounts as in DVT. With respect to clinical utility the low exclusion rate may be considered a major limitation. However, the low exclusion rate of about 20% in our study also relates – at least in part – to the rather high prevalence of DVT in our series of outpatients.

Because the assessment of circulating plasma fibrin monomer levels differs significantly using different assays, our results are valid only for the test we used in this study but may not apply for other currently available fibrin monomer assays (13, 14). In addition, further clinical trials are needed to elucidate the cut-off points and to determine whether management decisions can be safely made based on the results of this assay.

In the actual format the Enzymun-Test FM<sup>®</sup> is designed for batch analysis and can be performed on a routine ELISA instrument system (ES 22, ES 33, ES 200 or ES 300) within a few hours. Clinical utility could be improved when the assay would suit for rapid testing on individual patients.

The results of our study are consistent with the results of other recent reports. Fibrin monomer testing was found to be a valuable diagnostic

tool for the early diagnosis of postoperative DVT (21). Moreover, soluble fibrin, assessed by an ELISA with an antibody specific for the sequence  $\gamma$  312-324 of fibrin, was demonstrated to be of potential clinical utility in the exclusion of suspected DVT and pulmonary embolism (7, 22).

In conclusion, this study shows that the new fibrin monomer assay (Enzymun-Test FM<sup>®</sup>) has a performance equal to a routine D-dimer ELISA in the exclusion of DVT in symptomatic outpatients and is of potential clinical utility. The results support the development of an assay format more prone to rapid individual testing. Based on these results a clinical management trial should be performed in which the decision about whether the patient will not be further investigated will be based on a normal FM-test result. Moreover, further studies are needed to establish definitely the appropriate cut-off level of the new FM-test for the exclusion of DVT in symptomatic outpatients.

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