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Hemostasis

Comparison of the diagnostic performance of three soluble fibrin monomer tests and a D-dimer assay in patients with clinically suspected deep vein thrombosis of the lower limbs

We assessed three soluble fibrin monomer (SFM) assays in 231 in and out-patients with clinically suspected deep-vein thrombosis. Thrombosis was confirmed or excluded by complete lower-limb ultrasound. SFM assay were less accurate than VIDAS D-dimer and in patients with small thrombosis or under anticoagulation. Specificity was lower for a similar sensitivity.

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Soluble fibrin monomers (SFM) reflect the action of thrombin on fibrinogen. Some publications suggest that tests of SFM have a similar efficacy as measuring D-dimer levels in the exclusion diagnosis of venous thrombo-embolism.[1-6] We assessed the accuracy of three tests measuring SFM complexes as compared with a well established rapid D-dimer ELISA in the diagnosis of deep-vein thrombosis (DVT), and the effect of thrombus extent and anticoagulant treatment. Small thromboses will induce few changes in coagulation and fibrinolysis. Anticoagulation will decrease thrombin generation while it has less effect on D-dimer production. As a consequence, less fibrin monomer will be formed. In this prospective study, we included consecutive in and outpatients with a clinically suspected first episode of lower limb DVT and no signs of pulmonary embolism (PE), after they had given their signed informed consent. Complete lower limb venous ultrasonography (US) was used to exclude or confirm DVT. A full examination of the proximal and distal (infra-popliteal) veins was performed as previously described.[7] A DVT was considered confirmed when both vein non-compressibility and direct visualization of the thrombus were present.

Plasma D-dimers were determined using the automated VIDAS-ELISA technique (bioMérieux, Marcy L'Etoile, France). SFM were assayed by three different techniques: Berichrom FM (Dade Behring, Marburg, Germany) and Coatest SF (Chromogenix IL, Milan, Italy) based on the ability of fibrin monomers to catalyze the activation of plasminogen by t-PA, and Fibrinostika SF (Organon Teknika, Durham NC, USA) based on an ELISA principle. All tests were performed by independent operators and interpreted blindly.

For each test, we analyzed sensitivity and specificity with 95% confidence intervals at different thresholds and construct receiver operating characteristic (ROC) curves for three DVT categories (all DVT, proximal DVT, DVT ≥ 4 cm) and for untreated and treated patient groups. Potentially significant differ-

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Table 1. Area under receiver operating characteristic (ROC) curves for VIDAS D-dimer and the three soluble fibrin monomer (SFM) assays for different categories of DVT (all DVT; proximal DVT; DVT ≥ 4cm) and for patients without or with anticoagulant treatment.

Test	*All DVT (n=77)*	*DVT ≥ 4 cm (n=66)*	*Proximal DVT (n=22)*	*No anticoagulant (n=114)*	*Anticoagulant (n=117)*
VIDAS D-dimer	0.77 [0.72-0.82]	0.76 [0.71-0.81]	0.74 [0.70-0.77]	0.82 [0.75-0.89]	0.7 [0.63-0.77]
Berichrom FM	0.62 [0.55-0.70] $p < 0.001$	0.63 [0.55-0.70] $p = 0.002$	0.67 [0.57-0.77] $p = 0.21$	0.73 [0.63-0.83] $p = 0.11$	0.53 [0.42-0.63] $p = 0.002$
Fibrinostika SF	0.69 [0.62-0.76] $p = 0.04$	0.71 [0.63-0.78] $p = 0.17$	0.71 [0.61-0.81] $p = 0.7$	0.73 [0.63-0.84] $p = 0.09$	0.66 [0.56-0.76] $p = 0.44$
Coatest SF	0.58 [0.50-0.66] $p < 0.001$	0.6 [0.51-0.68] $p < 0.001$	0.58 [0.44-0.72] $p = 0.03$	0.64 [0.53-0.75] $p = 0.002$	0.51 [0.40-0.63] $p = 0.002$

FM: fibrin monomer. SF: soluble fibrin. DVT: deep vein thrombosis. [ ]: 95% confidence interval. Each SFM test was compared with VIDAS D-dimer by considering the area under the curve which represents a global test accuracy. The test with the greater area is better. A test whose 95% confidence interval area includes the 0.5 value is not informative. Potential differences between the areas under the curve were assessed by the χ^2 test. The difference was significant at the 5% level (p value < 0.05). At the time of diagnosis, 91 patients were under prophylactic anticoagulation with low-molecular-weight-heparin (n=81) or unfractionated-heparin (n=10) and 26 patients were under therapeutic anticoagulation with oral anticoagulants (n=7), low-molecular-weight-heparin (n=11) or unfractionated heparin (n=8).

Table 2. Diagnostic performance of the VIDAS D-dimer assay and the three soluble fibrin monomer tests as compared to complete lower limb venous ultrasound.

	Sensitivity [95% CI]	*Specificity [95% CI]*	*PPV [95% CI]*	*NPV [95% CI]*
D-dimer 500 ng/mL	0.96 [0.89-0.99]	0.27 [0.20-0.34]	0.40 [0.33-0.47]	0.93 [0.82-0.98]
D-dimer 400 ng/mL	0.97 [0.91-0.99]	0.24 [0.18-0.31]	0.39 [0.32-0.46]	0.95 [0.83-0.99]
Berichrom FM	0.99 [0.93-1.00]	0.06 [0.04-0.11]	0.34 [0.28-0.41]	0.91 [0.62-0.98]
Fibrinostika SF	0.97 [0.91-0.99]	0.12 [0.08-0.18]	0.36 [0.29-0.42]	0.90 [0.71-0.97]
Coatest SF	0.99 [0.93-1.00]	0.09 [0.05-0.14]	0.35 [0.29-0.42]	0.93 [0.68-0.99]

CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; ng: nanogram. Predictive values were obtained for a DVT prevalence of 33.33 %. The soluble fibrin monomer assays had a lower specificity and exclusion rate than the D-dimer at cut-off levels that provide a similar sensitivity. The difference in the exclusion efficiency between D-dimer and SFM becomes theoretically higher as the clinical probability increases (with a lower NPV for both tests), and vice versa.

ences between the areas under the curve (AUC) were assessed by the χ^2 test at the 5% level.[8] We also determined the optimal threshold for each SFM from the ROC curves at the best sensitivity value: 8 μg/mL for Behring FM, 20 ng/mL for Fibrinostika SF and 35 SF units for Coatest SF. The VIDAS D-dimer cut-off was fixed at 500 ng/mL. Data were analyzed using Stata software (Statacort, 1999. Release 6.0 College Station, TX: Stata Corporation, USA) and CIA software version 2.0.0 (University of Southampton, UK). From June 1998 to March 1999, 231 patients were included in this study. The major clinical characteristics of these patients were: mean age 61±18 years, sex ratio 0.89, 178 inpatients and 53 outpatients, 117 patients (51%) under therapeutic (11.3%) or prophylactic (39.4%) anticoagulation, time elapsed between clinical onset and diagnosis 9.7±16.8 days. US showed a DVT in 77 patients (prevalence 33.33%), which was proximal in 22 and distal in 55. In 11 patients, the thrombus size was < 4 cm.

The AUC values for VIDAS D-dimer were in general higher than those for the SFM tests. Thrombus extent had an effect on global test accuracy. Analysis of the AUC demonstrated that VIDAS D-dimer performed better than any SFM for the diagnosis of *all DVT*, than Berichrom FM and Coatest SF for *DVT ≥ 4 cm*, and only Coatest SF for *proximal DVT* (Table 1). Fibrinostika SF performed better than Coatest SF for all DVT categories and better than Berichrom FM for *DVT ≥ 4 cm*.

Anticoagulant use also had some effect on the results. The D-dimer was more accurate than Coatest SF in patients without anticoagulant treatment and more accurate than both Berichrom FM and Coatest SF in patients on anticoagulants. No difference was demonstrated with Fibrinostika SF either in the treated or untreated groups of patients (Table 1).

Among the SFM assays, Fibrinostika SF represented the best alternative to VIDAS D-dimer even in patients on anticoagulants. However, all SFM had a lower specificity (6-12%) than the D-dimer test (24-27%) and a lower exclusion rate at cut-off levels that provide a similar sensitivity (96-99%) and negative predictive value (90-93%) (Table 2). Various methodological points can be discussed. Complete US was the reference diagnostic method. Although the ability and utility of this investigation for detecting isolated calf DVT remain controversial, it has proved to be accurate[9] and effective,[7,10] and is now widely used in practice in many centers. Because it is non-invasive, we were able to include patients consecutively. The study was performed in an unselected population of patients. The inclusion of in-patients makes extrapolation difficult to the out-patient setting. It is possible that the high prevalence of distal DVT in our population and the long delay between the clinical onset and diagnosis accounted for the poorer performance than that reported in the literature. VIDAS D-dimer assay remains preferable to SFM measurements and in patients with small thromboses or those already receiving anticoagulation.

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Key words: D-dimer, SFM, DVT diagnostic performance.

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Disorders of Hemostasis

Molecular characterization of factor X deficiency associated with borderline plasma factor X levels

Borderline plasma factor X (FX) levels might complicate the diagnosis of FX deficiency. An asymptomatic individual with 73% FX activity was identified to be heterozygous for the Val342Ala mutation. Expression studies suggested that this substitution is responsible for a CRM+ FX variant with normal activation but modestly reduced catalytic function.

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Factor X (FX) deficiency is a rare coagulation disorder, inherited as an autosomal recessive trait and characterized by a variable clinical presentation which correlates poorly with the laboratory phenotype.[1] A severe bleeding phenotype is usually associated with homozygous or doubly heterozygous conditions.[2] Heterozygous FX deficiency is generally asymptomatic, and in most cases is identified incidentally during pre-operative screening. The diagnosis of heterozygous FX deficiency and an estimate of the prevalence of this deficiency can be furher complicated by FX levels borderline to the normal range. Borderline levels may represent a general problem for the diagnosis of both hemorrhagic and thrombotic risk conditions. We have previously addressed this issue in asymptomatic FVII deficiency by exploiting a molecular genetic approach.[3]

In this study, we investigated a 51-year old man presenting with 73% FX activity in a prothrombin (PT)-based assay (normal range 77-123%) during a pre-surgery screen. FX antigen levels were not available.

DNA sequencing of the FX gene[4] revealed that the subject was heterozygous for an alanine (GCG) to valine (GTG) substitution at position 342 (cDNA numbering).[5]

Val342 (c160, chymotrypsin numbering) is conserved in FVII, FIX and FXII and is substituted by Ile in protein C. The presence of topologically equivalent mutations in FIX (Val to Phe) and protein C (Ile to Phe) associated with coagulation deficiencies supported the causative role of the Val342Ala substitution. To assess the effect of the Val342Ala mutation on FX function, the 342AFX variant was expressed in human embryonic kidney 293 cells (HEK293) and studied.

Construction of the expression vector, transient transfection of HEK293, determination of antigen levels and functional assays of the r342A-FX variant in conditioned medium were conducted as previously described.[6] The forward primer for FX cDNA mutagenesis was 5'GCTCAAGATGCTGGAGGCGCCCTACGTGGACCG3'. Antigen levels of r342A-FX (418±154 ng/mL) in medium were not significantly different from those of recombinant wild type (rWt)-FX (593±214 ng/mL), thus suggesting that the Val342Ala change does not impair FX biosynthesis or secretion.

Table 1. Activity of the r342A-FX in FX depleted plasma.

Thrombin generation activity	
extrinsic activation	52±4
intrinsic activation	50±6
Amidolytic activity	
extrinsic activation	64±3
Coagulant activity	
PT-based assay	35

Values (% of rWt-FX) are reported as mean ± standard deviation.

In a PT-based assay, the r342A-FX coagulant activity (Table 1) was 35% of rWt-FX activity and was consistent with that measured in the plasma of the heterozygous subject.

Activation of r342A-FX in conditioned medium with increasing concentrations of FVIIa-tissue factor (TF) complex was estimated through Western blot analysis (Figure 1A). At the lowest concentration of activator used the amount of FXaα form was comparable for both variants, thus suggesting normal activation of the r342A-FX molecule. Accordingly, a similar increase in FXa fluorogenic activity of both recombinant proteins was observed at increasing concentration of FVIIa/TF complex (Figure 1B).

The r342A-FX activity was further studied towards thrombin or a peptidyl substrate in FX depleted plasma to reproduce the *in vivo* conditions more precisely. Reduced thrombin generation activity was observed upon both extrinsic (52±4%) and intrinsic activation (50±6%) (Table 1). Once activated by the extrinsic pathway, r342A-FX showed a similarly reduced activity (64±3%) towards a peptidyl fluorogenic substrate. These findings suggested that the mutation was responsible for a dysfunctional FX variant. However, to confirm a conventional CRM+ phenotype the FX antigen evaluation in plasma would be required.

The reduction in activity by both activation pathways, and the parallel reduction in amidolytic and thrombin generation activity predict that the Val342Ala substitution alters the active site rather than the macromolecular interactions in the prothrombinase complex. These experimental observations are supported by the position of Val342, far from exosites known to participate in factor Va interactions.[7] The reduced generation of the γ-form (Figure 1A), deriving from the autocatalytic activity of the r342A-FXaβ form, might further reflect the partially compromised catalytic function of this variant.

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