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Thrombin split products (prothrombin fragment 1 + 2) in urine in patients with suspected deep vein thrombosis admitted for radiological verification

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ABSTRACT

Introduction: The appearance of prothrombin fragment 1 + 2 (F1 + 2) in urine has been associated with postoperative hypercoagulability and thromboembolism. We wanted to assess if F1 + 2 was released in urine (uF1 + 2) in patients with procoagulant disorders, and if higher levels were found in patients with radiological verified deep vein thrombosis (DVT).
Materials and methods: Consecutive patients were interviewed on comorbidities and medications. An unselected total cohort (n = 534) and a control cohort (n = 177) were analysed. A urine sample (10 ml) was collected and snap frozen before levels of uF1 + 2 were measured with an ELISA kit. Visualisation of DVT was done with compression ultrasound, supplied with venography when feasible. All patients were followed up for 3-6 months.
Results: DVT was diagnosed in 108/534 patients. Statistical significant higher uF1 + 2 levels were found in patients with DVT ($p < 0.001$), in DVT positive patients with ongoing malignancy ($p = 0.034$) and in pregnant women compared to the control cohort ($p < 0.001$). Non-significant increased urine concentrations were found in DVT positive vs. DVT negative patients with infections and traumas.
Conclusions: Levels of uF1 + 2 was associated with DVT both in the total cohort and in the control cohort as well as in most patients with coexisting conditions.

Introduction

Several disorders activate the coagulation system resulting in thrombin generation that may result in development of deep vein thrombosis (DVT). D-dimer is at present an important bio-marker used to demonstrate or exclude ongoing coagulation activity [1]. However, blood sampling is needed which makes the test cumbersome for routine use. A test based on spot urine would be more feasible.

Conversion of prothrombin to thrombin split off prothrombin fragment 1 + 2 (F1 + 2) that is a small moelcule excreted in the urine and which can be quantified with an enzyme-linked immuno-sorbent assay (ELISA) [2,3]. Analysis of F1 + 2 in urine (uF1 + 2) is a new method under development to assess coagulation activation.

Radiological screening studies on selected non-symptomatic standard-operated orthopaedic patients showed a good correlation between uF1 + 2 and postoperative DVT [4,5]. The present study is taking this analysis a step further to non-selected patients admitted to the Department of Radiology with clinically suspected DVT. We wanted to evaluate if quantification of uF1 + 2 could be used as a marker of ongoing coagulation activity in patients with and without imaging verified DVT.

Materials and Methods

From April 2007 to November 2009, consecutive patients admitted to the Department of Radiology were asked to participate in the study. We planned to include at least 100 patients with DVT. Written informed consent was obtained. The study was conducted in accordance with the Declaration of Helsinki and approved by the regional Ethics Committee,

Abbreviations: CI, confidence interval; DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assay; F1 + 2, prothrombin fragment 1 + 2; NPV, negative predictive value; PPV, positive predictive value; uF1 + 2, urine prothrombin fragment 1 + 2.

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the Norwegian Social Science Data Services and the Regional Data Protection Officer.

Inclusion criteria were patients admitted with clinical suspected lower extremity DVT for diagnostic imaging. Exclusion criteria were refusal to provide informed consent and age <18 years.

All patients were interviewed on duration of symptoms, comorbidities and use of medications.

Laboratory Analysis

A urine sample (10 ml) was collected before the radiological procedure and divided in two aliquots. The samples were snap frozen and stored at -80 °C until analysis after completion of the planned number of patients.

Urine levels of F1 + 2 were measured using a commercial available ELISA kit (Enzygnost F1 + 2, Monoclonal; Dade Behring, Marburg, Germany) and a BEP 2000® analyzer (Dade Behring, Marburg, Germany), in accordance with the manufacturers instructions. The lowest and highest detectable levels of uF1 + 2 was 20 and 1200 pmol L^{-1}, respectively.

Radiological Examinations

Examinations of the vessels were conducted with B-mode compression ultrasound (Toshiba Aplio 80 and Toshiba Aplio XG) visualizing the femoral, popliteal and deep calf veins. In case of non-conclusive ultrasound, unilateral venography was performed as described by Rabinov et al. [6]. All patients with verified DVT were referred to the Department of Internal medicine for treatment with low-molecular-weight heparin and warfarin for 3-6 months.

Follow Up

All patients were followed up to obtain information on new venous onsets, arterial thromboembolic events or other conditions that could trigger thrombin activity. Patients without DVT were interviewed after 3 months. Those with DVT were followed up 3 months after termination of the anticoagulation treatment.

Statistical Analysis

The total cohort was divided in two groups i.e. those with and those without DVT. A control cohort was defined as patients without current comorbidities and treatment with anticoagulants.

Data analysis was conducted using SPSS version 19 (IBM, Armonk, New York, USA). Descriptive statistics were given as percent, number of patients, median with 25-75 percentile and mean with 95% confidence interval (CI) as appropriate.

Due to asymmetric distribution the non-parametric Mann-Whitney *U* test was used to assess differences in uF1 + 2 levels in the DVT negative and positive patients. The results were considered statistically significant if the two-sided *p*-value was ≤ 0.05.

Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of uF1 + 2 were calculated with a uF1 + 2 cut-off value of 20 pmol L^{-1}.

Results

During the study period, 534 patients were consecutively included (260 men and 274 women). Median age was 60 (range 18-93) years. DVT was confirmed in 108 (20.2%) patients. Venography was performed in 52 (9.7%) patients due to inconclusive ultrasound. A previous history of venous thromboembolic events was seen in 99 (18.5%) patients and 18 (3.4%) had cardiovascular diseases.

Median uF1 + 2 levels in the total cohort was 28.1 pmol L^{-1} (25-75 percentile 20.0-80.5). The baseline characteristics of the DVT negative and the DVT positive patients are shown in Table 1. Patients with DVT had statistically significant higher uF1 + 2 levels compared with those without DVT, median 56.8 vs. 24.3 pmol L^{-1} ($p < 0.001$).

In subgroups of patients with infections and traumas (including surgery) during the past three months, higher levels were found in those with DVT compared to those without DVT, without being statistically significant. However, in patients with active cancer and DVT, statistically significant higher uF1 + 2 levels were found compared to those with active cancer without DVT. Similar differences were detected in patients having ongoing treatment with anticoagulants. Nine pregnant women had elevated levels of uF1 + 2 compared to the control cohort (median 71.4 pmol L^{-1}, 25-75 percentile 33.0-286.3, $p < 0.001$) (Table 1).

The control cohort without current comorbidities or treatment with anticoagulants consisted of 177 patients (91 men and 86 women) with median age 55 (range 19-90) years. Median uF1 + 2 levels were 20.0 pmol L^{-1} (25-75 percentile 20.0-39.0). Table 2 shows the uF1 + 2 levels according to gender and DVT status. DVT was diagnosed in 39 (22.0%) patients. Comparing those with and without DVT, statistically significant higher levels of uF1 + 2 were found (median 52.6 vs. 20.0 pmol L^{-1} respectively, $p < 0.001$) (Table 2). In this group unexplained high uF1 + 2 levels (range 53.1-833.6 pmol L^{-1}) were found in 12 (6.8%) patients.

Duration of DVT symptoms was known in 154 (87.0%) patients in the control cohort, 37 (24.0%) of whom had radiologically confirmed DVT. 28 (75.7%) reported symptoms for less than seven days and 9 (24.3%) for a longer duration. The group with short-term symptoms had lower uF1 + 2 levels (median 41.5 pmol L^{-1}, 25-75 percentile 20.0-173.0) than those with a longer duration (median 61.3 pmol L^{-1}, 25-75 percentile 40.1-331.0), but the difference was not statistically significant ($p = 0.173$).

The corresponding sensitivity, specificity, NPV and PPV using the lowest detectable uF1 + 2 level (20 pmol L^{-1}) are given for both cohorts in Table 3. The sensitivity and NPV in both cohorts were almost the same.

Discussion

This clinical study on heterogenous patients with suspected DVT admitted to Department of Radiology, showed that the urine levels of F1 + 2 was significantly higher in patients with imaging confirmed DVT vs. those without. This was true both in the total cohort, in subgroups with active cancer, in patients undergoing treatment with anticoagulants and in the control cohort, i.e. patients without current comorbidities and treatment with anticoagulants. These observations

Table 1
uF1 + 2 levels in the total cohort according to gender and DVT results (n = 534).

		n (%)	uF1 + 2[1]	*p*-value[2]
Gender	Males	260 (48.7)	33.0 (20.0-105.3)	0.077
	Females	274 (51.3)	25.7 (20.0-64.6)	
Total	DVT negative	426 (79.8)	24.3 (20.0-67.3)	<0.001
	DVT positive	108 (20.2)	56.8 (20.0-181.5)	
Anticoagulation	DVT negative	147 (27.5)	22.0 (20.0-55.7)	0.001
	DVT positive	30 (5.6)	58.8 (26.1-185.8)	
Active cancer	DVT negative	27 (5.1)	68.0 (29.8-199.0)	0.034
	DVT positive	10 (1.9)	215.6 (83.9-500.3)	
Infection	DVT negative	30 (5.6)	118.4 (46.2-392.5)	0.444
	DVT positive	2 (0.4)	640.3 (80.5-1200)	
Trauma	DVT negative	74 (13.9)	30.1 (20.0-109.9)	0.609
	DVT positive	24 (4.5)	45.3 (20.0-99.5)	

uF1 + 2 = urine prothrombin fragment 1 + 2.
DVT = deep vein thrombosis.
[1] Values shown are median (pmol L^{-1}) with 25-75 percentiles in parentheses.
[2] Mann-Whitney *U* test.