Venous thrombosis in the elderly: risk factors and consequences

Wang, H.

CHAPTER 3

D-dimer, thrombin generation and risk of a first venous thrombosis in the elderly

Huijie Wang, Frits R. Rosendaal, Mary Cushman, Astrid van Hylckama Vlieg

Res Pract Thromb Haemost. 2021 Jun;5(5):e12536.

ABSTRACT

Background

A high D-dimer level and parameters of the thrombin generation (TG) potential are associated with the risk of a first venous thrombosis (VT) in young and middle-aged populations.

Objectives

To investigate whether D-dimer and TG potential (lag-time, time-to-peak (ttPeak), peak thrombin, endogenous thrombin potential (ETP) and velocity index), are associated with the risk of a first VT in those aged 70 years and older.

Methods

We included 215 patients with a first VT and 358 controls, all aged>70 years, from the Age and Thrombosis, Acquired and Genetic risk factors in the Elderly (AT-AGE) study. To assess the risk of VT, odds ratios (OR) with 95% confidence intervals (CI) were estimated using logistic regression analysis.

Results

D-dimer and all TG parameters except lag-time were associated with an increased risk of VT in a dose-response manner. Comparing the 4th with the 1st quartile (for ttPeak comparing the 1st with the 4th quartile), risk estimates were: 7.8 (CI95: 4.0-15.0) for peak, 2.0 (CI95:1.2- 3.3) for ttPeak, 9.1 (CI95:4.4-18.9) for ETP, and 11.5 (CI95:5.7-23.3) for velocity index. Comparing the highest quartile of D-dimer with the lowest, the risk was 7.7-fold increased (CI95: 4.0-14.8). Furthermore, all factors also increased the risk of VT after dichotomizing at more extreme cut-off values. The risk of VT was further increased in the presence of multiple prothrombotic TG parameters and elevated D-dimer level or in combination with prothrombotic mutations.

Conclusions

D-dimer and TG parameters (except lag time) are associated with the risk of first VT in elderly population.

INTRODUCTION

Venous thrombosis (VT), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE±DVT), is a multi-causal disease [1]. The incidence of VT is 1-2 per 1000 persons per year and increases exponentially with age up to about 1 per 100 persons per year in the very elderly [2]. Many genetic and acquired risk factors for VT affect the coagulation and fibrinolytic system, thus resulting in a hypercoagulable state [3, 4].

The most commonly used assays for hypercoagulability testing include D-dimer and thrombin generation assays (TGA) [5]. Several studies have investigated the association between these measures and VT risk in young and middle-aged populations. Higher D-dimer was consistently associated with an increased VT risk [5-10]. Apart from the less often reported velocity index, which appears consistently associated with VT risk [11, 12], results for other TG parameters (endogenous thrombin potential (ETP), peak thrombin, lag time, time to peak (ttPeak)) were inconsistent [5, 6, 8-10, 13-20]. As reviewed by Pabinger et al., the ETP and peak thrombin were associated with the risk of a first event of VT [8]. Later papers illustrated lag time, ttPeak and velocity index were also associated with VT risk [5, 9- 12]. In contrast, recent findings showed no association with the risk of VT for ETP [6, 9, 11] , peak thrombin[9-11], lag time [15] and ttPeak[13]. As an ex vivo and in vitro indicator of thrombin generation, D-dimer and ETP were reported to be related to the risk of recurrent VT, and combining those two indicators improved the prediction of recurrent VT [21]. Elevated peak thrombin in combination with the Factor V Leiden mutation was also shown to lead to a further increase in VT risk, however, adjustment for D-dimers levels attenuated this risk [17].

Few studies assessed the association between hypercoagulability variables and the risk of VT, specifically in the elderly. In many studies, older individuals were part of the study population. However, no or very limited subgroup analyses were described or sample sizes of the elderly were too small for meaningful analyses [5, 10-12, 15, 16]. Haas et al. reported that ETP, lag time and peak thrombin were associated with the risk of DVT in people aged 75 years and older [13]. The Cardiovascular Health Study (CHS) investigated peak thrombin and D-dimer, which were associated with an increased risk of VT in adults aged 65 years and older [7, 17].

The aim of this study was to assess the association between hypercoagulability (D-dimer and parameters of TG: Peak thrombin, ETP, Lag time, ttPeak, velocity index) and the risk of a first VT (DVT and PE±DVT) in the elderly. Additionally, we studied the risk of VT associated with the combination of prothrombotic thrombin generation parameters, elevated D-dimer and prothrombotic mutations.

METHODS

Study design

All analyses were performed in the Age and Thrombosis, Acquired and Genetic risk factors in the Elderly (AT-AGE) Study, which is a two-center population-based case-control study in Leiden, the Netherlands and Burlington, Vermont, US, designed to study risk factors for VT in an older population. Patients aged 70 years and older with an objectively diagnosed, first episode of VT (DVT or PE±DVT) were included from the files of the anticoagulation clinics in Leiden and Haarlem. Both proximal and distal DVT patients were enrolled in the study. Patients with active malignancy, a history of VT, or severe cognitive impairment (measured with Mini-Mental State Exam (MMSE)) were excluded. Similarly, patients aged 70 years and older, with the same in-and exclusion criteria as in Leiden, were enrolled in Burlington, Vermont, USA. In Burlington, sequential patients were identified through testing in imaging centers. Both study locations covered large geographical areas. In total, 403 patients were included in this study and 433 control subjects were included from primary care practices in the same geographic areas as the patients. The same in- and exclusion criteria as for the patients were applied.

Participation

All participants were visited by a trained research assistant at their homes for an interview and a venipuncture. Data collection was similar in Leiden and Burlington (USA). Patients were visited twice; as soon as possible after the VT event and one year after the event. Control subjects were visited once. During the first home visit, an interview was conducted to ascertain VT event information, medical history, family history, and lifestyle habits, i.e., factors that (possibly) played a role in the development of thrombosis. Weight, height, and blood pressure were measured. Citrated blood was collected in Sarstedt tubes® using the aspiration method (citrate, pre-dosed with 0.106 molar solution (equivalent to 3.2% trisodium citrate) with a mixing ratio of 1:10 (sample 1)). For the patients only, a second home visit about 1 year after diagnosis of the VT, a second venipuncture was performed to collect citrated blood in the absence of anticoagulants or the acute phase response (sample 2). 401 Patients and 431 controls had complete interview data. Since both D-dimer and TG parameters are affected by use of oral anticoagulants, we used sample 2 for the patients and sample 1 for the controls.

Not all patients were available for the second home visit one year after the VT as 21 had died, and 30 declined to participate in the second home visit. In addition, blood collection or assay measurement was unsuccessful in 38 patients, leaving 312 patients. Of these, 93 were still taking vitamin K antagonists at the time of the second visit, therefore they were also excluded from the analysis. 4 Patients had a missing value for one or more

parameters of TG or D- dimer. In total, 215 patients who had complete results for all studied parameters were included in the analyses.

For 24 controls blood collection failed and 38 controls were taking a vitamin K antagonist at the time of blood sampling, thus leaving 369 controls with a blood sample not on oral anticoagulants. Of the controls, 11 had a missing value for one or more parameters of TG or D-dimer levels, resulting in 358 controls who had complete results for all studied parameters and who were included in the analyses.

Provoked VT was defined as thrombosis after hospitalization (including major surgery), fracture, plaster cast, splint, minor injuries of the lower extremities (such as a sprained ankle or contusion of the lower leg) or transient immobility at home ≥4 successive days in the three months before the index date (defined as the date of VT diagnosis for the patients and the date of the home interview for the control subjects). A proximal DVT was defined as involvement of inferior vena cava, iliac, femoral or popliteal veins and a distal DVT was defined as involvement of calf, tibial or gastrocnemius veins. All participants provided written informed consent. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and by the Committee of Human Research of the University of Vermont.

Blood processing and laboratory measurements

All blood samples were collected and processed within four hours. Tubes were centrifuged for 10 min 2500 RPM at 18°C, aliquoted, and frozen at -80°C. All aliquots used for the current analyses had not been thawed before. D-dimer was measured using the HemosIL D- dimer assay (Werfen, Barcelona, Spain), an automated latex enhanced immunoassay on the ACL TOP® Family Systems (Werfen, Barcelona, Spain). Thrombin generation parameters were measured in duplicate using the thrombin generation assay (TGA), a global coagulation test that reproduces the kinetics of thrombin formation, using the Calibrated Automated Thrombogram (CAT)® (Diagnostica Stago, Asinères, France) according to the manufacturer's specifications [22]. Plasma samples were mixed with assay reagents (tissue factor and phospholipids) and the fluorescent signal indicating thrombin generation was monitored in a Fluoroskan Ascent fluorometer (Thermo Scientific, Waltham, MA, USA). Parameters were calculated with the Thrombinoscope Software Program (Thrombinoscope BV, Maastricht, the Netherlands) [23]. The following parameters were included: peak thrombin, which represents the maximum concentration of thrombin formed at any time, the endogenous thrombin potential (ETP), which depicts the total amount of thrombin generated over time and reflects the total enzymatic activity of thrombin, the time to peak (ttPeak), which indicates the time required to reach the maximum amount of thrombin formed, the lag time, which measures the length of time

between the start of the assay and the initiation of TG, which represents the equivalent of the clotting time, and the velocity index, which is defined as [peak height/(time to peak-lag time)] indicating the rate of TG [10, 24].

Statistical analysis

We studied the association between D-dimer levels and parameters of the TG potential and the risk of a first VT in elderly, both as continuous variables and after stratification into categories. Cut-off values to stratify both D-dimer and TG parameters (lag time, ttPeak, peak thrombin, ETP and velocity index) were based on the levels measured in control subjects. High levels of D-dimer, ETP, peak thrombin and velocity index, and low levels of lag time and ttPeak were treated as risk groups. D-dimer, ETP, peak thrombin and velocity index were dichotomized at the 90^{th} percentile, while lag time and ttPeak were dichotomized at the 10^{th} percentile to study the risk of VT associated with extreme levels. Furthermore, the levels were stratified into quartiles to assess the presence of a dose-response relation with the risk of VT. The risk of a first VT was assessed by calculating odds ratios (ORs) with corresponding 95% confidence interval (CIs) after adjustment for age, sex, BMI (continuous), smoking status (current smokers versus never/ever smokers) and study center. The risk of VT was assessed for all VT combined and separately for deep vein thrombosis of the leg (DVT) and pulmonary embolism (PE±DVT) and for provoked and unprovoked events.

We also studied the risk of VT associated with the combination of prothrombotic thrombin generation parameters, elevated D-dimer and prothrombotic mutations (prothrombin G20210A mutation (PT20210A) and factor V Leiden mutation (FVL)). For this analysis, D- dimer, TG parameters were dichotomized at the 75th percentile as measured in controls. Combined analyses of TG parameters, D-dimer, and prothrombotic mutations were adjusted for age, sex, study center, body mass index(BMI) and smoking.

IBM SPSS 23.0 for Windows (SPSS Inc, Chicago, Ill) was used for data analysis.

RESULTS

Table 1 shows the characteristics of the patients and control subjects. The mean age of the controls was 77.3 years (range:70.3-94.2), similar to that of the patients (mean age: 78.1 years; range: 70.0-100.9). In both patients and controls, the majority were women (57.2% of patients and 55% of controls). Smoking status was similar in patients and controls; 10.7% of patients and 13.1% of controls were current smokers. BMI was similar for patients and controls (mean difference: 0.1 kg/m^2; 95% CI: -0.6 to 0.9). Of all patients,

108 (50.2%) had DVT without PE, while 107 (49.8%) were diagnosed with PE with or without DVT. Only 5 (2.3%) patients were diagnosed with both a PE as well as a DVT and 102 (47.4%) patients were diagnosed with a PE alone. Of the patients with DVT, 28 (13.0%) patients had distal DVT and 73 (34.0%) patients had proximal DVT. All provoking risk factors occurred more often in patients than in controls.

Table 1 Characteristics of the study population

Characteristics	**Patients N=215**	**Controls N=358**
Age, mean(range)	78.1 (70.0-100.9)	77.3 (70.3-94.2)
Men, N(%)	92 (42.8)	161 (45.0)
BMI, mean(kg m^{-2}) (range)	27.0 (17.3-43.2)	26.8 (17.0-49.7)
Smoking		
Current, N(%)	23 (10.7)	47 (13.1)
Former+Never, N(%)	192 (89.3)	311 (86.9)
Type VT, N(%)		
Deep vein thrombosis (DVT)	108 (50.2)	-
Distal DVT#	28 (13.0)	-
Proximal DVT#	73 (34.0)	-
Pulmonary embolism +/- deep vein thrombosis (PE ± DVT)	107 (49.8)	-
PE only	102 (47.4)	-
PE + DVT	5 (2.3)	-
Provoked VT*	106 (50.0)	-
Unprovoked VT*	106 (50.0)	-
Provoked factors		
Hospital admission, N(%)	68 (31.6)	18 (5.0)
Fracture, N(%)	15 (7.0)	1 (0.3)
Plaster cast, N(%)	12 (5.6)	3 (0.8)
Immobilisation, N(%)	19 (9.0)	4 (1.1)
Minor injury, N(%)	28 (13.1)	29 (8.1)
Comorbidities		
Heart failure, N(%)	4 (1.9)	9 (2.5)
Angina, N(%)	20 (9.3)	25 (7.0)
Myocardial infarction, N(%)	21 (9.8)	38 (10.6)
Cerebral bleeding, N(%)	4 (2.0)	4 (1.1)
Transient ischemic attack, N(%)	21 (9.8)	34 (9.7)
Cerebral infarction, N(%)	7 (3.3)	18 (5.0)

N=number, VT=venous thrombosis, BMI=body-mass index
*3 missings for provoked and unprovoked VT.
for 7 patients, the exact veins involved in the thrombosis were not reported.
Minor injury: defined as an injury of the lower extremities(hip, knee, ankle or foot) such as a sprained ankle or contusion of the lower leg that started within the three months window.

Mean D-dimer and TG parameters in patients and control subjects are shown in Table 2. D- dimer was higher in patients than in controls (mean difference: 442 ng/mL; 95%CI: 221-663). All TG parameters showed a more prothrombotic profile in the patients compared with control subjects (e.g., mean differences: ETP: 238.4 nM.min (95% CI: 171.1-305.8), peak thrombin: 28.2 nM (95% CI: 18.9-37.4), lag time: -0.0 min (95% CI: ((-0.4) - 0.3), ttPeak: - 0.5 min (95%CI: ((-0.9) - (-0.1)), and velocity index: 6.1 $nM.min^{-1}$ (95%CI: 2.8-9.4) for velocity index). Mean levels of D-dimer and all TG parameters were similar in provoked and unprovoked VT patients.

Table 2 Levels of D-dimer and Thrombin generation parameters at baseline.

Parameter	All patients N=215	Provoked VT patients N=106	Unprovoked VT patients N=106	Controls N=358	Mean difference (all patients-controls) (95%CI)
Hemos IL D-dimer(ng/mL)					
Mean(95%CI)	1260 (1097-1423)	1240 (1004-1475)	1303 (1069-1538)	818 (668-968)	442 (221-663)
Thrombinoscope TG(CAT)					
ETP(nM.min), mean(95%CI)	1401.8 (1332-1471.6)	1339.1 (1285.9-1392.3)	1409.8 (1354-1465.6)	1163.3 (1132-1194.6)	238.4 (171.1-305.8)
Peak(nM), mean(95%CI)	129.4 (119.5-139.4)	121.5 (114-129.1)	129.5 (122.2-136.8)	101.3 (97.3-105.2)	28.2 (18.9-37.4)
Time-to-peak(min), mean(95%CI)	15.2 (14.9-15.5)	15.4 (15-15.8)	15 (14.6-15.5)	15.7 (15.4-15.9)	-0.5 ((-0.9)-(-0.1))
Lag time(min), mean(95%CI)	8.8 (8.5-9)	8.8 (8.5-9.2)	8.6 (8.3-9)	8.8 (8.6-9)	-0.0 ((-0.4)-(0.3))
Velocity index, mean(95%CI)	22.5 (19.4-25.6)	20.4 (18.2-22.5)	22 (20-23.9)	16.4 (15.3-17.5)	6.1 (2.8-9.4)

N=number, CI=confidence interval, ETP=endogenous thrombin potential, CAT=calibrated automated thrombography

The risk of VT associated with one standard deviation (SD) increment was 1.4 (95%CI: 1.1- 1.8) for D-dimer, 2.9 (95%CI: 2.2-3.9) for peak thrombin, 2.8 (95%CI: 2.1-3.7) for ETP, and 3.0 (95%CI:2.2-4.2) for velocity index; The risk of VT associated with one SD decrease was 1.4 (95%CI: 1.1-1.7) for ttPeak, 0.9 (95%CI: 0.7-1.1) for lag time.

Table 3 shows the main results for all potential risk factors after dichotomization. Compared with lower levels, D-dimer, peak thrombin, ETP, and velocity index above the 90th percentile were associated with increased risk of VT, with ORs ranging from 2.1-4.3, also after adjustment for confounding. Shorter ttPeak (<10th percentile) was associated with a 2.4 (95%CI: 1.4-4.2)-fold increased risk compared with longer ttPeak. There was no association between lag time and VT risk. D-dimer levels and parameters of TG (except for lag time) were associated with the risk of both DVT and PE±DVT and with both provoked and unprovoked VT. The risk estimates were more pronounced for unprovoked events than for provoked events (e.g., ETP was associated with a 4.9-fold (95%CI: 2.8-8.6) increased risk of unprovoked VT and a 2.8-fold (95%CI: 1.6-5.1) increased risk of provoked VT). The effect of adjustment for all confounders individually is shown in supplemental table 1. Further adjustment for comorbidities did not affect the risk estimates.

Table 3 Risk of a first venous thrombosis associated with D-dimer levels and TG stratified by types of thrombosis.

	Crude OR (CI95)	OR*All VT (CI95)	OR DVT* (CI95)	OR PE±DVT* (CI95)	OR provoked* (CI95)	OR unprovoked* (CI95)
D-dimer						
≤ P_{90}	1(ref)	1(ref)	1 (ref)	1 (ref)	1(ref)	1(ref)
> P_{90}	2.4 (1.5-3.9)	2.1 (1.3-3.4)	2.8 (1.6-5.0)	1.6 (0.9-3.1)	1.9 (1.0-3.6)	2.3 (1.3-4.3)
Thrombin generation						
Peak thrombin						
≤P_{90}	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
>P_{90}	2.7 (1.7-4.4)	3.7 (2.2-6.2)	3.1 (1.6-5.8)	4.3 (2.3-8.2)	2.7 (1.4-5.2)	4.9 (2.7-9.2)
Time to peak						
≥P_{10}	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
<P_{10}	1.9 (1.2-3.2)	2.4 (1.4-4.2)	2.4 (1.2-4.6)	2.2 (1.1-4.3)	1.6 (0.8-3.4)	3.2 (1.7-6.0)
Lag time						
≥P_{10}	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
<P_{10}	0.7 (0.4-1.4)	0.7 (0.4-1.3)	1.1 (0.5-2.3)	0.4 (0.1-1.0)	0.6 (0.2-1.4)	0.8 (0.4-1.8)
ETP						
≤P_{90}	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
>P_{90}	3.8 (2.4-6.0)	3.8 (2.3-6.1)	3.3 (1.9-5.8)	4.4 (2.4-7.8)	2.8 (1.6-5.1)	4.9 (2.8-8.6)
Velocity index						
≤P_{90}	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
>P_{90}	2.4 (1.5-3.8)	4.3 (2.4-7.9)	3.2 (1.6-6.5)	5.5 (2.7-11.3)	4.0 (2.0-8.2)	4.4 (2.2-8.9)

VT=venous thrombisis, DVT=deep vein thrombosis, PE±DVT= pulmonary embolism,CI=confidence interval, ETP= endogenous thrombin potential, CAT=calibrated automated thrombography

*Adjusted for age, sex, study center, body mass index (BMI) and smoking

Cut-off values: D-dimer 1410 ng/mL (P90), peak thrombin 154 nM (P90), time to peak 13 min (P10), Lag time 7 min (P10), ETP 1519 nM.min (P90), velocity index 29 $nM.min^{-1}$ (P90)

Table 4 shows the risk of venous thrombosis after stratifying the TG parameters and D-dimer into quartiles. D-dimer and all TG parameters except lag time were associated with the risk of VT in a dose-response manner. Comparing the 4^{th} (highest) with the 1^{st} quartile (for ttPeak comparing the 1st with the 4^{th} quartile), risk estimates were 7.8 (95%CI: 4.0-15.0) for peak thrombin, 2.0 (95%CI: 1.2-3.3) for ttPeak, 9.1 (95%CI: 4.4-18.9) for ETP and 11.5 (95%CI: 5.7-23.3) for velocity index. Compared with D-dimer in the lowest quartile (<338ng/ml), the risk was 1.5 (95%CI: 0.8-3.2), 4.2 (95%CI: 2.2-8.2), and 7.7 (95%CI: 4.0-14.8)-fold increased for the 2^{nd}, 3^{rd}, and 4^{th} quartile, respectively.

Table 4 Risk of venous thrombosis associated with D-dimer and TG parameters(quartile)

Percentile	Patients N=215	Controls N=358	OR* (CI95)
D-dimer			
≤P_{25}	16	89	1 (ref)
P_{25}-P_{50}	24	90	1.5 (0.8-3.2)
P_{50}-P_{75}	62	90	4.2 (2.2-8.2)
>P_{75}	113	89	7.7 (4.0-14.8)
Thrombinoscope TG (CAT)			
Peak thrombin			
≤P_{25}	15	89	1 (ref)
P_{25}-P_{50}	33	90	2.2 (1.1-4.4)
P_{50}-P_{75}	72	90	4.3 (2.2-8.4)
>P_{75}	95	89	7.8 (4.0-15.0)
Time to peak			
≤P_{25}	72	90	2.0 (1.2-3.3)
P_{25}-P_{50}	55	90	1.2 (0.7-2.1)
P_{50}-P_{75}	45	92	0.9 (0.5-1.5)
>P_{75}	43	86	1 (ref)
Lag time			
≤P_{25}	55	89	0.8 (0.5-1.4)
P_{25}-P_{50}	54	90	0.9 (0.5-1.5)
P_{50}-P_{75}	54	92	0.9 (0.5-1.4)
>P_{75}	52	87	1 (ref)
ETP			
≤P_{25}	10	89	1 (ref)
P_{25}-P_{50}	42	90	3.9 (1.9-8.3)
P_{50}-P_{75}	58	90	4.9 (2.3-10.4)
>P_{75}	105	89	9.1 (4.4-18.9)
Velocity index			
≤P_{25}	13	89	1 (ref)
P_{25}-P_{50}	44	90	3.7 (1.8-7.7)
P_{50}-P_{75}	61	90	4.8 (2.4-9.7)
>P_{75}	97	89	11.5 (5.7-23.3)

CI=confidence interval, ref=reference, ETP= endogenous thrombin potential, CAT=calibrated automated thrombography

*Adjusted for age, sex, study center, body mass index(BMI) and smoking for D-dimer and thrombin generation parameters.

Cut=off values:

D-dimer : P25 338.8 ng/mL, P50 516.3 ng/mL, P75 848.7 ng/mL

Peak thrombin: P25 72.1 nM, P50 94.8 nM, P75 125.0 nM

Time to peak: P25 14.1 min, P50 15.7 min, P75 17.0 min

Lag time: P25 7.6 min, P50 8.5 min, P75 9.7 min

ETP: P25 942.7 nM.min, P50 1174.7 nM.min , P75 1381.4 nM.min

Velocity index: P25 9.3 $nM.min^{-1}$, P50 13.5 $nM.min^{-1}$, P75: 19.3 $nM.min^{-1}$

As important thrombin generation parameters, peak thrombin and ETP were associated with a significantly increased risk of VT separately. Therefore, we studied the combined effect of high levels of peak thrombin and ETP, D-dimer and prothrombotic mutations on the risk of VT (Table 5 and 6). Individuals with high peak thrombin alone had a 3.5-fold increased risk of VT (OR: 3.5, 95%CI: 2.1-5.9) and individuals with a high D-dimer level alone had a 3.7- fold increased risk of VT (OR: 3.7, 95%CI: 2.3-6.2), both compared with individuals with both low D-dimer and peak thrombin levels (<75th percentile). Individuals with both peak thrombin and D-dimer levels above 75th percentile had a 7.5-fold increased risk of VT (OR: 7.5, 95%CI: 4.2-13.4). Similar risk patterns were observed between ETP and D-dimer, i.e., the VT risk was highest when both ETP and D-dimer were high (>75th percentile).

Table 5 Risk of venous thrombosis of combined analysis of TG parameters (peak and ETP) and D-dimer.

TG parameters	D-dimer	Patients N=215	Controls N=358	OR* (CI95)
Peak				
-	-	58	212	1(ref)
+	-	44	57	3.5(2.1-5.9)
-	+	62	57	3.7(2.3-6.2)
+	+	51	32	7.5(4.2-13.4)
ETP				
-	-	51	211	1(ref)
+	-	51	58	3.4(2.0-5.5)
-	+	59	58	4.1(2.5-6.8)
+	+	54	31	6.8(3.9-11.9)

CI=confidence interval, ref=reference, ETP= endogenous thrombin potential
*Adjusted for age, sex, study center, body mass index(BMI) and smoking
D-dimer, ETP and peak are dichotomized at 75th percentile measured in controls

The risk of VT was also higher for all prothrombotic markers (D-dimer, ETP and peak thrombin) in the presence of prothrombotic mutations. Compared with individuals without prothrombotic mutations and D-dimer levels below the 75th percentile, the OR of VT in the presence of prothrombotic mutations and high D-dimer levels was 5.3 (95%CI: 1.9-14.6). Compared with those without the mutations and low peak levels (<75th percentile), the OR of VT with both mutations and high peak levels was 3.8 (95%CI: 1.3-11.0). Again, similar risk patterns were observed for ETP and prothrombotic mutations.

Table 6 Risk of venous thrombosis of combined analysis of TG parameters (peak and ETP), D-dimer and prothrombotic mutations.

D-dimer and TG parameters	Prothrombotic mutations	Patients N=215	Controls N=358	OR* (CI95)
D-dimer				
-	-	92	254	1(ref)
-	+	10	14	2.0 (0.8-4.7)
+	-	99	83	3.3 (2.2-5.0)
+	+	14	6	5.3 (1.9-14.6)
Peak				
-	-	106	256	1(ref)
-	+	14	13	2.5 (1.1-5.6)
+	-	85	82	3.3 (2.2-4.9)
+	+	10	7	3.8 (1.3-11.0)
ETP				
-	-	100	255	1(ref)
-	+	10	14	1.7 (0.7-4.0)
+	-	91	83	2.7 (1.8-4.0)
+	+	14	6	5.5 (2.0-15.5)

CI=confidence interval, ref=reference, ETP= endogenous thrombin potential
Prothrombotic mutations included prothrombin G20210A mutation and factor V Leiden mutation.
*Adjusted for age, sex, study center, body mass index(BMI) and smoking
D-dimer, ETP and peak are dichotomized at 75th percentile measured in controls

DISCUSSION

In this case-control study among elderly, D-dimer and TG parameters (peak thrombin, ttPeak, ETP, and velocity index) were associated with the risk of VT in a dose-response manner. Similar risk patterns were observed for DVT and PE±DVT and for provoked and unprovoked VT separately, albeit the relative risks of VT were more pronounced for unprovoked events than for provoked events. Compared with patients with low TG (low peak or low ETP) and low D-dimer, the risk of VT was highest among patients with both high TG and high D-dimer. Furthermore, for all prothrombotic markers (peak thrombin, ETP, and D- dimer), the risk was highest in the presence of a prothrombotic mutation (FVL or PT20210A).

A limited number of studies specifically addressed the association between D-dimer or TG parameters and the risk of VT in the elderly. Similar to our results, their results showed that ETP, lag time, peak thrombin and D-dimer levels were associated with the risk of VT in older individuals [7, 13, 17]. Lag time was studied in one previous case-control study in the elderly by Haas and colleagues, and was associated with an increased risk

of VT[13]. We did not find this association. In agreement with the current finding, this study reported no association between ttPeak and the risk of VT. However, the study by Haas et al. consisted of only 30 elderly patients, and therefore had limited power [13].

While evidence in the elderly is limited, associations of TG parameters and D-dimer with VT have been studied frequently in young and middle-aged populations. Higher D-dimer level was associated with VT risk in numerous studies, which is in line with our results in the elderly [5, 6, 8-10]. However, results were inconsistent for most TG parameters (ETP, peak thrombin, lag time, ttPeak) [5, 6, 8-13, 15, 16, 18-20], while only velocity index showed consistent results in previous studies [11, 12]. Similar to our results, ETP was associated with the risk of VT in most studies [5, 8, 10, 12, 15, 16, 18-20], while no association was observed between ETP and risk of VT in a few [6, 11]. In our study, a shortened lag time was not associated with the risk of VT. In contrast, several studies reported lag time was significantly prolonged in patients with suspected VT [6, 9-11] and a prospective study showed an increased risk of VT associated with a shortened lag time [12]. However, even for studies among young and middle aged populations where more evidence is available, it is difficult to compare study results regarding thrombin generation due to differences in the type of substrate used, sample preparation, data processing, presence or absence of thrombomodulin, tissue factor trigger concentration, and activated protein C [17, 25]. We showed an association between a shortened ttPeak and the risk of VT, while other studies indicated a prolonged ttPeak was significantly higher in VT patients [9-11]. Elevated peak thrombin was associated with an increased risk of VT in both our study and previous studies [5, 8, 12, 15, 18, 20]. In accordance with our results, the velocity index was also demonstrated to be associated with the risk of VT in several papers [11, 12].

We report that the risk of VT was highest when both ETP and D-dimer levels were elevated. Eichinger et al. reported a similar pattern for the risk of recurrent VT associated with ETP and D-dimer in patients >18 years old with an objectively confirmed VT[21]. Both findings show that patients with a first VT or recurrent VT can be stratified by in vitro (ETP) and ex vivo (D-dimer) indicators of TG, reflecting the ongoing process of TG. In our study, peak thrombin was positively associated with the risk of VT and participants with both elevated peak thrombin and factor V Leiden were at further increased risk of VT, which was inconsistent with a study by Lutsey et al. [17].

Some limitations should be acknowledged. Our study participants are predominately Caucasian, so results cannot be generalized to other ethnicities. All the study parameters were measured one year after the VT event in patients and may not reflect the coagulable state in these patients before the index date (i.e., reverse causation). However, blood sampling took place more than a year after the venous thrombosis so it is very unlikely

the levels were still affected. Some patients died and some refused to participate at the second home visit (12.7% in patients). In addition, a few samples had to be excluded (e.g., due to failed blood collection or unsucessful assay measurements (9.5% in patients and 5.6% in controls) or people taking vitamin K antagonists (23.2% in patients and 8.9% in controls)), thus resulting in a reduced sample size and potential selection bias. However, only 21 patients died and patients who declined or in whom the assays were not sucessful, were most likely unrelated to their D- dimer or thrombin generation level. Furthermore, as thrombin generation and D-dimer levels are not measured in routine clinical care and are therefore not part of the decision making in continuing or discontinuing oral anticoagulant use, this is, again, most likely random missingness which, if anything, leads to an underestimation of the true risk.

A strength of our study is that: this is one of the largest studies to investigate the association between hypercoagulability and risk of first VT (including DVT/PE±DVT and provoked/unprovoked VT) in individuals aged 70 years and older. Furthermore, we had measurements on two different global assays measuring coagulation, i.e., D-dimer levels and parameters of TG and sample size allowed a combined analysis.

In conclusion, we demonstrated that D-dimer and TG parameters (except lag time) are associated with the risk of first VT in elderly population. Assessing risk of first VT can be optimized by combining TG parameters (peak thrombin and ETP) with D-dimer and prothrombotic mutations.

REFERENCES

1. White RH. The epidemiology of venous thromboembolism. Circulation. 2003;107(23 suppl 1):I4-8.
2. Næss IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrøm J. Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost. 2007;5(4):692-699.
3. Smalberg JH, Kruip MJHA, Janssen HLA, Rijken DC, Leebeek FWG, de Maat MPM. Hypercoagulability and hypofibrinolysis and risk of deep vein thrombosis and splanchnic vein thrombosis. Arterioscler Thromb Vasc Biol. 2011;31(3):485-493.
4. Nakashima MO, Rogers HJ. Hypercoagulable states: an algorithmic approach to laboratory testing and update on monitoring of direct oral anticoagulants. Blood Res. 2014;49(2):85-94.
5. van Hylckama Vlieg A, Baglin CA, Luddington R, MacDonald S, Rosendaal FR, Baglin TP. The risk of a first and a recurrent venous thrombosis associated with an elevated D-dimer level and an elevated thrombin potential: results of the THE-VTE study. J Thromb Haemost. 2015;13(9):1642-1652.
6. Chaireti R, Jennersjo C, Lindahl TL. Thrombin generation and D-dimer concentrations in a patient cohort investigated for venous thromboembolism. Relations to venous thrombosis, factor V Leiden and prothrombin G20210A. The LIST study. Thromb Res. 2009;124(2):178-184.
7. Cushman M, Folsom AR, Wang LU et al. Fibrin fragment D-dimer and the risk of future venous thrombosis. Blood. 2003;101(4):1243-1248.
8. Pabinger I, Ay C. Biomarkers and venous thromboembolism. Arterioscler Thromb Vasc Biol. 2009;29(3):332-336.
9. Hunt BJ, Parmar K, Horspool K, Shephard N, Nelson-Piercy C, Goodacre S. The DiPEP (Diagnosis of PE in Pregnancy) biomarker study: An observational cohort study augmented with additional cases to determine the diagnostic utility of biomarkers for suspected venous thromboembolism during pregnancy and puerperium. Br J Haematol. 2018;180(5):694-704.
10. Wexels F, Dahl OE, Pripp AH, Seljeflot I. Thrombin generation in patients with suspected venous thromboembolism. Clin Appl Thromb Hemost. 2017;23(5):416-421.
11. Riva N, Vella K, Hickey K et al. Biomarkers for the diagnosis of venous thromboembolism: D-dimer, thrombin generation, procoagulant phospholipid and soluble P-selectin. J Clin Pathol. 2018;71(11):1015-1022.
12. D'Alessio A, Marchetti M, Tartari CJ et al. Long term low molecular weight heparin anticoagulant therapy modulates thrombin generation and D-dimer in patients with cancer and venous thromboembolism. Cancer Invest. 2017;35(7):490-499.
13. Haas FJLM, Schutgens REG, Kluft C, Biesma DH. A thrombin generation assay may reduce the need for compression ultrasonography for the exclusion of deep venous thrombosis in the elderly. Scand J Clin Lab Invest. 2011;71(1):12-18.
14. Smalberg JH, Kruip MJHA, Janssen HLA, Rijken DC, Leebeek FWG, de Maat MPM. Hypercoagulability and hypofibrinolysis and risk of deep vein thrombosis and splanchnic vein thrombosis: similarities and differences. Arterioscler Thromb Vasc Biol. 2011;31(3):485-493.
15. ten Cate-Hoek AJ, Dielis AW, Spronk HM et al. Thrombin generation in patients after acute deep-vein thrombosis. Thromb Haemost. 2008;100(2):240-245.
16. Tripodi A, Martinelli I, Chantarangkul V, Battaglioli T, Clerici M, Mannucci PM. The endogenous thrombin potential and the risk of venous thromboembolism. Thromb Res. 2007;121(3):353-359.

17. Lutsey PL, Folsom AR, Heckbert SR, Cushman M. Peak thrombin generation and subsequent venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE) study. J Thromb Haemost. 2009;7(10):1639-1648.

18. Billoir P, Duflot T, Fresel M, Chrétien MH, Barbay V, Le Cam Duchez V. Thrombin generation profile in non-thrombotic factor V Leiden carriers. J Thromb Thrombolysis. 2019;47(3):473-477.

19. van Hylckama Vlieg A, Christiansen SC, Luddington R, Cannegieter SC, Rosendaal FR, Baglin TP. Elevated endogenous thrombin potential is associated with an increased risk of a first deep venous thrombosis but not with the risk of recurrence. Br J Haematol. 2007;138(6):769-774.

20. Tappenden KA, Gallimore MJ, Evans G, Mackie IJ, Jones DW. Thrombin generation: a comparison of assays using platelet-poor and -rich plasma and whole blood samples from healthy controls and patients with a history of venous thromboembolism. Br J Haematol. 2007;139(1):106-112.

21. Eichinger S, Hron G, Kollars M, Kyrle PA. Prediction of recurrent venous thromboembolism by endogenous thrombin potential and D-dimer. Clin Chem. 2008;54(12):2042-2048.

22. Spronk HM, Dielis AW, De Smedt E et al. Assessment of thrombin generation II: Validation of the Calibrated Automated Thrombogram in platelet-poor plasma in a clinical laboratory. Thromb Haemost. 2008;100(2):362-364.

23. Orsi FA, Biedermann JS, Kruip MJHA, et al. Rosuvastatin use reduces thrombin generation potential in patients with venous thromboembolism: a randomized controlled trial. J Thromb Haemost. 2019;17(2):319-328.

24. Tripodi A. Thrombin generation assay and its application in the clinical laboratory. Clin Chem. 2016;62(5):699-707.

25. Kintigh J, Monagle P, Ignjatovic V. A review of commercially available thrombin generation assays. Res Pract Thromb Haemost. 2018;2(1): 42-48.

SUPPLEMENTARY TABLES

Supplemental table 1 Risk of a first venous thrombosis associated with D-dimer levels and TG in different models(choose 90th percentile)

	Patients N=215	Controls N=358	Model 1 OR(CI95)	Model 2 OR(CI95)	Model 3 OR(CI95)	Model 4 OR(CI95)	Model 5 OR(CI95)
D-dimer							
≤ 500 ng.mL^{-1}	36	168	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
> 500 ng.mL^{-1}	179	190	4.4(2.9-6.7)	4.5(2.9-6.8)	4.5(2.9-6.9)	4.4(2.8-6.9)	4.6(2.9-7.4)
≤ 1000 ng.mL^{-1}	123	291	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
> 1000 ng.mL^{-1}	92	67	3.2(2.2-4.7)	3.2(2.2-4.8)	3.1(2.1-4.6)	3.0(2.0-4.5)	3.3(2.1-5.1)
≤ P_{90}	170	323	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
> P_{90}	45	35	2.4(1.5-3.9)	2.4(1.5-3.9)	2.2(1.3-3.6)	2.1(1.3-3.5)	2.5(1.4-4.2)
Thrombin generation							
Peak thrombin							
≤ P_{90}	166	323	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
> P_{90}	49	35	2.7(1.7-4.4)	2.7(1.7-4.4)	3.6(2.1-6.0)	3.7(2.2-6.3)	4.1(2.2-7.5)
Time to peak							
≥ P_{10}	179	324	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
< P_{10}	36	34	1.9(1.2-3.2)	1.9(1.1-3.1)	2.4(1.4-4.1)	2.5 (1.4-4.3)	1.5(0.8-2.8)
Lag time							
≥ P_{10}	199	323	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
< P_{10}	16	35	0.7(0.4-1.4)	0.8(0.4-1.4)	0.7(0.3-1.2)	0.7(0.4-1.3)	0.6(0.3-1.2)
ETP							
≤ P_{90}	152	323	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
> P_{90}	63	35	3.8(2.4-6.0)	4.0(2.5-6.3)	3.6(2.3-5.8)	3.7(2.3-6.0)	3.8(2.3-6.3)
Velocity index							
≤ P_{90}	171	323	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
> P_{90}	44	35	2.4(1.5-3.8)	2.3(1.5-3.8)	4.5(2.5-8.1)	4.4(2.4-7.9)	3.6(1.8-7.1)

N=number, CI=confidence interval, ETP= endogenous thrombin potential, CAT=calibrated automated thrombography
Model1: Unadjusted OR
Model2: Adjusted for age and sex
Model3: Adjusted for age, sex and study center
Model4: Adjusted for age, sex, study center and body mass index(BMI)
Model5: Adjusted for age, sex, study center, body mass index(BMI), smoking and comorbidities(heart failure, angina, myocardial infarction, cerebral bleeding, transient ischemic attack and cerebral infarction).
Cut-off values:
D-dimer: P_{90} 1410 ng/mL
Peak thrombin: P_{90} 154 nM
Time to peak: P_{10} 13 min
Lag time: P_{10} 7 min
ETP: P_{90} 1519 nM.min
Velocity index: P_{90} 29 nM.min^{-1}