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ORIGINAL ARTICLE

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

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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 with 95% confidence intervals (CIs) 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 fourth with the first quartile (for ttPeak comparing the first with the fourth quartile), risk estimates were: 7.8 (95% CI, 4.0-15.0) for peak, 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. Comparing the highest quartile of D-dimer with the lowest, the risk was 7.7-fold increased (95% CI, 4.0-14.8). Furthermore, all factors also increased the risk of VT after dichotomizing at more extreme cutoff 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.

KEYWORDS
D-dimer, elderly, risk, thrombin generation, venous thrombosis

Essentials

- Venous thrombosis (VT) risk associated with D-dimer or thrombin generation in the elderly is unclear.
- A case-control study was performed among the elderly (Age and Thrombosis, Acquired and Genetic Risk Factors in the Elderly [AT-AGE] study).
- D-dimer and thrombin generation parameters (except lag time) were associated with VT risk.
- Risk patterns were similar for provoked/unprovoked VT and for deep vein thrombosis/pulmonary embolism separately.

1 | INTRODUCTION

Venous thrombosis (VT), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE ± DVT), is a multicausal disease.[1] The incidence of VT is 1 to 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 thrombin generation (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,[8] the ETP and peak thrombin were associated with the risk of a first event of VT. Later papers illustrating 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 TG, 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 (FVL) 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[13] reported that ETP, lag time, and peak thrombin were associated with the risk of DVT in people aged ≥75 years. The Cardiovascular Health Study investigated peak thrombin and D-dimer, which were associated with an increased risk of VT in adults aged ≥65 years.[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 TG parameters, elevated D-dimer, and prothrombotic mutations.

2 | MATERIALS AND METHODS

2.1 | 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, United States, designed to study risk factors for VT in an older population. Patients aged ≥70 years 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 patients with proximal DVT and patients with distal DVT were enrolled in the study. Patients with active malignancy, a history of VT, or severe cognitive impairment (measured with the Mini-Mental State Exam) were excluded. Similarly, patients aged ≥70 years, with the same inclusion and exclusion criteria as in Leiden, were enrolled in Burlington, Vermont. In Burlington, sequential patients were identified through testing in imaging centers. Both study locations covered large geographic 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 inclusion and exclusion criteria as for the patients were applied.

2.2 | 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. Patients were visited twice: as soon as possible after the VT event and 1 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, that is, 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, predosed 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). A total of 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 1 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. Four 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 3 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 the inferior vena cava or 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.

2.3 | Blood processing and laboratory measurements

All blood samples were collected and processed within 4 hours. Tubes were centrifuged for 10 minutes at 2500 *g* 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). TG parameters were measured in duplicate using the TGA, a global coagulation test that reproduces the kinetics of thrombin formation, using the Calibrated Automated Thrombogram (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 TG was monitored in a Fluoroskan Ascent fluorometer (Thermo Fisher 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; ETP, which depicts the total amount of thrombin generated over time and reflects the total enzymatic activity of thrombin; 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 and 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]

2.4 | Statistical analysis

We studied the association between D-dimer levels and parameters of the TG potential and the risk of a first VT in the elderly, both as continuous variables and after stratification into categories. Cutoff values to stratify both D-dimer and TG parameters (lag time, tt-Peak, 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 90th percentile, while lag time and ttPeak were dichotomized at the 10th 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, body mass index (BMI; continuous), smoking status (current smokers vs never/ever smokers) and study center. The risk of VT was assessed for all VT combined and separately for DVT of the leg and PE ± DVT and for provoked and unprovoked events.

We also studied the risk of VT associated with the combination of prothrombotic TG parameters, elevated D-dimer, and prothrombotic mutations (prothrombin G20210A mutation [PT20210A] and FVL). For this analysis, D-dimer and 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, BMI, and smoking.

SPSS 23.0 for Windows (IBM, Armonk, NY, USA) was used for data analysis.

3 | 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 and a DVT, and 102 (47.4%) patients were diagnosed with a PE alone. Of the patients with DVT, 28 (13.0%)

TABLE 1 Characteristics of the study population

Characteristics	Patients N = 215	Controls N = 358
Age, y, mean (range)	78.1 (70.0-100.9)	77.3 (70.3-94.2)
Men, N (%)	92 (42.8)	161 (45.0)
BMI, kg m^{-2}, mean (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(%)		
DVT	108 (50.2)	...
Distal DVT[a]	28 (13.0)	...
Proximal DVT[a]	73 (34.0)	...
PE ± DVT	107 (49.8)	...
PE only	102 (47.4)	...
PE + DVT	5 (2.3)	...
Provoked VT[b]	106 (50.0)	...
Unprovoked VT[b]	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)
Immobilization, 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)

Note: 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.

Abbreviations: BMI, body mass index; DVT, deep vein thrombosis; PE, pulmonary embolus; VT, venous thrombosis.

[a]For 7 patients, the exact veins involved in the thrombosis were not reported.

[b]Three missing for provoked and unprovoked VT.

patients had distal DVT, and 73 (34.0%) patients had proximal DVT. All provoking risk factors occurred more often in patients than in controls.

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 (eg, 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 minutes [95% CI, −0.4 to 0.3]; ttPeak, −0.5 minutes [95% CI, −0.9 to −0.1]; and velocity index, 6.1 nM.min^{-1} [95% CI, 2.8-9.4]). Mean levels of D-dimer and all TG parameters were similar in patients with provoked VT and patients with unprovoked VT.

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, and 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 to 4.3, also after adjustment for confounding. Shorter ttPeak (<10th percentile) was associated with a 2.4-fold (95% CI, 1.4-4.2) 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

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)
HemosIL D-dimer, ng/mL					
Mean (95% CI)	1260 (1097-1423)	1240 (1004-1475)	1303 (1069-1538)	818 (668-968)	442 (221 to 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 to 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 to 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 to −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 to 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 to 9.4)

Abbreviations: CAT, calibrated automated thrombography; CI, confidence interval; ETP, endogenous thrombin potential.

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

	Crude OR (95% CI)	OR[a] All VT (95% CI)	OR DVT[a] (95% CI)	OR PE±DVT[a] (95% CI)	OR provoked[a] (95% CI)	OR unprovoked[a] (95% CI)
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)
TG						
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)

Note: Cutoff 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).

Abbreviations: CAT, calibrated automated thrombography; CI, confidence interval; DVT, deep vein thrombosis; ETP, endogenous thrombin potential; PE, pulmonary embolism; TG, thrombin generation; VT, venous thrombosis.

[a]Adjusted for age, sex, study center, body mass index, and smoking.

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 (eg, 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 Table S1. Further adjustment for comorbidities did not affect the risk estimates.

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

Percentile	Patients N = 215	Controls N = 358	OR[a] (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)

Note: Cutoff 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}.

Abbreviations: CAT, calibrated automated thrombography; CI, confidence interval; ETP, endogenous thrombin potential; OR, odds ratio; TG, thrombin generation.

[a]Adjusted for age, sex, study center, body mass index, and smoking for D-dimer and TG parameters.

Table 4 shows the risk of VT 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 fourth (highest) with the first quartile (for ttPeak comparing the first with the fourth 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 (<338 ng/mL), the risk was 1.5 (95% CI, 0.8-3.2), 4.2 (95% CI, 2.2-8.2), and 7.7-fold (95% CI, 4.0-14.8) increased for the second, third, and fourth quartiles, respectively.

As important TG 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 (Tables 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 the 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, that is, the VT risk was highest when both ETP and D-dimer were high (>75th percentile).

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.

4 | DISCUSSION

In this case-control study among the 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,[13] and was

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[a] (95% CI)
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)

Note: D-dimer, ETP, and peak are dichotomized at the 75th percentile measured in controls.

Abbreviations: CI, confidence interval; ETP, endogenous thrombin potential;OR, odds ratio; TG, thrombin generation.

[a]Adjusted for age, sex, study center, body mass index, and smoking.

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[a] (95% CI)
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)

Note: Prothrombotic mutations included prothrombin G20210A mutation and factor V Leiden mutation. D-dimer, ETP and peak are dichotomized at the 75th percentile measured in controls.

Abbreviations: CI, confidence interval; ETP, endogenous thrombin potential;OR, odds ratio; TG, thrombin generation.

[a]Adjusted for age, sex, study center, body mass index, and smoking.

associated with an increased risk of VT. 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[13] consisted of only 30 elderly patients and therefore had limited power.

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 that 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 TG 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 that 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[21] reported a similar pattern for the risk of recurrent VT associated with ETP and D-dimer in patients aged >18 years with an objectively confirmed VT. 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 FVL 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 White, so results cannot be generalized to other ethnicities. All the study parameters were measured 1 year after the VT event in patients and may not reflect the coagulable state in these patients before the index date (ie, 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 (12.7% in patients) refused to participate at the second home visit. In addition, a few samples had to be excluded (eg, due to failed blood collection or unsuccessful 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, patients whom died (21 patients), declined or had failed assay results were mostly unrelated to their D-dimer or TG level. Furthermore, as TG 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. Furthermore, we had measurements on two different global assays measuring coagulation; that is, 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 the elderly population. Assessing risk of first VT can be optimized by combining TG parameters (peak thrombin and ETP) with D-dimer and prothrombotic mutations.

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AUTHOR CONTRIBUTIONS

HW: analysis and interpretation of data, preparation of manuscript. FRR, MC, and AvH: concept and design, interpretation of data, critical revising of the manuscript. All authors approved the final version of the article.

RELATIONSHIP DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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