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Venous thrombosis in the elderly: risk factors and consequences

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PART I

**The association between risk factors
and the risk of venous thrombosis
in the elderly**





CHAPTER 2

Procoagulant factor levels and risk of venous thrombosis in the elderly

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ABSTRACT

Background

Venous thrombosis (VT) incidence increases markedly with age. Coagulation factors are also positively associated with age.

Objective

To study whether higher levels of coagulation factors II (prothrombin),VIII, IX and XI are associated with risk of a first VT in the elderly.

Methods

401 patients and 431 control subjects aged 70 and older were included in the Age and Thrombosis, Acquired and Genetic risk factors in the Elderly (AT-AGE) study. Blood was collected one year after the event in patients and in all control subjects for measurement of coagulation factors. To assess the risk of VT, odds ratios were calculated after stratification of coagulation factors in quartiles and at the 90th percentile, adjusting for potential confounders (age, sex, body mass index, and study center).

Results

Mean age was 78 years (range: 70-100 years). The ORs of VT for factors in the top quartile compared with the lowest quartile were 4.5 (CI95: 2.7-7.3) for factor VIII, 2.4 (CI95: 1.1-5.2) for factor IX, and 1.7 (CI95: 1.0-2.9) for factor XI. High prothrombin was not associated with an increased VT risk. There was no dose-response association between the number of high coagulation factors and VT risk. The population attributable risk (PAR) of VT was 37.6%, 23.3% and 12.4% for factor VIII, IX, and XI, respectively.

Conclusion

In this study of elderly, higher factors VIII, IX and XI but not prothrombin, were positively associated with the risk of VT.

INTRODUCTION

The incidence of venous thrombosis (VT) in the general population is 1-2 per 1000 per year, rising sharply with age to 1 per 100 annually in the very old [1]. Increasing age is by far the most important risk factor for VT and the reasons for this are largely unknown. Because of the multi-causal character of VT, it is likely that multiple factors contribute to the risk of VT in the elderly [2].

Some acquired risk factors for thrombosis, e.g. immobilization, severe medical disorders, malignant disease, and heart failure, are more prevalent in the elderly than in younger populations [3-5], which explains part of the increase in incidence with age. However, after adjustment for this increased prevalence and possible accumulation of risk factors in the elderly, the risk of VT is still positively associated with age [3, 4].

Since the levels of coagulation factors VIII, IX and XI all increase with age [5-9] and are associated with VT risk [10-15], they are candidates for consideration as age-specific VT risk factors. To date, associations between coagulation factors and VT risk have been mainly established in studies that included patients under 70 years of age, since the elderly are often excluded from clinical studies on etiology and management because of comorbidities, short life expectancies, and logistical difficulties [16].

Few studies addressed the association between procoagulant factors and risk of VT in the elderly. The Cardiovascular Health Study (CHS) which is a population-based cohort study with people aged 65 years or older in the United States indicated factors VIII and XI were each positively associated with the risk of VT [12, 17]. No association was observed between prothrombin and factor IX and the risk of VT [15, 18]. A hospital-based case-control study published by Oger et al, with patients 70 years and older, concluded that higher factor VIII activity was associated with VT [19]. While the cohort of CHS contains a large sample of elderly individuals, the number of patients with VT in that study as well as in the case-control study by Oger et al was small. Therefore replication of these findings in a larger sample is indicated. Knowledge on the associations between procoagulant factors and the risk of VT in the elderly may guide physicians in the identification of high risk individuals and subsequent targeted prophylactic treatment in those at high risk.

The aim of this study was to assess whether high levels of coagulation factors previously reported in relation to VT risk in young and middle-aged populations were also associated with the risk of a first VT in the elderly. We evaluated coagulation factors II (prothrombin), VIII, IX and XI in a large population-based case-control study including 401 patients and 431 controls aged 70 years and older.

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 older people. As previously reported [20], patients aged 70 years and older with an objectively diagnosed, first episode of VT, i.e., deep vein thrombosis of the leg or a pulmonary embolism (DVT or PE (with or without DVT)) were included from the files of the anticoagulation clinics in Leiden and Haarlem. Diagnostic tests included compression ultrasonography, Doppler ultrasound, impedance plethysmography and contrast venography for diagnosis of deep vein thrombosis and perfusion and ventilation lung scanning, spiral computer tomography and pulmonary angiography for pulmonary embolism. 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 inclusion 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 cover large geographical areas. In total, 403 patients and 433 individuals without VT from primary care practices in the same geographic areas were included in this study. The same inclusion and exclusion criteria as for the patients were applied to controls. For this analysis we included 401 patients and 431 controls with complete interview data.

Participation

All participants were visited by a trained research assistant at their homes for an interview and a venipuncture. Data collection was performed identically in Leiden and Burlington (USA). Patients were visited twice, i.e., as soon as possible after the VT event and again one year after the event; control subjects were visited only once. During the first home visit, an interview was conducted to ascertain VT event information, medical history, family history, and lifestyle habits. Weight, height, and blood pressure were also 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)). During the second home visit in patients only, 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).

Not all patients were available for the second home visit at one year after the VT as 21 of them had died after the first visit and 24 declined to participate in the second home visit. Furthermore, in 44 patients and 26 controls, blood collection failed or the coagulation

assays were unsuccessful, leaving 312 patients and 405 controls for the analyses of the non-vitamin K dependent coagulation factors (factor VIII and XI). Vitamin K antagonists were still used by 149 patients at the time of the second home visit, and they were excluded from the analysis of vitamin K dependent coagulation factors, as were 36 controls who used these anticoagulants at their home visit. Thus, 163 patients and 369 controls could be included for the analyses of the vitamin K dependent coagulation factors (factor II and IX).

VT events were classified as provoked and unprovoked. Provoked VT was defined as thrombosis after hospitalization, (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).

All participants provided written informed consent. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center (protocol number: P08.066) and by the Committee of Human Research of the University of Vermont (protocol number: CHRMS M09-008).

Blood collection and laboratory measurements

All blood was collected and processed within four hours. Blood tubes were centrifuged for 10 min 2500 RPM at 18°C, aliquoted, and frozen at -80°C. Plasma samples which had not been previously thawed were retrieved for controls and at one year after the thrombotic event for the patients. The coagulation factor levels were measured as activity levels in one laboratory on the ACL TOP (Werfen, Barcelona, Spain).

In every run, two commercial controls, i.e., a normal and a low control, and normal pooled plasma, were included. Using these three quality control (QC) samples, the coefficients of variation (CV) ranged between 5.8-7.3% for factor II, 5.9-11.0% for factor VIII, 3.9-4.9% for factor IX, 3.7-4.3% for factor XI.

Statistical analysis

First, we assessed the risk of VT associated with the levels of all coagulation factors as continuous variables. Secondly, we categorized coagulation factor levels into quartiles based on the distribution in controls and also dichotomized levels at the 90th percentile. In addition to the assessment of the risk of VT associated with individual coagulation factors, we assessed the risk of VT associated with the combined effect of factor IX and its co-factor FVIII because the formation of the intrinsic tenase complex is an essential event in the procoagulant reactions that lead to clot formation [21].

We investigated the association between the number of coagulation factor levels which were high (defined as $>P75$, and including only those factors individually associated with VT risk) and the risk of VT to assess whether the burden of elevated procoagulant factors adds to VT risk. We separately analyzed associations between coagulation factors and provoked and unprovoked VT, and between coagulation factors and the type of VT (DVT only or PE with or without DVT). This study is a case-control study sampled from a dynamic population. In this study setting, the odds ratio is a perfect estimation of the rate ratio (relative risk), and can be interpreted as such [22].

Population attributable risks (PAR) were estimated as $PAR = pd \cdot (OR - 1) / (OR)$, in which pd refers to the proportion of patients exposed to the risk factor of interest. The PAR indicates the proportion of the total incidence of VT that can be attributed to the specific risk factor.

Sensitivity analysis

Sensitivity analyses with imputed data for missing coagulation factor levels replicated all main analyses. We used multiple imputation for 89 patients and 26 controls with missing data on coagulation factor levels. For an additional 149 patients who were still using vitamin K antagonists at the time of both blood collections, the levels of vitamin K dependent factors II and IX were imputed. Controls ($n=36$) who were using vitamin K antagonists for other indications than VT were excluded.

In the imputation model, missing coagulation factor levels of controls and patients were imputed separately. We imputed missing coagulation factors levels in controls using the information on age, sex, BMI, study center, provoking factors (hospital admission, fracture, plaster cast, immobilization and minor injury), and for the patients, in addition to all the parameters above, we also accounted for coagulation factor levels measured in samples obtained during the first visit. IBM SPSS 23.0 for Windows (SPSS Inc, Chicago, Ill) was used for all data analysis.

RESULTS

Table 1 shows the characteristics of patients and control subjects. The mean age in controls was 77.5 years (range: 70.2 – 96.3), which was similar to patients (mean age: 78.7; range: 70.0-100.9). In the patients, there were more women than men (58.5% versus 41.5%). Of all patients, 166 (41.4%) had DVT without PE, 213 (53.1%) were diagnosed with PE without a DVT diagnosis, and 22 (5.5%) had both PE and DVT. Of the patients, 131 (32.7%) had had a hospital admission within three months before the index date compared with 29 (6.7%) controls. Other acquired risk factors including fractures, plaster cast use,

immobilization, and minor injuries within three months before the index date occurred more often in patients than controls. In our study population, 96.2% of the participants were Caucasian.

Table 1. Baseline characteristics

	Controls	Patients
N	431	401
Men, N(%)	209(48.5)	166 (41.5)
Age, mean(range)	77.5(70.2-96.3)	78.7(70.0-100.9)
BMI, mean(kg m ⁻²)(range)	27.0(17.0-49.7)	27.2 (14.5-45.4)
Type of VT, N(%)		
Deep vein thrombosis (DVT)	-	166 (41.4)
Pulmonary embolism (PE)	-	213 (53.1)
PE+DVT	-	22 (5.5)
Hospital admission, N(%)	29 (6.7)	131 (32.7)
Surgery, N(%)	16 (3.7)	73 (18.2)
Fracture, N(%)	3 (0.7)	27 (6.7)
Plaster cast, N(%)	4 (0.9)	21 (5.2)
Immobilization, N(%)	8 (1.9)	39 (9.7)
Minor injury, N(%)	33 (7.7)	49 (12.2)

N=number , VT=venous thrombosis, BMI=body-mass index

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.

Table 2 shows the associations between coagulation factor levels and characteristics of controls. Mean coagulation levels in women were slightly higher than in men. Furthermore, The levels of all coagulation factors did not differ much between age subgroups. None of the coagulation factors showed a consistent trend across age or BMI categories.

In the analysis of the association between levels of coagulation factors as continuous variables and the risk of VT, the odds ratios of VT associated with an increase in ten units of the coagulation factors were 0.74 (CI95: 0.66-0.90) for prothrombin, 1.22 (CI95: 1.10-1.22) for factor VIII, 1.22 (CI95: 1.10-1.34) for factor IX and 1.10 (CI95:1.01-1.22) for factor XI. This model assumes a linear increase in the risk of venous thrombosis associated with coagulation factor levels. As this model may not correctly describe the data, we also stratified the levels into quartiles using the lowest quartile as the reference category.

Table 2. Associations between baseline characteristics and coagulation factors in controls.

	N (%)	Factor II mean(SD) IU/dL	Factor VIII mean(SD) IU/dL	Factor IX mean(SD) IU/dL	Factor XI mean(SD) IU/dL
Sex					
Men	209 (49)	83 (26)	134 (37)	109 (35)	102 (23)
Women	222 (51)	95 (21)	135 (29)	118 (26)	115 (20)
Age(y)					
70-75	175 (40)	92 (22)	129 (31)	113 (26)	110 (21)
75-80	129 (30)	88 (25)	136 (35)	115 (36)	108 (26)
80-85	85 (20)	86 (27)	141 (36)	113 (32)	107 (22)
>85	42 (10)	86 (27)	140 (30)	115 (35)	104 (19)
BMI, kg/ m ² *					
17-27	240 (55.7)	89 (24)	132 (33)	111 (32)	107 (23)
27-37	169 (39.2)	89 (26)	137 (32)	116 (29)	110 (22)
37-47	12 (2.8)	94 (13)	150 (26)	141 (20)	120 (16)
>47	2 (0.5)	91 (5)	113 (20)	126 (4)	122 (36)

N=number, SD=standard deviation, BMI=body-mass index

*number of missing is 8(1.8%)

Using quartiles of the coagulation factor levels, all except factor II were positively associated with the risk of VT. Compared with the lowest quartile, the odds ratios were 4.5 (CI95: 2.7- 7.3), 2.4 (CI95: 1.1-5.2), and 1.7 (CI95: 0.9-2.9) in the highest quartiles of factors VIII, IX and XI, respectively. In contrast, factor II was not associated with an increased risk of VT (OR 0.3, CI95: 0.1-0.6) (Table 3). The ORs were not substantially different when we dichotomized the coagulation factors at the 90th percentile (Table 4).

Table 3. Coagulation factor levels and risk of VT.

Coagulation factor	Patients**	Controls**	Model 1 OR (95CI)	Model 2 OR (95CI)	Model 3 OR (95CI)	Model 4 OR (95CI)
Factor II						
<P ₂₅	25	63	1(ref)	1(ref)	1(ref)	1(ref)
P ₂₅ -P ₅₀	55	99	1.4(0.8-2.4)	1.3(0.7-2.3)	1.0(0.6-1.9)	0.9(0.5-1.7)
P ₅₀ -P ₇₅	60	116	1.3(0.7-2.4)	1.3(0.7-2.3)	0.9(0.5-1.7)	0.8(0.4-1.6)
>P ₇₅	23	91	0.6(0.3-1.1)	0.5(0.3-1.1)	0.3(0.1-0.7)	0.3(0.1-0.6)
Factor VIII						
<P ₂₅	33	101	1(ref)	1(ref)	1(ref)	1(ref)
P ₂₅ -P ₅₀	45	111	1.2(0.7-2.1)	1.2(0.7-2.0)	1.3(0.7-2.2)	1.2(0.7-2.1)
P ₅₀ -P ₇₅	83	93	2.7(1.7-4.4)	2.5(1.5-4.0)	2.8(1.7-4.6)	2.5(1.5-4.2)
>P ₇₅	151	101	4.4(2.7-7.0)	4.2(2.6-6.8)	4.7(2.9-7.6)	4.5(2.7-7.3)
Factor IX						
<P ₂₅	14	63	1(ref)	1(ref)	1(ref)	1(ref)
P ₂₅ -P ₅₀	31	96	1.5(0.7-3.0)	1.6(0.8-3.2)	1.3(0.6-2.7)	1.3(0.6-2.8)
P ₅₀ -P ₇₅	53	113	2.1(1.1-4.1)	2.5(1.3-5.1)	1.7(0.9-3.5)	2.1(1.0-4.2)
>P ₇₅	65	97	2.8(1.5-5.5)	3.2(1.5-6.6)	2.3(1.2-4.6)	2.4(1.1-5.2)
Factor XI						
<P ₂₅	46	101	1(ref)	1(ref)	1(ref)	1(ref)
P ₂₅ -P ₅₀	86	108	1.8(1.2-2.9)	1.9(1.2-3.0)	1.6(1.0-2.6)	1.7(1.0-2.7)
P ₅₀ -P ₇₅	86	96	2.2(1.4-3.6)	2.3(1.4-3.7)	1.9(1.1-3.2)	1.9(1.1-3.1)
>P ₇₅	94	100	2.1(1.2-3.5)	2.0(1.2-3.4)	1.8(1.0-3.0)	1.7(1.0-2.9)

N=number, OR=odds ratio, CI=confidence interval, ref=reference group, VT=venous thrombosis.

**312 patients and 406 controls for non-vitamin K dependent factor VIII, 312 patients and 405 controls for non-vitamin K dependent factors XI, 311 patients, 163 patients and 369 controls for vitamin K dependent factors(factor II and IX).

Model 1: Adjusted for age and sex

Model 2: Adjusted for age,sex and body mass index(BMI)

Model 3: Adjusted for age,sex and study center

Model 4: Adjusted for age, sex, body mass index(BMI) and study center

Cut-off values for quartiles:

Factor II- P₂₅ 86 IU/dL P₅₀ 94 IU/dL P₇₅ 103 IU/dL

Factor VIII- P₂₅ 112 IU/dL P₅₀ 132 IU/dL P₇₅ 152 IU/dL

Factor IX- P₂₅ 104 IU/dL P₅₀ 117 IU/dL P₇₅ 132 IU/dL

Factor XI- P₂₅ 93 IU/dL P₅₀ 108 IU/dL P₇₅ 123 IU/dL

Table 4. Associations of coagulation factor levels in the top decile with VT.

Coagulation factor	Patients **	Controls **	Model 1 OR (95CI)	Model 2 OR (95CI)	Model 3 OR (95CI)	Model 4 OR (95CI)
Factor II						
<P ₉₀	154	331	1(ref)	1(ref)	1(ref)	1(ref)
>P ₉₀	9	38	0.5(0.2-1.1)	0.5(0.3-1.2)	0.4(0.2-0.9)	0.4(0.2-1.0)
Factor VIII						
<P ₉₀	240	364	1(ref)	1(ref)	1(ref)	1(ref)
>P ₉₀	72	42	2.5(1.7-3.9)	2.6(1.7-4.0)	2.9(1.9-4.5)	2.9(1.9-4.5)
Factor IX						
<P ₉₀	122	325	1(ref)	1(ref)	1(ref)	1(ref)
>P ₉₀	41	44	2.3(1.4-3.8)	2.4(1.5-4.0)	2.1(1.3-3.4)	2.1(1.2-3.5)
Factor XI						
<P ₉₀	259	365	1(ref)	1(ref)	1(ref)	1(ref)
>P ₉₀	53	40	1.9(1.2-3.0)	1.8(1.2-2.9)	1.9(1.2-3.0)	1.8(1.1-2.9)

N=number, OR=odds ratio, CI=confidence interval, ref=reference group,VT=venous thrombosis.

**312 patients and 406 controls for non-vitamin K dependent factor VIII, 312 patients and 405 controls for non-vitamin K dependent factor XI, 163 patients and 369 controls for vitamin K dependent factors(factor II and IX).

Model 1: Adjusted for age and sex

Model 2: Adjusted for age, sex and body mass index(BMI)

Model 3: Adjusted for age, sex and study center

Model 4: Adjusted for age, sex, body mass index(BMI) and study center

Cut-off values for 90th percentile of coagulation factors:

Factor II- P₉₀ 110 IU/dL

Factor VIII- P₉₀ 176 IU/dL

Factor IX- P₉₀ 143 IU/dL

Factor XI- P₉₀ 135 IU/dL

To assess whether the burden of elevated procoagulant factors added to VT risk, we considered the coagulation factors which were individually associated with VT risk, i.e., factors VIII, IX and XI, as shown in Table 5. Using the 75th percentile as a cut-off as the risk of VT increased above 75th percentile for all the included factors, we compared individuals with one or more high coagulation factors with individuals without any high coagulation factor as a reference category. The OR was 1.8, 2.3, and 1.9 when one, two, or three coagulation factors were >75th percentile compared with none of the coagulation factors >75th percentile, indicating no clear trend for an increasing risk of VT associated with the number of high coagulation factors could be observed. Overall, similar risk patterns were observed for provoked and unprovoked VT and for DVT and PE±DVT (Table 5).

Table 5. The risk of VT associated with increasing number of coagulation factors (Factor VIII, Factor IX and Factor XI, all coagulation factors dichotomized at P₇₅).

Number of elevated factors	Controls N	Patients N	OR overall* (CI95)	OR provoked* (CI95)	OR unprovoked* (CI95)	OR DVT* (CI95)	OR PE±DVT* (CI95)
0	118	219	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
1	97	105	1.8(1.2-2.6)	1.4(0.9-2.3)	2.1(1.3-3.5)	1.9(1.2-3.2)	1.7(1.1-2.7)
2	64	52	2.3(1.4-3.6)	1.6(0.9-2.9)	3.1(1.8-5.5)	2.6(1.4-4.9)	2.0(1.1-3.5)
3	33	28	1.9(1.0-3.5)	1.8(0.9-3.8)	2.0(0.9-4.3)	1.8(0.8-4.1)	1.9(0.9-3.8)

*OR adjusted for age, sex, body mass index(BMI) and study center N=number, VT=venous thrombosis, ref=reference group

In the combined analysis of co-factors VIII and IX, compared with individuals with factors VIII and IX below the 75th percentile, individuals with high factor IX alone had no increased risk of VT (OR 1.0, CI 95 0.5-1.7) and individuals with high factor VIII alone had a 4.2-fold increased risk of VT (OR 4.2, CI95 2.7-6.4). Participants with both factor VIII and factor IX above the 75th percentile were not at further increased risk compared with those with either high factor VIII alone or high factor IX alone (Table 6).

Table 6. Association with high coagulation factors FVIII and FIX (coagulation factors dichotomized at P₇₅)

Factor VIII	Factor IX	Controls N	Patients N	OR overall* (CI95)
-	-	136	260	1(ref)
-	+	25	45	1.0(0.5-1.7)
+	-	97	50	4.2(2.7-6.4)
+	+	54	51	1.9(1.1-3.1)

*OR adjusted for age, sex, body mass index(BMI) and study center N=number, VT=venous thrombosis, ref=reference group

The population attributable risk (PAR) of VT was 37.6% for factor VIII (>P75), 23.3% for factor IX (>P75), and 12.4% for factor XI (>P75).

Results of the sensitivity analyses using multiple imputation of missing levels of coagulation factors were similar to the main analysis except for the analysis of the burden of elevated coagulation factors where, in the sensitivity analysis, a trend for an increasing risk of VT associated with the number of high coagulation factors was observed (see supplemental tables 1-4).

DISCUSSION

In this study, high levels of coagulation factors VIII, IX and XI, but not prothrombin, were associated with the risk of VT in elderly individuals. The risks were increased, with adjusted ORs of VT between 2 and 4 for levels in the top quartile compared with the lowest quartile. In the main analysis, there was no clear dose-response association between the number of elevated coagulation factors and the risk of VT. Similar risk patterns were observed for provoked and unprovoked VT and for DVT and PE separately. Unexpectedly, we found no association between high prothrombin levels and risk of VT.

The CHS study reported similar results in those aged ≥ 65 , with an association between procoagulant factors VIII and XI and VT during an average 7.8 years of follow-up [17], whereas no association was observed between prothrombin level and the risk of VT [18]. We previously reported the frequency of carriers of prothrombin G20210A in the patients and controls included in this study, which was 2.3% and 1.6%, respectively [23]. The risk of VT was a 1.4-fold (95%CI 0.5-3.9) increase in carriers of the prothrombin mutation compared with non-carriers [23], indicating that also the prothrombin 20210A mutation is, at most, weakly associated with the risk of VT in the elderly. Furthermore, factor IX was associated with the risk of VT in univariate and multivariate analysis in CHS, but not after adjustment for body mass index and diabetes [15]. Similar results were reported in the Atherosclerosis Risk In Communities (ARIC) study, which examined adults aged 45 to 64 years [15, 17, 18].

In our study, we included a higher proportion of women than men. Previous research shows that incidence rates are higher in women during childbearing years, while incidence rates after 45 years are generally higher in men. However, at old age, this difference appears much less pronounced. In the paper by Naess et al., describing a population from central Norway (the HUNT2-study)[1], it was shown that after the age of 70, the incidence of DVT and PE is similar or even higher in women than in men (e.g., the incidence DVT for 85+year-old men: 4.05 (2.59-6.36), for women: 4.73 (3.54-6.31); the incidence PE for 85+year-old men: 1.49 (0.71-3.13), for women: 2.67 (1.82-3.93)). In line with these numbers, in our study population, we included consecutive patients with VT, which included a slightly larger percentage of women than men.

Our results showed that the levels of all coagulation factors did not differ much between age subgroups, and none of the coagulation factors showed a consistent trend across age in control groups. Most studies describing the age trend in coagulation factor levels consider the age range from 18-70 years old. In our data set, we included people aged 70 years and older, and, therefore, we were interested in whether this trend also continued at old age.

We did not see a further increase in coagulation factor levels when comparing individuals aged 70-75 with those >85, indicating a threshold effect after 70 [24]. Nonetheless, the mean coagulation factor levels in controls of our study population are high, which is in accordance with the previously described age increase in younger individuals.

Inherent to a study design in which analyses are restricted to elderly individuals, a potential limitation may be the presence of index event bias. Due to index event bias, some risk factors may seem unrelated to the outcome or risk estimates may even reverse, i.e., risk factors may seem protective[25]. This bias could therefore explain the absence of an association between high prothrombin levels and the risk of VT or even the seemingly protective effect. For index event bias to affect the risk estimates, the risk factor of interest should be associated with survival until the age of 70. Furthermore, there should be other risk factors associated with survival until the age of 70, which in turn are also associated with the outcome, i.e., VT. The latter is possible, e.g., comorbidities associated with mortality as well as the risk of VT. However, the association between high coagulation factors and survival until old age is, if anything, weak, which suggests that index event bias in this study is unlikely. Moreover, the risk estimates for the other procoagulant factors were in the expected direction. As elevated levels of prothrombin have been associated with an increased venous thrombosis risk multiple times, the seemingly protective effect of prothrombin levels in our study is most likely due to chance. In CHS study, prothrombin levels did not play a role in thrombosis in the elderly, and we therefore come to a similar conclusion, i.e., that prothrombin levels were not associated with an increased risk of VT in elderly.

While our observed associations were modest with odds ratios slightly lower than those reported in young and middle-aged populations, in this age group, the PARs associated with high levels of coagulation factors were not trivial. This is because the absolute risk of VT increases sharply with age, which emphasizes the importance of these risk factors in the elderly population.

Our study has several other limitations. First, 96.2% of the participants were Caucasian, so we cannot generalize our findings to other ethnicities. Second, blood samples were obtained after the VT, so it remains a theoretical possibility that coagulation factors were high as a consequence of thrombosis. However, because the blood samples were obtained a year after the VT event, observed associations are unlikely to be due to acute-phase effects. Due to the fact that some patients were using oral anticoagulants during the second home visit, sample size for the analysis of the vitamin K dependent coagulation factors was limited in some subgroup analysis. However, this is unlikely to have affected the results as sensitivity analysis after multiple imputation lead to similar results.

The main strength of our study is that it is one of the largest studies on VT risk in the elderly (individuals aged 70 years and older). Recruiting elderly patients can be difficult, but the use of home visits here facilitated participation. Furthermore, we measured multiple coagulation factors, enabling us to directly compare risk estimates and assess the risk of VT associated with multiple high coagulation factors.

Knowledge of the associations between procoagulant factors and the risk of VT in the elderly may guide physicians in identifying high-risk individuals and subsequent targeted prophylactic treatment in those at high risk. It remains difficult to draw conclusions on the causality of the association. Future studies could focus on a broader range of genetic variants that have been associated with levels of coagulation factors in young and middle-aged populations.

In conclusion, this study demonstrates that elevated levels of factor VIII, IX and XI, but not prothrombin, are associated with the risk of VT in the elderly.

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SUPPLEMENTARY TABLES

Supplemental table 1. Coagulation factor levels and risk of VT after imputation of missing data.

Coagulation factor	Patients**	Controls**	Model 1 OR(95CI)	Model 2 OR (95CI)	Model 3 OR (95CI)	Model 4 OR (95CI)
Factor II						
<P ₂₅	72.6	71.5	1(ref)	1(ref)	1(ref)	1(ref)
P ₂₅ -P ₅₀	141.4	103.5	1.3(0.8-2.3)	1.4(0.8-2.3)	1.2(0.6-2.1)	1.2(0.6-2.1)
P ₅₀ -P ₇₅	138.7	120.5	1.1(0.7-1.8)	1.1(0.7-1.9)	0.9(0.5-1.5)	0.9(0.5-1.5)
>P ₇₅	48.3	95.5	0.5(0.3-0.9)	0.5(0.3-0.9)	0.4(0.2-0.6)	0.4(0.2-0.7)
Factor VIII						
<P ₂₅	40.6	104.7	1(ref)	1(ref)	1(ref)	1(ref)
P ₂₅ -P ₅₀	55	117	1.2(0.7-2.0)	1.2(0.7-2.0)	1.2(0.7-2.1)	1.2(0.7-2.1)
P ₅₀ -P ₇₅	102.3	98.9	2.6(1.6-4.2)	2.6(1.6-4.3)	2.7(1.6-4.4)	2.7(1.6-4.4)
>P ₇₅	203.1	110.4	4.5(2.9-7.0)	4.6(2.9-7.1)	5.0(3.2-7.9)	5.0(3.1-7.8)
Factor IX						
<P ₂₅	35.7	68.5	1(ref)	1(ref)	1(ref)	1(ref)
P ₂₅ -P ₅₀	72.1	100.8	1.4(0.8-2.5)	1.4(0.8-2.6)	1.3(0.7-2.3)	1.3(0.7-2.3)
P ₅₀ -P ₇₅	129.7	119.6	2.1(1.2-3.7)	2.2(1.3-3.8)	1.9(1.1-3.2)	1.9(1.1-3.2)
>P ₇₅	163.5	102.1	3.0(1.7-5.3)	3.2(1.8-5.7)	2.7(1.5-4.7)	2.6(1.5-4.7)
Factor XI						
<P ₂₅	58.4	108.9	1(ref)	1(ref)	1(ref)	1(ref)
P ₂₅ -P ₅₀	111.4	114.3	1.9(1.2-3.1)	1.9(1.2-3.1)	1.7(1.0-2.8)	1.7(1.0-2.8)
P ₅₀ -P ₇₅	110.9	102.8	2.1(1.3-3.4)	2.1(1.3-3.4)	1.9(1.2-3.1)	1.9(1.2-3.0)
>P ₇₅	120.3	105	2.3(1.4-3.8)	2.3(1.4-3.8)	2.0(1.2-3.4)	2.0(1.2-3.3)

N=number, OR=odds ratio, CI=confidence interval, ref=reference group.

**401 patients and 431 controls for non-vitamin K dependent factors VIII and XI.

**401 patients and 391 controls for vitamin K dependent factors II and IX.

Model 1: Adjusted for age and sex

Model 2: Adjusted for age, sex and body mass index(BMI)

Model 3: Adjusted for age, sex and study center

Model 4: Adjusted for age, sex, body mass index(BMI) and study center

Supplemental table 2. Associations of coagulation factor levels in the top decile with VT after imputation of missing data.

Coagulation factor	Patients**	Controls**	Model 1 OR (95CI)	Model 2 OR (95CI)	Model 3 OR (95CI)	Model 4 OR (95CI)
Factor II						
<P ₉₀	382	350.9	1(ref)	1(ref)	1(ref)	1(ref)
>P ₉₀	19	40.1	0.4(0.2-0.9)	0.4(0.2-0.9)	0.4(0.2-0.8)	0.4(0.2-0.8)
Factor VIII						
<P ₉₀	302	384.8	1(ref)	1(ref)	1(ref)	1(ref)
>P ₉₀	99	46.2	2.7(1.7-4.1)	2.7(1.7-4.1)	3.0(1.9-4.7)	2.9(1.8-4.6)
Factor IX						
<P ₉₀	296.1	344.2	1(ref)	1(ref)	1(ref)	1(ref)
>P ₉₀	104.9	46.8	2.5(1.6-3.9)	2.6(1.6-4.0)	2.4(1.5-3.7)	2.3(1.5-3.7)
Factor XI						
<P ₉₀	335.5	389.1	1(ref)	1(ref)	1(ref)	1(ref)
>P ₉₀	65.5	41.9	1.8(1.2-2.8)	1.8(1.2-2.8)	1.8(1.1-2.8)	1.8(1.1-2.7)

N=number, OR=odds ratio, CI=confidence interval, ref=reference group.

**401 patients and 431 controls for non-vitamin K dependent factors VIII and XI, 401 patients and 391 controls for vitamin K dependent factors II and IX.

Model 1: Adjusted for age and sex

Model 2: Adjusted for age, sex and body mass index(BMI)

Model 3: Adjusted for age, sex and study center

Model 4: Adjusted for age, sex, body mass index(BMI) and study center

Supplemental table 3. The risk of VT associated with increasing number of coagulation factors (Factor VIII, Factor IX and Factor XI, all coagulation factors dichotomized at P₇₅) after imputation of missing data.

Number of elevated factors	Patients N	Controls N	OR overall* (CI95)	OR provoked* (CI95)	OR unprovoked* (CI95)	OR DVT* (CI95)	OR PE±DVT* (CI95)
0	40	101	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
1	72	104.7	1.7(1.0-2.8)	1.8(0.9-3.4)	1.5(0.7-3.1)	1.7(0.8-3.5)	1.6(0.9-3.1)
2	109.2	141.1	2.0(1.2-3.3)	1.8(0.9-3.5)	2.1(1.1-4.3)	2.2(1.1-4.3)	1.8(0.9-3.3)
3	179.8	84.2	5.1(3.1-8.5)	4.5(2.3-8.6)	5.8(3.0-11.2)	4.9(2.5-9.5)	5.2(2.8-9.6)

N=number, OR=odds ratio, CI=confidence interval, VT=venous thrombosis, ref=reference group

*OR adjusted for age, sex, body mass index(BMI) and study center

Supplemental table 4. The risk of VT associated with high coagulation factors FVIII and FIX (coagulation factors dichotomized at P_{75}) after imputation of missing data.

Factor VIII	Factor IX	Patients	Controls	*OR overall (CI95)
-	-	155.1	272.4	1(ref)
-	+	42.8	48.2	1.4(0.8-2.4)
+	-	82.6	56	2.8(1.8-4.4)
+	+	120.7	54.4	3.7(2.4-5.9)

*OR adjusted for age, sex, body mass index(BMI) and study center N=number, VT=venous thrombosis