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Unfavorably altered plasma clot properties in women with a HERDOO2 score equal to or greater than 2 and prediction of recurrent venous thromboembolism

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KEY WORDS

clot, deep vein thrombosis, fibrin, HERDOO2, recurrence

ABSTRACT

INTRODUCTION The HERDOO2 rule can help identify patients in whom anticoagulation can be safely discontinued. Unfavorably altered fibrin clot properties predict recurrent venous thromboembolism (VTE).

OBJECTIVES We aimed to assess a possible association between fibrin clot properties and the HERDOO2 score in women after unprovoked VTE.

PATIENTS AND METHODS Eighty women younger than 70 years after a first unprovoked VTE separately and combined with 32 women after hormone-related VTE were followed for a median of 48.5 months (interquartile range, 37.5–67 years). Plasma fibrin clot permeability (K_s), lysability, turbidity measurements, and thrombin generation were assessed 3 months after the index event in relation to the HERDOO2 score.

RESULTS Nineteen women (23.8%) with a HERDOO2 score equal to or higher than 2 were characterized by lower K_s (–6.8%), indicating formation of more compact clots, impaired fibrinolysis as evidenced by a reduced maximum rate of D-dimer release from clots (D-D_{rate}, by 6.8%), and prolonged clot lysis time (CLT, by 23.8%). No increased thrombin generation or differences in the remaining fibrin clot properties were observed. When combined with estrogen-related VTE, the same trends were observed. D-D_{rate} and CLT correlated with the HERDOO2 score ($r = –0.28$, $P = 0.01$ and $r = 0.35$, $P = 0.002$, respectively) in 80 women with unprovoked VTE. Unfavorable clot phenotype, defined as $K_s \leq 6.55 \times 10^{-9}$ cm^2 and CLT >99.5 minutes, was associated with high risk of recurrence in the HERDOO2 rule ($P = 0.02$).

CONCLUSIONS We showed that middle-aged women after unprovoked VTE with high risk of recurrence based on the HERDOO2 rule are characterized by formation of denser fibrin clots and impaired fibrinolysis.

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INTRODUCTION Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common and potentially life-threatening disease.[1] The risk of recurrence is higher after unprovoked VTE, and is estimated at about 10% at 1 year and 30% at 5 years.[2] In patients with a first unprovoked VTE and low or moderate risk of bleeding, anticoagulant therapy longer than 3 months is recommended (no scheduled discontinuation), while in patients with high bleeding risk 3 months of anticoagulant treatment is suggested.[2] Anticoagulant treatment was reported to prevent VTE recurrence during its use and only to delay recurrence after its discontinuation.[3] Most real-life patients discontinue anticoagulation a few months after the VTE event.[4] Anticoagulation-related bleeding due to lifestyle (eg, alcohol abuse and smoking) may contribute to this phenomenon.[5] It has been reported that the risk of VTE recurrence is

1.75-fold higher in men and 2-fold higher in patients with elevated D-dimer levels.[2]

It is suggested that anticoagulant treatment can be safely stopped if the risk of recurrent VTE is lower than 5% during a year since discontinuation.[6] The prediction of recurrent VTE following discontinuation is still a challenge. Several strategies regarding anticoagulation withdrawal after the first unprovoked VTE were proposed, including the Vienna Prediction Model, the Dash score, or the HERDOO2 rule.[7,8] The latter identifies women at low risk of recurrence (women meeting a maximum of 2 of the following criteria: hyperpigmentation, edema or redness [HER] in either leg, increased D-dimer levels ≥250 ng/ml; obesity with body mass index ≥30 kg/m^2; or older age ≥65 years), in whom anticoagulants can be stopped after 5 to 7 months since an unprovoked VTE event.[8] In a prospective study, Rodger et al[9] observed that the rate of recurrence after discontinuation of anticoagulant therapy was 3% per patient-year (95% CI, 1.8%–4.8%) in low-risk women (HERDOO2 score of 0 or 1) and 7.4% per patient-year (95% CI, 3%–15.2%) in high-risk women. D-dimer levels of 500 ng/ml or lower and peak thrombin generation of less than 400 nM measured after treatment discontinuation have been also proposed to identify patients at low risk of recurrent VTE.[2,8,10]

Evidence indicates that formation of more compact clots, relatively resistant to lysis, mediated by both environmental and genetic factors, may predispose to VTE[11] (particularly unprovoked VTE)[12] and may have a prognostic value in VTE prediction.[13,14] The postthrombotic syndrome and associated recurrent VTE have been reported to be related with prothrombotic plasma clots.[15] To our knowledge, it is unknown whether the HERDOO2 rule is associated with unfavorable fibrin clot properties and increased thrombin generation. For this reason, we sought to assess the association between the HERDOO2 rule and fibrin clot properties assessed 3 months after the VTE event and to investigate whether this rule could be helpful in choosing patients with an increased risk of recurrent VTE in our cohort of women after unprovoked VTE.

PATIENTS AND METHODS

We screened 368 consecutive outpatients (age ≤70 years) after a first unprovoked VTE episode who were referred for further clinical evaluation, including the decision on extended anticoagulant therapy between October 2008 to June 2010. The methodology and patient characteristics were described in our previous report.[13] We excluded patients with known malignancy (n = 12), chronic kidney disease stage 4 or 5 (n = 4), severe thrombophilia, including antiphospholipid syndrome, antithrombin deficiency, and homozygous factor V Leiden or prothrombin 20210A mutations (n = 24), pregnancy (n = 1), international normalized ratio (INR) of more than 1.2 (n = 2), acute coronary syndrome or ischemic stroke within the previous 3 months (n = 2), and acute infection or exacerbated chronic inflammation (n = 3). Men who did not meet the above exclusion criteria (n = 155) were also excluded. Finally, we excluded women with VTE provoked by trauma, hospitalization, or pregnancy/postpartum (n = 55). The current analysis included 112 women with unprovoked and hormone-related VTE. The diagnosis of DVT was confirmed by a positive finding on color duplex ultrasound, and of PE, by positive results on high--resolution spiral computed tomography. Initially, all patients were treated with unfractionated or low-molecular-weight heparin at therapeutic doses, and then vitamin K antagonists (VKAs) were continued for at least 3 months (hormone--related DVT) or 6 months (unprovoked DVT). Unprovoked VTE was defined as the absence of cancer history, surgery under general anesthesia, major trauma, plaster cast or hospitalization in the last month, and pregnancy or delivery in the last 3 months. Hormone-related VTE was defined as VTE associated with the use of oral contraceptives or hormone replacement therapy.

Women were classified into one of the two groups with a high (≥2 points) or low (0 or 1 point) risk of recurrent VTE based on the HERDOO2 rule.[8,9]

The university's bioethical committee approved the study, and all participants provided informed consent in accordance with the Declaration of Helsinki.

Follow-up Patients were followed on a 6-month basis (a visit at the center or a telephone contact). The primary endpoint of the study was objectively documented symptomatic recurrent VTE. The end of follow-up was defined as the date of a recurrent thromboembolic event or death. The minimal follow-up for patients without recurrent VTE was 30 months.

Laboratory investigations Fasting blood samples were drawn at 8 to 10 AM, after 12 to 15 weeks of anticoagulant treatment since DVT diagnosis. Patients treated with VKAs were switched to low-molecular-weight heparin for 10 to 14 days, and blood samples were collected 16 to 24 hours after the last injection. Then, patients continued anticoagulation with VKAs. Blood cell count, lipid profile, glucose, creatinine, and INR were determined by routine laboratory techniques. Fibrinogen was measured by the Clauss method, and C-reactive protein (CRP), by nephelometry (Siemens, Munich, Germany). D-dimer, tissue plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1) antigens were assayed (American Diagnostica Inc., Stamford, Connecticut, United States). Permeation of plasma fibrin clots in the assay involving addition of tissue factor was assessed using a pressure-driven system and presented as $K_{s,}$ as described previously.[16] We recorded a lag phase before the start of fibrin polymerization initiated by adding a Tris buffer containing human

thrombin (Sigma-Aldrich, St. Louis, Missouri, United States) and $CaCl_2$ to plasma samples, slope of the polymerization curve, and the maximum absorbance at plateau.[12,17] Two methods were implemented to assess the efficiency of clot lysis, as described previously.[18,19] The calibrated automated thrombogram (CAT) was performed (Thrombinoscope, BV, Maastricht, the Netherlands).[20] Briefly, plasma samples were mixed with recombinant relipidated tissue factor and phospholipids. A starting reagent contains $CaCl_2$ and fluorogenic substrate. The Fluoroskan Ascent® microplate fluorometer (Thermo Fisher Scientific, Vantaa, Finland) was used to read the fluorescence intensity. The peak thrombin, endogenous thrombin potential, and the time to thrombin peak were assessed. We determined factor V Leiden, prothrombin 20210A, as well as 2 additional mutations, FXIII Val34Leu and α fibrinogen Thr312Ala polymorphisms, as described previously.[12]

Statistical analysis Continuous variables were presented as mean (SD) or median (interquartile range [IQR]), while categorical and qualitative variables, as number (percentage). The normality of variable distribution was checked with the Shapiro–Wilk test. The Fisher exact test, Spearman rank correlation test, Student *t* test, Welch *t* test, and Mann–Whitney test were used as appropriate. The univariate Cox proportional hazards models and logistic regression for risk factors for recurrent VTE were performed and expressed as hazard ratios (HRs) or the odds ratio (OR) with 95% CI. The association between a prothrombotic phenotype and a HERDOO2 score of 2 or higher was analyzed using the χ^2 test. The level of significance for the 2-sided tests was set below 0.05. All data management and analyses were performed using Statistica 13 (StatSoft Inc., Tulsa, Oklahoma, United States) and R package (R Core Team, 2016).

RESULTS A total of 112 women, including 80 women with unprovoked VTE (TABLE 1) and 32 women with hormone-related VTE after a first VTE episode, were enrolled into the study. The mean (SD) age of patients was 44.8 (10.8) years.

The groups did not differ in baseline characteristics except for a higher proportion of DVT combined with PE in patients with unprovoked VTE (37.5% vs 9.4%, $P < 0.001$). Women with a HERDOO2 score of 2 or higher were less commonly treated with statins (TABLE 1).

High risk of recurrent VTE was observed in 19 women (23.75%), and low risk, in 61 (76.25%). High-risk patients were characterized by higher body mass index (by 18%) and higher levels of glucose (8%), triglycerides (52.8%), CRP (58.3%), fibrinogen (10.7%), D-dimer (15.1%) (TABLE 1). Other routine laboratory tests, including tPA, PAI-1, peak thrombin, and endogenous thrombin potential, were similar in both groups.

When women with unprovoked VTE and hormone-related VTE were analyzed together, the same trends were observed between high- and low-risk groups (n = 112; data not shown).

The HERDOO2 rule and fibrin clot properties Among women after unprovoked VTE (n = 80), those classified as high risk according to the HERDOO2 rule had significantly lower K_s (by 6.8%), reduced D-D_{rate} (6.8%), and prolonged CLT (23.8%) compared with the remaining patients (TABLE 1, FIGURE 1), also after adjustment for fibrinogen (except for K_s; $P = 0.22$). After inclusion of women with hormone-related VTE, the same trends were observed. Women treated with statins did not differ from the remaining patients in fibrin clot properties except for shorter CLT (82.6 [16.5] min and 90.8 [17.1] min, respectively, $P = 0.03$).

D-D_{rate} and CLT were correlated with the HERDOO2 score in women after unprovoked VTE ($r = -0.28$, $P = 0.01$ and $r = 0.35$, $P = 0.002$, respectively). We observed a positive association between the HERDOO2 score of 2 or higher and prothrombotic phenotype ($P = 0.02$) in women with unprovoked VTE, but the association was no longer observed after including women with hormone-related VTE in the analysis ($P = 0.07$).

There was no difference in fibrin clot properties and thrombin generation between women with and without 1 point for the HER component (TABLE 2). Patients with D-dimer levels of 250 ng/ml or higher (66.3%) had prolonged time to peak thrombin compared with those with the levels below 250 ng/ml (4.8 [4.3–5.3] min vs 4.2 [3.9–5.3] min; $P = 0.02$). They also had reduced D-D_{rate} and prolonged CLT (TABLE 2). Obese patients (21.3%) had higher ΔAbs, lower D-D_{rate}, and longer CLT compared with the remaining patients (TABLE 2).

Follow-up Three women with unprovoked VTE and 1 woman with hormone-related VTE were lost to follow-up. All patients were followed for a median of 48.5 months (IQR, 37.5–67 months).

In 19 high-risk women after unprovoked VTE (23.75%), 10 recurrent VTE events occurred (1.15% recurrent VTE per patient-year; 95% CI, 0.44%–1.86%). Among 61 low-risk women (76.25%), there were 21 recurrent VTE episodes during follow-up (0.73% recurrent VTE per patient-year; 95% CI, 0.42%–1.04%). Women after unprovoked VTE with a HERDOO2 score of 2 or higher did not have an increased risk of recurrent VTE compared with the remaining patients (OR, 2.12; 95% CI, 0.75–6.01; TABLE 3). The same was observed after the inclusion of patients with hormone-related VTE (OR, 2.26; 95% CI, 0.9–5.67).

The prothrombotic clot phenotype, defined as $K_s \leq 6.55 \times 10^{-9}$ cm^2 and CLT >99.5 minutes, was associated with a higher risk of recurrent VTE separately for women after unprovoked VTE (OR, 5.33; 95% CI, 1.29–22.02) and after the inclusion of hormone-related VTE women (OR,

TABLE 1 Baseline characteristics of women after unprovoked venous thromboembolism depending on the HERDOO2 score (continued on the next page)

Variable	All women (n = 80)	HERDOO2 ≥2[a] (n = 19)	HERDOO2, 0 or 1[b] (n = 61)	*P* value (a vs b)
Age, y, mean (SD)	44.7 (12.1)	44.7 (14.1)	44.7 (11.6)	0.99
BMI, kg/m^2, median (IQR)	26.2 (24.2–29.4)	30.2 (29.7–33)	25.6 (23.8–27)	<0.001
Time of anticoagulation, mo, median (IQR)	12 (11–13.5)	13 (12–14)	12 (10–13)	0.17
Current smokers, n (%)	20 (25)	2 (10.5)	18 (30)	0.13
Family history of VTE, n (%)	11 (13.8)	2 (10.5)	9 (14.8)	0.64
Compression therapy, n (%)	54 (67.5)	16 (84.2)	38 (62.3)	0.07
Comorbidities, n (%)				
Hypertension	26 (32.5)	7 (36.8)	19 (31.1)	0.64
Diabetes	1 (1.3)	0 (0)	1 (1.6)	0.57
Heart failure	1 (1.3)	0 (0)	1 (1.6)	0.57
Medications, n (%)				
Aspirin	11 (13.8)	2 (10.5)	9 (14.8)	0.64
Sulodexide	6 (7.5)	1 (5.3)	5 (8.2)	0.67
ACEI	12 (15)	4 (21.1)	8 (13.1)	0.40
β-blocker	3 (3.8)	2 (10.5)	1 (1.6)	0.07
Statin	40 (50)	5 (26.3)	35 (57.4)	0.02
Laboratory parameters, median (IQR) (unless indicated otherwise)				
Creatinine, µmol/l	69.4 (61–75.5)	68 (61–71)	70.0 (61–78)	0.6
Glucose, mmol/l, mean (SD)	5.1 (0.7)	5.4 (0.7)	5 (0.7)	0.02
Triglycerides, mmol/l	1.13 (0.73–1.61)	1.62 (0.7–2.4)	1.06 (0.76–1.44)	0.04
Total cholesterol, mmol/l	5.05 (4.22–5.65)	4.79 (4.33–5.37)	5.08 (4.2–5.78)	0.25
LDL cholesterol, mmol/l (SD)	3 (0.74)	2.94 (0.66)	3.01 (0.77)	0.69
HDL cholesterol, mmol/l	1.53 (1.24–1.74)	1.4 (1.19–1.78)	1.55 (1.28–1.72)	0.57
C-reactive protein, mg/l	1.3 (0.8–2)	1.9 (1.2–2.5)	1.2 (0.8–1.8)	0.005
International normalized ratio	0.99 (0.9–1.04)	1 (0.95–1.03)	0.98 (0.9–1.05)	0.72
Fibrinogen, g/l	2.8 (2.4–3.6)	2.99 (2.6–4.55)	2.7 (2.4–3.4)	0.04
D-dimer, ng/ml	286.5 (240.5–337.5)	298 (260–451)	259 (232–322)	0.01
TPA, ng/ml	10 (7–11.7)	10.3 (7.8–11.9)	10 (7–11.6)	0.57
PAI-1, ng/ml	12.3 (9–16.9)	12.9 (9.1–16.7)	12.3 (9–16.9)	0.96
Peak thrombin, nM	243.6 (204–302)	279. 3 (219.4–305.4)	240 (201.6–299.6)	0.22
ETP, nM × min	1534.2 (230.2)	1581 (224.6)	1520 (232)	0.31
Time to thrombin peak, min	4.67 (4.21–5.33)	4.6 (4.2–6)	4.7 (4.2–5.3)	0.64
Genotyping, n (%)				
Factor V Leiden	15 (18.7)	3 (15.8)	12 (19.7)	0.99
Prothrombin 20210A	4 (5)	1 (5.3)	3 (4.9)	0.99
Factor XIII Val34Leu	40 (50)	12 (63.2)	28 (45.9)	0.19
α-fibrinogen Thr312Ala	29 (36)	8 (42.1)	21 (34.4)	0.53
Fibrin clot features, median (IQR)				
K_s, 10^{-9} cm^2	7.40 (6.55–8.0)	6.9 (5.9–7.8)	7.4 (6.8–8.10)	0.05
Lag phase, s	41.5 (38.5–45)	40 (35–45)	42 (39–45)	0.27
ΔAbs	0.81 (0.76–0.86)	0.85 (0.79–0.9)	0.81 (0.75–0.85)	0.06
D-D_{max}, mg/l	4.2 (3.7–4.4)	4.31 (3.7–4.59)	4.07 (3.67–4.39)	0.37
D-D_{rate}, mg/l/min	0.071 (0.067–0.078)	0.068 (0.065–0.07)	0.073 (0.069–0.08)	0.001
CLT, min	86.5 (72–99.5)	99 (85–106)	80 (70–98)	0.005

SI conversion factors: to convert time to thrombin peak to seconds, multiply by 60; CLT to seconds, by 60; D-D_{rate} to mg/l/s, divide by 60.

Abbreviations: ΔAbs, difference between maximum and minimum absorbance in the turbidimetric clotting assay; ACEI, angiotensin-converting enzyme inhibitor; BMI, body mass index; CLT, clot lysis time; D-D_{max}, maximum D-dimer levels in the lysis assay; D-D_{rate}, maximum rate of increase in D-dimer levels in the lysis assay; ETP, endogenous thrombin potential; HDL, high-density lipoprotein; HERDOO2, hyperpigmentation, edema or redness in either leg, increased D-dimer levels, obesity, or older age; IQR, interquartile range; K_s, fibrin clot permeability coefficient; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator; VTE, venous thromboembolism

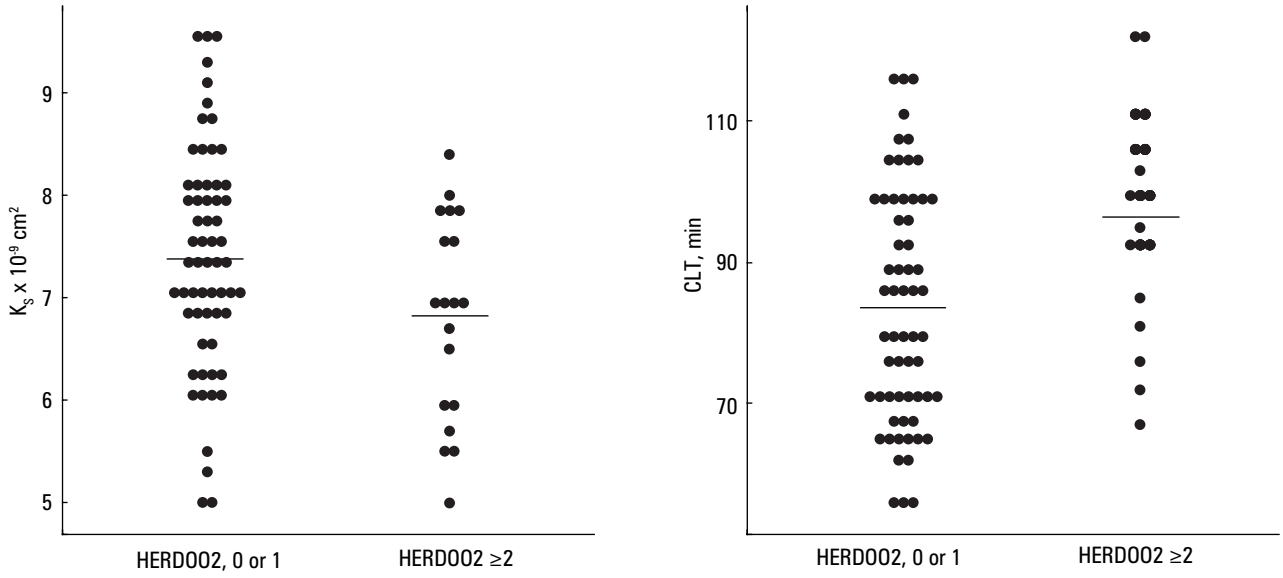

FIGURE 1 Fibrin clot permeability coefficient and clot lysis time (CLT) for patients after unprovoked venous thromboembolism classified as low (0 or 1 point; n = 61) and high risk (≥2 points; n = 19) according to the HERDOO2 rule. Horizontal lines represent means in each group.
Abbreviations: see **TABLE 1**

TABLE 2 Comparison of plasma fibrin clot properties according to the components of the HERDOO2 rule among patients after unprovoked venous thromboembolism

HERDOO2 component	Plasma fibrin clot properties					
	K_s, × 10^{-9} **cm²**	Lag phase, s	ΔAbs	D-D_{max}, mg/l	D-D_{rate}, mg/l/min	CLT, min
HER						
Yes (n = 13)	7 (0.9)	41.6 (6.3)	0.79 (0.76–0.88)	4 (3.7–4.4)	0.072 (0.009)	92 (75–103)
No (n = 67)	7.3 (1.1)	42.1 (5.1)	0.82 (0.75–0.86)	4.2 (3.7–4.4)	0.072 (0.007)	86 (72–99)
P value	0.42	0.75	0.92	0.96	0.89	0.73
D-dimer, ng/ml						
≥250 (n = 53)	7.1 (1.1)	41 (37–45)	0.82 (0.75–0.87)	4.2 (3.6–4.4)	0.071 (0.066–0.074)	92 (77–103)
<250 (n = 27)	7.5 (1.1)	43 (40–45)	0.81 (0.77–0.85)	4.3 (3.8–4.4)	0.076 (0.068–0.081)	75 (70–90)
P value	0.1	0.15	0.98	0.4	0.02	0.003
Obesity						
Yes (n = 17)	6.9 (1.2)	40.5 (4.8)	0.85 (0.79–0.9)	4.3 (4.1–4.4)	0.069 (0.066–0.07)	96.2 (16)
No (n = 63)	7.4 (1.1)	42.5 (5.4)	0.81 (0.74–0.85)	4 (3.6–4.4)	0.072 (0.067–0.08)	84.1 (16.7)
P value	0.13	0.17	0.02	0.13	0.04	0.009

Data are presented as mean (SD) or median (interquartile range).

No comparisons regarding age ≥65 years are shown due to a limited number of patients older than ≥65 years (n = 4).

SI conversion factors: see **TABLE 1**

Abbreviations: HER, hyperpigmentation, edema or redness in either leg; others see **TABLE 1**

6.04; 95% CI, 1.94–18.8). Women with unprovoked VTE compared with hormone-related VTE did not have an increased risk of VTE recurrences (HR, 1.61; 95% CI, 0.74–3.51).

DISCUSSION In this study we showed that unfavorably altered fibrin clot properties, but not thrombin generation, occur in women after a first unprovoked VTE with a HERDOO2 score of 2 or higher. Our findings indicate that abnormal fibrin clot properties, including increased clot density and lysability, could at least partially explain the association between high scores calculated using the HERDOO2 rule and an increased risk of VTE recurrence reported in several studies. We previously demonstrated that the prothrombotic plasma clot phenotype might help identify patients at higher risk of VTE.[13,14] Similar, although less pronounced, alterations were detected in the current analysis. However, we failed to show the predictive value of the HERDOO2 in our cohort of relatively young women. Nevertheless, the association cannot be excluded because of the wide ranges of CI values (separately in women with unprovoked VTE and when analyzed together with women with hormone-related VTE).

TABLE 3 Risk of venous thromboembolism recurrence according to the HERDOO2 components, HERDOO2 rule, and prothrombotic phenotype in women with unprovoked venous thromboembolism (n = 80)

HERDOO2 component	VTE recurrence (n = 31)	No VTE recurrence (n = 49)	OR (95% CI)
HER, n (%)			
No	26 (83.9)	41 (83.7)	Reference
Yes	5 (16.1)	8 (16.3)	0.99 (0.30–3.34)
D-dimer ≥250 ng/ml, n (%)			
No	6 (19.4)	21 (42.9)	Reference
Yes	25 (80.6)	28 (57.1)	3.12 (1.09–8.98)
Obesity, n (%)			
No	23 (74.2)	40 (81.6)	Reference
Yes	8 (25.8)	9 (18.4)	1.55 (0.52–4.56)
Age ≥65 years, n (%)			
No	29 (93.5)	49 (100)	–
Yes	2 (6.5)	0 (0)	–
HERDOO2 score, points, n (%)			
0 or 1	21 (67.7)	40 (81.6)	Reference
≥2	10 (32.3)	9 (18.4)	2.12 (0.75–6.01)
Prothrombotic phenotype			
No	23 (74.2)	46 (93.9)	Reference
Yes	8 (25.8)	3 (6.1)	5.33 (1.29–22.02)
HERDOO2 score ≥2 and prothrombotic phenotype, n (%)			
No	27 (87.1)	47 (95.9)	Reference
Yes	4 (12.9)	2 (4.1)	3.48 (0.6–20.28)

Abbreviations: OR, odds ratio; others, see TABLES 1 and 2

The components of the HERDOO2 rule, including postthrombotic syndrome, increased D-dimer, obesity, and older age, have been reported to be independent risk factors of recurrent VTE.[2,8,21] In the previous study, we demonstrated that the occurrence of postthrombotic syndrome is associated with lower K_s, reduced D-D_{rate}, and longer CLT,[15] and all these parameters predicted recurrent VTE.[13,15] This could explain how the HER item could contribute to the association between prothrombotic phenotype and high risk according to the HERDOO2 rule. We did not observe HER-related differences in fibrin clot characteristics; however, patients who receive 1 point for HER tend to have lower K_s and longer CLT. Moreover, Rodger et al[8] found that HER assessed after 5 to 7 months of oral anticoagulant treatment was the strongest predictor of recurrent VTE. This observation might refer to older women after unprovoked VTE, while the current study was performed in young and middle-aged women.

In our study obese women after unprovoked VTE were characterized by higher ΔAbs and lower D-D_{rate}, while other fibrin clot features were similar in obese and nonobese individuals. It has been observed that switching obese patients to low-fat diet resulted in shorter CLT but no change in K_s.[22] Recently, we reported that both higher ΔAbs and reduced D-D_{rate} were risk factors for recurrent VTE.[13] It has been demonstrated that obese patients had higher concentrations of fibrinogen.[23] This could partially explain why in our study the difference in K_s was no longer observed after adjustment for fibrinogen, a main determinant of clot characteristics,[24] when patients were divided according to the HERDOO2 rule. The current study provides additional evidence for prothrombotic alterations in obesity, including hypofibrinolysis.

The key finding of the present study is slightly lower K_s (by 6.8%) and markedly prolonged CLT (by 23.8%) observed in high-risk women after unprovoked VTE, which supports the concept that the prothrombotic fibrin clot phenotype has important contribution to a prothrombotic state following VTE. Importantly, no evidence of increased thrombin generation using the CAT assay was noted, indicating that another mechanism underlies changes in clot structure and function. Reduced D-D_{rate} (by 6.8%) that was also observed in high-risk women and reflected impaired transport of tPA in fibrin networks and its slower degradation even at higher tPA concentrations confirms that higher HERDOO2 scores are associated with hypofibrinolysis. A similar abnormality has been reported to predispose to residual vein obstruction,[25] which might increase the risk of DVT recurrence.

Patients characterized by a combination of risk factors of recurrent VTE could be predisposed to forming more compact plasma fibrin clots associated with impaired susceptibility to lysis. The correlations between D-D_{rate}, CLT and the HERDOO2 score shown in our study could support the concept that the predictive value of the HERDOO2 rule is partially driven by alterations in fibrin clot properties. However, whether the same alterations can be seen in elderly women after unprovoked VTE remains to be established. In our cohort, women with high HERDOO2 scores were less commonly treated with statins. It has been reported that statins could improve fibrin clot properties[17,26] and could have a beneficial effect on the risk of VTE recurrence.[27,28] We observed faster fibrinolysis in women receiving statins, which supports the role of statins as modifiers of clot properties.

Unlike some other researchers,[8,29] we did not classify hormone- or pregnancy-related VTE as unprovoked in this study, even if the risk of recurrent VTE is increased in women with hormone-related VTE compared with those with VTE provoked by surgery or trauma.[29] We found that women after VTE related to the use of oral contraceptives or hormone replacement therapy did not differ in the risk of VTE recurrences compared with women after unprovoked VTE, which is in line with previous studies.[30,31] However, subjects after VTE related to estrogen use had a lower recurrence risk by 29% compared with nonusers (adjusted HR, 0.71; 95% CI 0.58–0.88) in the study of 4170 women aged 15 to 64 years.[32] Importantly, in women with factor V Leiden mutation, discontinuation of oral contraceptives increased K_s and shortened CLT.[33]

We observed a lower rate of recurrent VTE in women compared with some previous reports on older patient populations.[34] Moreover, we failed to find an increased risk of recurrent VTE in women with a HERDOO2 score of 2 or higher. This observation might result from a few discrepancies between studies. First, the number of individuals in our study was limited. We assessed the HERDOO2 score after 12 to 15 weeks of anticoagulant therapy, while the HERDOO2 rule was originally calculated after 5 to 7 months of anticoagulation.[9] Women in our high-risk group were younger than those reported by Rodger et al[9] (mean age, 44.7 years and 65.5 years, respectively). Low-risk women aged 50 years or older had a higher recurrence rate (5.7/100 patient-years vs 2/100 patient-years).[9] Moreover, we measured D-dimer concentrations using a different assay from that used in the study by Rodger et al.[9] Finally, unlike Rodger et al,[9] we excluded patients with previous provoked VTE. Taken together, the current study suggests that the value of the HERDOO2 rule can be limited among women aged 65 years or younger, even if prothrombotic alterations can be detected in blood drawn from patients at high risk of VTE recurrence using this rule. Still, long-term anticoagulant treatment in patients after unprovoked VTE is recommended,[2] and the HERDOO2 rule is a clinical tool that can help identify younger women who might benefit from long-term anticoagulation.[35]

Several study limitations should be considered. The number of study participants was limited, and we observed a low incidence rate of recurrent VTE events. Moreover, only patients suspected of VTE recurrence based on the signs and symptoms were evaluated by imaging, so asymptomatic VTE events might have been missed. We determined each variable at a single time point, so changes over time of some of them cannot be excluded. All patients after a first VTE episode discontinued VKA treatment during follow-up. It is unclear whether in patients after unprovoked VTE, treatment with non–vitamin K antagonist oral anticoagulants (instead of heparins and VKAs) as well as statin administration may impact the predictive value of the HERDOO2 rule as well as fibrin clot properties.[28,29,36]

In conclusion, we demonstrated that the prothrombotic clot phenotype is associated with a HERDOO2 score of 2 or higher in women after unprovoked VTE. This observation could provide a possible explanation for the predictive value of this scoring system in women after VTE; however, the rule appears to have a limited usefulness in the prediction of recurrent VTE in middle-aged women. It is tempting to speculate that in this female population the assessment of clot density and lysability might be more useful than the HERDOO2 rule. However, large-scale studies are needed to corroborate this hypothesis.

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CONTRIBUTION STATEMENT SM and JC interpreted data and wrote the article; EB performed statistical analysis. AU designed the study, recruited patients, collected data, and approved the article for submission. SM and JC contributed equally to the study. All authors edited and approved the final version of the manuscript.

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