

Thrombotic Variables and Risk of Idiopathic Venous Thromboembolism in Women Aged 45-64 Years

Relationships to Hormone Replacement Therapy

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Key words

Venous thromboembolism, thrombophilia, oestrogens

Summary

Hormone replacement therapy (HRT) has been shown to increase the relative risk of idiopathic venous thromboembolism (VTE) about threefold in several observational studies and one randomised controlled trial. Whether or not this relative risk is higher in women with underlying thrombophilia phenotypes, such as activated protein C (APC) resistance, is unknown. We therefore restudied the participants in a case-control study of the relationship between the use of HRT and the occurrence of idiopathic VTE in women aged 45-64 years. After protocol exclusions, 66 of the cases in the original study and 163 of the controls were studied. Twenty haematological variables relevant to risk of VTE were analysed, including thrombotic states defined from the literature. The relative risk of VTE showed significant associations with APC resistance (OR 4.06; 95% CI 1.62, 10.21); low antithrombin (3.33; 1.15, 9.65) or protein C (2.93; 1.06, 8.14); and high coagulation factor IX (2.34; 1.26, 4.35), or fibrin D-dimer (3.84; 1.99, 7.42). HRT use increased the risk of VTE in women without any of these thrombotic states (OR 4.09; 95% CI 1.26, 13.30). A similar effect of HRT use on the relative risk of VTE was also found in women with prothrombotic states. Thus for example, the combination of HRT use and APC resistance increased the risk of VTE about 13-fold compared with women of similar age without either APC resistance or HRT use (OR 13.27; 95% CI 4.30, 40.97).

We conclude that the combination of HRT use and thrombophilias (especially if multiple) increases the relative risk of VTE substantially; hence women known to have thrombophilias (especially if multiple) should be counselled about this increased risk prior to prescription of HRT. However, HRT increases the risk of VTE about fourfold even in women without any thrombotic abnormalities: possible causes are discussed.

Introduction

In premenopausal women, the risk of venous thromboembolism (VTE) increases during pregnancy and the puerperium (1) and use of combined oral contraceptive pills (2), possibly due to complex effects of oestrogens and progestogens upon haematological variables related to blood coagulation, fibrinolysis and rheology (1, 3). In peri- and postmenopausal women, recent evidence from several epidemiological studies and one clinical trial suggests that use of hormone replacement therapy (HRT) increases the risk of idiopathic VTE about threefold (4-9). The mechanisms are not established, but as with oral contraceptives may reflect complex effects of HRT upon haematological variables (10-12), including activated protein C (APC) resistance (12). Blood coagulation screening has been suggested to identify underlying prothrombotic states (thrombophilias), at least in women considering HRT who have a personal or family history of venous thromboembolism (13). With increasing use of HRT, however, widespread screening would involve major costs for potential benefits which have not been defined.

To identify the associations of prothrombotic states with VTE, and their interactions with HRT for risk of VTE, we studied 20 haematological variables related to thrombotic risk in a follow-up of a hospital-based case-control study of idiopathic VTE in women aged 45-64 years (4).

Subjects and Methods

The original case-control study has been reported previously (4). Briefly, cases were 103 women aged 45-64 years who were admitted to hospital between April 1990 and December 1994 in the old Oxford Regional Health Authority area with a main diagnosis of first-episode of deep vein thrombosis (DVT) or pulmonary embolism (PE); and who had no past history of stroke, myocardial infarction, or certain cancers; or of pregnancy, surgical operation, severe trauma or illness necessitating bed rest for longer than one week in the 6 weeks prior to admission. Hospital controls (178 in number) were recruited from among women admitted to hospital with a diagnosis judged to be unrelated to HRT use in the study age group. Up to two controls were recruited per case, matched by 5-year age group, district of admission and date of admission. Exclusion criteria were as for cases.

For the follow-up study, all surviving cases and controls were invited for interview in their general practitioner's surgery or at home, between June 1995 and July 1996. After explanation and informed consent, a questionnaire was administered which enquired about intercurrent illnesses including VTE, current drug use including HRT and anticoagulants, and smoking habit. Height and weight were recorded as before (4).

20 ml of venous blood was sampled from an antecubital vein and anticoagulated with trisodium citrate (0.11 M: 9:1 v:v) or K_2 EDTA (1.5 mg/ml). K_2 EDTA blood samples were sent to the Glasgow laboratory by first-class post for assays of whole-blood and plasma viscosities and haematocrit as described previously (14), and for extraction of DNA for subsequent genetic analyses.

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Citrated plasma samples were centrifuged the same day, aliquotted and stored locally at –50° C prior to courier transport to the Glasgow laboratory on dry ice. Plasma activities of fibrinogen (Clauss assay); coagulation factors VII, VIII and IX; and coagulation inhibitors (antithrombin, protein C and protein S), were measured as previously described (15); as were the activated partial thromboplastin time (APTT) and activated protein C ratio (12) (a low APC ratio indicates APC resistance). The coagulation activation markers, prothrombin fragment F1+2 and thrombin-antithrombin complexes (TAT) were measured using ELISAs (Behringwerke, Marburg, Germany) (15). Plasma levels of von Willebrand factor antigen (vWF), fibrin D-dimer antigen, tissue plasminogen activator antigen (tPA) and plasminogen activator inhibitor activity (PAI) were measured as previously reported (16). Prothrombin time (PT) and PT-derived fibrinogen were measured in an ACL 300 research coagulometer (Instrumentation Laboratory, Warrington) (17) using the manufacturer's standards.

Statistical analysis. Between the time of the original study (4) and the follow-up study, several cases or controls lost their matched partners, due to non-availability for re-study, or to development of exclusion criteria from the original study (certain cancers, coronary heart disease, stroke, oral contraceptive use; or VTE in controls). Rather than exclude these unmatched women, a stratified, unmatched analysis, was performed. Generalised linear models were fitted, controlling for the matching variables from the initial study: age, area of admission to hospital, and time of diagnosis (4).

Thrombophilias were defined as levels of APC ratio (12), antithrombin, protein C or protein S below the laboratory's 95th percentile for age- and sex-matched controls. Other thrombotic states were derived from the literature as follows: fibrinogen ≥4 g/l (17, 20, 22); factor VIII ≥150 iu/dl (18); the same cut-off point for factor VIII was assigned for vWF, factor VII, and factor IX which have similar population distributions (15), APTT ≤25 s (24), and D-dimer ≥250 ng/ml (24). Obesity (body mass index ≥30 kg/m^2) was also studied in view of its association with VTE in women (25).

The study was approved by local Research Ethics Committees, and written, informed consent was obtained from all subjects.

Results

80 cases and 171 controls from the original case-control study (4) attended for re-study. 14 cases were excluded from analysis because of cancer (2), coronary heart disease (1), or current oral anticoagulant use (11) which affects several assays of thrombotic variables, leaving 66 cases. 8 controls were excluded because of intercurrent VTE (2), cancer (3), coronary heart disease (1) or current OC use (2), leaving 163 controls. In the case group, 18 of 32 HRT users at the time of their thromboembolic event had stopped HRT use at the time of the follow-up study, while 2 of 34 non-users had commenced HRT use. In the control group, the corresponding numbers were 5 of 39 HRT users and 18 of 124 non-users. At the time of re-study, 50 eligible cases were not using HRT, 6 were using combined oral oestrogen and progestogen preparations, 5 were using unopposed oral oestrogen, and 5 were using patches. Of the 163 eligible controls, the corresponding numbers were 111, 21, 20 and 10, plus one woman taking Livial. As in the original study (4), cases had a higher body mass index than controls (mean difference = 1.4 kg/m^2, p = 0.06). There were no significant (p <0.05) differences in smoking habit between cases and controls (data not shown).

We first examined the data to see if we could detect any effect of current HRT use on any of the thrombotic variables. For this purpose, we compared the 50 cases who were not using HRT at the time of blood sampling with the 16 cases who were, and the 111 controls who were not using HRT at the time of blood sampling with the 52 who were. The results are shown in Tables 1 and 2. No statistically significant differences were observed. Current HRT use was thus not taken into account in subsequent analyses.

Table 3 shows the mean levels of thrombotic variables in cases and controls, after adjustment for age, area and date of admission to the study. Cases of VTE had significantly higher activated protein C (APC) resistance (defined as a lower APC ratio) and von Willebrand factor antigen, as well as significantly lower plasma antithrombin activity. Notable but non-significant associations were observed for fibrinogen (PT-derived assay), factor VIII, factor IX, protein C, and D-dimer.

Table 4 shows the association of VTE with defined thrombotic states with the variables treated as dichotomies. The odds ratios are shown adjusted for age, area and date of admission to the study and additionally adjusted for HRT use at the time of the index event as well. It can be seen that inclusion of the last mentioned variable has very little effect on the odds ratios. The strongest association of VTE was with APC resistance (APC ratio ≤2; OR 4.06 [95% CI 1.62, 10.21]; p = 0.003). Low levels of antithrombin (≤90 iu/dl; OR 3.33 [1.15, 9.65] p = 0.03) or protein C (≤80 iu/dl OR 2.93 [1.06, 8.14]; p = 0.04) were also associated with VTE, as were high levels of D-dimer (≥250 ng/ml; OR 3.84 [1.99, 7.42], p = 0.006) and factor IX (≥150 iu/dl; OR 2.34 [1.26, 4.35]; p = 0.007). Non-significant associations (OR 1.40-1.81) were observed for fibrinogen, factor VIII, vWF and APTT. The association with obesity (BMI ≥30 kg/m^2) was also non-significant

Table 1 Mean (95% CI) levels of thrombotic variables among cases of VTE according to use of HRT at time of blood sampling, and in controls according to use of HRT at time of blood sampling. Coagulation factors, coagulation times and APC ratio

	Cases		Controls	
HRT use at time of blood sample	No (n=50)	Yes (n=16)	No (n=111)	Yes (N=52)
Fibrinogen (g/l)				
- Clauss	3.63(3.38,3.89)	3.70(3.20,4.27)	3.49(3.28,3.71)	3.33(3.08,3.59)
-PT derived	3.78(3.48,4.09)	3.63(3.05,4.25)	3.51(3.26,3.78)	3.29(3.00,3.61)
Factor VII (iu/dl)	128(118,138)	130(109,150)	131(123,140)	124(113,135)
Factor VIII (iu/dl)	141(118,168)	170(119,243)	145(132,161)	135(119,153)
vWF (iu/dl)	143(122,167)	146(106,200)	137(122,154)	127(110,146)
Factor IX (iu/dl)	140(124,156)	159(126,196)	140(130,152)	144(131,158)
PT (s)	13.5(13.2,13.8)	13.3(12.6,13.9)	13.3(13.1,13.6)	13.4(13.1,13.7)
APTT(s)	28.2(27.1,29.5)	27.0(24.9,29.4)	27.7(27.0,28.6)	28.0(27.0,29.1)
APC ratio	2.22(2.08,2.38)	2.31(2.02,2.64)	2.39(2.30,2.48)	2.48(2.37,2.61)

	Cases		Controls	
HRT use at time of blood sample	No (n=50)	Yes (n=16)	No (N=111)	Yes (N=52)
Antithrombin (iu/dl)	109(103,115)	117(105,129)	116(112,120)	112(107,118)
Protein C (iu/dl)	110(100,120)	117(96,137)	122(115,129)	120(111,129)
Protein S (%pool)	118(108,129)	116(94,138)	133(124,141)	125(115,136)
F1+2 (pmol/ml)	2.04(1.81,2.29)	1.89(1.44,2.39)	2.08(1.85,2.32)	2.34(2.03,2.66)
TAT (ng/ml)	4.38(3.70,5.19)	4.17(2.96,5.88)	3.86(3.25,4.59)	3.30(2.65,4.12)
D-dimer (ng/ml)	169(133,215)	218(134,356)	157(134,184)	150(123,183)
PAI (%pool)	85.2(75.6,95.9)	72.4(56.5,92.2)	81.8(71.4,93.5)	71.9(60.6,85.1)
tPA (ng/ml)	8.03(7.04,9.17)	6.41(4.91,8.37)	7.63(6.68,8.72)	6.25(5.30,737)
Viscosity (mPa.s)				
- blood	3.04(2.94,3.15)	3.05(2.85,3.26)	3.11(3.00,3.21)	3.03(2.91,3.15)
- plasma	1.333(1.304,1.363)	1.341(1.281,1.404)	1.345(1.321,1.370)	1.314(1.285,1.344)
Haematocrit(%)	43.4(42.6,44.3)	44.1(42.3,45.9)	43.7(42.8,44.6)	43.7(42.6,44.8)

Table 2 Mean (95% CI) levels of thrombotic variables among cases of VTE according to use of HRT at time of blood sampling, and in controls according to use of HRT at time of blood sampling. Coagulation inhibitors, activation markers, fibrinolysis and rheology variables

Table 3 Mean (95% confidence interval) for haematological variables in cases and controls after adjustment for age, area and date of admission to study

	Cases (n = 66)	Controls (n = 163)	p value
Fibrinogen (g/l)			
- Clauss	3.48 (3.30, 3.67)	3.40 (3.28, 3.51)	0.46
- PT derived	3.58 (3.37, 3.80)	3.36 (3.22, 3.50)	0.09
Factor VII (iu/dl)	137 (129, 144)	132 (128, 137)	0.37
Factor VIII (iu/dl)	145 (132, 161)	136 (127, 144)	0.24
vWF (iu/dl)	142 (128, 157)	125 (118, 134)	0.05
Factor IX (iu/dl)	150 (139, 160)	138 (132, 145)	0.07
PT (sec)	13.4 (13.2, 13.6)	13.4 (13.3, 13.6)	0.86
APTT (sec)	27.9 (27.2, 28.7)	28.1 (27.7, 28.6)	0.62
APC ratio	2.34 (2.26, 2.44)	2.46 (2.40, 2.52)	0.04
Antithrombin (iu/dl)	110 (106, 114)	115 (113, 118)	0.03
Protein C (iu/dl)	110 (103, 117)	121 (117, 126)	0.07
Protein S (% pool)	126 (119, 134)	128 (123, 132)	0.79
F1 ± 2 (pmol/ml)	1.97 (1.80, 2.15)	2.00 (1.89, 2.12)	0.76
TAT (ng/ml)	3.79 (3.31, 4.34)	3.84 (3.52, 4.19)	0.87
D-dimer (ng/ml)	160 (138, 185)	140 (128, 153)	0.13
PAI (% pool)	83.3 (73.8, 93.8)	78.4 (72.6, 84.5)	0.41
tPA (ng/ml)	7.79 (6.95, 8.73)	7.09 (6.60, 7.62)	0.18
Viscosity (mPa.s)			
- blood	3.02 (2.94, 3.11)	3.04 (2.98, 3.09)	0.80
- plasma	1.329 (1.308, 1.351)	1.331 (1.318, 1.345)	0.90
Haematocrit (%)	43.3 (42.5, 44.1)	43.7 (43.2, 44.2)	0.33

Note: values shown are back-transformed after analysis on log scale (Clauss fibrinogen, factor VIII, vWF, APC ratio, TAT, D-dimer, tPA, blood and plasma viscosity), square root scale (PT derived fibrinogen, factor IX, F1+2), reciprocal scale (APTT) and after raising to the power 1/10 (PAI)

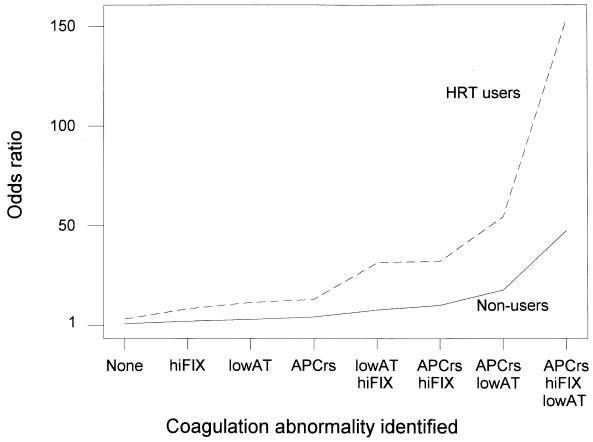

Fig. 1 Odds ratios for risk of idiopathic venous thromboembolism (taking non-users of HRT with no identified coagulation abnormality as the baseline group), in non-users of HRT (solid line) and users of HRT (broken line). hiFIX = high factor IX; low AT = low antithrombin; APCrs = activated protein C resistance

(OR 1.39). No association of VTE was observed with high factor VII or low protein S activities.

101 women had no potential thrombotic risk factor for VTE, taking into account only those factors with a significant effect in Table 4, that is either a "low" APC ratio, antithrombin or protein C level; or a "high" level of factor IX or D-dimer. Of these, 17 were cases (8 were HRT users at the index event) and 84 were controls (20 were HRT users at the index event). The odds ratio for the association of HRT with VTE in this sub-group was 4.09 (95% CI 1.26, 13.30; p = 0.02), very similar to the odds ratio in the whole group (3.37; 1.79, 6.34; p = 0.0002).

Multiple logistic regression analysis was undertaken and the number of thrombotic variables with a significant relationship with VTE was reduced from 5 to 3. Protein C became non-significant given APC ratio (p = 0.18); while D-dimer became non-significant (p = 0.11) given APC ratio and factor IX. A statistical model was developed to predict the odds ratio for VTE for single and combined abnormalities of APC resistance, low antithrombin and high factor IX; in the presence and absence of HRT use at the index event (Fig. 1). In the absence of HRT use, when the baseline risk of VTE was defined as 1 in women without any of the three coagulation abnormalities, the risk rose slightly in the presence of one abnormality; increased considerably in the presence of two abnormalities; and increased still further in the presence of all three abnormalities to an odds ratio of 47.5 (95% CI 8.95, 252). The figure predicts that, at any level of risk attributable to the effects of thrombotic variables, HRT use consistently increases this risk approximately threefold, that is the odds ratios are independent and multiplicative. In the extreme case of HRT use in a woman with APC resistance, antithrombin deficiency, and high factor IX, the predicted odds ratio for VTE is 153 (95% CI 23.5, 1001).

Table 4 Odds ratios for venous thromboembolism when variables are treated as dichotomies

Variable	Cut-point	Number (%)[1] Cases	Controls	Odds ratio (95% conf. interval) Basic adjustment[2]	p value	Adjusted for HRT[3]	p value
Fibrinogen							
- Clauss	≥ 4	16 (25%)	34 (21%)	1.31 (0.65, 2.63)	0.45	1.40 (0.68, 2.89)	0.37
- PT derived	≥ 4	13 (26%)	22 (19%)	1.79 (0.79, 4.06)	0.17	1.81 (0.77, 4.25)	0.17
Factor VII	≥ 150	20 (31%)	49 (30%)	1.12 (0.60, 2.13)	0.71	1.06 (0.55, 2.05)	0.87
Factor VIII	≥ 150	33 (51%)	66 (40%)	1.59 (0.88, 2.88)	0.12	1.67 (0.91, 3.09)	0.10
vWF	≥ 150	30 (46%)	58 (36%)	1.45 (0.80, 2.65)	0.22	1.50 (0.81, 2.80)	0.20
Factor IX	≥ 150	33 (51%)	53 (33%)	2.25 (1.24, 4.10)	0.008	2.34 (1.26, 4.35)	0.007
APTT	≤ 25	13 (20%)	20 (12%)	1.82 (0.84, 4.00)	0.13	1.66 (0.74, 3.73)	0.22
APC ratio	≤ 2	14 (22%)	10 (6%)	4.45 (1.83, 10.82)	0.001	4.06 (1.62, 10.21)	0.003
Antithrombin	≤ 90	9 (14%)	9 (6%)	3.18 (1.17, 8.66)	0.02	3.33 (1.15, 9.65)	0.03
Protein C	≤ 80	9 (14%)	10 (6%)	2.42 (0.92, 6.39)	0.07	2.93 (1.06, 8.14)	0.04
Protein S	≤ 90	7 (11%)	13 (8%)	1.22 (0.45, 3.29)	0.69	1.01 (0.37, 2.80)	0.98
BMI	≥ 30	13 (20%)	29 (18%)	1.17 (0.56, 2.46)	0.67	1.39 (0.64, 3.00)	0.40

[1] At or beyond the cut-point [2] Adjusted for age, area and date of admission to study [3] Adjusted also for HRT status in original study

Discussion

The present report appears to be the first systematic study to investigate the relationships between HRT use, idiopathic VTE, and thrombophilias (defined as changes in haemostatic phenotypes associated with increased risk of VTE) in peri- and post-menopausal women. Our findings that the risk of idiopathic VTE is increased in such women by either activated protein C (APC) resistance, or low antithrombin or protein C activity, are consistent with those of the Leiden Thrombophilia Study, a case-control study of men and women aged 16-73 years (21, 23), as well as with those of other epidemiological, family and genetic studies (26). Positive but non-significant trends for an association of VTE with high fibrinogen or factor VIII/vWF levels were observed, which are consistent with results from the Leiden Study (21, 22). We observed no association of low protein S or high factor VII with VTE, as in the Leiden Study (21, 22).

We report two novel laboratory associations of idiopathic VTE: high levels of coagulation factor IX, and of fibrin D-dimer. The association of high factor IX levels with VTE is biologically plausible, because of the association of VTE and laboratory evidence of hypercoaguability following infusion of prothrombin complex concentrates in factor IX-deficient haemophiliacs (27). Fibrin D-dimer is a marker of increased turnover (formation and lysis) of cross-linked fibrin, which is associated with incident venous (24) and arterial (16) thrombosis. However the association of D-dimer with VTE became non-significant after allowing for the effects of APC resistance and factor IX. Elevated D-dimer levels are normalised by warfarin, which lowers levels of vitamin-K-dependent coagulation factors including factor IX (28, 29); hence it is possible that D-dimer levels are a marker of other hypercoagualable states (APC resistance, high factor IX) or of previous VTE.

Our prediction from the statistical model that the risk of idiopathic VTE increases with combined APC resistance and low antithrombin accords with the increased risk observed in persons with combined relevant genetic abnormalities (26). The model also predicts that the combination of either of these coagulation inhibitor deficiencies with high factor IX levels increases risk of VTE. In accordance with this prediction, infusion of prothrombin complex concentrates in patients with liver failure (who have low plasma antithrombin levels) was associated with a high risk of thrombosis (30). The combination of high factor IX with deficiencies in both coagulation inhibitor systems (APC resistance plus antithrombin deficiency) is predicted to carry a very high risk of VTE; however data to substantiate this high predicted risk will be difficult to obtain because of the rarity of this combination of risk associations.

What is the relevance of these associations of prothrombotic states with VTE to prescription of HRT in women aged 45-64 years? Our data suggest that the effect of HRT use is to increase, approximately three- to fourfold, the *relative* risk of VTE, *regardless* of underlying prothrombotic states. This implies that the *absolute* risk of VTE will be strongly affected by the presence of underlying prothrombotic states, especially if multiple.

The Leiden Study observed a multiplicative effect of oral contraceptive use and the factor V Leiden mutation (an important determinant of APC resistance) on risk of venous thromboembolism (odds ratio 34.7; 95% CI 7.8-154) compared with women without this mutation who were not users of oral contraceptives (23). In the present study, we have found a similar multiplicative effect of HRT use and APC resistance in peri- and post-menopausal women (odds ratio 13.27; 95% CI 4.30, 40.97) compared with women without this phenotype who were not users of HRT. It has recently been reported from the Leiden Study that the APC resistance phenotype is associated with DVT, independently of the factor V Leiden mutation (31). Likewise, the ECAT DVT study observed that the APC resistance phenotype was an independent predictor of DVT after hip replacement surgery, on multivariate analysis (32).

In studies of biological effects, HRT has been associated not only with increased APC resistance (12); but also with lower plasma antithrombin levels (10, 11). Recently, Bloemenkamp et al. (33) reported that in women with a history of DVT, current *oral contraceptive* use was associated with more pronounced effects on APC resistance, antithrombin, protein C and factor VII levels than in women without a history of DVT. In the present study, we did not observe significant differences between VTE cases and controls in the effects of current HRT use on any thrombotic test studied (Tables 1 and 2). It must be stressed, however, that the present study was small and had little power to detect small differences.

We observed that HRT increased the risk of idiopathic VTE approximately fourfold in women without any of the thrombotic abnormalities we took into consideration. This finding suggests that HRT use is not simply precipitating VTE in predisposed, thrombophilic women, but is exerting a more general effect on risk of VTE, at any level of thrombotic risk factors. Possible mechanisms include venodilatation, which has

been associated with risk of postoperative DVT (34). It is interesting to note that VTE is commoner in premenopausal women than in men of similar age (35), and to speculate that the "increased risk" of VTE with HRT may be not so much an adverse effect of a drug, but rather a perpetuation of the premenopausal oestrogenic state. From the viewpoint of total cardiovascular risk, such a state may in general confer a threefold increased risk of VTE, which would be outweighed by the one-third decreased risk of myocardial infarction observed in epidemiological studies if this effect can be confirmed in controlled trials. Nevertheless, our findings suggest that the absolute risk of VTE may be high in women with certain thrombophilias who use HRT: routine counselling on relative thrombotic risks in such women may have to be modified. We suggest that further studies be performed to determine more precisely the interactions of HRT use and predisposing factors for VTE: we are currently studying the influence of relevant genotypes, as well as the phenotypes reported in the present paper. The relative thrombotic risks of HRT and effects of different HRT preparations should also be studied, as well as the possible benefits, risks and costs of coagulation screening prior to HRT prescription. At present, it appears reasonable to confine such screening to the minority of women (under 1%) who have a past history or family history of VTE (13).

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Conflicts of Interest

MPV holds research consultancies with Novo Nordisk Pharmaceuticals (Kliofem) and Eli Lilly and Company Ltd (EVISTA).

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